# BEHAVIOR CHANGES IN GRAZING DAIRY COWS DURING THE TRANSITION PERIOD ARE ASSOCIATED WITH RISK OF DISEASE

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# ABSTRACT

There is growing interest in the use of behavior data derived from accelerometers as a potential measure of animal health, however, research determining the optimal use of these devices and the interpretation of data derived from them, is lacking, particularly in grazing systems. The aims of this thesis were to understand: 1) data management considerations that need to be taken into account when using accelerometer devices to measure behavior in a research setting; 2) environmental and other potentially-confounding variables that can influence cow behavior and, therefore, the interpretation of behavior data; 3) 'normal' behavior of clinically-healthy grazing dairy cows during the transition period, and; 4) changes to behavior of grazing dairy cows experiencing varying degrees of hypocalcemia and hyperketonemia. To do this, data from 4 separate parent experiments were collated to generate a database containing detailed phenotype data, including, but not limited to, measures of cow performance (e.g., milk production and composition, body weight and body condition score), cow health (e.g., energy and protein metabolites, minerals, liver enzymes, and immune markers in blood), and cow behavior (e.g., lying behavior and activity derived from triaxial accelerometers).

My review of the appropriate use of leg-mounted accelerometers to monitor lying behaviors of dairy cows indicated that applying editing criteria to remove errors in lying behavior data caused by erroneous movements of the leg (e.g., scratching and kicking) can improve the accuracy of data derived from accelerometers for recording daily lying bouts (LB); however, has little to no impact on the accuracy of lying time. Lying behavior data must be edited using a suitable LB criterion where the interest is in studying both lying time and LB.

My results indicated that inclement weather, parity, and physiological state are important variables that influence behavior in their own right and must be considered in subsequent analyses. Interestingly, when comparing my results with lying behaviors previously reported in housed cows, my results indicated that grazing dairy cows engage in similar lying behaviors to housed cows before and at the time of calving, while postcalving, grazing cows spend less time lying. Furthermore, grazing dairy cows displayed greater behavioral synchrony (i.e., cows engaged in the same behaviors simultaneously) compared with reports in housed cows. These postcalving differences highlight the importance of assessing behavior within the farming system of interest. My results also indicated that cows alter their behavior in response to ill health, whereby grazing dairy cows experiencing clinical hypocalcemia (without paresis) and hyperketonemia [with severe negative energy balance (NEB)] altered their behavior before, at the time of, and after disease diagnosis compared with healthy cows.

My results indicated that behavioral differences between cows classified into 3 blood calcium groups [clinically-hypocalcemic (without paresis), subclinically-hypocalcemic, and normocalcemic] were transient. On the day of calving, clinically-hypocalcemic cows (without paresis), were less active, spent more time lying, and had more frequent LB compared with subclinically-hypocalcemic and normocalcemic cows; however, changes in behavior were short lived and were no longer present by 2 d postcalving. My results indicate that observed differences in behavior associated with hypocalcemia are small and may not be biologically significant as a metric to discriminate between hypocalcemic and normocalcemic cows. On the contrary, changes in behavior over time and within cow may allow differences between hypocalcemic and normocalcemic cows to be more easily discerned than using mean

values of lying behavior and activity at a specific time point. My findings indicated that a relative increase in the number of steps taken within cow compared with a baseline period 2 wk precalving was positively associated with blood calcium concentrations postcalving.

Further, my results indicated the behavioral differences between cows classified into 3 energy status groups [Hi–Hi = high non-esterified fatty acids (NEFA) and high β-hydroxybutyrate (BHB); Hi–Lo = high NEFA and low BHB, and; Lo–Lo = low NEFA and low BHB] occurred up to 2 wk before calving. During the 2 wk before calving, cows identified as Hi–Hi were more active, spent less time lying, and had fewer LB than the other 2 energy status groups. Interestingly, similar to the hypocalcemia work, my results indicated that a relative increase in the number of steps taken within cow during the 2 wk before calving was associated with lower odds of developing hyperketonemia with NEB; therefore, greater increases in activity before calving were associated with improved health outcomes postcalving in both studies. My results suggest that relative changes in behavior, in particular, step activity, might be an improved metric to discriminate between clinically-healthy grazing cows and cows experiencing a subclinical metabolic disease.

My research provides an improved understanding of the associations between cow behavior and health, particularly for grazing dairy cows. This information provides a base for further exploring the potential for behavior and activity measures to identify cows experiencing ill health during the transition period. Future work should focus on continuing to improve our understanding of associations between behavior and disease, particularly in grazing dairy cows. Using within-cow behavior measures and determining how these data could be interpreted so that farmers could be alerted to sick animals and make actionable decisions on farm, should be the focus of future studies.

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"If it doesn't challenge you, it won't change you".

– Fred DeVito

I give much of my success during my PhD to the belief that any challenge I faced was an opportunity for personal growth and learning. Failure is not evidence of unintelligence or a judgement of character, but an opportunity to develop intelligence and resilience. While I am proud of the person I have become in the journey to obtaining my PhD, I will be forever grateful to the people that have supported me during my life and academic journey.

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# PREFACE – THESIS LAYOUT, FORMATTING, AND PUBLISHING

I have undertaken this thesis in the form of publishable experimental chapters, using the format of *PhD by Publication*. Each of the chapters reports on a distinct aspect of the research topic. At the end of the dissertation, my inductive and deductive reasoning are integrated in the General Discussion (Chapter 9).

Chapters have either been published or prepared for submission; therefore, some repetition of methodology and discussion occurs. Chapters reproduced from a journal article, including the current publication status, are described on the title page of each chapter. I have outlined the layout of the chapters below. Formatting of the Chapters is according to the *Journal of Dairy Science* publishing requirements and the language used throughout this dissertation is American English, because most of the Chapters were prepared for submission to this journal. Chapters 4 and 5 were prepared for journals with different formatting requirements, including the use of UK English. For consistency, Chapters 4 and 5 have been formatted according to the *Journal of Dairy Science* and converted to American English for this dissertation.

All publications relating to the chapters of this thesis are summarized below.

**Chapter 1. General Introduction**

**Chapter 2. *Biological Literature Review*: Understanding dairy cow behavior**

**Chapter 3. Evaluating the appropriate use of wearable accelerometers in research to monitor lying behaviors of dairy cows.** S. J. Hendriks, C. V. C. Phyn, J. M Huzzey, K. R. Mueller, S-A. Turner, D. J. Donaghy, and J. R. Roche.

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**Chapter 5. Effect of weather on activity and lying behaviour in clinically healthy grazing dairy cows during the transition period.** S. J. Hendriks, C. V. C. Phyn, S-A. Turner, K. R. Mueller, B. Kuhn-Sherlock, D. J. Donaghy, J. M. Huzzey, and J. R. Roche.

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**Chapter 7. Associations between lying behavior and activity and hypocalcemia in grazing dairy cows during the transition period.** S. J. Hendriks, J. M. Huzzey, B. Kuhn-Sherlock, S-A. Turner, K. R. Mueller, C. V. C. Phyn, D. J. Donaghy, and J. R. Roche.

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**Chapter 9. General Discussion**

**Appendices: 1–20.**

**Appendix 1. *Invited Keynote Speaker:* The use of precision technologies to predict calving and ill health in transition dairy cows.** S. J. Hendriks, C. V. C. Phyn, S-A. Turner, K. R. Mueller, B. Kuhn-Sherlock, D. J. Donaghy, J. M. Huzzey, and J. R. Roche.

Presented at *Australia and New Zealand College of Veterinary Scientists Science Week: Cattle Chapter*, Gold Coast, Australia on 4 July 2019 and Abstract Published in *Proceedings of the New Zealand and Australia College of Veterinary Scientists: Cattle Chapter*, 2019. Page 21.

Analyses undertaken for Chapters 4, 5, 6, 7, and 8 were completed using an existing historical database. The data were provided by DairyNZ Ltd. from 4 parent experiments that were previously undertaken under the supervision and guidance of Prof. J. R. Roche, Dr. S. Meier, and Dr. C. V. C. Phyn. Many thanks to the DairyNZ Lye and Scott Farm staff and DairyNZ technical staff for their support in the conduct of the parent experiments.

I collated the data from these 4 parent experiments into a single database for my analyses. I undertook the coding, editing, and summarizing of the original behavior data at California Polytechnic State University (San Luis Obispo, CA) under the guidance of Asst. Prof. J. M. Huzzey. I also undertook the exploratory and statistical analyses under the guidance of Dr. B. Kuhn-Sherlock. I undertook and completed all experimental design work, interpretation and analysis of data, interpretation of results, and manuscript write-up, with support from Doctors J. M. Huzzey, K. R. Mueller, B. Kuhn-Sherlock, S-A. Turner, D. J. Donaghy, C. V. C. Phyn, and J. R. Roche.

The reference list is a combined reference list for all chapters and appendices to minimize unnecessary repetition and the formatting of the reference list is according to the instructions for authors outlined by the *Journal of Dairy Science*. To explain the progression of the thesis ideas and for ease of the reader, summary paragraphs conclude each chapter.

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# COMMON ABBREVIATIONS

The unrestricted use abbreviations are used without definition throughout this dissertation; the unrestricted use elsewhere abbreviations are defined in the abstract and are used without definition elsewhere. The author-derived abbreviations are defined in the text in parentheses in bold font. The unrestricted use elsewhere and author-derived abbreviations are defined in the captions for tables and figures or in numbered footnotes below tables. Abbreviations are re-defined in each chapter.

***Unrestricted Use***

| | |
|---|---|
| ANOVA | Analysis of variance |
| IL | Interleukin |
| $NAD^+$/NADH | Nicotinamide Adenine Dinucleotide (Oxidized/Reduced) |

***Unrestricted Use Elsewhere***

| | |
|---|---|
| BCS | Body Condition Score |
| BHB | β-hydroxybutyrate |
| BW | Body Weight |
| CI | Confidence Interval |
| CoA | Coenzyme A |
| CP | Crude Protein |
| DIM | Days in Milk |

| | |
|---|---|
| DM | Dry Matter |
| DMI | Dry Matter Intake |
| EBV | Estimated Breeding Value |
| ECM | Energy-Corrected Milk |
| SCC | Somatic Cell Count |
| TMR | Total Mixed Ration |

***Author-Derived***

| | |
|---|---|
| AGR | Albumin:Globulin Ratio |
| AIC | Akaike Information Criterion |
| ALB | Albumin |
| AMS | Automatic Milking System |
| AST | Aspartate Aminotransferase |
| BrW | Breeding Worth |
| CBW | Calf Birth Weight |
| CK | Clinical Ketosis |
| GDH | Glutamate Dehydrogenase |
| GLO | Globulin |
| Hp | Haptoglobin |
| HYK | Hyperketonemia |

| | |
|---|---|
| HF | Holstein-Friesian |
| HF x J | Holstein-Friesian Jersey Crossbred |
| J | Jersey |
| $k_g$ | efficiency of energy use for the gravid uterus |
| $k_{GD}$ | efficiency of energy use for weight gain in dry cows |
| $k_{GL}$ | efficiency of energy use for weight gain in lactating cows |
| $k_L$ | efficiency of energy use for the synthesis of milk |
| LB | Lying Bout |
| LSM | Least Squares Means |
| $\rho_c$ | Lin's Concordance Correlation Coefficient |
| PC | Principal Component |
| PCA | Principal Component Analysis |
| PMNC | Polymorphonucleated Cells |
| PW | Production Worth |
| ME | Metabolizable Energy |
| NEB | Negative Energy Balance |
| NEFA | Non-Esterified Fatty Acids |
| NSAID | Nonsteroidal Anti-Inflammatory Drugs |
| ROS | Reactive Oxygen Species |

| | |
|---|---|
| $r_p$ | Pearson Correlation Coefficient |
| $r_s$ | Spearman Rank Correlation |
| $R^2$ | Coefficient of Determination |
| SCK | Subclinical Ketosis |
| SD | Standard Deviation |
| Se | Sensitivity |
| SE | Standard Error |
| SED | Standard Error of the Difference |
| Sp | Specificity |
| TAC | Total Antioxidant Capacity |
| TAG | Triacylglyceride |
| TP | Total Protein |

## UNITS AND TERMS

| | |
|---|---|
| calcium | Ca |
| calorie (gram) | cal |
| celsius | °C |
| centimeter(s) | cm |
| chloride | Cl |
| crossed with, times | x |
| day(s) | d |
| gram(s) | g |
| gravity | *g* |
| hour(s) | h |
| joules | J |
| kilo | k (prefix) |
| kilocalorie(s) | kcal |
| kilogram(s) | kg |
| liter(s) | L |
| Mega | M (prefix) |
| magnesium | Mg |
| meter(s) | m |

| | |
|---|---|
| meters, square | $m^2$ |
| micro | µ (prefix) |
| milli | m (prefix) |
| milliliter | mL |
| millimolar (concentration) | m*M* (= mmol/L) |
| millimole (mass) | mmol |
| minute(s) | min |
| molar (concentrations) | *M* |
| n | number of records(animals) |
| no. | number |
| pico | p (prefix) |
| picogram(s) | pg |
| probability | *P* |
| revolutions per minute | rpm |
| second(s) | s |
| sodium | Na |
| unit(s) | U |
| week(s) | wk |
| year(s) | yr |

# CHAPTER 1. GENERAL INTRODUCTION

The transition period is the 6 wk encompassing calving (i.e., 3 wk before and after calving; Grummer, 1995; Drackley, 1999). Approximately 75% of dairy cow diseases occur in the first month after calving (Ingvartsen, 2006; LeBlanc et al., 2006). These diseases can have detrimental impacts on cow performance, lifetime productivity (Fourichon et al., 1999; Rajala-Schultz et al., 1999), and animal welfare (von Keyserlingk et al., 2009), and often result in economic losses due to lost production, increased labor and treatment costs, and cow mortality (Beaudeau et al., 2000). In addition to the on-farm impacts, failure to address animal health and welfare issues is creating poor consumer perceptions about the dairy industry (Robbins et al., 2016), in particular, where it leads to premature mortality and culling (Beaudeau et al., 2000; De Vries et al., 2010; Compton et al., 2017). Poor consumer perceptions are a growing issue as consumers are becoming increasingly interested in how their food is produced (Cembalo et al., 2016). The attitudes of consumers towards animals supports that the dairy industry must commit to ensuring the optimal treatment of animals under their care (Robbins et al., 2016; Weary and von Keyserlingk, 2017). As a result, researchers are investigating ways to improve on-farm health management and the treatment of animals by developing tools that could assist in the identification of sick animals, with a focus on the transition period. One area of interest to both farmers and researchers is the use of precision monitoring tools that can record the behavior of individual animals and provide information on that animals' state of health.

Historically, health management has focused on the identification of sick animals through visual behavioral cues, known as sickness behaviors, to diagnose and treat disease (Petersson-Wolfe et al., 2017). Behavioral cues rely on skilled farm staff with

sufficient time to observe animals; therefore, early and correct identification of sick animals using behavioral cues can be difficult. Identifying sick animals may be particularly difficult in large herds common to many countries including New Zealand, Australia, and North America and more recently, the Middle East, China, and South America (Roche et al., 2017b; Whitlock et al., 2017; Beggs et al., 2018). In these situations, staff are often less trained in animal husbandry and have limited time to monitor individual cows (Bewley et al., 2001; Schulze et al., 2007). Therefore, a proactive approach to health management using behavior-monitoring technology to identify sick animals is of significant interest to farmers (Bewley et al., 2017).

The ability to monitor individual animals through behavior-recording technologies also moves away from the more traditional approach of group management of animals, and instead, focuses on monitoring individual animals (Schulze et al., 2007). The data recorded from these monitoring technologies are meaningless, however, unless transformed into interpretable information that farmers can use to identify that an individual is sick or at risk of becoming sick (Rutten et al., 2013). Consequently, an expanding body of literature is developing our understanding of the associations between behavior and disease and how behavior may have the ability to be used to identify sick animals (Weary et al., 2009).

Behavior-monitoring technology has the potential to improve the efficacy of the diagnosis, treatment, and management of sick animals; but first, successful on-farm implementation of such technologies requires quantitative research to improve our understanding of behavior changes that occur due to illness (Weary et al., 2009; Proudfoot and Huzzey, 2017). Since the early 2000s, associations between behavior and disease have been investigated for dystocia, clinical and subclinical ketosis, lameness, mastitis,

endometritis, and subclinical hypocalcemia in housed cows (Petersson-Wolfe et al., 2017; Proudfoot and Huzzey, 2017). Nevertheless, despite this increase in knowledge, information specific to grazing dairy cows is limited.

This PhD research aims to understand the 'typical' behavior of grazing dairy cows and associations between behavior and disease during the transition period in grazing dairy cows, to determine whether measures of behavior could be useful to discriminate between clinically-healthy and subclincially-ill cows during this time.

# CHAPTER 2. *LITERATURE REVIEW*: UNDERSTANDING DAIRY COW BEHAVIOR

## 2.1 HISTORY OF THE RECOGNITION OF ANIMAL BEHAVIOR

'Behavior' is the cognitive and innate actions of an animal, or group of animals, in response to internal (physiological) or external (environmental) stimuli, motivated by the animals' need to survive (Hart, 1988). Since the dawn of animal agriculture, the caretakers of animals have learned to detect disease and observe normal and abnormal behaviors (Weary et al., 2009). Publications by Aristotle (~300 BC) included detailed information about observations of, and ideas about, animal behavior. The ancient Greeks and Aristotle observed abnormal behaviors presented as a result of disease (Hart, 1988). Further, Hippocrates (~400 BC) wrote about and described these behaviors and provided precise accounts of the fever response ('The Book of Prognostics'; Treatise: "On the Sacred Disease"; Atkins, 1984). These accounts indicate that the fever response has long been known to man, although until the late 1980s was seen as a simple sign of undesirable debilitation as a result of disease (Hart, 1988).

Benjamin L. Hart first summarized a variety of research findings in a review article to argue that the fever response is part of a complex survival adaptation essential for the animal to overcome a pathogen challenge (Hart, 1988). Since then, researchers have confirmed that the fever response is a highly-coordinated physiological response, where the immune system communicates directly with the brain to elicit a behavioral response to improve the likelihood that an animal will combat disease (Hart, 1988; reviewed by Dantzer and Kelley, 2007).

The observation of sickness behaviors typically occurs at the time of or after the animal has been diagnosed with a clinical or subclinical illness or infection (Weary et al., 2009); although, recent studies have identified changes in behavior that occur before the diagnosis of illness or infection and Proudfoot and Huzzey (2017) ascribed these behaviors as 'early indicators of disease'. Common sickness behaviors include reduced appetite, depression, lethargy, restlessness, discomfort, and fever (Hart, 1988). More recently, researchers have begun to explore changes in feeding, exploratory, social, and sexual behaviors, as sickness behaviors and early indicators of disease (Huzzey et al., 2007; Proudfoot and Huzzey, 2017; Sahar et al., 2020).

All common farm animals are stoic because they are a prey species and typically mask signs of vulnerability (Weary et al., 2009); therefore, sick animals may display subtle changes in behavior, especially if the illness makes them an easier target for predation (Fitzpatrick et al., 2002; Weary et al., 2009), or the behavior is influenced by the severity of the disease (Sepúlveda-Varas et al., 2014). Furthermore, identifying sick animals requires highly-skilled staff with sufficient time to observe animals (Verkerk and Hemsworth, 2010); therefore, the rising availability of wearable behavior-monitoring technologies are encouraging research in the field of animal ethology, to improve our understanding of sickness behavior and potential uses for behavior to identify disease (Weary et al., 2009).

## 2.2 TYPICAL LYING BEHAVIOR AND ACTIVITY IN DIFFERENT LIFE STAGES OF DAIRY COWS

Dairy cows are highly motivated to lie and will trade-off other behavioral activities to maintain consistent lying times during the day (Munksgaard et al., 2005).

Lying is an important component of cow comfort and health and an indicator of welfare (Munksgaard and Simonsen, 1996; von Keyserlingk et al., 2012). Changes to an animal's physiological state and management related changes across all life stages (Huzzey et al., 2005; Munksgaard et al., 2005), alongside large within-cow variation, can influence their behavior (von Keyserlingk et al., 2012). Cow, farm, and management related factors are discussed later in this review (see 2.3 Factors Affecting Lying Behavior and Activity).

The transition period, the calving event, early, mid-, and late lactation and the dry period are key life stages of a dairy cow and are defined under each sub-heading in this review. To improve our understanding of what constitutes 'typical' behavior, lying behavior and activity across different life stages in dairy cows, particularly during the transition period, have been investigated. In the following section, I compare lying behavior and activity for dairy cows in housed and grazing systems during each life stage.

### *2.2.1 Transition Period*

The transition period of dairy cows is the 3 wk before and after calving (Grummer, 1995; Drackley, 1999). During the transition period, dairy cow behavior and changes in behavior appear to be most marked compared with other life stages. The change from a nonlactating to a lactating state that occurs during the transition period, including the calving event itself, appear to influence these changes in behavior (Grummer, 1995; Noakes et al., 2001; Huzzey et al., 2005). Increased physiological, metabolic, and nutritional demands imposed (Grummer, 1995; Drackley, 1999) and the multitude of management related changes experienced during this time (Huzzey et al., 2005; Calderon and Cook, 2011; Sepúlveda-Varas et al., 2014), may influence the changes in behavior during this period. Studies investigating behavioral changes during the transition period have largely focused on changes occurring immediately before, on the day of, and after

the calving event. In this section, I describe the behavior during the transition period (e.g., –21 to –3 d pre- and 3 to 21 d postcalving) in both housed and grazing dairy cows. Further, I describe the changes in behavior that occur on the day of calving and 2 d pre- and postcalving in a later section (see 2.2.2 Calving Event).

***Lying Time.*** Daily lying times (h/d) for housed cows varies, but reported values range from 10.5 to 13.5 h/d precalving and 9.35 to 11.1 h/d postcalving (Huzzey et al., 2005; Chapinal et al., 2009; Proudfoot et al., 2009a; Proudfoot et al., 2010; Calderon and Cook, 2011; Borchers et al., 2017; Piñeiro et al., 2019). To my knowledge, only 2 studies have investigated precalving lying behavior in dairy cows kept on pasture, although, cows were fed TMR (Black and Krawczel, 2016; Rice et al., 2017), and 1 study has investigated postcalving lying behavior in dairy cows grazing pasture (Sepúlveda-Varas et al., 2014). Precalving daily lying times for dairy cows on pasture have been reported by Black and Krawczel (2016) and Rice et al. (2017). Both studies reported a mean precalving lying time of ~10.3 h/d, while Sepúlveda-Varas et al. (2014) reported that postcalving, daily lying times range from 7.50 to 8.50 h/d and are lower than values reported in housed cows. These differences may be due to different demands on the time budgets of cows under different systems (Proudfoot and Huzzey, 2017). These demands could be a result of access to resources that require more travel and increased walking, such as water and feed, or time spent actively grazing, including feed accessibility and prehension (Munksgaard et al., 2005). The increased daily activity reported in nonlactating cows on pasture compared with nonlactating housed cows supports this hypothesis (Black and Krawczel, 2016).

Studies in cows kept in groups indoors have reported a decrease in daily lying time from the pre- to postcalving period by ~1 to 3 h/d (Huzzey et al., 2005; Proudfoot et

al., 2010; Piñeiro et al., 2019). In lactating cows, differences in the time associated with milking (Tucker et al., 2007a), and milking-related activities, place demands on the time budgets of cows that nonlactating cows do not experience, which would affect the time available for lying. Therefore, the onset of lactation may explain the differences in lying time observed in lactating compared with nonlactating cows (Huzzey et al., 2005; Kok et al., 2017; Proudfoot and Huzzey, 2017). Milking-related activities may place greater demands on the time budgets of grazing dairy cows, where cows typically walk long distances to and from the milking parlor compared with housed cows (Beggs et al., 2018). But it is not possible to determine whether this is the case, due to a lack of descriptive information available about the typical behavior of dairy cows grazing pasture, both pre- and postcalving.

***Lying Bouts.*** A lying bout (**LB**) is the period between 2 standing events. The number of daily LB (no./d) for housed cows varies, but has been reported to range from 9.5 to 12.4 no./d precalving and 10.5 to 13.1 no./d postcalving (Huzzey et al., 2005; Chapinal et al., 2009; Proudfoot et al., 2010; Calderon and Cook, 2011; Borchers et al., 2017; Piñeiro et al., 2019). In cows on pasture and fed TMR, Rice et al. (2017) reported a mean number of precalving daily LB of 10/d, while Sepúlveda-Varas et al. (2014) reported a range of postcalving daily LB values from 8.4 to 9.7 no./d in dairy cows grazing pasture (Sepúlveda-Varas et al., 2014). Although Black and Krawczel (2016) did not report daily LB values for cows kept on pasture and fed TMR in the text, the values presented in figures pre- and postcalving, appear similar to those reported by Sepúlveda-Varas et al. (2014) and Rice et al. (2017). Lying bouts appear to be similar in housed and grazing cows.

Most studies investigating lying behavior reported daily lying time and number of LB; although, due to the close association of these 2 measures with LB duration, few studies reported mean LB duration. Mean LB duration for cows housed indoors and outdoors has been reported to range from 70.6 to 96.9 min/bout precalving (Calderon and Cook, 2011; Black and Krawczel, 2016; Rice et al., 2017) and from 50.8 to 73.2 min/bout postcalving (Calderon and Cook, 2011; Sepúlveda-Varas et al., 2014; Black and Krawczel, 2016). Information about LB duration and the variation within cow may be useful as indicators of poor health or compromised welfare (Solano et al., 2016); therefore, it would be beneficial to present this information in future studies. Studies describing the lying behavior and activity of grazing dairy cows is limited. To my knowledge, no studies in dairy cows grazing pasture have reported lying behaviors (e.g., lying time, LB, and LB duration) both pre- and postcalving. Future research should consider describing the lying behavior of grazing dairy cows to determine whether large differences in lying time occur pre- and postcalving and to gain a better understanding of the time budgets of grazing compared with housed cows.

### *2.2.2 Calving Event*

Cows progressively alter their behavior throughout the transition period, although the most dramatic changes in lying behavior and activity occur from 24 h before to 12 h after giving birth (Huzzey et al., 2005; Jensen, 2012; Borchers et al., 2017; Rice et al., 2017; Barraclough et al., 2020). Several studies have described the changes in lying behavior and activity surrounding the calving event for cows calving indoors (Huzzey et al., 2005; Miedema et al., 2011a, b; Jensen, 2012; Black and Krawczel, 2016; Ouellet et al., 2016; Borchers et al., 2017) and outdoors (Black and Krawczel, 2016; Rice et al., 2017). In this section, I describe the changes in behavior that occur during the calving

event, and immediately pre- and postcalving in cows kept indoors and outdoors. For this review, I have defined the calving event as the period –2 to +2 d relative to the day of calving (d 0).

***Days Before Calving.*** In the few days before calving, subtle changes in behavior occur, where cows spend less time lying down, have more frequent transitions between lying and standing and take more steps (Huzzey et al., 2005; Ouellet et al., 2016; Borchers et al., 2017). The reduction in time spent lying, along with increased steps and LB, suggests that cows may become more uncomfortable as they prepare for the calving event.

***24 h Before Calving.*** Substantial changes in lying behavior (Huzzey et al., 2005; Maltz and Antler, 2007; Calderon and Cook, 2011; Miedema et al., 2011a, b; Jensen, 2012; Black and Krawczel, 2016; Ouellet et al., 2016; Borchers et al., 2017; Rice et al., 2017) and activity (Miedema et al., 2011a, b; Jensen, 2012; Black and Krawczel, 2016) occur in the final 24 h before calving compared with the days before, where lying time is further reduced, and number of steps taken and LB further increase compared with the days before (Jensen, 2012; Ouellet et al., 2016; Rice et al., 2017). These behaviors are associated with the first stage of labor when the calf is moving into its appropriate position for birth and the dam's cervix begins to dilate (Noakes et al., 2001).

More specifically, hourly and bihourly changes occur during the 24 h before calving and are more highly variable than daily changes (Miedema et al., 2011a; Jensen, 2012; Borchers et al., 2017; Rice et al., 2017). Overall, daily lying time decreases on the day of calving and lowest lying times occur 8 to 12 h before calving (Ouellet et al., 2016); although cows lie more in the hours immediately preceding calving, whereby lying time increases 2 to 4 h before calving (Jensen, 2012; Borchers et al., 2017). During the second stage of labor (~0 to 2 h before calving), the calf is actively pushed through the birth

canal, which explains the recumbent behavior displayed (i.e., increased lying down) to facilitate delivery (Noakes et al., 2001; Schuenemann et al., 2011).

Concurrently, steady bihourly changes in LB frequency and the number of steps taken occur, where an increase in LB frequency and the number of steps taken occurs from 18 h and 6 to 8 h before calving, respectively (Jensen, 2012; Ouellet et al., 2016; Borchers et al., 2017; Barraclough et al., 2020). Finally, in the final 2 to 6 h before calving, LB frequency and the number of steps taken increases sharply (Miedema et al., 2011b; Jensen, 2012; Ouellet et al., 2016; Borchers et al., 2017; Barraclough et al., 2020). Restlessness and discomfort around 2 to 8 h before calving are likely to be associated with the first stage of labor, where an increase in the duration and frequency of myometrial contractions (Noakes et al., 2001) is likely to be largely responsible for the peak in LB frequency and more steps taken (i.e., repeated lying down and then standing up and pacing) (Huzzey et al., 2005; Wehrend et al., 2006; Jensen, 2012; Borchers et al., 2017). Changes in behavior during this time may also be due, in part, to cows searching for a suitable location to calve and secluding themselves from the herd (Lidfors et al., 1994; Proudfoot et al., 2014).

Subsequent research has used this knowledge about changes in behavior occurring before and at the time of calving to determine whether lying behavior or activity can predict imminent calving in indoor systems (Ouellet et al., 2016; Borchers et al., 2017; Miller et al., 2020). The ability for lying behavior, activity, or both, to accurately predict calving, is of interest and would be particularly valuable in large herds (i.e., seasonal calving systems), where it is difficult for farmers to individually monitor the progress of calving and cows requiring assistance with delivery (Jensen, 2012; Petersson-Wolfe et al., 2017).

***24 h After Calving.*** Following the calving event, the cow must recover and rejoin the herd as a lactating cow. In the hours immediately after calving, cows spend less time lying down, which coincides with high levels of sniffing and licking their calves, which decreases from ~6 h postcalving (Edwards and Broom, 1982; Jensen, 2012). Following this period of maternal behavior, an overall increase in time spent lying and feeding and a decrease in activity and LB in the 24 h postcalving occurs (Steensels et al., 2012). Lying time further increases during the 4 d postcalving in cows housed in individual box stalls (Jensen, 2012); while lying time decreases in the first week postcalving in cows housed in groups under freestall conditions (Proudfoot et al., 2010). Cows housed individually would have less competition for resources than would occur in a group environment, where dairy cows are typically separated from the calf and moved into a milking herd within 24 h of calving (Jensen, 2012).

The differences in lying times reported may suggest that when given the opportunity, cows spend more time resting to recover during the first few days postcalving (Jensen, 2012; Proudfoot and Huzzey, 2017). The greater lying times reported in cows housed individually compared with those kept in groups immediately postcalving is an important consideration, as reduced resting and feeding in the postcalving period has been associated with increased risk of disease such as hypocalcemia, hyperketonemia (**HYK**), and displaced abomasum (Goldhawk et al., 2009; Proudfoot et al., 2010; Suthar et al., 2013). It is plausible that regrouping after parturition may further exacerbate this increased risk of disease due to social disruptions (von Keyserlingk et al., 2008). Differences in behavior due to the management and regrouping of cows postcalving and potential effects of regrouping on recovery from calving and successful transition warrants further investigation in both housed and grazing cows.

Daily and 24-hourly changes in lying behavior and activity before, at the time of, and after calving, have been extensively described in housed cows (Proudfoot and Huzzey, 2017). On the contrary, daily and 24-hourly changes in lying behavior and activity before calving have not been investigated in dairy cows grazing pasture, under seasonal calving systems. Further research to determine changes in lying behavior and activity that occur in grazing dairy cows pre- and postcalving, and compared with housed cows, is needed.

### *2.2.3 Early and Mid- to Late Lactation and the Early Dry Period*

Stage of lactation may be important in determining the amount of time that cows spend lying down (Chaplin and Munksgaard, 2001; Blackie et al., 2006). Following the transition period, cows experience slight alterations in lying behavior (Løvendahl and Munksgaard, 2016; Stone et al., 2017) as they adjust to BCS loss and increasing milk production, accompanied by increasing energy requirements during early to mid-lactation (Morton and McBride, 2004; Roche et al., 2007a, b). Typically, individual cows establish a baseline lying behavior and activity, and relative changes are minimal in mid- to late lactation (Maselyne et al., 2017). During mid- to late lactation, large deviations from baseline lying behavior within cow are typically linked to management related changes, injury (Zambelis et al., 2018), or adverse health events (Walker et al., 2008; Thompson et al., 2019). Large deviations in behavior can also occur periodically due to estrus during late lactation (Walker et al., 2008; Silper et al., 2015a; b). The dry period is considered a rest period for the cow (Kok et al., 2017), where cows engage in less physical activity in the last 2 months of pregnancy. Changes in behavior during this period are linked to pregnancy, the cessation of lactation and the elimination of time associated with milking (Tucker et al., 2007a). In this section, I describe lying behavior during early, mid- to late

lactation and the early dry period. For this review, I define early lactation as the period after the end of the transition period ~22 d postcalving until 50 DIM and mid- to late lactation is defined as >50 DIM. I define the early dry period as the nonlactating period up until 3 wk precalving.

Lying behavior appears stable throughout early and mid- to late lactation and in the early dry period; however, between the different life stages, slight differences in overall lying times and LB occur, along with cow and herd-level variation due to external factors. Lying times from early lactation through to the early dry period range from 9.2 to 16.5 h/d (Dechamps et al., 1989; Haley et al., 2000; Blackie et al., 2006; Chapinal et al., 2009; Løvendahl and Munksgaard, 2016; Kok et al., 2017; Stone et al., 2017; Thompson et al., 2019; van Hoeij et al., 2019). Lying time tends to be slightly longer in mid- to late lactation and the early dry period than in early lactation, in both housed cows and cows at pasture (Phillips and Leaver, 1985; Chaplin and Munksgaard, 2001; Vasseur et al., 2012; Westin et al., 2016; Maselyne et al., 2017; O'Driscoll et al., 2019). It is worth noting that lying time does not simply increase from the onset of lactation, rather it significantly decreases at the beginning of lactation until approximately 1 month after calving, after which it steadily increases (Maselyne et al., 2017). Interestingly, this follows the opposite trend as a standard lactation curve for milk yield and the same trend as changes in energy balance (Maselyne et al., 2017). Authors have hypothesized several reasons for the differences in lying behavior at these different life stages.

Lower lying times in early lactation could be due to a reduction in time available for lying, due to the cows spending more time feeding (Jensen et al., 2005; Vasseur et al., 2012) to meet the higher energy requirements imposed by the demands of lactation (Blackie et al., 2006; Løvendahl and Munksgaard, 2016; Stone et al., 2017). The increase

in time spent feeding in cows fed TMR from early to mid-lactation supports this hypothesis (DeVries et al., 2003), although this might vary across systems (Norring et al., 2012; Vasseur et al., 2012; Norring et al., 2014).

Increasing amounts of milk in the udder leading to discomfort (Jensen et al., 2005; Norring et al., 2012; Vasseur et al., 2012) and time constraints associated with milking, have also been attributed as possible causes for lower lying times during early lactation (Beggs et al., 2018). Increased lying times during the early dry period have been attributed to the exponential increase in fetal weight occurring during late gestation (Jainudeen and Hafez, 2000); however, an increase in time available for lying due to a reduction in time associated with milking in the nonlactating cow (Tucker et al., 2007a) may also explain the increased time spent lying in the early dry period. It is difficult to determine, with certainty, what drives the variation in behavior from early lactation until the early dry period in dairy cows, as the literature in this area is sparse and further work is needed.

## 2.3 FACTORS AFFECTING LYING BEHAVIOR AND ACTIVITY

Individual and herd-level variation in lying behavior and the magnitude of change is multifactorial and is influenced by interactions between cow, farm, and management-related factors (Fregonesi and Leaver, 2001; Munksgaard et al., 2005; Westin et al., 2016). Substantial within-cow and herd and between-cow and herd variation in lying behavior exist, and this variation should be taken into consideration when comparing behavior measurements from different farms or groups of animals (Ito et al., 2009; von Keyserlingk et al., 2012; Kok et al., 2017).

Cow-related factors include age and parity (Steensels et al., 2012; Lobeck-Luchterhand et al., 2015; Thompson et al., 2019), breed (Stone et al., 2017), milk

production (Løvendahl and Munksgaard, 2016; Piñeiro et al., 2019), BCS (Calderon and Cook, 2011; Matthews et al., 2012), and social behavior (Huzzey et al., 2006; Huzzey et al., 2007; Proudfoot et al., 2009b). These include farm and management-related factors such as housing, underfoot surface conditions (Norring et al., 2008; Ledgerwood et al., 2010), management (Legrand et al., 2009; Deming et al., 2013; Al-Marashdeh et al., 2019), time spent engaged in milking-associated activities (Hart et al., 2013; Beggs et al., 2018), competition for resources (Fregonesi et al., 2007; von Keyserlingk et al., 2008; Charlton et al., 2014), weather (Tucker et al., 2007b; Thompson et al., 2019), and photoperiod (Dechamps et al., 1989; Overton et al., 2002; Fregonesi et al., 2007; Schirmann et al., 2012). In the following section, I briefly describe the cow, farm, and management-related factors that influence lying behavior and activity in dairy cows and are relevant to this thesis. A description of cow, herd, and farm-level variation currently reported in literature will follow (see 2.3.5 Individual, Herd, and Farm-Level Variation).

### *2.3.1 Breed, Production, Parity, and BCS*

Behaviors expressed may also differ due to cow factors such as the influence of breed, milk production, age and parity, and BCS. Cows of different breeds and parities may vary in their behavioral responses due to differences between milk yield and composition, BW, BCS, and DMI, and their interactions (Kristensen et al., 2015); therefore, these factors may need to be considered in combination (Neave et al., 2017).

***Breed.*** Most studies investigating the use of behavior-monitoring technologies have used Holstein cows because they are the predominant dairy breed in housed systems (Capper et al., 2009); however, Holstein and Friesian crossbreeds, Jerseys and other breeds (e.g., Ayrshire, Brown-Swiss, and Danish Holstein-Friesian among others) are common in European confinement systems (Cunningham, 1983) and grazing systems

(VanRaden and Sanders, 2003). Stone et al. (2017) reported that their study was the first to investigate differences in lying behavior among Holstein-Friesian, Jersey, and Holstein-Friesian x Jersey dairy cattle breeds, but failed to demonstrate any associations between lying behaviors and breed. Stone et al. (2017) explained that the inclusion of breed, parity, and milk yield in their models might have confounded their results and masked any effects of breed. Further studies investigating lying behavior and activity of differing dairy cow breeds are needed to improve our current understanding and might be particularly important for systems adopting the use of crossbreeds and breeds other than Holsteins.

***Milk Production.*** Despite a lack of association between lying behavior and breed reported, several studies have reported a decrease in lying time with increasing milk yield (Vasseur et al., 2012; Kok et al., 2017; Stone et al., 2017), and an increase in feeding time with increasing milk yield in housed cows (Fregonesi and Leaver, 2001; Bewley et al., 2010; Løvendahl and Munksgaard, 2016). Authors hypothesize that higher-yielding cows and cows with greater energy requirements have to spend more time feeding, creating a trade-off between feeding and time spent lying down (Fregonesi and Leaver, 2001; Roche et al., 2009; Bewley et al., 2010; Steensels et al., 2012; Kok et al., 2017). Others have speculated that increased udder pressure in higher-yielding cows may cause discomfort when lying down and partly explain the reduction in lying time (Jensen et al., 2005; Norring et al., 2012; Kok et al., 2017). Further research is needed to understand the associations between milk production, feeding, and lying behavior and whether this poses any risks to higher-yielding cows.

***Age and Parity in Lactating Cows.*** Reports of associations between lying behavior and parity when comparing primiparous with multiparous cows have been

inconsistent. Several studies have reported increased lying time (Stone et al., 2017; Piñeiro et al., 2019), fewer LB (Vasseur et al., 2012; Neave et al., 2017; Duncan and Meyer, 2019), or a combination of both with increasing parity (Calderon and Cook, 2011; Steensels et al., 2012; Sepúlveda-Varas et al., 2014; Lobeck-Luchterhand et al., 2015; Westin et al., 2016; Barraclough et al., 2020), while others have no association (Krohn and Munksgaard, 1993; Chaplin and Munksgaard, 2001; Bewley et al., 2010; Duncan and Meyer, 2019).

Several of these studies have demonstrated that after controlling for differences in housing, BW, and milk production, parity effects on lying behavior remain. Sepúlveda-Varas et al. (2014) speculated that in lactating cows, lower lying times in younger cows could be due to younger cows being less socially dominant (Neave et al., 2017); therefore, if being less socially dominant means that they are often positioned towards the end of the milking order, they would have to spend more time waiting for milking and this may reduce the time available for lying and other activities. Although others have reported no association between milking order and parity (Beggs et al., 2018), and because Sepúlveda-Varas et al. (2014) did not monitor milking order, this hypothesis lacks support.

Another theory is that the increased lying time in multiparous cows is due, in part, to multiparous cows spending more time ruminating while lying (Stone et al., 2017), having more difficulty standing up and lying down, or having a less disrupted rest pattern than primiparous cows (Vasseur et al., 2012). A possible cause of disruption of lying in primiparous cows may be competition for space to lie down in freestalls where young, less-dominant cows are displaced more often from lying stalls (Fregonesi et al., 2007; Vasseur et al., 2012; Neave et al., 2017). In early lactation, first-lactation cows may spend

more time exploring as they experience and adapt to a new environment and routine (e.g., milking) (Vasseur et al., 2012; Neave et al., 2017). Understanding how social ranking and competition and motivation for access to different resources may disrupt normal lying behavior requires further investigation.

***Age and Parity in Nonlactating Cows.*** In nonlactating cows, differences in lying behavior between primiparous and multiparous cows are more consistent, with results showing that primiparous cows lay down less and become more active in the days leading up to calving than multiparous cows (Owens et al., 1985; Wehrend et al., 2006; Titler et al., 2015; Borchers et al., 2017), and also transition more frequently between lying and standing positions (Calderon and Cook, 2011; Titler et al., 2015). This increase in activity indicates that primiparous cows may be more restless before calving, although greater rates of dystocia in primiparous cows could also be involved (Matthews et al., 2012; Calderon and Cook, 2011). There is a theme of reduced lying time in younger cows reported across several systems and both pre- and postcalving; however, some inconsistencies exist and this warrants further investigation. Whether lower lying times in younger cows increases health risks and compromises welfare, also requires further investigation. Parity may be an important consideration for describing behavioral changes during different life stages and due to illness.

***BCS.*** The associations between lying behavior and BCS are unclear. Previous studies investigating the associations between lying behavior and BCS have reported a reduction in daily lying time with decreasing BCS (Matthews et al., 2012; Westin et al., 2016). Calderon and Cook (2011) reported no differences in daily lying time due to BCS, but thin cows (BCS <3.0; on a 5-point scale; 1 = emaciated, 5 = obese; Roche et al., 2004) had fewer LB and longer LB durations compared with moderate (BCS 3.0 to 3.75), or fat

cows (BCS ≥4.0) during the transition period. Several authors have hypothesized possible reasons for the reduction in lying time with decreasing BCS. Westin et al. (2016) reported that the reduction in lying time and LB might reflect discomfort due to the lack of cushioning (due to lower body fat) when the thin cows in housed conditions are lying on hard surfaces. In grazing cows, Matthews et al. (2012) speculated that lower BCS cows might spend more time grazing which reduces the time available to lie, while others speculated that exhaustion or (subclinical) metabolic disorders might discourage cows from engaging in energy-expensive behaviors, therefore, reducing the number of LB in thin cows (Itle et al., 2015; Kok et al., 2017). In contrast, Bewley et al. (2010) reported that BCS (range: BCS <2.75 to ≥3.25; on a 5-point scale; Roche et al., 2004) did not affect lying behavior. Few studies have considered the potential effect of BCS and changes in BCS on lying behavior and activity and risk of illness, and this warrants further investigation.

### *2.3.2 Inclement Weather*

Weather can have adverse effects on behavior and cow comfort; therefore, understanding how it can influence the behavior of cows is important. Animals do not need to alter their metabolic processes to maintain homeostasis within the thermoneutral zone because it does not affect metabolic rate (NRC, 2001); but at upper critical temperatures animals must engage in thermogenesis, and at lower critical temperatures animals must engage in activities to dissipate excess heat (NRC, 2001; Polsky and von Keyserlingk, 2017).

In outdoor systems, cows might be especially affected by environmental factors such as rain, wind, and cold temperatures, which can affect their behavior and physiology (Tucker et al., 2007b; Schütz et al., 2010). Few studies have investigated the associations

between lying behavior and cold and wet conditions in grazing cows kept on open pasture without an insulated space to lie (Redbo et al., 2001; Thompson et al., 2019). Two studies have reported that cows spend more time standing when exposed to cold and wet conditions while kept outdoors on a woodchip surface (Tucker et al., 2007b) and exposed to artificial rainfall and wind (Webster et al., 2008). The increase in time spent standing during inclement weather may reflect a lack of cow comfort, reducing the desire to lie, or a strategy for the cows to minimize heat loss and improve their thermoregulation ability (Bøe, 1990; Tucker et al., 2007b). While other studies have reported more time spent lying during exposure to cold and wet conditions (Gonyou et al., 1979; Redbo et al., 2001), these studies provided access to bedding, which likely provided an insulating base for the animal to lie down. Therefore, whether the animal spends more time lying or standing may be influenced by the underfoot conditions (Gonyou et al., 1979; Bøe, 1990), with a wet, muddy, or frozen surface contributing to a reduction in lying time (Tucker et al., 2007b; Schütz et al., 2010; Chen et al., 2017).

The associations between cold and wet weather and lying behavior and activity are inconsistent. Clear relationships have not been established in grazing dairy cows kept on open pasture during the transition period, without an insulated space to lie and under natural conditions, consistent with conventional grazing systems. To my knowledge, there are no studies that have reported the associations between activity (e.g., the number of steps taken) and cold and wet conditions in grazing cows. Future work should investigate whether inclement weather could be a potentially-confounding variable in animal behavior models in grazing cows kept outdoors on pasture surfaces.

### *2.3.3 Photoperiod*

Lying times in lactating dairy cattle follow a diurnal pattern, inverse to that of feeding behavior (Fregonesi et al., 2007; Schirmann et al., 2012). A cow's day is largely divided into time spent lying, feeding, and ruminating (Phillips and Leaver, 1985; O'Connell et al., 1989), with time also spent standing, standing idle, grooming, displaying aggressive behavior, and engaged in milking-associated activities (Phillips and Leaver, 1985; Tucker et al., 2007a; Gomez and Cook, 2010; Norring et al., 2012). In lactating grazed cows and housed cows, there are considerable similarities in the temporal distribution of lying behavior (O'Connell et al., 1989; Overton et al., 2002), yet, there are marked differences in the level of behavioral activities and herd synchrony (O'Connell et al., 1989; Singh et al., 1993). Research supports that the temporal pattern of lying is similar in both dry and lactating cows where cows are housed (Dechamps et al., 1989). To my knowledge, there does not appear to be any data available regarding temporal lying behavior in nonlactating grazing dairy cows and this warrants further investigation.

Two major periods of lying are observed to occur in the lactating cow, the first in the middle of the day before evening milking and the second from sunset to sunrise (O'Connell et al., 1989; Miller and Wood-Gush, 1991; Overton et al., 2002; Fregonesi et al., 2007; Schirmann et al., 2012; O'Driscoll et al., 2019). Irrespective of grazed or housed, greater proportions of lying and the longest LB usually occur at night and last for between 1.5 to 4.8 h (Dechamps et al., 1989; O'Connell et al., 1989; Singh et al., 1993; Chaplin and Munksgaard, 2001; Overton et al., 2002; Ouellet et al., 2016), and feeding during the hours of darkness are minimal in both housed (Collings et al., 2011; Schirmann et al., 2012) and grazing cows (Phillips and Leaver, 1985; O'Connell et al., 1989; Sheahan et al., 2013).

The lowest lying times correspond to peaks in feeding behavior in both grazed and housed cows. In grazing cows, the major grazing events are mainly confined to the hours between sunrise and sunset, with the major grazing bouts occurring in the early morning and late afternoon to early evening, corresponding with the periods after the morning and afternoon milkings (Phillips and Leaver, 1985; O'Connell et al., 1989; Fregonesi et al., 2007; Gregorini, 2011; Sheahan et al., 2013). Sheahan et al. (2011; 2013) described this as diurnal grazing behavior with crepuscular tendencies, due to the influence of sunrise and sunset on feeding patterns (O'Connell et al., 1989). On the contrary, cows housed indoors express less synchronization (e.g., behavioral asynchrony where cows kept in groups are performing different behaviors to their herd mates) of behavior than cows kept on pasture (O'Connell et al., 1989; Miller and Wood-Gush, 1991; Singh et al., 1993; DeVries et al., 2003; Fregonesi et al., 2007), because in housed dairy cows fed TMR, fresh feed delivery and frequency of feeding have a large influence on peaks in feeding behavior and subsequently, other behaviors (Miller and Wood-Gush, 1991; DeVries et al., 2003; DeVries and von Keyserlingk, 2005; Schirmann et al., 2012).

Under conditions that increase competition for resources as indicated by higher levels of aggressive behavior to gain access to feed and space to lie down (DeVries and von Keyserlingk, 2005), behavioral asynchrony may be exacerbated. Animals may need to feed and rest at different times to avoid excessive aggression and to access shared resources (Miller and Wood-Gush, 1991; Singh et al., 1993; Overton et al., 2002; Fregonesi et al., 2007). Further, under conditions that upset natural diurnal rhythms, for example, lighting in the housing facility, behavioral asynchrony may be exacerbated; however, further research to affirm this association is needed (O'Connell et al., 1989; Singh et al., 1993; Phillips et al., 1998).

Due to the importance of lying and feeding as components of cow comfort, health, and welfare, the disruption of lying and feeding behavior due to social competition and artificial light in housed systems may predispose housed cows to increased risk of ill health and compromised welfare not experienced by grazing cows. Whether the level and distribution of behavioral synchrony and disruption of normal behavior impacts the health and welfare of grazing and housed cows, has not yet been fully explored. Investigating behavioral synchrony and asynchrony may be an alternative and novel approach to studying behavior in dairy cows to detect cows at risk of ill health or with compromised welfare and should be considered in future work.

### *2.3.4 Individual, Herd, and Farm-Level Variation*

Cow, farm, or management-related factors such as those discussed previously (Section 2.3) should be taken into consideration when comparing behavior measurements from different farms or herds of animals and between animals within herds. Interestingly, despite large differences in behavior reported across a range of farm and management systems, several studies have reported that the variation in behavior among overall herd means is less than the range of means among individual cows within herds (Dechamps et al., 1989; Ito et al., 2009; von Keyserlingk et al., 2012; Charlton et al., 2016). In housed cows, Ito et al. (2009) reported a difference in the range of means among individual cows within herd of 4.2 to 19.5 h/d and 1 to 28 no./d for daily lying time and LB, respectively, and individual means ranged from 22 to 342 min/bout for the mean LB duration. The difference in the range of overall herd means was 9.5 to 12.9 h/d and 7 to 10 no./d for daily lying time and LB, respectively, and herd means ranged from 65 to 112 min/bout for the mean LB duration (Ito et al., 2009). Although data are still limited in housed cows, to my knowledge, no studies have investigated the variation in individual and herd-level

means for lying behavior in grazing dairy cows. Future research describing the variation in lying behaviors of grazing dairy cows would be valuable.

Although research investigating the consistency of lying time within cow across different life stages is limited, Liboreiro et al. (2015) reported that individual cows are consistent in activity 3 wk pre- and postcalving and Huzzey et al. (2005) reported that individual cows are consistent in time spent lying 10 d pre- and postcalving when housed. Additionally, a study by Vasseur et al. (2012) reported that cows are not consistent in time spent lying between early and mid-lactation but were more consistent in time spent lying between mid- and late lactation. Cows that lie less in early lactation are not the same cows that lie less in mid-lactation and some of the factors mentioned previously and discussed in Section 2.3 could be responsible for this variation. Also, sickness behaviors due to subclinical illness could contribute to this variation (Ito et al., 2009; Vasseur et al., 2012) and will be discussed in Section 2.4.

Due to the large individual variation in lying behavior, it could be difficult to assess essential lying behavior using benchmark values as health or welfare metrics on farm (Chaplin and Munksgaard, 2001; Ito et al., 2009; Vasseur et al., 2012), particularly for cows managed under different conditions and in different life stages. The large individual cow variation in lying behavior could also reduce the statistical power of tests relying on between-cow comparisons; therefore, future studies should focus on studying within-cow changes, as relative changes in behavior are likely to be more sensitive than individual or herd-level means (Ito et al., 2009; Proudfoot and Huzzey, 2017).

## 2.4 ASSOCIATIONS BETWEEN BEHAVIOR AND HYPOCALCEMIA AND HYPERKETONEMIA

Approximately 75% of disease in dairy cows occurs within the first month after calving (Ingvartsen, 2006; LeBlanc et al., 2006) and, as a result, transition-cow health has been a hot topic in literature, and our understanding of physiology, metabolism, nutrition, management, and immune regulation is continually growing in dairy cows under housed and grazing systems (Nydam et al., 2017). Recently, researchers have recognized the potential of behavior as a phenotype that could provide insight into transition-cow health and optimize management (Weary et al., 2009), because it is highly physiologically regulated. Also, behavior-monitoring technologies are becoming increasingly more available, with a range of commercially-available technologies that can record a range of behaviors, and some farmers are already recording data using these technologies on farm (Borchers and Bewley, 2015). As a result, many studies have investigated the relationships between a range of behaviors and illnesses to provide a proof of concept that differences in behavior could be valuable indicators of transition-cow disease, and the literature in this area is continually growing (González et al., 2008; Jawor et al., 2012).

Associations between transition-cow disease and social, drinking, feeding, stepping, lying, and standing behavior have been studied in housed systems, however, studies undertaken in grazing systems are scarce. Several studies have investigated metabolic transition-cow disorders and include hypocalcemia (Jawor et al., 2012; Liboreiro et al., 2015), HYK [or subclinical ketosis (**SCK**) or clinical ketosis (**CK**)] (González et al., 2008; Goldhawk et al., 2009; Itle et al., 2015; Liboreiro et al., 2015; Kaufman et al., 2016; King et al., 2017; Rodríguez-Jimenez et al., 2018; Stangaferro et al., 2016a; van Hoeij et al., 2019), retained fetal membranes (Liboreiro et al., 2015) and

negative energy balance (**NEB**) (Adewuyi et al., 2006), while infectious diseases investigated include endometritis and metritis (Urton et al., 2005; Huzzey et al., 2007; Stangaferro et al., 2016b; King et al., 2017; Barragan et al., 2018; Neave et al., 2018). Other studies have been less specific in their groupings and rather than focusing on a specific disease, categorized cows as 'sick' according to a diagnosis with 1 or more metabolic or infectious diseases including, for example, milk fever, mastitis, retained placenta, SCK, or metritis, or according to a diagnosis of any recorded infection (Proudfoot et al., 2014; Sepúlveda-Varas et al., 2014; Jensen and Proudfoot, 2017; King et al., 2017).

Feeding, drinking, and social behaviors as indicators of disease have been studied in housed systems (i.e., Huzzey et al., 2007; González et al., 2008; Goldhawk et al., 2009); however, feeding and drinking behaviors are difficult to measure accurately in grazing cows, and, therefore, may not be suitable behavioral indicators for grazing systems currently. Social behavior has received the least amount of attention in research and may be an important consideration related to disease risk (Sepúlveda-Varas et al., 2013). Social behavior is typically monitored in housed systems using video technology, which would also be an unsuitable measure for commercial use in pasture-based grazing systems where cows are moved between paddocks frequently (Roche et al., 2005).

Lying behavior and activity as indicators of transition-cow disease have also been studied in housed cows (i.e., Jawor et al., 2012; Itle et al., 2015; Kaufman et al., 2016) with only 1 study in grazing cows (excl. lameness; Sepúlveda-Varas et al., 2014). The availability of advanced and robust technologies that can accurately measure these behavioral parameters makes them a practical option for use in commercial pasture-based grazing systems (Borchers et al., 2016). I review the research to date using accelerometer

technology and report on the appropriate use of accelerometer technology for research purposes to monitor lying behaviors of dairy cows in a technical review in Chapter 3.

In this section, I review the studies that have investigated the associations between behavior and hypocalcemia and HYK that are common diseases in grazing dairy cows during the transition period. Lying behavior and activity are the behaviors of interest due to their potential application in pasture-based systems. Associations of lying behavior and activity before, at the time of, and after disease diagnosis and hypocalcemia and HYK in cows kept in conventional freestall systems are presented in Tables 2.1 and 2.2. Sickness behaviors refer to those behavioral changes occurring at the time of, or after disease diagnosis, while early indicators of disease refer to those behavioral changes occurring before disease diagnosis.

### *2.4.1 Hypocalcemia*

Hypocalcemia usually occurs when a dairy cow is unable to adapt to the increased physiological demand to mobilize calcium and maintain calcium homeostasis at the onset of lactation (Goff, 2008). During the precalving period, adequate amounts of calcium are typically available in the diet and calcium absorption occurs through the gastrointestinal tract (Horst et al., 1994; Goff, 2008). The onset of lactation can cause a large drain of calcium into the mammary gland and if the cow is unable to respond by increasing the inflow of calcium rapidly, a deficiency can occur (Ramberg et al., 1970; Horst et al., 1994). Most cows will experience some degree of hypocalcemia during this period; however, blood calcium (**Ca**) concentrations can return to normal after 2 to 3 d where macromineral adaptation has occurred (Ramberg et al., 1970; Horst et al., 1994; Goff, 1999).

Cows are often identified as clinically-hypocalcemic where blood Ca concentrations <1.4 mmol/L (cited in Roche and Berry, 2006; Martín-Tereso and Martens, 2014), and subclinically-hypocalcemic where blood Ca concentrations <2.0 mmol/L within the first 12 to 24 h postcalving (Oetzel, 2004; Goff, 2008; Reinhardt et al., 2011). Low blood Ca concentrations are not always associated with clinical symptoms common to a severe form of hypocalcemia known as milk fever (Martín-Tereso and Martens, 2014). Thus, while it is possible to visually detect milk fever due to the appearance of clinical symptoms (Horst et al., 1997), cows experiencing subclinical or clinical hypocalcemia (without paresis) may be more difficult to detect.

Milk fever is a risk factor for the development of metabolic, infectious, and reproductive disorders and reduced productivity (Goff, 2008; DeGaris and Lean, 2009; Martinez et al., 2012). While the short- and long-term effects of subclinical hypocalcemia on health are less well defined, the prevalence of subclinical hypocalcemia in housed cows (range: 25 to 54%) is of concern to farmers (Reinhardt et al., 2011). As a result, research using quantitative measures of behavior has been undertaken in cows under housed systems to explore new approaches to identify cows at risk of developing hypocalcemia (Weary et al., 2009; Neves et al., 2017; Proudfoot and Huzzey, 2017). Associations between behaviors such as activity, lying, feeding, rumination, and drinking have been reported in housed cows diagnosed with hypocalcemia (Jawor et al., 2012; Liboreiro et al., 2015). Only 1 study has reported changes in lying behavior before and after diagnosis of subclinical hypocalcemia (Jawor et al., 2012), while others have reported no association between lying behavior and subclinical hypocalcemia (Piñeiro et al., 2019), and activity and subclinical hypocalcemia before, at the time of, or after disease diagnosis (Liboreiro et al., 2015) (Table 2.1).

**Table 2.1. Studies reporting associations between lying and standing behavior and activity and hypocalcemia.**

Associations of lying and standing behavior and activity before, at the time of, and after disease diagnosis and hypocalcemia in housed cows. No studies have been undertaken in cows grazing pasture. [1]Early indicators of disease refer to those behavioral changes occurring before disease diagnosis. [2]Sickness behaviors' refer to those behavioral changes occurring at the time of or after disease diagnosis. Ca = calcium.

| Study; Sample Population | Treatment Comparison(s) | Early Indicators of Disease[1] | Sickness Behaviors[2] |
|---|---|---|---|
| Jawor et al. (2012);<br>30 cows, 1 farm | Hypocalcemia (blood Ca ≤1.8 mmol/L) vs. Healthy (blood Ca >1.8 mmol/L) within 24 h postcalving | Standing time precalving ↑<br>Standing bouts: No association | Standing time postcalving ↓<br>Standing bouts: No association |
| Liboreiro et al. (2015);<br>296 cows, 1 farm | Hypocalcemia (blood Ca <8.55 mg/dL within 72 h postpartum); univariate analysis using continuous data | Activity: No association | Activity: No association |
| Piñeiro et al. (2019);<br>1,052 cows; 3 farms | Hypocalcemia (blood Ca <2.0 mmol/L) vs. Healthy (blood Ca ≥2.0 mmol/L) within 48 h postcalving | Lying time: No association | |

***Sickness Behaviors.*** Jawor et al. (2012) reported that cows with subclinical hypocalcemia spent ~2.7 h less time standing on the day after calving compared with healthy cows (Table 2.1). While there is evidence that sick cows may spend more time lying down to conserve energy to assist the recovery process (Hart, 1988; Dantzer and Kelley, 2007), Jawor et al. (2012) hypothesized that the decrease in time spent standing may be due to impaired skeletal muscle contractility, which could be affected by low blood Ca, compromising movement (Murray et al., 2008).

***Early Indicators of Disease.*** Jawor et al. (2012) reported that cows experiencing subclinical hypocalcemia spent almost 3 h more time standing during the 24 h before calving compared with healthy cows. The authors speculated that greater time spent standing might have been driven by discomfort associated with prolonged labor due to weak uterine contractions, which could be affected by low blood Ca, compromising myometrial smooth muscle cell contractility (Bernal, 2003).

Whether differences in behavior are evident after disease diagnosis may be influenced, in part, by the severity of the disease (Sepúlveda-Varas et al., 2014). The lack of association between behavior and hypocalcemia in the studies by Liboreiro et al. (2015) and Piñeiro et al. (2019) may have been influenced, in part, by the threshold cut-points used to categorize the cows into groups, and the timing of hypocalcemia classification (Neves et al., 2017; 2018). Future research should carefully consider the timing of blood sampling and the use of appropriate cut-points to classify cows into distinct categories, to avoid masking potential differences in behavior.

### *2.4.2 Hyperketonemia*

Negative energy balance is universal in dairy cows in early lactation, but when adaptive mechanisms fail to cope with this state, clinical and SCK can occur (Herdt,

2000). During early lactation, dairy cows must mobilize fat from adipose tissue to meet their energy requirements due to the onset of lactation and the inability to consume sufficient feed (Nielsen and Ingvartsen, 2004). As a result of the mobilization of fat, concentrations of non-esterified fatty acids (**NEFA**) increase, and these are then further oxidized by the liver via β-oxidation to acetyl-CoA to supply energy (LeBlanc, 2010; Abdelli et al., 2017). If the liver is unable to process all the available NEFA, this can lead to hepatic lipidosis (i.e., fatty liver disease) and incomplete oxidization of fat in the liver. Incomplete oxidization of fat leads to the accumulation of ketone bodies, which are the intermediate metabolites of fatty acid oxidization (LeBlanc, 2010). Ketone bodies (predominantly BHB, but also acetone and acetoacetate) can be used as an alternative fuel source to glucose by the heart, brain, liver, and mammary tissue (Dohoo and Martin, 1984; Nielsen and Ingvartsen, 2004; LeBlanc, 2010). Excessive ketone production and low tissue uptake can lead to increased concentrations of ketones in circulation and lead to HYK, more commonly known as ketosis (Nielsen and Ingvartsen, 2004).

Ketosis typically occurs immediately after calving and can be categorized into 2 main types; however, each type has a different etiology, and, therefore, should be managed differently (Oetzel, 2004). Type II ketosis is typically due to preexisting fatty liver before and at calving where increased fatty acid uptake and triacylglycerol storage in the liver occurs in over-conditioned cows (Ospina et al., 2010; Seifi et al., 2011). Type II ketosis tends to cause elevated BHB concentrations in the first 5 to 15 DIM and elevated concentrations of NEFA pre- and postcalving (Oetzel, 2004; LeBlanc, 2010). Type I ketosis typically occurs about 3 to 6 wk postcalving and is due to severe NEB where cows are typically producing large quantities of milk, but are unable to meet their energy requirements from dietary intake (Oetzel, 2004; LeBlanc, 2010). Glucose concentrations

typically become low due to the demand for glucose exceeding the gluconeogenesis capacity of the liver (Ingvartsen, 2006; LeBlanc, 2010). Despite Type I and Type II ketosis being well defined, there is overlap between the categories where cows with Type II ketosis may have persistent SCK that continues into 3 or more weeks postcalving (Oetzel, 2004).

Clinical ketosis has been defined using a threshold blood BHB concentration of ≥3.0 mmol/L (Oetzel, 2004), while threshold levels for defining SCK using blood BHB concentrations range from ≥1.0 to 1.5 mmol/L (Duffield et al., 1997; Oetzel, 2004; Duffield et al., 2009; LeBlanc, 2010; Compton et al., 2014; 2015). While SCK may occur at blood BHB concentrations of ≥1.0 mmol/L, some of the decisions to set thresholds for SCK appear to be somewhat arbitrary (Duffield et al., 2009). Increased blood ketones are part of the adaptive response to NEB and, therefore, thresholds for defining SCK should be defined based on levels at which animals are at greater risk of production, reproduction, or health issues (Duffield et al., 2009). Several authors have reported greater risk of displaced abomasum (LeBlanc et al., 2005; Duffield et al., 2009), CK (Oetzel, 2004; Duffield et al., 2009), metritis (Duffield et al., 2009), reduced reproductive outcomes (Walsh et al., 2007b), prolonged postpartum anovulation (Walsh et al., 2007a), increased severity of mastitis (Suriyasathaporn et al., 2000), and lower milk production in early lactation (Duffield et al., 2009; LeBlanc, 2010) in cows experiencing SCK postcalving.

The incidence of SCK across dairy systems is of concern to farmers, where based on 1 or more blood BHB concentrations ≥1.2 mmol/L during early lactation ranges from 11 to 37% in Europe (Suthar et al., 2013) and 40 to 60% in North America (Duffield, 2000; McArt et al., 2012). In a study undertaken in New Zealand under intensive grazing

management, based on 1 or more blood BHB ≥1.2 mmol/L within 5 wk postcalving, the herd level incidence of SCK was 68% (Compton et al., 2015). Therefore, monitoring SCK and CK could be beneficial.

Plasma NEFA and BHB are measures of successful adaptation to NEB (LeBlanc, 2010; McArt et al., 2013; Abdelli et al., 2017). Plasma BHB is the gold standard measure used to diagnose HYK (Duffield et al., 2009); whereas blood NEFA concentration 7 to 10 d before expected calving date is a reliable measure to predict HYK (LeBlanc, 2010; Ospina et al., 2010; Rodríguez-Jimenez et al., 2018). Because intensive blood testing regimes are impractical for testing large groups of cows, both BHB and NEFA measures are limited in their application on farm. As a result, research has been undertaken to explore new approaches to identify cows at risk of developing HYK and other diseases using quantitative measures of behavior in housed cows (Goldhawk et al., 2009; Itle et al., 2015; Liboreiro et al., 2015; Proudfoot and Huzzey, 2017). Associations between behaviors, such as activity, lying, feeding, rumination, and drinking have been reported in housed cows diagnosed with SCK and CK (González et al., 2008; Goldhawk et al., 2009; Soriani et al., 2012; Itle et al., 2015; Liboreiro et al., 2015; Piñeiro et al., 2019; Sahar et al., 2020). Several studies have reported associations between lying behavior and activity at the time of, before, and after disease diagnosis and SCK and CK (Table 2.2).

**Table 2.2. Studies reporting associations between lying and standing behavior and activity and subclinical and clinical ketosis.** Associations of lying and standing behavior and activity before, at the time of, and after disease diagnosis and subclinical ketosis (SCK) and clinical ketosis (CK) in housed cows. No studies have been undertaken in cows grazing pasture. [1]BHB = β-hydroxybutyrate; DIM = days in milk; n = number of cows in study. [2]Early indicators of disease refer to those behavioral changes occurring before disease diagnosis. [3]Sickness behaviors refer to those behavioral changes occurring at the time of or after disease diagnosis.

| Study; Sample Population | Treatment Comparison(s)[1] | Early Indicators of Disease[2] | Sickness Behaviors[3] |
|---|---|---|---|
| Edwards and Tozer (2004); 1,445 cows, 3 farms | CK vs. Healthy | Activity ↓ | Activity ↑ |
| Itle et al. (2015); 30 cows, 1 farm | CK (3x blood BHB ≥1.2 mmol/L and 1 or more samples ≥2.9 mmol/L) vs. Healthy (blood BHB <1.2 mmol/L) within 3 wk postcalving | Standing time ↑<br>Standing bouts ↓<br>Bout duration ↑ | Standing time: No association<br>Standing bouts: No association<br>Bout duration: No association |
| Liboreiro et al. (2015); 296 cows, 1 farm | SCK (1x blood BHB >1.0 mmol/L) within 3 wk postcalving; univariate analysis using continuous data | Activity: no association | Activity ↓ |
| Kaufman et al. (2016); 236 cows, 4 farms | SCK (1x blood BHB ≥1.2 mmol/L) vs. Healthy (1x blood BHB <1.2 mmol/L and no other health problems) within 3 wk postcalving | Lying time ↑ ($P$ = 0.08)<br>Lying bouts: No association<br>Bout duration: No association | Lying time ↑<br>Lying bouts: No association<br>Bout duration: No association |

**Table 2.2.** Continued over page.

**Table 2.2 (Continued).** Associations of lying and standing behavior and activity before, at the time of, and after disease diagnosis and subclinical ketosis (SCK) and clinical ketosis (CK) in housed cows. No studies have been undertaken in cows grazing pasture. [1]BHB = β-hydroxybutyrate; DIM = days in milk; n = number of cows in study. [2]Early indicators of disease refer to those behavioral changes occurring before disease diagnosis. [3]Sickness behaviors refer to those behavioral changes occurring at the time of or after disease diagnosis.

| Study; Sample Population | Treatment Comparison(s)[1] | Early Indicators[2] | Sickness Behaviors[3] |
|---|---|---|---|
| Stangaferro et al. (2016a); 1,121 cows, 1 farm | Ketosis defined as decreased appetite, test at or above 'moderate' using a urine ketone strip test (KetoStic, Bayer Diagnostics, Tarrytown, NY), and reduced milk production from expected vs. non-diseased (cows not diagnosed with a health disorder within 10 DIM) | Activity ↓ (n = 44)<br>Activity: No association (n = 5) | Activity ↓ (n = 44)<br>Activity: No association (n = 5) |
| Rodríguez-Jimenez et al. (2018); 24 cows, 1 farm | SCK (mean blood BHB <1.4 mmol/L) vs. Healthy (mean blood BHB ≥1.4 mmol/L) within 15 DIM at 8 time points | Lying time ↑<br>Lying bouts: No association<br>Bout duration: No association | Standing time: No association<br>Lying bouts ↑ ($P$ = 0.06)<br>Bout duration: No association |
| Piñeiro et al. (2019); 1,052 cows; 3 farms | SCK (1x blood BHB ≥1.2 mmol/L) vs. Healthy (blood BHB <1.2 mmol/L and no other health problems) within 2 wk postcalving | Lying time ↑ (day of disease diagnosis unknown) | Lying time ↑ (day of disease diagnosis unknown) |

**Table 2.2.** Continued over page.

***Sickness Behaviors.*** Longer lying times postcalving have been associated with SCK (Table 2.2). Cows diagnosed with SCK spent more time lying after disease diagnosis compared with healthy cows (Kaufman et al., 2016). Piñeiro et al. (2019) reported an increase in lying time within 2 to 4 d postcalving, whereas Kaufman et al. (2016) reported an increase in lying time during wk 3 and 4 postcalving. Due to the study design by Piñeiro et al. (2019), the authors were unable to diagnose the onset of ketosis; therefore, it is difficult to determine whether the increase in lying time is a sickness behavior caused by ketosis, or an early indicator of disease and possibly a predisposing behavior. Kaufman et al. (2016) were able to determine that the mean day of diagnosis of ketosis was 7 DIM, after which, the increased time spent lying occurred. These authors hypothesized that these cows spent more time lying down after disease diagnosis because they were ill and potentially conserving energy to recover (Hart, 1988; Johnson, 2002; Dantzer and Kelley, 2007), or conserving energy needed for milk production due to their state of NEB, or a combination of both (Kaufman et al., 2016).

Interestingly, in the study by Itle et al. (2015), clinically-ketotic cows spent more time standing and had fewer and longer standing bouts on the day of calving. While increased time spent standing is not a common sickness behavior, if cows are spending more time standing idly and reducing their overall activity, this may reflect an energy-conserving behavior (Proudfoot and Huzzey, 2017), in agreement with other studies investigating SCK (Liboreiro et al., 2015; Kaufman et al., 2016; van Hoeij et al., 2019). On the contrary, Edwards and Tozer (2004) reported that cows increased activity when experiencing CK; however, it is worth noting, that both nervous and excitable or lethargic behaviors are characteristic symptoms of CK (blood BHB $\geq$ 3.0 mmol/L) (Fox, 1971) and this could explain the contrasting behaviors reported in these studies.

Alternatively, others have demonstrated significant reductions in feeding behavior and DMI after the diagnosis of SCK (Goldhawk et al., 2009; Rodríguez-Jimenez et al., 2018), and this is a well recognized sign of CK in dairy cows (Baird, 1982). Whether reduced activity and increased time spent lying could further exacerbate the NEB due to reductions in DMI in cows already experiencing SCK, warrants further investigation.

***Early Indicators of Disease.*** Two studies reported longer lying times before disease diagnosis in cows diagnosed with SCK (Kaufman et al., 2016; Rodríguez-Jimenez et al., 2018) while, in contrast, 1 study reported longer standing times before disease diagnosis in cows diagnosed with CK (Itle et al., 2015) (Table 2.2). Cows diagnosed with CK during the week after calving stood, on average, 2 h/d longer during the week before calving and 5 h longer on the day of calving compared with nonketotic cows (Itle et al., 2015). Itle et al. (2015) suggested that longer standing times may increase NEB if it is a consequence of social rank or competition for access to feed and lying spaces. Cows that later developed CK may have been socially subordinate (Kaufman et al., 2016; Sahar et al., 2020) and spent more time waiting for a space at the feed bunk or lying stalls (Fregonesi et al., 2007; Proudfoot et al., 2009b) rather than engaging in competitive behaviors, and, therefore, spent more time standing. If longer wait times resulted in a reduction in time spent feeding (Huzzey et al., 2006; Goldhawk et al., 2009), and cows experience greater distress (Munksgaard and Simonsen, 1996), this may put these cows at greater risk for CK. The work of Sahar et al. (2020) supports this hypothesis, where cows that spent more time eating and engaging in a greater number of agonistic interactions during the precalving period were more likely to remain healthy compared with cows that developed HYK, metritis, or both postcalving.

In contrast, opposite associations were observed in studies investigating the lying behavior of cows diagnosed with SCK, where cows spent more time lying down (i.e., less time standing). Rodríguez-Jimenez et al. (2018) hypothesized that this might be due to a lack of competition influencing the behaviors observed. Edwards and Tozer (2004) and Kaufman et al. (2016) both hypothesized that cows that spend more time lying down (Kaufman et al., 2016; Rodríguez-Jimenez et al., 2018) and are less active before disease diagnosis (Edwards and Tozer, 2004; Stangaferro et al., 2016a; King et al., 2017) may also be consuming less feed. In subsequent research, Rodríguez-Jimenez et al. (2018) supported this hypothesis and reported an association of SCK with greater lying time, less time at the feed bunk, and less DMI around calving time. Other studies have also reported a reduction in feeding behaviors and DMI before diagnosis of SCK and CK (González et al., 2008; Goldhawk et al., 2009; Sahar et al., 2020). Future research should consider understanding the cause and effect associations between lying, feeding, and social behaviors before and after disease diagnosis and SCK and CK (Rodríguez-Jimenez et al., 2018).

Interestingly, several studies have attempted to understand the associations between activity before diagnosis of HYK in housed cows (Edwards and Tozer, 2004; Liboreiro et al., 2015; Stangaferro et al., 2016a). While Edwards and Tozer (2004) reported reduced activity before diagnosis of CK postcalving (Table 2.2), Liboreiro et al. (2015) reported no association between precalving activity and SCK postcalving. Interestingly, Stangaferro et al. (2016a) reported differences in the activity displayed by individual cows diagnosed with ketosis, where some animals had reduced activity (n = 44) while others had no change in activity when compared with non-diseased cows (n = 5; Table 2.2). Stangaferro et al. (2016a) hypothesized that the severity of the disorder

influenced this difference in activity level in cows diagnosed with ketosis. But, if an animal at risk of developing SCK or CK cannot be distinguished from non-diseased animals using the behavior of interest, this creates its own set of challenges. A review by Proudfoot and Huzzey (2017) also identified a need for future research to understand individual differences in cow behavior due to disease risk. Therefore, future work should focus on understanding behaviors that could identify disease, how different behaviors could be used in combination, and how within-cow behavioral changes are associated with disease.

Understanding changes in behavior at the time of and after disease diagnosis may have several applications on farm. Quantifiable behavioral changes may be useful for improved and earlier disease diagnosis, earlier intervention and treatment, monitoring of recovery and the efficacy of treatment programs, and aid in the design of appropriate environments and care for sick animals (Weary et al., 2009; Proudfoot and Huzzey, 2017). Understanding changes in behavior before disease diagnosis and the drivers of these changes will improve our understanding of whether behaviors as early indications of disease are caused by a subsequent illness, are a predisposing factor, or are an effect of a pre-existing illness (Proudfoot and Huzzey, 2017). If the behavior is caused by the disease, quantifiable behavioral changes may allow earlier detection and higher rates of detection of illness (Weary et al., 2009), allowing more effective veterinary treatments. If the behavior predisposes the animal to disease, it may allow management changes to be implemented to reduce the risk for susceptible cows. Improved and earlier identification of illness could have substantial positive effects on cow survival and performance, improved individual animal care, reduced rates of culling and premature mortality, and improved animal welfare.

## 2.5 AIMS AND OBJECTIVES

Subclinical disease in transition dairy cows that goes largely undetected in conventional farming systems contributes to reduced cow performance and compromised welfare. Sickness behavior is a well-regulated physiological response and may assist in the early detection of ill health. The development of electronic monitoring devices (e.g., accelerometers) provides a unique opportunity to allow the remote monitoring of animal behavior that has previously been difficult to measure at high resolutions, particularly in commercial farming systems. Existing research in housed systems show promising results in the use of accelerometer devices to differentiate between diseased and non-diseased dairy cows using behavior information; however, research in pasture-based grazing systems is minimal. Several considerations need to be taken into account to further our understanding of the associations between behavior and disease and the appropriate use of accelerometer devices in grazing dairy cows. The working hypothesis of this dissertation is that greater disease risk will be associated with increased lying time and decreased activity before, at the time of, and after the onset of disease in grazing dairy cows, and that these changes would be detectable using accelerometer devices. If changes in behavior are detected, this could provide insightful information for farmers to monitor individual animals at risk of disease during the transition period.

The main objectives of this PhD thesis were to:

1) Review studies that have validated lying behavior derived from accelerometer devices and determine the considerations for selecting a device and editing and interpreting the data (Chapters 3 and 4)
2) Investigate the associations between lying behavior and activity and environmental (Chapter 5) and cow factors (Chapter 6) to determine

potentially-confounding variables when analyzing and interpreting behavior data in grazing dairy cows.

3) Describe the changes in daily and 24-hourly lying behavior and activity that are 'typical' for clinically-healthy grazing dairy cows during the transition period (Chapter 6).
4) Investigate the associations between lying behavior and activity and hypocalcemia in grazing dairy cows to determine if lying behavior and activity could be used to differentiate between cows experiencing normocalcemia and varying degrees of hypocalcemia (Chapter 7).
5) Investigate the associations between lying behavior and activity and HYK or NEB, or both in grazing dairy cows to determine if lying behavior and activity could be used to differentiate between cows with experiencing NEB, with or without HYK compared with cows not experiencing NEB or HYK (Chapter 8).

## 2.6 SUMMARY

The biological literature review provides evidence that the lying behavior and activity of dairy cows has the potential to act as an indicator of health and welfare. My review indicates that the existing research in grazing dairy cows is limited, especially where the interest is in using lying behavior and activity to identify transition-cow disease. The cow, farm, and management-related factors influencing the variation in lying behavior and activity and the typical behavior of clinically-healthy grazing dairy cows, and changes in behavior occurring during different life stages and due to transition-cow disease have yet to be investigated in grazing dairy cows. An integral part of my work was using lying behavior and activity data derived from accelerometer devices to investigate these aforementioned themes further; therefore, before undertaking any

analyses, I reviewed the studies that have validated accelerometers for measuring lying behavior in dairy cows and provided a comprehensive overview of the considerations that researchers should take into account to ensure that accelerometers are used appropriately, and subsequently, I applied my findings to my dataset (Chapter 3).

# CHAPTER 3. EVALUATING THE APPROPRIATE USE OF WEARABLE ACCELEROMETERS IN RESEARCH TO MONITOR LYING BEHAVIORS OF DAIRY COWS

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## 3.1 ABSTRACT

Until recently, animal behavior has been studied through close and extensive observation of individual animals and has relied on subjective assessments. Wearable technologies that allow the automation of dairy cow behavior-recording currently dominate the precision dairy technology market. Wearable accelerometers provide new opportunities in animal ethology using quantitative measures of dairy cow behavior. Recent research developments support that quantitative measures of behavior may provide new objective on-farm measures to assist producers in predicting, diagnosing, and managing disease or injury on farms and allow producers to monitor cow comfort and estrus behavior. These recent research developments and the significant increase in the availability of wearable accelerometers have led to a growing interest of both researchers and producers in this technology. This review aimed to summarize the studies that have validated lying behavior derived from accelerometers and to describe the factors that should be considered when using accelerometers attached to the leg and neck-worn collars used to describe lying behavior (e.g., lying time and lying bouts) in dairy cows for research purposes. Specifically, we describe accelerometer technology, including the instrument properties and methods for recording motion, the raw data output from accelerometers, and methods developed for the transformation of raw data into meaningful and interpretable information. We highlight differences in validation study outcomes for researchers to consider when developing their own experimental methodology for using accelerometers to record lying behaviors in dairy cows. Finally, we discuss several factors that may influence the data recorded by accelerometers and highlight gaps in the literature. We conclude that researchers using accelerometers to record lying behaviors in dairy cattle should (1) select an accelerometer device that, based

on device attachment and a sufficiently high sampling rate, is appropriate to record the behavior of interest; (2) account for cow, farm, and management-related factors that could influence the lying behaviors recorded; (3) determine the appropriate editing criteria for the accurate interpretation of their data; (4) support their chosen method of recording, editing, and interpreting the data by referencing an appropriately-designed and accurate validation study published in literature; (5) report, in detail, their methodology to ensure that others can decipher how the data were captured and understand potential limitations of their methodology. We recommend that standardized protocols be developed for collecting, analyzing, and reporting lying behavior data recorded using wearable accelerometers for dairy cattle.

## 3.2 INTRODUCTION

Wearable electronic monitoring technologies could change the features of intensive, large-scale dairy farming by allowing the individualized monitoring and management of animals (Bewley et al., 2017). Since the early 2000s, there has been a substantial increase in published literature investigating the use of wearable electronic monitoring technologies for adoption in commercial farming environments (Weary et al., 2009; Mottram, 2015). Worldwide, intensive dairy management systems have experienced increasing herd sizes, while skilled and experienced labor has become less available, with less ability and time to monitor individual animals (Mottram, 2015; Bewley et al., 2017; Whitlock et al., 2017).

Changes in animal behavior, specifically, changes in lying behavior of dairy cows is well recognized as an indicator of injury (e.g., lameness; González et al., 2008), ill health (e.g., metabolic and infectious disease; Calderon and Cook, 2011; Itle et al.,

2015; Neave et al., 2018), and cow comfort and welfare (Weary et al., 2009), and can be used to detect estrus (Dolecheck et al., 2015). Historically, lying behavior was monitored in experimental work through visual and video observations (Rutten et al., 2013) and on farm, through visual observations and manual assessment; however, manual assessment of animal behavior is subjective and open to observer interpretation (Weary et al., 2009; Borchers et al., 2016) and is time- and resource-intensive (Stafford and Gregory, 2008; Richeson et al., 2018). In contrast, wearable electronic monitoring technologies have evolved substantially due to the development of more-accessible hardware and software (Turner et al., 2000; Brown et al., 2013) and can overcome many of the limitations of traditional methods of behavior-monitoring (Frost et al., 1997; O'Driscoll et al., 2008). The use of accelerometers allows behavior to be measured remotely [e.g., HOBO (Onset Computer Corporation, Pocasset, MA; Ledgerwood et al., 2010) and IceQube (IceRobotics Ltd., Edinburgh, Scotland; Kok et al., 2015)], for extensive periods (Borchers et al., 2016), and in 'real time', to give detailed information about lying behavior (Brown et al., 2013).

The increasing accessibility and rapidly-growing market of technologies that can measure lying behavior in dairy cows (Bewley et al., 2017) and their potential practical application on farm, supports that these technologies are of high interest to both researchers and producers (Munksgaard and Simonsen, 1996; Richeson et al., 2018). The technologies of interest for the present review are those that use accelerometers attached to the leg or mounted via collars around the neck to measure lying behavior in dairy cattle (Martiskainen et al., 2009). Lying behaviors that can be derived from accelerometers include lying time, lying bouts (**LB**), LB duration, and lying laterality (Gibbons et al., 2012).

When reviewing studies using the same type of accelerometer under similar farming systems, a lack of user understanding of the appropriate use of accelerometer devices was apparent (e.g., discrepancies existed in the protocols used for collecting, analyzing, and reporting data between studies using the same type of accelerometer in cows under similar systems; Appendix 2 – Supplemental Material and Supplemental Table 1). To obtain accurate measures of lying behavior using accelerometers in dairy cows, thought should be given to developing standardized protocols for collecting, analyzing, and reporting data across studies (Anderson et al., 2013), including understanding the management, editing, and analysis of large datasets (Chen and Bassett, 2005; Ledgerwood et al., 2010; Brown et al., 2013). Several methodological issues affect the accuracy of accelerometer-derived lying behavior and should be considered by all users.

In this review, we describe the instrument properties and methods for recording lying behavior using accelerometers to improve the understanding of the importance of sampling frequency and sampling interval selection to record the behavior of interest. Our review highlights several factors for researchers to consider when deploying the devices in the field in dairy cows and when editing large datasets derived from accelerometers. We discuss potential limitations in the studies validating accelerometer-derived lying behavior that researchers need to evaluate when developing their own experimental methodology. Finally, the need to create a robust set of criteria and an adequate explanation of those criteria in the methodology of studies using accelerometers in dairy cows is discussed.

## 3.3 LITERATURE SEARCH

The web-based literature databases searched were Web of Science (http://wokinfo.com) and Scopus (https://www.scopus.com). Scientific articles (both peer-reviewed and non-peer-reviewed), conference proceedings, and abstracts written in English were considered. Search terms were accelerometer(s), automatic, automatic monitoring, behavior (behaviour), behavior monitoring (behavior-monitoring), cattle, cow(s), dairy, dairy cow(s), data logger(s), logger(s), lying behavior, recording lying, sampling interval(s), technology, technical note, three-dimensional (3-D), two-dimensional (2-D), validation, validating. Inclusion criteria were that the paper must report on lying behavior measured by accelerometers attached by collars or leg bracelets in dairy cows.

This review focuses on validation studies that have used identical loggers on opposite legs, direct observation, or video recording to validate accelerometer-derived lying behaviors. Studies comparing the performance of 2 differing technologies for validation are not considered in this review or reported in tables due to the different sampling frequencies and sampling intervals between devices potentially-confounding the data. Lying behaviors that are referred to throughout and can be measured by accelerometers and were the focus of this review are lying time, LB and LB duration. Lying time can be defined as a durational activity and is typically measured as the time an animal spends engaged in lying within a set time (e.g., 24-h period; h/d) and LB can be defined as transitional activities and are instances where animals transition from standing to lying positions and then back to standing (Ledgerwood et al., 2010). Lying bouts are measured as the number of times an animal transitions (e.g., number of bouts/d). Further, the time of a single bout of lying can be defined as the LB duration (e.g.,

min/bout). Although the total duration of an activity is valuable for understanding the time budgets of animals, transitional behavior can provide valuable information that total durational activities cannot explain (Elischer et al., 2013; Kok et al., 2015).

From our literature search, thirteen studies have validated a range of accelerometers for durational (daily lying and standing time) and transitional activities (e.g., LB) under a range of conditions, sampling frequencies and intervals, and editing criteria. The purpose of this review was not to give an overall recommendation for the use of accelerometers. Instead, we provide a concise summary of the validation studies that have been undertaken in adult cattle for others to reference to discern the appropriate experimental methodology based on their study design and objectives. General information about these studies is described and summarized in Supplemental Tables 2, 3, and 4 in Appendices 3, 4, and 5, respectively, of this thesis, including information about the validation study design, the accelerometers used, and behaviors validated.

## 3.4 ACCELEROMETER TECHNOLOGY

Wearable accelerometers can remotely and efficiently collect detailed data related to measures of animal behavior (Brown et al., 2013). The accelerometer measures changes in velocity over time and is attached to the animal in a specific location so that the orientation of the device can provide detailed information about movement and body position relating to the behavior of interest (Scheibe and Gromann, 2006). A summary of the devices referred to throughout this review, the manufacturer details, the behaviors recorded, attachment of the device, sampling intervals available for selection to record the behavior of interest, data retrieval, and on-board data storage is provided in Table 3.1.

**Table 3.1. Description of the devices referred to throughout this review.**

The name of the technology, manufacturer, behavior recorded, device attachment, sampling interval(s), data retrieval and on-board data storage of the devices referred to throughout this review.

| Technology[1] | Manufacturer | Behavior Recorded[2] | Attachment[3] | Sampling Interval(s) | Data Retrieval[4] | On-board Storage[5] |
|---|---|---|---|---|---|---|
| Actiwatch | Cambridge Neurotechnology | Activity | Leg band | 2 s to 15 min | Manual | ≥1.5 d |
| AfiAct | Afimilk (ceased manufacture) | Activity, LB, and LT | Leg band | 1 min to 2 h | Manual | - |
| AfiTagII | Afimilk | LB and LT | Leg band | 15 min | UHF | 22 h |
| ACT | Lely | Activity, EAT, LT, and RM | Collar | 2 h | UHF | 24 h |
| CowScout Leg Sensor | GEA Farm Technologies | Activity, LB, and LT | Leg band | 15 min | UHF | 24 h |
| Halter | Halter USA Inc. | Activity | Collar | - | Cellular | - |
| HOBO | Onset Computer Corporation | *g* force or degrees of tilt on the 3-axes (user classification required) | User-designed attachment | Fast mode: 0.01 to 0.99 s<br>Normal mode: 1 s to 18 h | Manual | ≥3.6 min<br>≥6 h |
| IceQube | IceRobotics Ltd. | Activity, LB, and LT | Leg band | 15 min, 1 h, 2 h, 1 d, and 1 wk | Manual | 9 d |

**Table 3.1.** Continued over page.

**Table 3.1 (Continued).** The name of the technology, manufacturer, behavior recorded, device attachment, sampling interval(s), data retrieval and on-board data storage of the devices referred to throughout this review.

| Technology[1] | Manufacturer | Behavior Recorded[2] | Attachment[3] | Sampling Interval(s) | Data Retrieval[4] | On-board Storage[5] |
|---|---|---|---|---|---|---|
| IceTag 2D | IceRobotics Ltd. (ceased manufacture 2008) | Activity, LB, and LT | Leg band | 1 s, 1 min, 15 min, 1 h, 2 h, 1 d, and 1 wk | Manual | 60 d |
| IceTag 3D | IceRobotics Ltd. | Activity, LB, and LT | Leg band | 1 s, 1 min, 15 min, 1 h, 2 h, 1 d, and 1 wk | Manual | 60 d |
| IDA Tracker | Connecterra | Activity, EAT, RM, and LT | Collar | - | UHF | 24 h |
| The Track a Cow | ENGS Systems | LB, LT, and visits to feed bunk | Leg band | 1 min | Cellular | 24 h |

[1]Technology: Actiwatch = The Actiwatch® Activity Monitoring System (Cambridge Neurotechnology, Cambridgeshire, UK), AfiAct = AfiAct® Pedometer Plus (ceased manufacture) and AfiTagII = AfiTag® II Pedometer (Afimilk, Kibbutz, Afikim, Israel), ACT = Automatic Milking System Activity Monitor (Lely, Maassluis, The Netherlands), CowScout = CowScout Leg Sensor (GEA Farm Technologies, Bönen, Germany), Halter = Halter Activity Monitor (Halter USA Inc., Auckland, New Zealand), HOBO = HOBO Pendant G Acceleration Data Logger (Onset Computer Corporation, Pocasset, MA), IceQube = IceQube Activity Monitor (IceRobotics Ltd., Edinburgh, Scotland), IceTag 2D = IceTag 2.004 Activity Monitor (ceased manufacture 2008), IceTag 3D = IceTag 3D Activity Monitor, (IceRobotics Ltd., Edinburgh, Scotland), IDA Tracker = IDA Tracker (Connecterra B.V., Amsterdam, The Netherlands), TAC = The Track a Cow (ENGS, Rosh Pina, Israel).

[2]LB = Lying bouts; LT = Lying time; EAT = Eating time; RM = Rumination time.

[3]Attachment refers to the way the device is attached to the cow. Collars refer to those worn around the neck of the cow. Leg band refer to those worn on the leg (typically the hind leg is preferred).

[4]Cellular = data is transferred automatically through the use of satellites; Manual = manual download is required using product-specific software; UHF = data is transferred automatically via ultra-high frequency data download technology using either fixed or portable wireless transceivers.

[5]On-board storage is the number of recording days of data storage available on the device based on the shortest possible sampling interval. For devices that allow the selection of longer sampling intervals, the number of recording days increases with the selection of a longer sampling interval (i.e., fewer data points recorded per day).

### *3.4.1 Device Attachment*

Accelerometers require attachment on a specific anatomical location and orientation on the body through the use of either collars (e.g., attached around the neck of the cow; Martiskainen et al., 2009), leg bands (e.g., attached to the lateral side of the front or hind limb; Müller and Schrader, 2003; Robert et al., 2009), harnesses (e.g., attached around the body of the cow; Champion et al., 1997), or user-designed device housing (e.g., attached using vet wrap; Rodríguez-Jimenez et al., 2018), depending on the behavior of interest (Ledgerwood et al., 2010; Bonk et al., 2013). Harnesses and user-designed device housing are typically used for research purposes if the accelerometer is not contained within an easily-attachable housing [e.g., The Actiwatch® Activity Monitoring System (**Actiwatch**; Cambridge Neurotechnology, Cambridgeshire, UK) and HOBO.

Accelerometer attachment can affect the outcome of a validation study and the behavior that can be recorded. The influence of accelerometer attachment varies; for example, attachment in the same location on opposite legs (e.g., left and right back legs) has little influence on the accuracy of data recorded by accelerometers (Munksgaard et al., 2006; Shepley et al., 2017). While accelerometer attachment on the front or back leg appears to have little influence on the accuracy of lying time information recorded (Borchers et al., 2016; Nielsen et al., 2018); however, when LB information is of interest, attachment on the front leg can reduce device performance by overestimating LB (Charlton et al., 2017). It appears that this is due to an increased number of false LB when the device is attached to the front leg compared with the back leg (Thorup et al., 2016). The ACT device is a neck-worn collar, where activity units are reported for a 2-h block rather than reporting specific information about the orientation of a limb. Elischer et al.

(2013) reported that lying is not distinguishable from standing when using this neck-worn accelerometer and, therefore, is not appropriate where the interest is in lying behavior. These examples emphasize the importance of considering the accelerometer device and options for device attachment, depending on the behavior of interest (Charlton et al., 2017).

### *3.4.2 Behavior Classification*

An accelerometer is a spring-like piezoelectric sensor (Brown et al., 2013) that generates a wave-like voltage signal that is proportional to the acceleration (change in velocity) it experiences over time (Dow et al., 2009). The sensor measures either a summed acceleration or the acceleration at defined intervals and is derived when the sensor is deformed by gravitational (due to changes in animal posture) as well as inertial acceleration (due to animal movement) (Shepard et al., 2008; Miwa et al., 2015). The acceleration is measured in the direction of a single plane of movement. In a two-dimensional accelerometer, the acceleration is measured along the X- and Y-axes; however, accelerometers have been developed to allow the acceleration to be recorded across 3 axes. In a three-dimensional accelerometer (**3D-accelerometer**), acceleration is recorded across the X-, Y-, and Z-axes that are aligned orthogonally to signal vertical, forward, and lateral movement (Ito et al., 2009; Brown et al., 2013). Omnidirectional accelerometers, where acceleration is measured along multiple planes in all directions are rarely used in research involving cattle with only 1 early study in dairy cows appearing in our literature search (e.g., Actiwatch, developed for medical research in humans; Müller and Schrader, 2003). To record lying behavior using devices attached to the leg, the 3D-accelerometer is fixed to the medial or lateral side of the front or hind limb of the cow (Darr and Epperson, 2009; Gibbons et al., 2012), which allows specific leg

orientations associated with lying and standing behavior (X- and Y-axes) to be recorded as well as lying laterality (Z-axis) (e.g., lying on the left or right side; Robert et al., 2009; Gibbons et al., 2012). The X-axis is perpendicular to the ground during standing events (Figure 3.1a), the Y-axis is perpendicular to the ground during lying events (Figure 3.1b) (Robert et al., 2009), and the Z-axis runs parallel to the ground pointing away from the sagittal plane (Darr and Epperson, 2009; Ito et al., 2009).

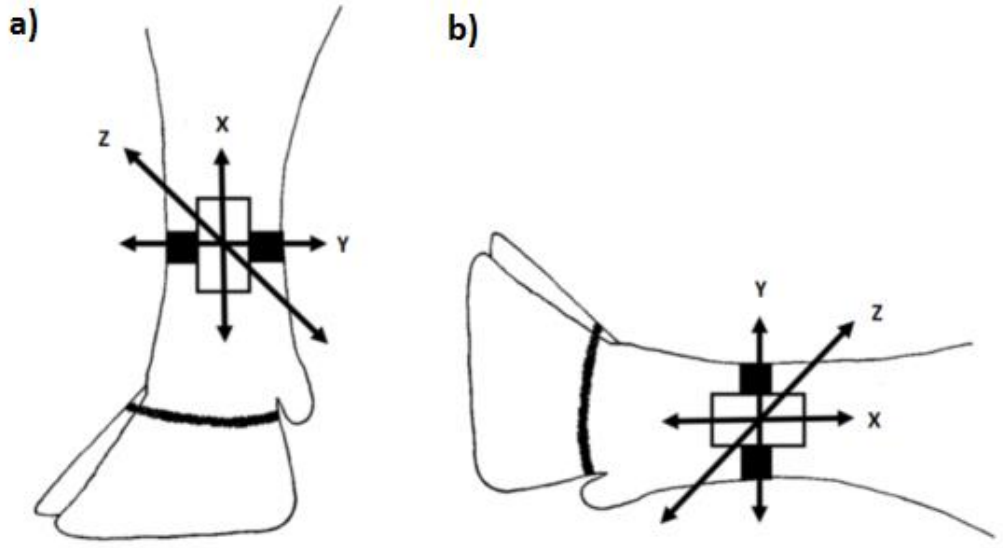


**Figure 3.1. A graphic representation of the planes of a three-dimensional accelerometer attached to the hind limb of a cow.**
Demonstration of the position of the three-dimensional accelerometer and illustration of measured X-, Y-, and Z-axes on the lateral aspect of the hind limb in a standing (a) and lying (b) position.

To record lying behavior using neck-worn collars, the 3-D accelerometer is attached at the top of or the side of the neck. Acceleration along the X-axis is associated with vertical movement of the cows' head (e.g., up and down), acceleration along the Y-axis is associated with horizontal movement of the cow's head (e.g., forwards and

backward) and acceleration along the Z-axis is associated with lateral movement of the cow's head (Figure 3.2; Tamura et al., 2019).

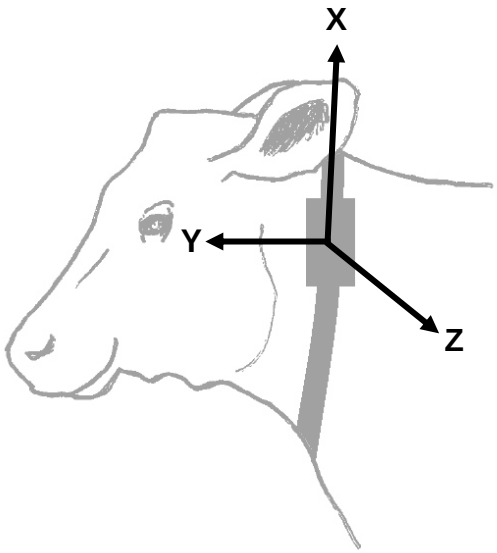


**Figure 3.2. A graphic representation of the planes of a three-dimensional accelerometer worn around the neck of a cow.**
Demonstration of the position of the three-dimensional accelerometer and illustration of measured X-, Y-, and Z-axes at the side of the cows' neck when the cow is standing.

Accelerometers record the change in velocity along each of the axes, separately, per unit time. While every device is different, the 3D-accelerometer will capture information about the acceleration along the 3 axes at a sampling frequency predetermined by the product manufacturer (Mitlöhner et al., 2001; Brown et al., 2013). Sampling frequencies for technologies that have been validated in literature range from 1 to 100 Hz (Mattachini et al., 2013; Borchers et al., 2016; Nielsen et al., 2018). While some devices, such as the HOBO, allow the user to program the sampling frequency (Range: 0.017 to 100 Hz), it is typically fixed in other devices (Table 3.1). A sampling frequency of 1 Hz corresponds to the capture of 1 acceleration measurement per second on each axis and, therefore, would correspond to 3 measurements per second, which

rapidly accumulates into millions of logged measurements over time (Brown et al., 2013). Consideration of a suitable sampling frequency when choosing a device is important because the sampling frequency is often specific to the device and typically cannot be altered (Chen and Bassett, 2005).

Unnecessarily high sampling frequencies should be avoided when the digital storage space is limited (Ropert-Coudert and Wilson, 2004; Halsey et al., 2009). For example, the HOBO has 64 Kbyte of internal memory (approximately 21.8K combined X-, Y-, and Z-axis readings; Onset, 2019) and this allows data storage for ~6 h using a 1-s sampling interval or 15 d using a 1-min sampling interval. Typically, devices without automated data download capability used for research purposes allow shorter sampling intervals (≥0.01 s) where the device may be removed more regularly [e.g., HOBO, IceTag 2.004® Activity Monitor (**IceTag 2D**; ceased manufacture 2008, IceRobotics Ltd.) and IceTag 3D Activity Monitor (**IceTag 3D**; IceRobotics Ltd.)], while devices used commercially often summarize the data within a longer sampling interval ≥15 min [e.g., The AMS Activity Monitor (**ACT**; Lely, Maassluis, The Netherlands) and IceQube] to maximize the memory capacity and battery life of the device (Table 3.1). However, devices developed for research and commercial purposes are increasingly incorporating automated data download features to reduce the need for such large on-board storage capacity.

Raw data are recorded on the on-board memory of the device. The 3D-accelerometer raw data output contains a wave-like signal with units in voltage that are recorded during the acceleration of the sensors and these signals can be used to determine the behavior occurring at each time point (Brown et al., 2013). In devices without software containing proprietary algorithms, prior to analysis, these wave-like

signals must be classified manually into specific behavioral categories through validation studies, which involves synchronization of the raw data output with observed behaviors to determine the specific signals corresponding with specific behaviors (Brown et al., 2013; Richeson et al., 2018). A graphic representation of the wave-like signals generated on each of the 3-axes (X-, Y-, and Z-axes) is depicted in Figure 3.3; this identifies different levels of voltage for lying and standing behaviors and the distinctive patterns that correspond with these behaviors.

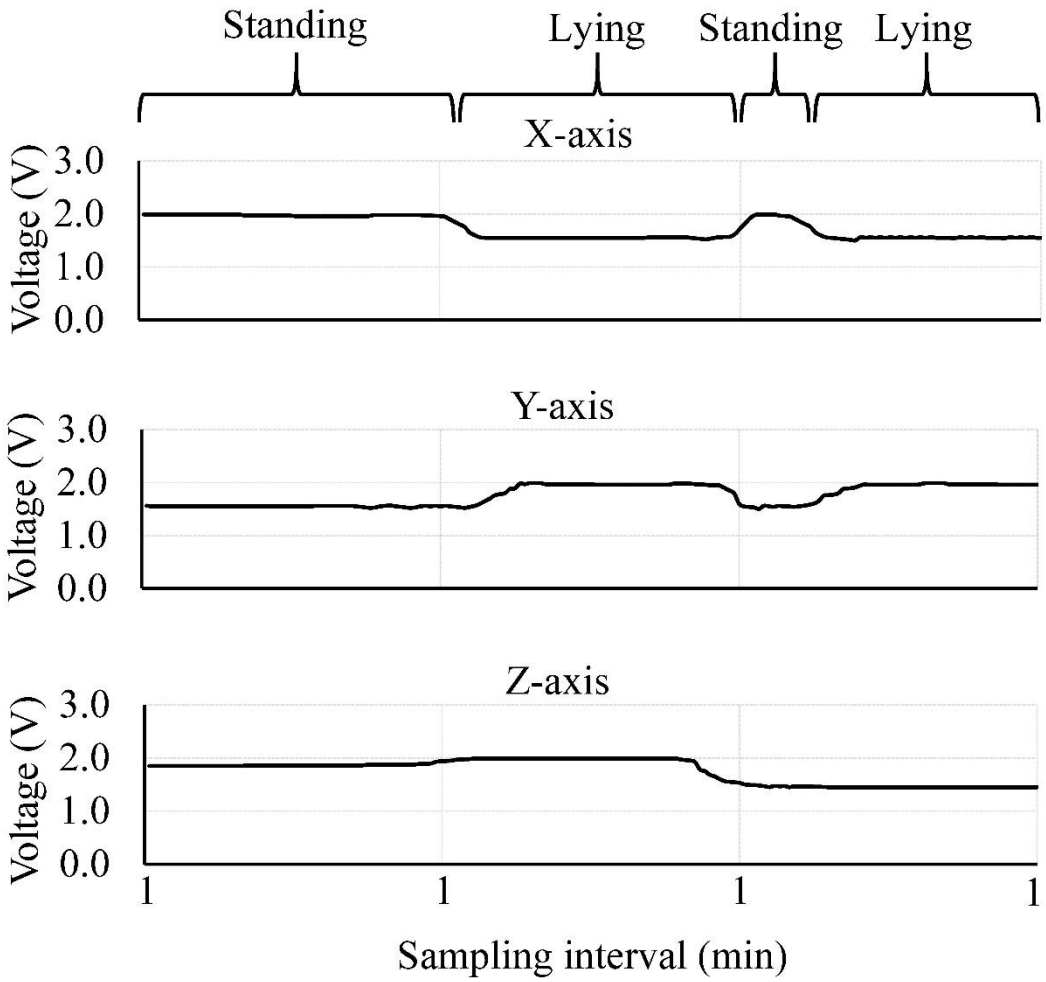


**Figure 3.3. A graphic representation of the wave-like signals generated by a three-dimensional accelerometer.**

A graphic representation of the wave-like signals generated by different levels of voltage recorded on each of the 3-axes (X-, Y-, and Z-axes) of a three-dimensional accelerometer.

To classify the waveform patterns for a specific behavior, they can be categorized using either a custom-designed classification system, or automatically using software developed by the manufacturer (Darr and Epperson, 2009; Brown et al., 2013). Many commercially-available devices [e.g., ACT, AfiAct® Pedometer Plus (**AfiAct**; Kibbutz, Afikim, Israel), CowScout (GEA Farm Technologies, Bönen, Germany), IceQube, and The Track a Cow (**TAC**; ENGS, Rosh Pina, Israel)] and some research devices (e.g., IceTag 2D and IceTag 3D) include software that categorizes the waveform patterns to

time (or count) of a specific behavior (e.g., steps, standing, lying) using a proprietary algorithm; however, much of the early research in the field of ethology required the development of a custom-designed classification system (e.g., Müller and Schrader, 2003). The Actiwatch and HOBO devices are both examples of devices that are reliant on the user to interpret the data (Müller and Schrader, 2003; Ledgerwood et al., 2010; Richeson et al., 2018), whereas the IceTag and IceQube devices are examples of devices that allow the user to download summary files using the software (IceManager 2010) provided by the manufacturer (IceRobotics Ltd.).

### *3.4.3 Data Retrieval*

Data retrieval includes either manual download using product-specific software after retrieval of the device (Brown et al., 2013) or automatically using ultra-high frequency data download technology (Table 3.1). Automated data retrieval allows the data to be downloaded through the use of either fixed (e.g., IceQube) or portable (e.g., IDA Tracker, Connecterra B. V., Amsterdam, The Netherlands) wireless transceivers (range: 5 to 1000+ m) or anywhere with cellular coverage through the use of satellites (e.g., Halter, Halter USA Inc., Auckland, New Zealand; Brown et al., 2013); however, the ability to manually or automatically download data is device-specific. The downloaded data are summarized according to the user-defined sampling interval (Robert et al., 2009) and can typically be further summarized once downloaded using the product software. For example, the IceTag (IceRobotics Ltd.) data can be summarized into 1 s, 1 min, 15 min, 1 h, 2 h, 1 d, and 1 wk recording intervals, whereas the IceQube can be summarized only into 15 min, 1 h, 2 h, 1 d, and 1 wk sampling intervals (Table 3.1; IceRobotics Ltd., 2017). While there are several accelerometers on the market with a range of sampling interval options and data retrieval and download options, when

choosing a device it is important to consider the data granularity required, the sampling duration required, ease of device retrieval, and the storage capacity or data offloading abilities of the device (Brown et al., 2013).

## 3.5 TRANSFORMATION OF DATA INTO INTERPRETABLE LYING BEHAVIOR

Classifying the waveform patterns into distinct behaviors allows the data to be further summarized into interpretable information in a summary file for each device. Lying behaviors are often classified into durational (e.g., daily lying time) and transitional behaviors (e.g., daily LB, LB duration, and lying laterality). The summary file contains information about the behavior recorded within each sampling interval and orders the data by a timestamp, which indicates the sequence in which the behaviors occurred (Bonk et al., 2013). For example, for the IceTag the IceManager 2010 (IceRobotics Ltd.) software generates 1 summary file per cow containing lying time (s), standing time (s), and the number of steps taken for 1-min sampling intervals. These summary files are, typically, further summarized to generate single behavioral values across a specific period of time (e.g., over a 24-h period to give daily lying time). Further, this summary output can then be used to manually calculate transitional behavior using a method similar to that published online by the University of British Columbia (e.g., HOBO devices; Ledgerwood et al., 2010; UBC, 2013) or may be obtained from the device software (e.g., IceManager 2010 software) to generate a second summary file containing all recorded LB with a start date, start time (hh:mm:ss), and duration (s). These additional files can also be further summarized to generate single behavioral values across a specific period

(e.g., LB are often summarized over a 24-h period to give total daily LB and mean LB duration).

## 3.6 VALIDATION OF ACCELEROMETERS

Validation of behavior data recorded using accelerometers is important to determine appropriate sampling frequencies, sampling intervals, and editing criteria for the accurate reporting of behavioral information under a range of conditions (Ouellet et al., 2016). Researchers using accelerometers must understand the limitations of validation studies when applying recommendations to their own data and future validation studies should address the current gaps in knowledge. It is clear when reviewing studies undertaken using the same types of accelerometers under similar systems that there is a lack of understanding by some users of the factors that should be considered when using accelerometers (Appendix 2 – Supplemental Materials and Supplemental Table 1). There are discrepancies in the protocols used for collecting, analyzing, and reporting data between studies using the same accelerometers in cows under similar systems, and these protocols are not always supported by validation studies (Appendix 2 – Supplemental Materials and Supplemental Table 1). For example, several studies using IceTag devices in lactating dairy cows in freestall barns have either failed to edit data (e.g., false LB were not discarded from the original data) or failed to report whether the data were edited prior to interpretation (Telezhenko et al., 2012; Kokin et al., 2014), or referenced validation studies in their methodologies that did not fit with the behavior or technology of interest (Gibbons et al., 2012). Therefore, the purpose of the following sections is to disentangle some of the information surrounding the factors that affect validation study outcomes and recommendations such as the gold standards chosen to validate the devices, causes of measurement error and variability, and, finally, accelerometer recording settings.

### *3.6.1 Gold Standards for Validation*

Validation is important to determine the accuracy of an accelerometer-derived behavior (whether an observed value agrees with the true value; Watson and Petrie, 2010). Validation studies involve taking 2 methods to assess the same variable or outcome and to evaluate how well they agree (Watson and Petrie, 2010). One method is regarded as the "gold standard," and the other method is commonly a quicker, cheaper, or otherwise more-efficient method that may replace the gold standard (Watson and Petrie, 2010). Validation of accelerometers compares the behavioral data summarized from the device with visual observations (e.g., direct observation or video recording), or by attaching 2 loggers per animal on opposite legs to identify agreement between the 2 devices (Müller and Schrader, 2003; Munksgaard et al., 2006; McGowan et al., 2007), or by comparing the performance of technologies already validated with technologies not previously validated (Mattachini et al., 2013; Bewley et al., 2017). The performance of the accelerometer reported varies based on the choice of the gold standard, the algorithms used to characterize the data, the variation in the behavior tested, and the size of the test dataset (e.g., number of data records; Rutten et al., 2013).

Researchers have used different gold standards, or similar gold standards, but using different experimental methods for the validation of accelerometers, which complicates comparisons between studies because they are rarely equivalent (Rutten et al., 2013). This issue can result in similar validation studies making different recommendations. For example, in the study by Rutter et al. (2014), direct observations as the gold standard were undertaken using a 5-min scan sampling approach [as described by Mitlöhner et al. (2001)]. A gross overestimation of LB from the IceTag 3D compared to the manual observation was observed. It was concluded that the majority of false LB

recorded were bouts of short duration and, therefore, the authors recommended caution when interpreting data from these accelerometers (Rutter et al., 2014). It is worth noting, however, that a LB duration of <5 min is plausible (Tolkamp et al., 2010) with LB duration ≤1 min reported in beef cattle on hilly rangeland (Ungar et al., 2018), and, therefore, the 5-min scan sampling approach used as the gold standard may have resulted in missed LB recordings. This may have exacerbated the overestimation of LB reported, due to an underestimation of LB in the gold standard measure. In contrast, in a study by McGowan et al. (2007), continuous direct observations, as the gold standard, were undertaken to validate the accuracy of LB recorded by IceTag 3D devices in cows on pasture and 100% agreement between visually-observed data and the device was reported. As a result, the study concluded that the IceTag 3D accurately recorded several types of behavior, suggesting useful applications as both a research and industry tool.

These differences in study recommendations using the same accelerometers under similar experimental conditions may confuse researchers interested in using accelerometers. While direct observation is typically a preferred gold standard, the dataset in the study by McGowan et al. (2007) may have been limited due to a small variation in the LB recorded due to the short data collection period (e.g., across 3 d; ~9.3 h of data was recorded). In particular, a lack of short LB in the test dataset would have made it difficult to validate the device for recording true short LB in the study, and as a result, may be, in part, responsible for the different findings reported by the 2 aforementioned studies. Therefore, a best-practice gold standard comparison should be chosen for a validation study; however, the method for comparing measured data with the gold standard should also be considered (e.g., we propose continuous visual observation as the best-practice gold standard to allow true short LB to be distinguished from false LB).

Verification of behavior data recorded by accelerometers with a known gold standard can provide useful information about measurement variability and measurement error that may exist and, therefore, the reliability of the data (Watson and Petrie, 2010). In this example, the test dataset should also be sufficiently large and varied enough to detect measurement errors in the data (e.g., false positives). Sample size estimation can be calculated to ensure sufficient statistical power (number of records needed to achieve sensitivity (**Se**) and specificity (**Sp**) >99%) if the expected proportion of false LB below a certain threshold is determined (e.g., 95% of LB ≤1 min were false in the study by Ungar et al. (2018) in IceTag devices) and we recommend researchers undertaking validation studies in the future take this into consideration.

### *3.6.2 Measures of Behavioral Data Agreement*

Several measures of agreement have been used in validation studies to evaluate the accuracy and reliability of behavior data recorded by accelerometers compared with a gold standard. The most common measures of agreement include accuracy, positive and negative predictive values, Se and Sp measures, Lin's concordance correlation coefficients ($\boldsymbol{\rho_c}$), Pearson correlation coefficients ($\mathbf{r_p}$), Spearman rank correlation ($\mathbf{r_s}$), and coefficients of determination ($\mathbf{R^2}$) (Appendices 4 and 5 – Supplemental Tables 3 and 4, respectively). A range of agreement statistics have been presented in validation studies; however, some studies do not report agreement statistics, reliability, Se, or Sp. Therefore, researchers need to assess whether the statistics presented are appropriate to support a reliable assessment of the measures presented.

Detection performance can be described by Se, Sp, and accuracy (Rutten et al., 2013), where the response of interest is dichotomous (e.g., falls into 1 of 2 categories; lying or standing). Sensitivity refers to the proportion of time that the device correctly

identifies the behavior of interest (e.g., the true lying time measured by the gold standard divided by the total lying time recorded by the accelerometer; Ledgerwood et al., 2010). Specificity refers to the proportion of time that the device correctly identifies the opposite behavior (e.g., the animal not lying and, therefore, standing). This statistic is defined as the true total standing time measured by the gold standard divided by the total standing time recorded by the accelerometer (Weiss and Koepsell, 2014). The perfect test has a Se and Sp equal to 100% (Watson and Petrie, 2010) and high Se and Sp are both desirable (Weiss and Koepsell, 2014). Accuracy refers to the proportion of all behaviors recorded by the device agreeing with the gold standard (Weiss and Koepsell, 2014). For example, Kok et al. (2015) reported accuracy to describe the ability for different LB criteria thresholds to allow IceQube devices to correctly classify true and false LB recorded and defined accuracy "as the sum of correctly discarded false LB records and correctly retained true LB records divided by the total amount of LB records" (p. 7913). Across studies, Se and Sp values >99% have been reported for recording lying time (Appendix 4 – Supplemental Table 3) and >98% for recording LB (Appendix 5 – Supplemental Table 4) when devices attached to the hind leg were validated and when either the 1-s or 1-min sampling intervals were selected (e.g., HOBO, IceTag 2D, and IceQube devices; Ledgerwood et al., 2010; Mattachini et al., 2013; Kok et al., 2015, respectively).

To our knowledge, no recommendations exist for device performance limits for accelerometers recording lying behavior, unlike the sensor limits set by the International Organization for Standardization (ISO, 2007) for other commercially-available sensors (e.g., for mastitis detection; Rutten et al., 2013). Similarly, if device performance limits (e.g., Se and Sp values >97% achieved in validation studies reviewed) were set for

accelerometers marketed for measuring lying time and LB, this would give the user a high level of confidence in the data recorded.

Alternatively, where the description of reliability is based on the data being viewed as numerical data rather than categorical, then $\rho_c$ may be calculated by determining the agreement between the 'gold standard' measure and the device (Watson and Petrie, 2010). For example, in the study by Nielsen et al. (2018) validating the IceTag 3D and CowScout devices, both video-labeled and IceTag behavior data were summarized into 15-min periods for individual cows to match the video observations with the behavior measured by the device. Lin's concordance correlation modifies the $r_p$ by assessing not only how close the data are to the line of best fit, but also how far that line is from the 45-degree line through the origin; where a 45-degree line represents perfect agreement (Watson and Petrie, 2010). Lin's coefficient is 1 when all points lie exactly on the line drawn through the origin (Watson and Petrie, 2010). McBride (2005) suggests that <0.90 indicates poor, 0.90 to 0.95 moderate, >0.95 to 0.99 substantial and >0.99 perfect agreement. Across studies reviewed, the $\rho_c$ values reported for recording lying time are >0.99 for the IceQube, IceTag 3D, CowScout (15-min sampling interval; Borchers et al., 2016; Nielsen et al., 2018)], TAC, and AfiAct (1-min sampling interval; Borchers et al., 2016)] (Appendix 4 – Supplemental Table 3). The validity of the measure of interest can also be quantified using Spearman's rank correlation, if the measure and gold standard are ordinal variable and if the data are not normally distributed, where a value equal 1 indicates perfect agreement (Weiss and Koepsell, 2014); however, only 1 validation study used this agreement statistic (Müller and Schrader, 2003).

In contrast, $r_p$ is often inappropriately used to evaluate for agreement between pairs of data points and is an incorrect measure of repeatability (Watson and Petrie, 2010)

Pearson correlation coefficients provide a measure of the linear correlation between 2 variables (the association and strength of a linear relationship). Typically, when test results are continuous, variables are drawn from a normally-distributed population and the $r_p$ may be used (Karras, 1997). The $R^2$ provides a measure of the variance of 1 variable that is predictable from the other variable. Pearson correlation coefficients and $R^2$ values do not give a measure of whether the data conform to a line of equality (Watson and Petrie, 2010; Zaki et al., 2013). Therefore, they are not the most appropriate methods for evaluating reliability between 2 methods, because the points may not cluster tightly about the line, although the correlation may still be significant (Zaki et al., 2013). Instead of presenting $r_p$ to indicate accuracy when comparing accelerometers with a gold standard, Ledgerwood et al. (2010) suggested using an $R^2 \geq 0.90$ and a slope and intercept not statistically different ($P < 0.05$) from 1 and 0, respectively, to indicate accuracy. When comparing studies, the accuracy, Se, Sp, and $\rho_c$ values are the preferred measures of reliability and future work should consider whether the agreement statistics presented in validation studies are suitable for the purpose of their work.

### *3.6.3 Measurement Error*

Measurement error exists when the observed values differ from the true values due to random and systematic error (Watson and Petrie, 2010). Random error is considered to be normally distributed and, therefore, these errors tend to balance out on average (Hibbert, 2007). Systematic error can be caused by incorrect interpretation of data, which influences the overall accuracy of the measured data (Watson and Petrie, 2010). Where systematic error and its causes are known, the error can be eliminated or minimized, a correction can be applied to compensate or an algorithm built into the software to make the adjustment (Hibbert, 2007). The editing criteria, sampling

frequency, sampling interval (Charlton et al., 2017), and software used for interpretation of the raw data are major determinants of the accuracy of behaviors recorded by accelerometers and should be considered when making comparisons between validation studies and different devices (Kok et al., 2015), and when determining the appropriate experimental method.

### *3.6.4 Systematic Error I: False Lying Bouts*

Lying bouts occur more frequently when an animal is experiencing physical discomfort, for example, during parturition (Huzzey et al., 2005), and due to the shorter duration of these movements (Chen and Bassett, 2005; Martin and Bateson, 2007), LB must be captured using a high sampling frequency (e.g., ≤2 min; Chen and Bassett, 2005, Martin and Bateson, 2007; Mattachini et al., 2013). This creates a dichotomy between detecting shorter LB that provide valuable information about the behavior of an animal while avoiding movements that are not true records of behavior due to spurious false recordings that are not biologically meaningful (Mattachini et al., 2013; de Weerd et al., 2015). A common systematic error that occurs in accelerometers is the generation of false, short lying events in the data (e.g., the accelerometer records lying behavior when the cow is standing) and can occur when the animal temporarily shifts its leg position during activities such as grooming, kicking, or grazing. These false, short lying events recorded by the accelerometer are known as false LB (O'Driscoll et al., 2008; Higginson et al., 2010; Tolkamp et al., 2010; Ungar et al., 2018).

Lying bouts of very short duration should be treated with suspicion and editing criteria applied to remove such errors from the dataset without removing valuable information that correctly describes the behavior of an animal (de Weerd et al., 2015). Users should carefully consider applying appropriate LB criterion to their data as part of

the data-editing process (Mattachini et al., 2013). A LB criterion is defined as a set minimum LB duration that the user deems is indicative of the true lying behavior and is used to adjust the dataset so that erroneous short LB are removed. A LB criterion is based on ethological considerations of lying behavior that could be representative of true behavior and the device of interest is tested against a range of chosen criteria in validation studies.

To our knowledge, the accelerometers used for research purposes (e.g., HOBO and IceTag 3D devices) require the user to remove false LB data through data editing rather than relying on algorithms built into the software to remove these false LB. Despite this known systematic error, however, several validation studies do not recommend the use of LB criterion to remove false positives from the dataset (e.g., McGowan et al., 2007; Felton et al., 2012; Borchers et al., 2016; Nielsen et al., 2018). In some validation studies, this is because the removal of false LB appears to have little effect on total daily lying time. For example, strong agreement has been reported for several sampling intervals ranging from 1 to 15 min where no LB criteria were applied to the dataset in the IceTag 2D, IceTag 3D, HOBO, TAC, and AfiTag® II Pedometer (**AfiTagII**; Afimilk, Kibbutz, Afikim, Israel) (Munksgaard et al., 2006; Ledgerwood et al., 2010; Mattachini et al., 2013; Borchers et al., 2016; Nielsen et al., 2018; Appendix 4 – Supplemental Table 3). Ungar et al. (2018) also reported that where IceTag 3D devices (1-s sampling interval) were tested in beef cattle on hilly rangeland, that despite a large proportion (42%) of false LB ≤1 min recorded, the false LB only accounted for 3% of total daily lying time. Therefore, it is possible to achieve high levels of agreement, where the interest is in lying time without applying a LB criterion to the data for sampling intervals from 1 to 15 min.

The application of the LB criterion is important when quantifying LB. Due to the detection of false LB, a threshold for the minimum duration of a LB (LB criterion) should be applied to the dataset to ensure that the maximum number of false LB are discarded and the maximum number of true LB are retained. A range of LB criteria have been tested in validation studies reviewed (range: 6 s to 30 min); however, the LB criteria commonly recommended tends to be ≤4 min as relatively few LB records have a duration less than 4 min (Ledgerwood et al., 2010; Tolkamp et al., 2010; Mattachini et al., 2013; Kok et al., 2015; Henriksen and Munksgaard, 2019) (Appendix 5 – Supplemental Table 4). The optimum threshold will be predominantly dependent on the sampling interval; therefore, it is important that the sampling interval used and appropriate LB criteria are considered together. Sampling interval selection is another source of systematic error, which should be evaluated when selecting an accelerometer and before the device is deployed in the field. After the data are retrieved from the accelerometer, the false LB should be removed.

### *3.6.5 Systematic Error II: Sampling Frequency and Sampling Interval*

A sampling interval contains a summary of the counts of behavior recorded by the device at the device's predetermined sampling frequency (Robert et al., 2009). For example, a sampling frequency of 1 Hz with a sampling interval of 1 min would produce 60 records within each interval (Dow et al., 2009) and this indicates the resolution of sampling. While the sampling interval of a device is usually product-specific, typically, the sampling interval is user-defined in research devices, where a range of sampling interval options (range = 1 s to 1 wk) are available for selection (e.g., IceTag 2D and IceTag 3D), and fixed in commercial devices (e.g., 2 h; **ACT**; Lely, Maassluis, The Netherlands). In some cases, it is not possible to program the device to further summarize the data into a defined sampling interval and, therefore, the data is summarized according

to the selected sampling frequency (e.g., HOBO). The sampling frequency and sampling interval both influence the resolution and amount of data obtained (Mattachini et al., 2013), and the level of data granularity required will depend on the research question and objectives of the study (Mitlöhner et al., 2001). Therefore, sampling frequency should be considered in conjunction with the sampling interval selection options.

Measurements of rapid movements (e.g., LB) require higher sampling frequencies than behaviors that animals spend a considerable amount of time engaged in (e.g., lying time; Martin and Bateson, 2007; Borchers et al., 2016). At lower sampling frequencies, the device must remain in a vertical or horizontal position for a longer period of time to register a standing or lying event (Borchers et al., 2016) and this reduces the level of accuracy because the time between samples is greater; however, lower sampling frequencies may be suitable if the interest is in recording durational behavior (e.g., daily lying time). A rule of thumb has been proposed that the sampling interval should be at least half that of the highest frequency movement being classified (Chen and Bassett, 2005); therefore, if the interest is in both lying time and LB behavior, a shorter sampling interval (higher resolution) should be selected [e.g., if it is plausible for the animal to engage in a 4 min bout of lying, the sampling interval required to record this behavior accurately would be ≤2 min (Chen and Bassett, 2005; Halsey et al., 2009; Ledgerwood et al., 2010; de Weerd et al., 2015). Therefore, the inability to select the sampling interval should be taken into consideration when choosing a device, depending on the intention of use.

While a higher-resolution sampling interval can provide important information about transitional behavior, as discussed in the previous section (see 3.11.2 Systematic Error II: False LB), it also requires the user to remove false-positive LB in data using a

validated LB criterion. In validation studies, applying different LB criteria to the data to discard false LB, across a range of sampling intervals under controlled experimental conditions, can allow the optimum combined sampling interval and editing criteria for specific behaviors to be tested and identified (de Weerd et al., 2015). Several studies have evaluated sampling intervals ranging from 1 s to 2 h alongside a range of editing criteria, from no editing to the removal of LB ≤30 min to determine the upper and lower limits of the accelerometer where the interest is in accurately recording lying time and LB in cows (Appendices 4 and 5 – Supplemental Tables 3 and 4). Several validation studies support that less frequent sampling (1- to 15-min sampling intervals) is suitable for recording daily lying time (Appendix 4 – Supplemental Table 3) (Martin and Bateson, 2007); however, short LB that do not last the duration of these longer sampling intervals (Chen and Bassett, 2005; de Weerd et al., 2015) are likely to be missed and the total number of LB can be substantially underestimated (Mattachini et al., 2013). Therefore, higher sampling frequencies (1 s to 1 min) are required to detect LB.

A range of true LB durations from 4 s to 4 h 20 min have been reported in lactating dairy cows under freestall housing (Kok et al., 2015). Therefore, it is not surprising that low-resolution sampling intervals ranging from 5 to 60 min appear to be inaccurate for recording LB behavior, assuming a LB duration of 4 s is plausible (Appendix 4 – Supplemental Table 3) (e.g., Ledgerwood et al., 2010; Mattachini et al., 2013). Sampling intervals ranging from 1 s to 2 min and a range of LB criteria have been investigated in dairy cows, and with the appropriate LB criterion applied to the data result in the highest levels of accuracy when the device is attached to the hind leg (Appendix 5 – Supplemental Table 4) (e.g., HOBO, IceTag 2D, IceTag 3D, and IceQube devices; Ledgerwood et al., 2010; Mattachini et al., 2013; Kok et al., 2015). While collars are sometimes used, leg

bracelets are more suited to measure lying behavior because they are fixed (Martiskainen et al., 2009; Ledgerwood et al., 2010; Mattachini et al., 2013) and this allows the dimension of movement and acceleration of the animal to be recorded without movement of the accelerometer on the animal obscuring measurements (Brown et al., 2013). Further, while studies validating the same or similar accelerometers recommend different LB criteria, it appears that recommendations should be applied under similar conditions to the validation study, due to differences in the variation of behavior across different systems.

***Sampling Intervals for Recording Daily Lying Time Only.*** Validation studies investigating the accuracy of accelerometers for measuring lying time when the sampling interval was ≥30 min have indicated that agreement between the gold standard measures and the devices of interest is poor (ACT and IceTag 2D; Siegford et al., 2012; Elischer et al., 2013; Mattachini et al., 2013) or moderate (HOBO; Borchers et al., 2016) (Appendix 5 – Supplemental Table 4). Using a lower sampling frequency results in fewer data points to estimate total daily lying times, leading to either gross overestimation or underestimation of lying time (Borchers et al., 2016). Some devices will use a scan sampling approach where data are captured at the beginning of each sampling interval (e.g., 1 sample every 1 h = 24 records/d); however, it is well known from previous research using manual observations of animal behavior that this approach lacks accuracy and precision in predicting the duration of lying behavior (Mitlöhner et al., 2001). Overall, the results indicate that sampling intervals ≥30 min would not be advisable to accurately record lying time in dairy cows, particularly where the durational totals are summations of infrequent scan samples (Elischer et al., 2013). It also emphasizes the importance of

considering whether the sampling interval of the device is suitable to record the behavior of interest.

Unbiased estimates of lying time can be obtained using sampling intervals ≤15 min due to the intervals being short enough relative to the duration of the behavior to allow accurate and precise predictions. In general, for sampling intervals ranging from 1 s to 15 min with no LB criterion applied to the data, the higher-resolution sampling intervals produce improved results (Munksgaard et al., 2006; Ledgerwood et al., 2010; Mattachini et al., 2013; Borchers et al., 2016). Although consideration is needed to determine whether such a high-frequency sampling regime (e.g., 1 s) is necessary and whether a lower frequency sampling regime could be used to describe the behavior under investigation (Ropert-Coudert and Wilson, 2004; Halsey et al., 2009). The sampling regime should be a compromise between recording the behavior of interest as accurately as possible while considering the constraints of the device (e.g., on-board storage and battery life). Despite this, however, typically, most studies are interested in several behavioral indices, not just the lying time. Therefore, the chosen sampling frequency, sampling interval, and LB criteria may be influenced by other behavioral measures of interest so that all behavioral indices can be accurately recorded (Martin and Bateson, 2007). Based on the literature reviewed, to accurately record daily lying time, a sampling interval ≤15 min is required (Appendix 4 – Supplemental Table 3); however, to accurately record daily LB a sampling interval ≤1 min is required (Appendix 5 – Supplemental Table 4).

## 3.7 MEASUREMENT VARIABILITY

Although variation in behavioral measurements is inevitable, differences in experimental conditions between studies validating accelerometers can create additional variation in the data and affect its distribution (Tolkamp et al., 2010). This variation can result in different outcomes and recommendations for the use of the accelerometer, which may cause confusion for researchers when deciding on an appropriate LB criterion (Watson and Petrie, 2010; Bewley et al., 2017). Measurement variability exists because behavioral variables are repeated measures within individuals over time and there will always be intra-individual (within individual) as well as inter-individual (between individuals) variability. Variability in lying behavior and the proportion of short LB in data are influenced by cow, farm, and management-related factors (Munksgaard et al., 2006; Watson and Petrie, 2010; Mattachini et al., 2013). Therefore, it is important to consider the animals and management system in validation studies to determine whether the LB criterion is appropriate (Kok et al., 2015).

Different recommendations have been reported by authors even though the same or similar type of accelerometers have been validated in cows of differing ages [e.g., calves and cows (Trénel et al., 2009; Ledgerwood et al., 2010)], under different management [e.g., grazing pasture (McGowan et al., 2007; Rutter et al., 2014), tie-stall (Felton et al., 2012), and freestall housing (Elischer et al., 2013; Kok et al., 2015)], different underfoot conditions [e.g., slatted floors and deep-bedded (Ledgerwood et al., 2010; Henriksen and Munksgaard, 2019)], and different physiological states [e.g., nonlactating and lactating (Trénel et al., 2009; Ledgerwood et al., 2010; Mattachini et al., 2013)]. For example, where the interest was in LB, Ledgerwood et al. (2010) reported that at a 30 s sampling interval, a higher LB criterion (≤60 s) was recommended for their

second experiment (Exp. 2) to achieve a similar level of Se and Sp compared with the ≤30 s LB criterion recommended for their first experiment (Exp. 1). It was speculated that different experimental conditions could have caused these differences due to a greater proportion of short LB in dry cows housed individually on a bedded pack in Exp. 2 (23.8% of LB <5 min) compared with the group of lactating cows housed in a freestall barn in Exp. 1 (1.5% of LB <5 min). While it is difficult to disentangle whether a single factor was responsible for influencing the outcome of the study by Ledgerwood et al. (2010), other studies have reported different outcomes in cows housed under different conditions.

Underfoot conditions can affect lying behavior recorded due to the bedding material affecting the angle of the leg with the device attached when the cow is standing and lying, which could lead to variation in the number of minutes recorded as lying and standing time or result in a larger number of false LB records in comparison to what was observed (Henriksen and Munksgaard, 2019). The study by Henriksen and Munksgaard (2019) reported that the accuracy of the AfiTagII for recording LB was improved in the cows kept on a slatted floor compared with dry cows kept on deep bedding (positive predictive value = 0.96 vs. 0.85, respectively). Typically, the devices are attached just above the fetlock and rely on the correct orientation of the limb to accurately record time spent standing or lying. The housing and underfoot conditions could affect the accuracy of the device and, therefore, should be considered when determining an appropriate LB criterion required to remove false LB. Ideally, the experimental conditions under which a device was validated should be similar to the experimental conditions under which the device is to be used in prospective studies to ensure the LB criterion is appropriate (Kok et al., 2015).

## 3.8 CONSIDERATIONS FOR FUTURE VALIDATION STUDIES

We have reviewed the studies published to date that have validated accelerometers for measuring lying behavior in dairy cows. Despite the abundance of recently-published studies investigating aspects of lying behavior and aspects of behavior affected by cow, farm, and management-related factors using accelerometers, methodological issues involving the appropriate use of many accelerometers for the accurate recording of lying behavior still exist. While some validation studies have been able to systematically evaluate the accuracy of particular accelerometers using a range of devices, sampling frequencies, sampling intervals, and LB criteria (e.g., Ledgerwood et al., 2010; Mattachini et al., 2013; Kok et al., 2015), many have failed to establish a robust methodology for measuring lying behavior in the device of interest (e.g., Müller and Schrader, 2003; Felton et al., 2012; Siegford et al., 2012; Charlton et al., 2017). Researchers should ensure the appropriate application of the accelerometer and carefully consider and report methods accurately to allow inter-study comparisons. At a minimum, the following should be reported: 1) the system under which the animals were managed (e.g., housing, underfoot conditions, feeding, etc.); 2) the accelerometers used and how the device was attached to the animal; 3) a description of the method, software and statistical packages used to classify the raw accelerometer data into specific lying behaviors and for interpreting the data; 4) the sampling frequency and sampling interval selected; and, 5) the LB criterion applied to remove false positives with reference to validation work.

Protocols for the validation of accelerometer-derived lying behaviors should be developed to build on those proposed in the current review. Protocols should determine appropriate gold standards and device performance limits similar to those outlined by

Kamphuis et al. (2013) for evaluating in-line mastitis detection systems. Following the development of robust protocols for validation, more studies are needed to improve the appropriate use of accelerometers and researchers are encouraged to consider future research to (1) validate accelerometers under conditions outside of the range of previously-published validation studies (e.g., different housing, physiological states, underfoot conditions, ages, etc.); (2) investigate a range of sampling intervals and LB criteria to determine the optimal sampling intervals and LB criteria for the prediction of lying behavior; (3) ensure sufficiently sized and varied datasets are collected to achieve sufficient statistical power and report findings using appropriate agreement statistics; and, (4) validate accelerometers in grazing dairy cows using criteria comparable to those undertaken in housed cows. In the future, we recommend that manufacturers provide information regarding the performance limits of the accelerometers marketed for recording lying behavior, to give the user a high level of confidence in their product and the data recorded.

## 3.9 CONCLUSIONS

The use of accelerometers to record behavior in dairy cows is still evolving, and it is important that authors of both validation studies and experimental publications carefully consider and report methods accurately to allow inter-study comparisons. This review highlights the importance of understanding the relationship between sampling frequency, sampling interval, and LB criteria and the data obtained from accelerometers to make better-informed decisions. It reflects how these factors, alongside cow, farm, and management-related factors can affect the outcome of validation studies and, therefore, influence recommendations. This review highlights the importance of research to establish classification rules to accurately record the behavior of interest, based on

scientific validations and standardized protocols, and to outline these “rules” in detail in the methodology of published work. Thorough reporting of methodology will support universal gold standards for the management and analysis of accelerometer data.

## 3.10 ACKNOWLEDGEMENTS

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## 3.11 SUMMARY

In Chapter 3, I collated and summarized all the published studies that had validated accelerometer-derived lying behavior across a range of devices that are available for both research and commercial purposes. It was evident that no standard best-practice method has been determined for the editing of lying behavior data derived from triaxial accelerometers in both housed and grazing systems and it appears that some confusion exists regarding their appropriate use. In particular, robust validation studies in grazing dairy cows are limited and no clear recommendations are available in literature to support researchers using accelerometers under grazing conditions. Consequently, in Chapter 4, I attempted to determine the most suitable method for editing and interpreting the data recorded by the IceTag and IceQube devices used in my subsequent experimental chapters based on the information available in literature and the learnings from Chapter 3.

# CHAPTER 4. TECHNICAL NOTE: A COMPARISON OF EDITING CRITERIA FOR LYING BEHAVIOR DATA DERIVED FROM THREE-DIMENSIONAL ACCELEROMETER DEVICES ON GRAZING DAIRY COWS

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## 4.1 ABSTRACT

Shorter, more-frequent lying bouts (LB) could be used to predict calving and as an indicator of animal discomfort and ill health. In this technical study, we reviewed the literature to describe criteria for removing false short LB, caused by minor movements, from accelerometer data using IceRobotics technology. Using an existing dataset of grazing cows, we compared unedited with edited accelerometer data after applying 3 different LB thresholds (LB <33 s, ≤2 min, and <4 min were removed) within IceQube and IceTag accelerometers. Daily lying time, LB (no./d), and LB duration were derived from either IceQube or IceTag devices for 146 and 159 multiparous cows, respectively. Very-short LB were more common in the IceTag than IceQube data. Applying a shorter LB criterion (<33 s) to the IceQube dataset produced minimal differences between unedited (LB = 8.8 ± 3.6 no./d; n = 64,512 lying records) and edited data (LB = 8.3 ± 3.4 no./d; n = 60,463). In contrast, we observed large differences between unedited (LB = 307 ± 293 no./d; n = 2,305,693) and edited data (LB = 8.8 ± 4.1 no./d; n = 66,139) when a longer LB criterion (≤2 min) was applied to the IceTag dataset. Removing short LB that are unlikely to represent true behavior will improve the interpretation of lying behavior data; however, prospective studies are needed to determine the most-suitable LB criterion.

## 4.2 INTRODUCTION

Activity-monitoring devices that measure cow behavior may allow remote and individualized management of animals which could improve dairy cow health and welfare. It is important, however, that the methodology chosen to edit behavior data is supported by an appropriate and robust validation study, where a high level of accuracy

is reported (Charlton et al., 2017). Several studies indicate that unedited data from IceTag and IceQube accelerometer devices (IceRobotics Ltd.) provide accurate records of daily lying time in cattle across a range of systems (McGowan et al., 2007; Mattachini et al., 2013; Ungar et al., 2018), but there is inconsistency in the literature regarding the appropriateness of editing criteria for lying bouts (**LB**), which are a potentially valuable indicator of cow health, welfare, and comfort.

Lying bouts can be defined as the period of lying between 2 standing events and can be short in duration; therefore, a high sampling frequency is required to capture LB accurately. This creates a dichotomy, however, because a high sampling frequency will also detect minor movements, such as kicking or scratching, which generate short LB (e.g., <4 min) in the dataset that are not reflective of true lying behavior (Mattachini et al., 2013). These short LB are a systematic error and should be discarded to improve data accuracy; however, there is no consensus for a LB editing protocol that researchers can follow when analyzing and interpreting lying behavior data from IceRobotics accelerometer devices.

We are interested in examining lying behaviors (e.g., lying time, LB, and LB duration) in grazing dairy cows during the transition period from late gestation to early lactation when animals are at greatest risk of adverse health events. To our knowledge, no researchers validating IceRobotics devices in grazing cows have recommended the removal of false LB from the dataset. We expect that data derived from IceRobotics devices will contain short LB that are unlikely to be representative of true behavior, as reported in housed cows, and will need to be discarded (Kok et al., 2015). Therefore, our first objective was to review published experiments that have validated IceRobotics devices to assess criteria used for editing behavioral data prior to analysis. Our second

objective was to use an existing dataset from transition dairy cows grazing pasture to retrospectively examine descriptive lying behavior data before and after applying editing criteria, and to justify the selection of the final criteria for subsequent research.

## 4.3 LITERATURE REVIEW

We reviewed the published literature for studies that used either the IceQube or IceTag devices manufactured by IceRobotics Ltd. (Edinburgh, Scotland). The IceQube and IceTag should be considered separately when determining an appropriate editing methodology due to their different sampling frequencies (4 and 16 Hz, respectively). We first reviewed studies that have used either device in grazing cows to evaluate editing criteria applied. Second, we reviewed studies validating either device to evaluate the experimental design and editing methodologies. Due to our interest in using both lying time and LB behavior derived from IceQube and IceTag devices, we focused specifically on studies that validated both behaviors.

Few studies undertaken in grazing cows or cows with access to pasture have examined both lying time and LB using IceTag devices and our literature search returned no studies using IceQube devices. One study in grazing cows (Umstatter et al., 2015) and 2 studies in which, cows had access to pasture (Black and Krawczel, 2016; Rice et al., 2017) determined lying time and LB; however, there was no single preferred method for managing the data. For example, Umstatter et al. (2015) discarded LB <4 min as recommended by Tolkamp et al. (2010), while others discarded LB ≤2 min as recommended by Munksgaard et al. (2006), Endres and Barberg (2007), and Bewley et al. (2010). While most researchers removed LB ≤2 min, the justification for this editing criterion was not based on validation studies (e.g., Endres and Barberg, 2007; Bewley et

al., 2010) or the validation study referenced did not provide a detailed description of the experimental design (e.g., Munksgaard et al., 2006) (Appendix 6 – Supplemental Table 5).

Variation in the time animals spend engaged in certain lying and standing behaviors under different systems can affect the outcome of the validation study (Ledgerwood et al., 2010); therefore, validation studies undertaken under similar conditions are preferred when determining the most appropriate editing methodology for subsequent research (Ledgerwood et al., 2010; Kok et al., 2015). Only 1 study has validated both lying time and LB behavior derived from IceTag devices in dairy cows grazing pasture (McGowan et al., 2007). Others have validated both behaviors from IceQube (Charlton et al., 2017) and IceTag devices in cows housed indoors and taken out to pasture between morning and afternoon milking (Rutter et al., 2014); but these studies have limitations. McGowan et al. (2007) reported that the unedited dataset recorded by the IceTag provided accurate lying time and LB values; however, the short data collection period (3 d; ~9.3 h recorded data in total), the timing of the study (during the dry period), and small sample size (n = 15) may have limited the variation within the test dataset and, in particular, its applicability to the lactating cow. In comparison, according to Rutter et al. (2014), LB behavior derived from the IceTag was grossly overestimated; however, the gold standard measure of manual behavior records used to validate the IceTag was inadequate due to the low recording resolution (5-min intervals). Furthermore, Charlton et al. (2017) validated the IceQube but did not report appropriate accuracy measures suggested by others (e.g., sensitivity (**Se**) and specificity (**Sp**) estimates or Lin's concordance correlation; Watson and Petrie, 2010). Contradictory reports exist, where Rutter et al. (2014) advised caution when interpreting the number of LB derived from

unedited data from IceTag devices in cows at pasture, but authors of 2 other studies concluded that the original unedited data from the devices gave accurate lying time and LB records (McGowan et al., 2007; Charlton et al., 2017).

Due to these contrasting recommendations and the limitations of studies in pastured cows, we then considered validation studies undertaken in housed cows, which have more robust methodologies (e.g., Ledgerwood et al., 2010; Mattachini et al., 2013). Mattachini et al. (2013) and Tolkamp et al. (2010) both reported good correspondence between the IceTag device and continuous video observations for lying time and LB, but differed in their editing criteria, recommending the removal of LB ≤2 min and <4 min, respectively. For the IceQube device, to our knowledge, only 1 validation study has reported lying time and LB measures, with authors recommending the removal of LB <33 s from the original data (Kok et al., 2015).

Based on our assessment of the literature, we chose LB editing criteria of <33 s, ≤2 min, and <4 min from previous validation studies to conduct our next phase of this study. We visually inspected our existing accelerometer dataset of transition dairy cows grazing pasture before and after applying the 3 different editing criteria to examine the within-device variability for IceQube and IceTag devices when short LB are removed.

## 4.4 COMPARISON OF THREE EDITING CRITERIA

### *4.4.1 Materials and Methods*

***Description of the dataset.*** A database, described in detail in Chapter 6 (Appendix 7 – Supplemental Table 6), was compiled from 4 separate parent experiments that investigated various management and cow-related factors during the transition period in grazing cows. Of 380 cows available from the 4 experiments, data from 311

multiparous mixed-age and breed (Holstein-Friesian, n = 216; Holstein-Friesian x Jersey, n = 93; and Jersey, n = 1) cows were selected for analysis. In total, 69 cows were removed from the analysis due to incomplete data [>10 d of data missing between –5 to +10 d relative to the day of calving (d 0)], inaccessible files, the device fell off during the experimental period, or the cow was removed from the study (Appendix 8 – Supplemental Table 7).

***Behavior data collection and editing.*** Each cow was fitted with 1 device, either an IceQube (n = 146) or IceTag (n = 159) on the lateral side of a hind leg. No effect of hind limb choice for sensor attachment on lying behavior has been reported (Munksgaard et al., 2006). IceQube and IceTag devices were equally spread across treatments within parent experiment. Both devices were contained within plastic housing secured by a leg bracelet (IceRobotics Ltd.) and captured data at a frequency of 4 Hz (IceQube) and 16 Hz (IceTag), respectively.

Through the position of the 3 axes of the devices, behavioral parameters were characterized. Lying behavior was recorded when the orientation of the hind leg was horizontal, and LB were defined as periods between the device changing from vertical to horizontal and back to vertical. These data were stored on the device (60 d on-board storage capacity) with data granularity at a sampling interval of one second. Data were removed and downloaded using the IceManager 2010 software (IceRobotics Ltd.) to generate a summary file containing all recorded LB, with a start date, start time (hh:mm:ss), and duration (s) and this was used to calculate daily LB (no./d) and mean LB duration (min/d). From the output dataset, the sampling dates for each individual cow were assigned an experimental day (**expday**) relative to d 0. Each cow's recording period

began 00:00 on the day following attachment as recommended by Bewley et al. (2010). This transformed dataset was the basis of subsequent analyses.

***Statistical analysis.*** Statistical analyses were undertaken using SAS version 9.4 (SAS Institute Inc., Cary, NC). Recorded data ranged from –40 to +162 d (mean ± standard deviation (**SD**); start expday = –19 ± 13 d and end expday = +43 ± 35 d) (Appendix 9 – Supplemental Figure 1). Using PROC FREQ, the number of daily behavior records per cow was determined and expday were discarded where data from fewer than 100 cows and 2 studies were available. The remaining data included 14,891 records from 305 cows during the period –21 to +35 d. Lying time was calculated within expday by summation of LB durations for individual cows using PROC SUMMARY. Daily LB were calculated using the number of observations (n) output for individual cows within expday using PROC SUMMARY and mean LB duration was calculated using the means statement in PROC SUMMARY to average the durations of all LB for individual cows within expday.

Based on the literature review, 3 different LB criteria were applied to this organized dataset where LB <33 s, ≤2 min, and <4 min were discarded. To compare behavior values from the unedited data and edited data, mean, SD, and 95% CI were calculated for daily lying time, LB, and LB duration using PROC SUMMARY for the period –21 to +35 d for the 2 devices (Table 4.1). Confidence intervals were examined to determine differences at $P < 0.05$ between editing criteria.

## 4.5 RESULTS AND DISCUSSION

Activity devices, such as those manufactured by IceRobotics Ltd., generate useful data that can be used to monitor cow behavior; however, short false LB may overstate the

lying behavior recorded by these devices (Kok et al., 2015). As such, data editing may be necessary to improve the accuracy of the data (Mattachini et al., 2013). Mean daily lying time and LB number and duration before and after applying different LB criteria to our dataset are presented in Table 4.1. In the unedited data, the IceTag had 36 times more lying records than the IceQube, indicating a very large number of short LB. Consequently, the mean daily lying time was 0.43 h greater in the IceTag than the IceQube device; however, both devices had mean values within the range (7.50 to 10.3 h/d) of lying times previously reported for healthy grazing dairy cows (Sepúlveda-Varas et al., 2014) and for cows on pasture and fed TMR (Black and Krawczel, 2016; Rice et al., 2017). There was no change in mean, SD, and 95% CI for daily lying time after the removal of short LB from the IceQube dataset using the 3 editing criteria, but mean daily lying time was reduced (by between 0.58 and 0.82 h/d) in the IceTag dataset after editing (Table 4.1). Mean daily lying time for the IceTag dataset was shortest when LB ≤2 min or <4 min were removed. False LB typically make up a small proportion of total lying time; for example, in the study by Ungar et al. (2018) removing LB ≤1 min eliminated 95% of the LB from the original data; however, LB ≤1 min only accounted for 3% of total lying time. Our results are consistent with reports that discrepancies between unedited data recorded by IceQube devices and direct observations are small when summarizing daily totals for lying time (Ledgerwood et al., 2010) and, therefore, applying LB criteria has little to no effect on daily lying time, especially for IceQube devices as reported previously (Kok et al., 2015). Larger discrepancies in the daily lying times in the IceTag datasets after editing indicates that short LB make up a larger proportion of total lying time, which may lead to overestimation in unedited data.

**Table 4.1. Descriptive data for the IceQube and IceTag in the original unedited data and after applying 3 editing criteria.**

Number of records (n), mean, standard deviation (SD), and lower and upper 95% confidence limits for the daily lying time (h/d), lying bouts (LB; no./d) and LB duration (min/bout) for the period –21 to +35 d relative to the day of calving (d 0) in grazing dairy cows wearing either IceQube or IceTag accelerometers (IceRobotics Ltd., Edinburgh, Scotland). Data are presented as original unedited data and 3 subsets of edited data where different criteria were applied to the original dataset to remove LB <33 s, ≤2 min, and <4 min.

| | IceQube | | | | | IceTag | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 95% Confidence Limits | | | | | 95% Confidence Limits | |
| | n | mean | SD | Lower | Upper | n | mean | SD | Lower | Upper |
| | | | | | Unedited data | | | | | |
| Lying time, h/d | 64,512 | 8.52 | 2.41 | 8.48 | 8.57 | 2,305,693 | 8.95 | 2.60 | 8.90 | 9.00 |
| LB, no./d | 64,512 | 8.80 | 3.62 | 8.74 | 8.87 | 2,305,693 | 304 | 293 | 299 | 310 |
| LB duration, min/bout | 64,512 | 58.1 | 51.2 | 57.7 | 58.4 | 2,305,693 | 1.76 | 12.9 | 1.75 | 1.78 |
| | | | | | LB <33 s discarded | | | | | |
| Lying time, h/d | 60,463 | 8.52 | 2.41 | 8.48 | 8.57 | 157,200 | 8.37 | 2.41 | 8.32 | 8.41 |
| LB, no./d | 60,463 | 8.25 | 3.39 | 8.19 | 8.32 | 157,200 | 20.8 | 21.5 | 20.4 | 21.2 |
| LB duration, min/bout | 60,463 | 62.0 | 50.6 | 61.6 | 62.3 | 157,200 | 24.1 | 43.5 | 24.0 | 24.3 |

**Table 4.1.** Continued over page.

**Table 4.1 (Continued).** Number of records (n), mean, standard deviation (SD), and lower and upper 95% confidence limits for the daily lying time (h/d), lying bouts (LB; no./d) and LB duration (min/bout) for the period –21 to +35 d relative to the day of calving (d 0) in grazing dairy cows wearing either IceQube or IceTag accelerometers (IceRobotics Ltd., Edinburgh, Scotland). Data are presented as original unedited data and 3 subsets of edited data where different criteria were applied to the original dataset to remove LB <33 s, ≤2 min, and <4 min.

| | IceQube | | | | | IceTag | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 95% Confidence Limits | | | | | 95% Confidence Limits | |
| | n | mean | SD | Lower | Upper | n | mean | SD | Lower | Upper |
| | | | | | LB ≤ 2 min discarded | | | | | |
| Lying time, h/d | 58,739 | 8.52 | 2.41 | 8.47 | 8.56 | 66,139 | 8.19 | 2.40 | 8.14 | 8.23 |
| LB, no./d) | 58,739 | 8.02 | 3.02 | 7.96 | 8.08 | 66,139 | 8.75 | 4.05 | 8.67 | 8.82 |
| LB duration, min/bout | 58,739 | 63.7 | 50.2 | 63.4 | 64.1 | 66,139 | 56.2 | 52.2 | 55.8 | 56.5 |
| | | | | | LB <4 min discarded | | | | | |
| Lying time, h/d | 56,887 | 8.50 | 2.41 | 8.46 | 8.55 | 56,866 | 8.13 | 2.40 | 8.09 | 8.18 |
| LB, no./d | 56,887 | 7.77 | 2.72 | 7.71 | 7.82 | 56,866 | 7.52 | 2.77 | 7.47 | 7.57 |
| LB duration, min/bout | 56,887 | 65.7 | 49.8 | 65.4 | 66.1 | 56,866 | 64.9 | 51.3 | 64.5 | 65.2 |

In contrast, data editing using LB thresholds can substantially improve accuracy when estimating daily LB number and duration (Ledgerwood et al., 2010; Kok et al., 2015). Mean daily LB number decreased and duration increased for the IceQube dataset when each successive LB criterion was applied; however, these changes were small compared with the large differences obtained when editing IceTag data (Table 4.1). In total, 11.8% of the LB records from the IceQube had a duration of <4 min (Figure 4.1a). When LB <33 s were discarded from the IceQube data, 5.6% more LB were retained compared with the LB criterion of <4 min. Kok et al. (2015) validated the IceQube by comparing sensors on each hind limb and reported that despite relatively few LB records with a duration of <4 min (7.2% of total LB records), about half of those were assumed to be true LB and a LB criterion of <33 s retained 2.5% more records than a LB criterion of <4 min. Removal of LB <33 s improved combined sensitivity and specificity estimates (Se = 99.3%; Sp = 97.7%) relative to removing LB <4 min (Se = 96.7%; Sp = 100%) due to the underestimation of up to 10 LB per d using a LB criterion of <4 min (Kok et al., 2015). Therefore, based on the interpretation of our data and the recommendation of Kok et al. (2015), the use of the <33-s LB criterion in the IceQube device is our preferred option.

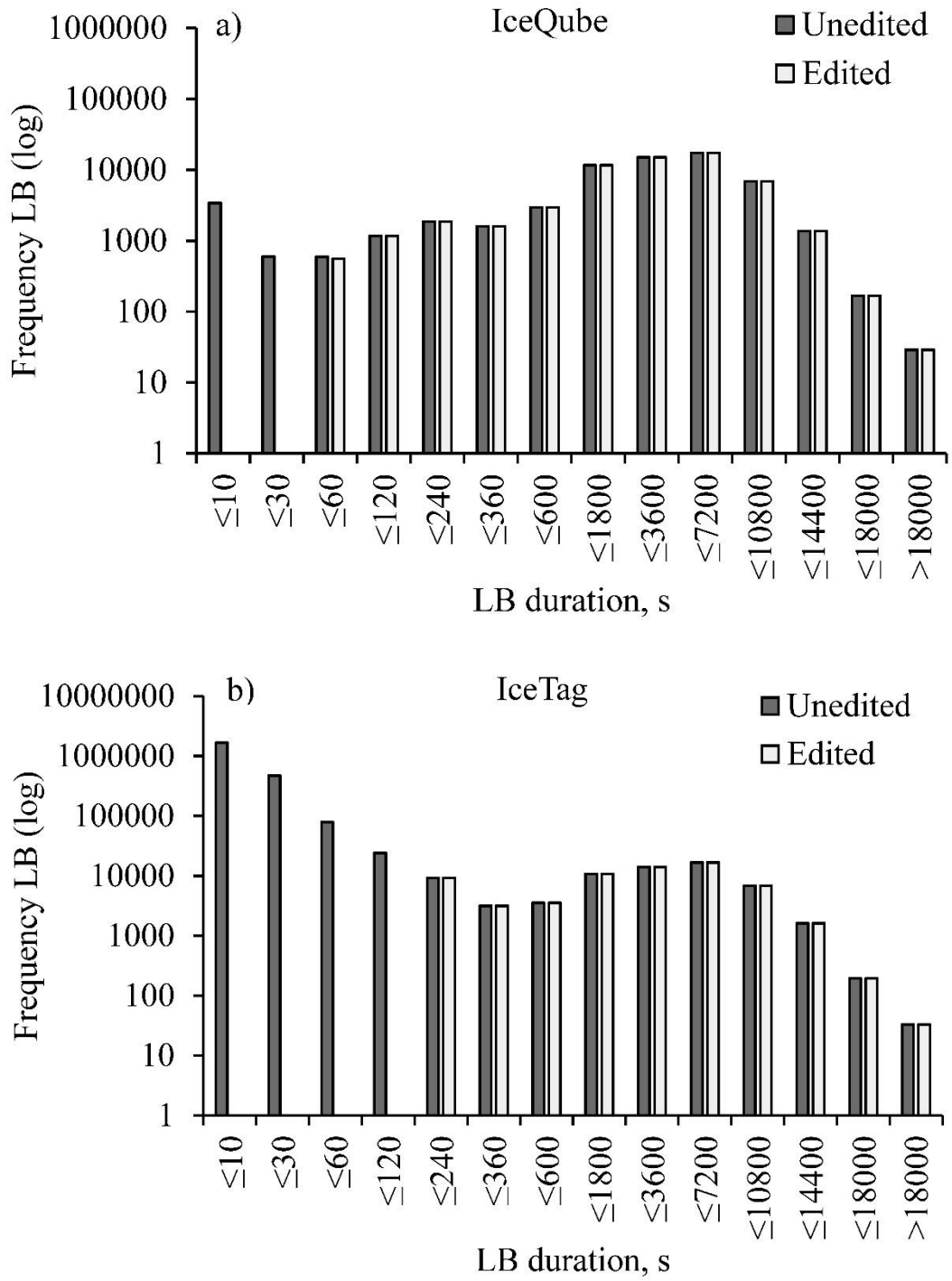


**Figure 4.1. Frequency distribution of lying bouts from unedited and edited IceQube and IceTag data.**

Frequency (logarithmic scale) of lying bouts (LB) within a range of bout durations (between ≤10 to >18,000 s) from unedited and edited data from IceQube (a) and IceTag (b) devices attached to the hind leg of transition dairy cows grazing pasture. To generate

the edited datasets, LB <33 s and ≤2 min were removed from IceQube and IceTag datasets, respectively. Each cow was fitted with 1 device, either an IceQube or IceTag.

The frequency distribution of IceTag data was comprised of 2 peaks, with a left skewed distribution of very large numbers of LB ≤240 s (≤4 min; Figure 4.1b). The removal of LB <33 s, ≤2 min, and <4 min eliminated 93%, 97%, and 97.5% of LB records, respectively (Table 4.1). Hence, short LB made up a considerable number of the LB records in the unedited IceTag data and although we cannot be certain from our data, it is unlikely that all of these records represented true LB (Tolkamp et al., 2010). Large numbers of erroneous short LB recorded by the IceTag may be explained, in part, by its high sampling frequency resulting in the detection of rapid behaviors and minor movements such as scratching and stepping (Tolkamp et al., 2010).

It is more realistic to choose a LB criterion that is likely to represent true behavior; therefore, we have justified our selected criteria based on LB values reported in literature. Discarding LB <33 s from our IceTag dataset (Table 4.1) still resulted in mean daily LB well outside of previously reported ranges of 9.50 to 13.1 no./d (Calderon and Cook, 2011; Borchers et al., 2017), indicating that a higher threshold was required. Removing LB ≤2 min or <4 min in the IceTag dataset resulted in 58 and 64% fewer total lying records, respectively, compared with removing LB <33 s (Table 4.1). Although, when a criterion of removing LB ≤2 min was used relative to <4 min, the mean and SD for daily LB number and durations were different between these editing criteria. A validation study of IceTag devices indicated that removing LB <4 min increased accuracy, where only 2% of the LB in the final data were false (Tolkamp et al., 2010); however, that study was undertaken in housed beef cows during late pregnancy, so care should be taken when extrapolating these results to transition dairy cows grazing pasture. The authors did not

recommend a shorter LB criterion because they did not record LB <4 min through video observation; however, others have reported that lactating dairy cows can spend <4 min lying in a single bout (Mattachini et al., 2013; Kok et al., 2015). Furthermore, studies undertaken in housed lactating cows using IceTag (Mattachini et al., 2013) and HOBO devices (Onset Computer Corporation, Pocasset, MA; Ledgerwood et al., 2010), which have a similar sampling interval (1 record per min), support the removal of LB ≤2 min. A suitable editing criterion should maximize the true records retained as well as minimize false records to ensure data accurately reflects lying behavior (Kok et al., 2015). It is possible for our data to contain true short LB <4 min, particularly during calving; therefore, the removal of LB ≤2 min is our preferred criterion for the IceTag dataset, to limit the risk of excluding true short LB durations.

Visual comparison of the temporal profile of daily LB number over the transition period between IceQube and IceTag devices with LB <33 s and ≤2 min removed, respectively, indicated a similar number of LB were achieved across the 2 devices (Figures 4.2a and b). It is evident from our data that the use of different LB editing criteria can have considerable effects on the output data of these devices (Figure 4.1). Based on our study, we cannot determine whether the editing criteria chosen, represented cow lying behavior at the same level of accuracy that has been reported in validation studies and the application of these editing criteria under different conditions to which they were tested is a limitation of our study; however, the final editing criteria chosen produced descriptive data that were consistent with previous literature and were biologically plausible.

Further investigations are required to determine inter-device agreement and the precision of accelerometer-derived data relative to true lying behaviors. Therefore, we recommend that future validation studies use an appropriate and robust experimental

protocol, which considers potentially false LB, to test the accuracy, sensitivity, and specificity of IceTag and IceQube devices for recording lying behavior in grazing cows.

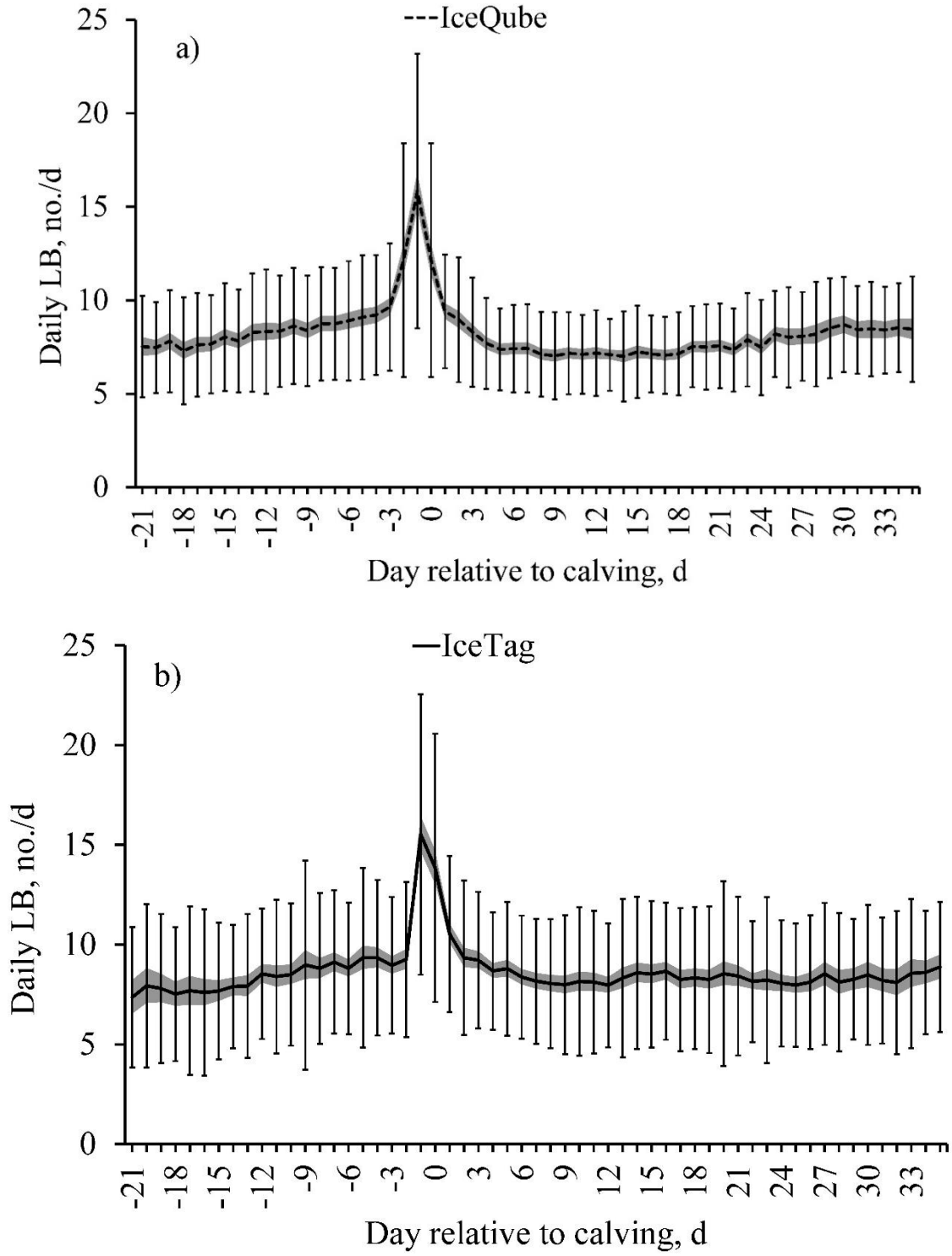


**Figure 4.2. Profile of daily lying bouts during the transition period in grazing dairy cows.**

Profile of mean daily lying bouts (LB; no./d) in grazing dairy cows during the period –21 to +35 d relative to the day of calving (d 0) where LB <33 s and ≤2 min were removed

from the IceQube (a) and IceTag (b) datasets, respectively. Each cow was fitted with 1 device, either an IceQube or IceTag. Error bars represent ± 1 standard deviation, and grey shaded areas represent 95% confidence intervals around the mean.

## 4.6 CONCLUSIONS

Short LB that are unlikely to represent true behavior in the original data recorded by IceQube and IceTag devices bias the daily number and duration of LB derived, but without materially affecting daily mean lying time for IceQube devices. Using previous reports validating IceQube and IceTag devices, along with an assessment of our dataset from transition cows grazing pasture, we chose from 3 editing criteria where LB <33 s, ≤2 min, and <4 min were discarded from the original data recorded by IceRobotics devices. Removing LB <33 s and ≤2 min from the data recorded by the IceQube and IceTag devices, respectively, was our preferred option. The removal of LB using these criteria reduced the within-device variation of LB. Future work is needed to validate a suitable LB criterion against a gold standard measure (e.g., visual or video observations) for IceQube and IceTag devices in grazing dairy cows.

## 4.7 ACKNOWLEDGEMENTS

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## 4.8 SUMMARY

In Chapter 4, I provided the justification and rationale for the editing criteria I chose and applied to the original data from the IceTag and IceQube devices deployed in the 4 parent experiments I collated for my database. I took a pragmatic approach to determine the most suitable methodology for editing the data based on what is known about dairy cow behavior, the recommendations reported for housed cows, and from visual inspection of my behavior data when left unedited and after applying different editing criteria. First, I undertook a literature review to determine if a recommended editing methodology existed from validation studies undertaken in grazing dairy cows. Following this, I determined 3 possible editing criteria, and then, visually inspected the descriptive data after applying these criteria to the original data from IceTag and IceQube devices. The intention of editing the data was to remove erroneous false LB that are generated in data derived from leg-mounted accelerometers due to short leg movements (e.g., scratching and kicking). My exploratory analysis indicated that removing LB <33 s and ≤2 min for the IceQube and IceTag, respectively, produced lying behavior values that reflect what is likely to be true behavior. I provided evidence that LB editing criteria can have considerable effects on the interpretation of the final dataset and should be chosen on a scientific basis. To further understand the factors that could affect the analysis of behavior data and could confound the data, I investigated the associations between lying behavior and activity and inclement weather in Chapter 5.

# CHAPTER 5. EFFECT OF WEATHER ON ACTIVITY AND LYING BEHAVIOR IN CLINICALLY HEALTHY GRAZING DAIRY COWS DURING THE TRANSITION PERIOD

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## 5.1 ABSTRACT

Lying behavior and activity were measured in healthy grazing dairy cows during the transition from late gestation to early lactation (i.e., the transition period). Behavior data derived from IceTag or IceQube (IceRobotics Ltd., Edinburgh, Scotland) triaxial accelerometers were collated from 311 cow parities of mixed-age and breed [Holstein-Friesian (HF), Jersey (J), and crossbred (HF x J)] cows from 4 experiments. The IceTag and IceQube devices captured lying and step data at 1- and 15-min intervals, respectively. Behavior was recorded during the transition period (d –21 prepartum to d +34 postpartum) to determine daily lying time, number of lying bouts (LB), mean LB duration, and number of steps taken. The effect of rainfall and air temperature on lying behavior and activity during 2 periods, namely, prepartum (d –21 to –3) and postpartum (d 3 to 34) was evaluated. Multiple-regression analysis determined that decreased air temperature and increased rainfall are associated with a decline in daily lying time, number of LB, and LB duration during both prepartum and postpartum periods. Exposure to both wet and cold conditions exacerbated the behavioral response. The results highlight the importance of considering the effects of air temperature and rainfall and the interaction of these 2 climate variables when analyzing lying behavior and activity. Further work is required to quantify the trigger points for this activity modulation to help understand the balance of welfare experiences in the life of a grazing cow.

## 5.2 INTRODUCTION

The transition period is defined as the 6 wk encompassing the calving event (Grummer, 1995; Drackley, 1999). Poor adaptation by cows to the associated metabolic changes occurring during this period is associated with an increased risk of ill health.

However, the identification of health problems is difficult for even the most highly skilled farm personnel (Stafford and Gregory, 2008). The use of electronic quantitative monitoring of lying behavior and activity to monitor transition-cow health (Weary et al., 2009) and the welfare of animals (Müller et al., 2018) is of interest, particularly, as herd sizes increase, which can result in less time to monitor individual cows, and as the public interest in the welfare of animals in grazing systems increases (Müller et al., 2018). Information resulting in improved management of individual dairy cows and the successful early detection of health problems will improve cow welfare and productivity as well as reduce health costs (Drackley, 1999; Loor et al., 2013). Electronic monitoring of lying behavior and activity allows these traits to be easily quantified in grazing systems (Borchers and Bewley, 2015). The information could allow researchers to determine the effects of extrinsic factors, such as particular management decisions or climatic differences, on behavior (Neave et al., 2017).

Several studies have focused on understanding the role of extrinsic factors in modifying animal behavior in housed dairy production systems, such as the type of housing (Legrand et al., 2009), management (Black and Krawczel, 2016), and illness (Huzzey et al., 2005; King et al., 2017). However, few studies have been undertaken in grazing systems where other considerations may need to be accounted for; for example, grazing dairy cows may experience periods of inclement weather. The risk is greater during the transition period, which typically coincides with cold and wet winter conditions in seasonal-calving grazing systems (Tucker et al., 2007b). It has been reported that grazing cows experience behavioral changes when exposed to inclement weather (Redbo et al., 2001; Tucker et al., 2007b; Webster et al., 2008). Lying time is a high-priority behavior in dairy cows and different physiological states can affect the time

budgets of dairy cows (Munksgaard et al., 2005); however, the relationships between inclement weather and lying behavior and activity have not been explored for grazing dairy cows during the transition period. Our objective was to investigate the relationships between climate variables and lying behavior and activity during the transition period in clinically-healthy grazing dairy cows, so as to provide information that could improve the analysis of behavioral data under grazing conditions.

## 5.3 MATERIALS AND METHODS

### *5.3.1 Animal Handling and Experimental Design*

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations in accordance with the New Zealand Animal Welfare Act (Ministry for Primary Industries, 1999). Data were extracted from 4 parent experiments [nonsteroidal anti-inflammatory drugs (**NSAID**), BCS, feed, and zeolite studies] undertaken across 4 seasons and 3 locations, namely, between June and September 2012, July and September 2013, June and September 2014, and June and August 2016, respectively. Comprehensive details and animal management from each experiment are described respectively, in Meier et al. (2014), Roche et al. (2015), Roche et al. (2017a), and Roche et al. (2018) (Appendix 7 – Supplemental Table 6).

### *5.3.2 Behavior Data Collection and Editing*

Behavioral parameters were extracted for analysis from the 4 parent experiments using subsets of cows that were rotationally grazed as described by Roche et al. (2005), from a total of 17 study treatments. Cows were fitted with electronic activity monitors for the period of d –23 prepartum to d +39 postpartum; however, only d –21 to +34 were used for the analysis. Of 380 cow parities available, a total of 311 cow parities from

mixed-age (mean ± standard deviation (**SD**); 4.5 ± 1.65 yr) and mixed-breed [Holstein-Friesian (**HF**), Jersey (**J**), and crossbred (HF × J)] multiparous cows were selected for analysis. Behavior data were recorded using an electronic activity monitor. Each cow was fitted with an IceTag (n = 162) or IceQube (n = 149; IceRobotics Ltd., Edinburgh, Scotland) on the lateral side of a hind leg. Both of these activity monitors use triaxial accelerometers and weigh ~200 g (IceTag; 65 x 60 x 30 mm; IceQube; 96 x 81 x 31 mm); both were contained within plastic housing. The IceTag and IceQube have been validated against visual observations for summarizing daily lying times (Mattachini et al., 2013; Borchers et al., 2016, respectively). Sixty-nine cows were excluded from the analysis due to invalid data [e.g., activity-monitor errors, incomplete data (≥3 consecutive days of data missing), or cows removed from the study]. The IceQube and IceTag devices captured animal activity through the position of the 3 axes of the activity monitor and the raw data were stored on the memory of the device. Data were removed and downloaded using the IceManager 2010 software (IceRobotics Ltd.) to generate 2 summary files per cow. One file consisted of recorded lying time (s), standing time (s), and number of steps for 1- and 15-min epoch intervals, for the IceTag and IceQube, respectively. This summary output was then used to calculate daily lying time (h/d) and daily number of steps taken (steps/d) for each cow. The other file contained all recorded lying bouts (**LB**), with a start date, start time (hh:mm:ss), and duration (s) and was used to calculate transitional behavior (e.g., daily LB (no./d) and mean LB duration (min/bout), where LB is defined as the period between the activity monitor changing from vertical to horizontal and back to vertical. A suitable threshold for the minimum duration of a LB record should be specified before LB data are analyzed to discard false records from the raw data that are caused by minor movements due to shifts in position, grooming, or grazing (O'Driscoll et al., 2008; Kok et al., 2015).

### *5.3.3 Weather*

Daily rainfall (mm) and daily air temperature (°C) recorded at 0900 h were retrieved from The National Climate Database (NIWA, 2018) for the duration of the 4 experiments. Data were retrieved from station agent Number 26,117 (37.8°S, 175.3°E) for the BCS, feed, and zeolite studies and from station agent Number 25,222 (39.6°S, 174.3°E) for the NSAID study (NIWA, 2018). The distance from the climate station to the study site for the BCS, feed, and zeolite studies was ~3 km and for the NSAID study the distance was <1 km.

### *5.3.4 Statistical Analyses*

Each cow was assigned to a group by concatenating study and treatment from the parent experiments. Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC), and unstructured and compound symmetry covariance structures were tested for all mixed models and that with the lowest Akaike information criterion (**AIC**) value used. Number of records and means for the daily LB and mean LB duration recorded by the IceTag and IceQube devices were calculated for all cows from –21 to +34 d relative to calving using PROC SUMMARY in SAS. In total, 2,321,879 LB records were available in the raw data and these were reduced to a total of 123,589 LB records in the filtered data. In the current study, based on previously determined thresholds for IceRobotics sensors, LB of <33 s and ≤2 min were discarded from the raw data recorded by the IceQube (Kok et al., 2015) and IceTag devices, respectively (Mattachini et al., 2013). The data recorded on the day that the data loggers were removed or fitted to the cows were not included in the behavior data analysis and outliers were removed only where the data point could be explained by an incorrect logger recording (i.e., <24 h of total activity recorded within an experimental day or total lying time equal

to 24 h; Bewley et al., 2010). These transformed datasets were the basis of subsequent analyses.

Number of records, and mean, median, minimum, and maximum for the daily rainfall and air temperature were calculated for the period of d –21 to +34 relative to calving by using PROC SUMMARY in SAS (Table 5.1). Daily rainfall and air temperature data were combined with behavior data for each cow according to calendar date. In total, 13,786 cow days were available for analysis. Multiple-regression analyses were undertaken using PROC MIXED to determine the effect of rainfall (RAIN, β1) and air temperature (AIR T, β2) and their interaction (RAIN x AIR T = β3) on lying behavior and activity, including day and group and their interaction as fixed effects and cow as a random effect. Multiple-regression equations for the dependent variables (daily lying time, number of LB, LB duration, and number of steps taken) were estimated for the 2 periods of **PRE** (d –21 to –3 prepartum) and **POST** (d 3 to 34 postpartum). On the basis of the AIC and *P*-value of <0.05 of the overall model, it was decided whether additional factors improved the model fit. The days immediately before and after calving (d –2 prepartum to d +2 postpartum) were not included in the analysis as this period should be considered independently of the periods' PRE and POST due to the substantial changes in behavior about the time of calving (Huzzey et al., 2005).

For main effects,

dependent variable = intercept + β1 x RAIN or, dependent variable = intercept + β2 x AIR T,

for additive effects,

dependent variable = intercept + β1 x RAIN + β2 x AIR T,

and for interactive effects,

dependent variable = intercept + β1 x RAIN + β2 x AIR T + β3 x RAIN x AIR T.

**Table 5.1. Descriptive data for the weather conditions pre- and postcalving.**
Number of records (n), mean, median, minimum, and maximum values for the weather conditions [daily rainfall (mm) and daily air temperature (°C)] during 2 periods: PRE (d –21 to –3 prepartum) and POST (d 3 to 34 postpartum).

| Weather factor | n | Mean | Median | Minimum | Maximum |
|---|---|---|---|---|---|
| Daily air temperature, °C | | | | | |
| PRE | 4,732 | 7.9 | 8.0 | –0.9 | 16.5 |
| POST | 8,372 | 8.7 | 9.0 | –0.8 | 15.4 |
| Daily rainfall, mm | | | | | |
| PRE | 4,732 | 3.8 | 0.2 | 0 | 52.6 |
| POST | 8,372 | 2.2 | 0.0 | 0.0 | 52.6 |

## 5.4 RESULTS AND DISCUSSION

We explored the association between behavioral parameters and weather variables in clinically-healthy grazed cows during the transition period. Daily lying time, number of LB, mean LB duration, and number of steps taken were influenced by inclement weather in both the PRE and POST periods. A summary of daily climate data recorded in PRE and POST periods is presented in Table 5.1. The daily rainfall ranged from 0.0 to 52.6 mm and air temperature recorded at 0900 h ranged from –0.8°C to 16.5°C for the periods of data collection across the studies. Multiple-regression model equations used to determine main, additive, and interactive effects of daily rainfall and air temperature on PRE and POST lying behavior and activity are presented in Table 5.2. The best-fit models are presented where main, additive, and interactive effects that were not significant at the

level $P < 0.05$ are not presented. Greater daily rainfall was associated with a reduction in daily lying time and lying time declined at a greater rate when the air temperature was lower during the PRE period due to an interactive effect; however, during the POST period daily lying time declined at the same rate irrespective of air temperature (Table 5.2). The results indicated that cows are likely to spend less time lying and, by definition, more time standing during inclement weather. Other authors have also reported shorter lying times for cattle exposed to colder or inclement weather when kept outdoors (Tucker et al., 2007b; Webster et al., 2008) and in indoor simulation experiments (Schütz et al., 2010). In contrast, while several studies have reported more time spent lying during exposure to colder temperatures (Gonyou et al., 1979; Redbo et al., 2001), the cows in these studies had access to bedding which is likely to have provided an insulating base on which the animal can lie. It has been suggested that underfoot conditions (Gonyou et al., 1979; Bøe, 1990) may affect whether the animal spends more time lying or standing, with a wet or frozen surface contributing to a reduction in the time cows lie down (Tucker et al., 2007b; Schütz et al., 2010). In the current study, cows were grazing and were not provided dry bedding and, therefore, the decrease in lying time may reflect a lack of cow comfort, reducing a desire to lie, or a strategy for the cows to minimize heat loss and improve their thermoregulation ability (Bøe, 1990; Tucker et al., 2007b).

**Table 5.2. Associations between lying behavior and activity parameters and inclement weather.**

Multiple-regression model equations used to determine the main, additive, and interactive effects of daily rainfall (RAIN, mm) and air temperature (AIR T, °C) on PRE (d –21 to –3 prepartum) and POST (d 3 to 34 postpartum) lying time (h/d), number of lying bouts (LB; no./d), LB duration (min/bout), and number of steps taken (steps/d). Model means and standard errors (SE) are presented.

| Parameter[1] | PRE | | | POST | | |
|---|---|---|---|---|---|---|
| | Estimate | SE | *P*-value | Estimate | SE | *P*-value |
| | | | Lying time, h/d | | | |
| Intercept | 9.08 | 0.56 | <0.001 | 8.29 | 0.51 | <0.001 |
| RAIN, mm | –0.28 | 0.02 | <0.001 | –0.06 | 0.01 | <0.001 |
| AIR T, °C | –0.06 | 0.01 | <0.001 | –0.02 | 0.01 | <0.001 |
| RAIN x AIR T | 0.02 | 0.01 | <0.001 | - | - | - |
| | | | Number of LB, no./d | | | |
| Intercept | 6.48 | 0.85 | <0.001 | 7.50 | 0.37 | <0.001 |
| RAIN, mm | –0.12 | 0.02 | <0.001 | –0.06 | 0.01 | <0.001 |
| AIR T, °C | –0.03 | 0.01 | <0.01 | - | - | - |
| RAIN x AIR T | 0.01 | 0.01 | <0.001 | - | - | - |
| | | | LB duration, min/bout | | | |
| Intercept | 90.2 | 6.61 | <0.001 | 66.7 | 5.77 | <0.001 |
| RAIN, mm | –0.68 | 0.21 | <0.01 | –0.78 | 0.22 | <0.001 |
| AIR T. °C | 0.11 | 0.10 | 0.278 | –0.13 | 0.08 | 0.130 |
| RAIN x AIR T | 0.06 | 0.02 | <0.01 | 0.06 | 0.02 | <0.01 |
| | | | Number of steps taken, steps/d | | | |
| Intercept | 3,016 | 240 | <0.001 | 4,166 | 176 | <0.001 |
| RAIN, mm | 22.0 | 7.63 | <0.01 | –84.8 | 12.4 | <0.001 |
| AIR T, °C | –19.8 | 3.70 | <0.001 | –0.60 | 3.92 | 0.878 |
| RAIN x AIR T | –1.55 | 0.68 | <0.05 | 6.75 | 1.13 | <0.001 |

Text under table continued on next page.

Text under table from previous page.

[1]Interactive, additive, and main effects that were not significant at the level $P < 0.05$ are not presented. RAIN = $\beta_1$, AIR T = $\beta_2$, RAIN x AIR T = $\beta_3$; for interactive effects, dependent variable = intercept + $\beta_1$ x RAIN + $\beta_2$ x AIR T + $\beta_3$ × RAIN x AIR T; for additive effects, dependent variable = intercept + $\beta_1$ x RAIN + $\beta_2$ x AIR T; for main effects, dependent variable = intercept + $\beta_1$ x RAIN.

Consistent with the reduction in lying time, cows also transitioned less frequently from standing to lying positions as indicated by the decrease in the number of LB as daily rainfall increased both PRE and POST. The decrease in the number of LB during the period PRE was exacerbated by lower temperatures as indicated by the interactive effect of air temperature and rainfall (Table 5.2). An increase in daily rainfall of ~15 mm during the POST period was associated with 1 fewer daily LB. A reduction in the number of LB and LB duration was associated with a concomitant reduction in daily lying time because these behavioral measures are interrelated. During the PRE and POST periods, there was an interactive effect whereby the mean LB duration decreased when the air temperature was lower and daily rainfall was greater; however, this effect diminished as the air temperature increased. To our knowledge, the present study is the first to have investigated the effect of air temperature and rainfall on LB number and duration during the day. Fisher et al. (2003) reported reductions in lying times driven by a reduction in the number of LB in cows that were stood-off pasture on a small wet paddock area under muddy conditions. Dairy cows are highly motivated to lie down (Jensen et al., 2005; Munksgaard et al., 2005), and welfare is seriously compromised when cows are deprived of lying time (Metz, 1985). Therefore, further research is required to understand the role of inclement weather in reducing dairy cow lying time, number of LB, and LB duration, and whether inclement weather and associated wet underfoot conditions compromise their welfare in grazing systems.

Colder weather was associated with greater activity during the PRE period, as the daily number of steps taken increased as air temperatures decreased and were further increased when daily rainfall was greater. However, during the POST period, this interaction was opposite, such that the daily number of steps taken decreased as air temperatures decreased, and were further decreased when daily rainfall was higher. The reason for this different interaction on the daily number of steps taken during the PRE and POST periods is unknown (Table 5.2). To the best of our knowledge, the daily number of steps taken due to inclement weather has not been previously reported in literature. Further research is required to understand the motivation to change activity due to adverse weather in nonlactating and lactating dairy cows grazing pasture, including potential changes to feed intake, paddock surface conditions, and grazing behavior.

In the current study, exposure to wet conditions alone did not reduce lying time to the same extent as occurred when cows were subject to both low air temperatures and rainfall. Therefore, accounting for the effects of air temperature and rainfall and the interaction of these 2 climate variables in behavioral analysis is an important consideration during the transition period. Climate variables and interactions between climate variables may influence behavior during other seasons to a greater or lesser extent due to differing weather patterns; however, further research is needed to understand these relationships.

## 5.5 CONCLUSION

The results of the current study indicated that when interpreting changes in behavior, climatic factors should be considered. Cows exposed to wet and cold conditions were more active, taking more steps prepartum, and less active, taking fewer steps

postpartum. Cows exposed to wet and cold conditions were spending less time lying, with shorter and fewer daily LB. There appears to be a direct thermal effect associated with behavior and changes in behavior in grazing dairy cows exposed to brief periods of cold and wet weather.

## 5.6 ACKNOWLEDGEMENTS

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## 5.7 SUMMARY

In Chapter 5, I determined the associations between lying behavior and activity and inclement weather in grazing dairy cows. There appears to be a direct thermal effect associated with behavior in dairy cows exposed to brief periods of inclement weather. Researchers should consider climatic factors when interpreting changes in behavior in grazing dairy cows. In Chapter 6, I described the lying behavior and activity of clinically-healthy grazing dairy cows. It is important first to understand what constitutes 'typical' lying behavior and activity and the potential causes of cow and herd-level variation that should be considered before assessing differences in behavior in sick animals.

# CHAPTER 6. LYING BEHAVIOR AND ACTIVITY DURING THE TRANSITION PERIOD OF CLINICALLY HEALTHY GRAZING DAIRY COWS

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## 6.1 ABSTRACT

Lying behavior and activity may provide useful information for the prediction of an imminent calving and the health of transition dairy cows; however, it is important first to understand what constitutes typical lying behavior and activity because this has not been defined for grazing dairy cows during the transition period. Our objective was to describe changes in lying behavior and activity in grazing dairy cows during the transition period using varying phenotypes typical of commercial dairy herds under grazing systems. Behavior data from IceTag or IceQube (IceRobotics Ltd., Edinburgh, Scotland) triaxial accelerometers were collected for 310 cow parities from multiparous, mixed-age (mean ± standard deviation; 4.5 ± 1.65 yr) and breed [Holstein-Friesian; n = 216, and Holstein-Friesian x Jersey; n = 94] grazing dairy cows from 4 parent experiments. The IceTags or IceQubes captured lying and activity data during the transition period (–21 to +34 d relative to calving) to allow the calculation of daily lying time (h/d), daily lying bouts (LB; no./d), mean LB duration (min/bout), and the number of steps taken (steps/d). Lying behavior and activity were analyzed using a repeated measures ANOVA during 3 periods: PRE (–21 to –3 d), POST (3 to 21 d), and the day of calving (d 0). Lying time was lower on d 0 (7.25 h/d) compared with PRE and POST lying times (10.3 and 8.58 h/d, respectively), with more frequent LB on d 0 (12.9 no./d) compared with the PRE and POST daily LB (8.15 vs. 7.74 no./d). Cows took more steps POST (4,424 steps/d) compared with d 0 and PRE (4,105 and 2,289 steps/d, respectively). Regression analysis determined that daily lying time decreased from –3 to 0 d (slope = –1.03 ± 0.07 h/d) and increased substantially from –2 to –1 d for daily LB (slope = 5.09 ± 0.54 no./d), which may be due to the calving event itself but also reflect restlessness. Daily lying time, daily LB, LB duration, and number of steps taken were substantially altered at the time of the

calving event in grazing dairy cows. Cows were more active, spent less time lying, and took more steps postcalving compared with precalving and it appears that this behavior may largely be due to activity associated with twice daily milking. Mean lying behavior and activity measures were more highly variable across individuals than across groups. Information available via activity monitors may contribute to the improvement of individual management of transition dairy cows and this research provides a benchmark for typical changes in behavior during the transition period in grazing systems.

## 6.2 INTRODUCTION

Lying is an important component of cow comfort and an indicator of welfare (Munksgaard and Simonsen, 1996). In housed systems, lying behavior has recently been recognized as an early indicator of health problems (Weary et al., 2009), and is also of interest in grazing systems for the improved management of individual dairy cows (Drackley, 1999). Technology has recently become available that allows lying behavior and activity to be easily quantified in grazing systems (Borchers and Bewley, 2015). Quantitative research that focusses on defining changes in the behavior of healthy cows is an important consideration when using behavior as an indicator of illness or welfare (Maselyne et al., 2017; Neave et al., 2017); however, there is a lack of detailed information available for changes in lying behavior and activity of grazing dairy cows, particularly during the transition period.

The transition period of dairy cows is the 6 wk encompassing calving (Grummer, 1995; Drackley, 1999), during which dairy cows must manage the exponential growth of the fetus, overcome the event of calving, and adapt to the increased physiological, metabolic, and nutritional demands imposed by the start of lactation (Grummer, 1995;

Drackley, 1999; Roche et al., 2013). Cows are exposed to a multitude of management related changes (Sepúlveda-Varas et al., 2014), and these changes in addition to physiological and cow factors can result in poor adaptation associated with increased health risk (Drackley, 1999; Roche et al., 2013). Understanding the effects of cow, physiological, and management factors on lying behavior and activity will improve our understanding of factors to take into consideration when using behavior as an indicator of health or welfare and help producers optimize the treatment of cows (Bewley et al., 2010).

Behaviors expressed may differ due to cow factors; limited studies have investigated both pre- and postcalving lying behavior and activity and the influence of breed, parity, and BCS in grazing dairy cows. Stone et al. (2017) first evaluated differences among dairy cattle breeds under housing conditions. No effect of breed was observed when comparing Holstein-Friesian (**HF**), Jersey (**J**), and HF x J dairy cattle breeds for lying behavior. One study has reported increased lying time with parity in grazing cows (Sepúlveda-Varas et al., 2014) and the findings of Calderon and Cook (2011) in housed cows supports this; however, others have reported no effect of parity on lying behavior in housed cows (Bewley et al., 2010). One study in late lactation grazing cows reported a decrease in lying time with decreasing BCS (Matthews et al., 2012). In contrast, Bewley et al. (2010) reported that BCS category did not affect lying behavior in housed cows in early lactation. To our knowledge, further studies have not been undertaken to investigate the effect of breed, parity, and BCS on lying behavior in grazing cows, and information in this area is lacking.

Behaviors expressed may also differ due to physiological factors and management (Kok et al., 2017). Physiological and management factors can constrain the time budgets

of cows and drive differences in time required lying, feeding, ruminating, and in lactating cows being milked (Jensen et al., 2005; Norring et al., 2012). Physiological state due to the change from dry to lactating state is likely to be a large determinant of behavior immediately pre- and postcalving; however, management (e.g., housed versus grazing) may influence the magnitude of the change. Lying behavior has been investigated in transition cows in housed systems and in cows on pasture. A study evaluating the postcalving lying behavior of a group of grazing cows highlighted differences in daily lying times when compared with lying times reported in housed cows and it was speculated that this could be due to external factors such as feed accessibility and time spent walking to and from the milking parlor (Sepúlveda-Varas et al., 2014). Only 2 studies (Black and Krawczel, 2016; Rice et al., 2017) have investigated the precalving lying behavior of dairy cows on pasture, but these animals were only moved onto pasture prior to calving as they were usually housed and fed TMR and they were kept in small groups (2 to 6 cows). Therefore, the results of these studies may not accurately reflect typical behavior of grazing cows where animals are required to meet energy requirements from pasture, have longer walking distances, and are typically kept in larger groups (Beggs et al., 2018). To our knowledge, cow, physiological, and management factors that influence dairy cow lying behavior and activity encompassing the 6 wk transition period and calving event have not been investigated in a substantial population of animals in grazing dairy systems. Our objectives were to determine (1) the daily and 24-hourly changes in lying behavior and activity, and (2) the associations between cow factors and lying behavior and activity, during the transition period of grazing dairy cows.

## 6.3 MATERIALS AND METHODS

### *6.3.1 Animal Handling, Experimental Design, and Management*

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations in accordance with the New Zealand Animal Welfare Act (Ministry for Primary Industries, 1999). Data for the present study were obtained from 4 separate parent experiments [nonsteroidal anti-inflammatory drugs (**NSAID**), BCS, feed, and zeolite studies; Appendix 7 – Supplemental Table 6]. These experiments were undertaken across 4 seasons and 3 locations: between June and September in 2012, 2013, 2014, and 2016. Cows were managed as a typical commercial herd of grazing cows under a spring-calving system, where the herds are managed on pasture throughout the transition period and rotationally grazed as described by Roche et al. (2005). The area allocated per cow precalving (23 to 60 $m^2$/cow) was typical of grazing systems and increased with time in all studies. Increasing pasture growth rates and pasture availability alongside increased feed demand as the seasonal calving period extends from winter to spring allowed the allocations of fresh pasture to increase by increasing the area allocated per cow and supplementary feed allocations were reduced (Roche et al., 2009). Nonlactating cows and lactating cows received allocations of fresh pasture daily in all studies. Fresh pasture offered was a mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture. Nonlactating cows received pasture silage in the BCS and feed studies, maize silage in the BCS and zeolite studies, and palm kernel expeller in the BCS study as supplementary feeds. Lactating cows received pasture silage in all studies and maize silage in the NSAID and BCS studies as supplementary feeds. Cows were offered on average 1.6 to 3.0 kg DM/cow per d as supplementary feeds and cows consumed a diet that was at least 75% fresh pasture across all 4 studies. During the

postcalving period, cows were milked twice daily in a rotary parlor. Total time spent standing and walking to and from the milking parlor ranged from ~40 to 90 min/d.

### *6.3.2 Cow Descriptions, Data Collection, and Analyses*

Behavioral parameters were extracted for analysis from the 4 parent experiments using subsets of cows fitted with electronic activity monitors. Of 380 total cow parities available in the 4 experiments, data from 310 cow parities were selected for analysis. Multiparous mixed-age [mean ± standard deviation (**SD**); 4.5 ± 1.65 yr] and mixed-breed (HF; n = 216 and HF x J; n = 94) cows were selected. All cows included in the study were multiparous (i.e., approaching their second or greater parity at the time of calving). The remaining 69 cow parities were removed from analysis due to invalid data (e.g., activity monitor errors, incomplete data, or cows removed from the study). One J cow was removed to avoid a breed group of n = 1. Behavioral data were available for the period –21 to +34 d relative to the day of calving (**d 0**) for the analysis.

### *6.3.3 Behavioral Data and Editing*

Behavioral data were recorded using an electronic activity monitor. Each cow was fitted with an IceTag or IceQube (IceRobotics Ltd., Edinburgh, Scotland) on the lateral side of a hind leg. Both of these activity monitors use triaxial accelerometers to characterize activity and weigh 190 g (IceQube; 96 x 81 x 31 mm) and 197 g (IceTag; 65 x 60 x 30 mm); both were contained within plastic housing. The IceQube and IceTag devices capture data at a frequency of 4 and 16 Hz, respectively (IceRobotics Ltd., 2017).

Behavioral parameters were measured through the position of the 3 axes of the activity monitor and these data were stored in the memory of the device (60 d on-board storage capacity). Lying time is recorded when the orientation of the hind leg is horizontal, and the step count is measured by the number of times the animal lifts its leg

and places it back down again. A lying bout (**LB**) is defined as the period between the activity monitor changing from vertical to horizontal back to vertical.

Data were removed and downloaded using the IceManager 2010 software (IceRobotics Ltd.) to generate 2 summary files per cow. One file consisted of recording lying time (s), standing time (s), and number of steps for 1- and 15-min epoch intervals, for the IceTag and IceQube, respectively. This summary output was then used to calculate daily lying time (h/d) and number of steps taken (steps/d) for each cow. The IceTag and IceQube have been validated against visual observations for summarizing daily lying times (Mattachini et al., 2013; Borchers et al., 2016). The other file contained all recorded LB, with a start date, start time (hh:mm:ss), and duration (s) and was used to calculate transitional behavior [e.g., daily LB (no./d) and mean LB duration (min/bout)].

From the output data sets, the sampling dates for each individual cow were assigned an experimental day relative to the day of calving (d 0). The data recorded on the day that the data loggers were removed or fitted to the cows were not included in the analysis and outliers were removed only where the data point could be explained by an incorrect logger recording (i.e., <24 h of total activity recorded within-day or total lying time equal to 24 h). These transformed data sets were the basis of subsequent analyses.

#### *6.3.4 Milk, BCS, BW, Breed, and Production*

Cows were milked twice daily and milk yield was measured daily from 1 to 35 DIM. Milk was sampled weekly on consecutive afternoon and morning milkings and a composite sample was analyzed for milk composition by infrared analysis (FT120, Foss Electric, Hillerød, Denmark). Energy-corrected milk yield was calculated as (Nielsen et al., 2009):

kg of ECM = [kg of milk x (383 x fat% + 242 x protein% + 780.8)]/3,140.

Weekly BW was recorded and BCS (scale of 1 to 10, where 1 is emaciated and 10 obese; Roche et al., 2004) was determined, following morning milking or at approximately 0800 h during the nonlactating period. All BCS assessors were trained and recalibrated at the start of the experiment following the procedures set out in Macdonald and Roche (2011). Animal evaluation data for cow breed, Breeding Worth (**BrW**), Production Worth (**PW**), and reliability were kindly provided by Livestock Improvement Corporation Ltd. (Hamilton, New Zealand; Table 6.1). Breeding worth and PW are estimated economic values of a combination of eight traits as indicators of robustness and production efficiency (Johnson et al., 2018). Breeding worth ranks cows on their expected ability to breed profitable and efficient replacements, whereas PW ranks cows on their lifetime performance (DairyNZ, 2018). In the current study, the economic values are expressed as dollars (NZ$) of net farm income per 5.0 t of DM relative to a 2000-born genetic base cow for the year 2016 so that cows across studies and years are comparable (DairyNZ, 2018). Reliability is a measure of the confidence of an animal's BrW being a measure of their true genetic merit. Breeding worth and PW values were used as proxy measures for milk production potential due to the experimental animals being involved in studies during previous seasons that may have affected their milk yield records.

**Table 6.1 Descriptive data for all cows included in the study.**

Mean, standard deviation (SD), minimum, and maximum values for cow performance of 310 cow parities and weather parameters for 14,942 experimental days.

| Parameter[1] | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| cBCS[2] | 4.73 | 0.52 | 3.75 | 5.75 |
| PW,[3] $/5 t DM | 110 | 82.6 | –109 | 362 |
| PW reliability,[3] % | 91.0 | 8.18 | 51.9 | 95.7 |
| BrW,[3] $/5 t DM | 111 | 40.5 | –34.0 | 209 |
| BrW reliability,[3] % | 52.2 | 2.57 | 37.7 | 59.9 |
| Rainfall, mm | 2.75 | 6.27 | 0.00 | 52.6 |
| Air temperature,[4] °C | 8.38 | 3.48 | –0.90 | 16.5 |

[1]Production Worth (PW), Breeding Worth (BrW), and covariate precalving body condition score (cBCS).
[2]Body condition score at –5 wk precalving (on a 1 to 10 scale; Roche et al., 2004).
[3]Genetic merit (New Zealand Animal Evaluation Ltd., Hamilton, New Zealand). Expressed as dollars (NZ$) of net farm income per 5 t of DM relative to a 2000-born genetic base cow for the year 2016.
[4]Air temperature recorded at 0900 h.

### *6.3.5 Weather*

Daily rainfall (mm; 24-h period) and daily air temperature (°C; recorded at 0900 h) data were retrieved from The National Climate Database (NIWA, 2018) for the duration of the 4 experiments (Table 6.1). Data were retrieved from station agent number 26,117 (37.8°S, 175.3°E) for the BCS, feed, and zeolite studies and from station agent number 25,222 (39.6°S, 174.3°E) for the NSAID study (NIWA, 2018). The distance from the climate station to the study site for the BCS, feed, and zeolite studies is ~3 km and for the NSAID study the distance is <1 km.

### *6.3.6 Statistical Analyses*

Each cow was assigned to a group by concatenating study and treatment from the parent experiments. Statistical analyses were performed using SAS 9.4 (SAS Institute

Inc., Cary, NC). Unstructured and compound symmetry covariance structures were tested for all mixed models and that with the lowest Akaike's information criterion value used.

***Milk, BW, BCS, and Parity Data.*** Means and SD for daily yields of milk and ECM for 1 to 35 DIM and for pre- (–5 to –1 wk) and postcalving (1 to 5 wk) BCS and BW were obtained using the PROC MEAN procedure. Covariate precalving BCS (**cBCS**) was determined as the BCS recorded for individual cows at –5 wk precalving. Parity was grouped as follows: parity 2 to 3 (n = 201), parity 4 to 5 (n = 70), parity 6 to 7 (n = 26), and parity 8+ (n = 13). Repeated measures ANOVA (PROC MIXED) were undertaken to determine the effect of breed and parity on yields of milk and ECM, including breed, cBCS, parity, and group as fixed and cow as random effects, respectively.

***Behavioral Parameters.*** Number of records, means, and SD for the daily number of LB and mean LB duration recorded by the IceQube and IceTag devices were calculated for all cows from –21 to +34 d relative to calving using PROC SUMMARY in SAS (Appendix 10 – Supplemental Materials and Supplemental Table 8). In the current study, based on previously determined thresholds for IceRobotics sensors, LB <33 s (Kok et al., 2015) and ≤2 min (Mattachini et al., 2013) were discarded from the raw data recorded by the IceQube and IceTag devices, respectively.

***Overall, Group, and Individual Cow Means and SD***. Daily means and SD were obtained for each experimental day on a per-cow basis, from which the overall means and group means were calculated using the PROC MEAN procedure for daily lying time, daily LB, mean LB duration, and number of steps taken for –21 to +34 d. A total of 14,942 d were available for analysis.

***Average Changes in Behavior Across Days.*** Relationships between lying parameters and daily number of steps taken and day were examined by piecewise regression analysis (PROC NLIN) to determine the breakpoints where the most significant changes in behavior occurred by day relative to calving. Starting parameters were estimated for each time-period by applying simple linear regressions at multiple time points and investigating the model fit as determined by the square root of mean square error and adjusted R-square. The starting parameters from the models with the best fit were then used to fit the piecewise regression model. These breakpoints output by the model were rounded to the nearest whole day, and subsequently, the data were split into 4 periods according to the behavior of interest. To determine whether cows changed their behavior across days, multiple-regression analyses were undertaken using PROC MIXED to determine the effect of day for all 4 behavior measures (daily lying time, daily LB, mean LB duration, and number of steps taken) during the 4 periods determined from the piecewise regression analysis. For all regression analyses, the intercept was tested for difference from zero, to determine whether behavior changed on average across days. Fixed effects of breed, parity, cBCS, PW, BrW, and group were included to account for possible differences relating to the behaviors of interest because these factors have been shown to affect behavior. Cow was included as a random effect. The BrW and PW parameters were included in the multiple-regression models per 10 unit increase. Because cows were outdoors and the experimental periods (across 4 yr) were different for the studies, rainfall and air temperature and their interaction were evaluated and included in the model (Chapter 5). Variables were checked for multicollinearity; however, no variables were highly correlated or had variance inflation factors greater than 10. Descriptive statistics for descriptive variables presented in Table 6.1 were calculated using PROC MEAN procedure.

***Effect of Period on Behavior Parameters***. Additional repeated measures ANOVA (PROC MIXED) were undertaken to summarize daily and 24-h behavior immediately pre- and postcalving (i.e., **PRE;** –21 to –3 d and **POST**; 3 to 21 d) and on the d 0. Farm staff collected newborn calves and their dams once daily and, therefore, there may be a discrepancy of up to 24 h for the recording of the day of calving. Daily lying time, daily LB, mean LB duration, and number of steps taken were calculated for 3 periods. The associations between the behavior parameters and period were investigated using a repeated measures ANOVA (PROC MIXED) with breed, cBCS, parity, group, period, and interactions between group and period as fixed, day as a repeated measure, and cow as random effects, respectively. The overall cBCS, breed, parity, and interactive breed by parity effects on lying behavior were analyzed using a repeated measures ANOVA (PROC MIXED); however, they are not reported because the effects were not significant at the level $P < 0.05$. The association between number of steps taken and parity and parity by period interaction was investigated during the 4 periods, generated from the piecewise regression described above, using a repeated measures ANOVA (PROC MIXED) with cBCS, breed, parity, period, and the interactions between parity and period as fixed, day as a repeated measure, and cow as random effects, respectively. There was no overall cBCS, breed, or interactive breed by parity effect on daily number of steps taken in the current study.

***Effect of Period and Time on Behavior Parameters.*** For the 24-h data, time was divided into 4-h blocks (i.e., 0200 to 0559 h, 0600 to 0959 h, 1000 to 1359 h, 1400 to 1759 h, 1800 to 2159 h, 2200 to 0159 h) for each of the 3 periods mentioned above. The effect of period and time and their interactions on the behavior parameters were analyzed using a repeated measures ANOVA (PROC MIXED) with breed, cBCS, parity, group,

period, and interactions between group and period as fixed, hour as a repeated measure, and cow as random effects, respectively. All repeated measures ANOVA models were pairwise comparison-adjusted using Tukey-Kramer. Means and standard error of the mean were obtained using PROC MEAN procedure for lying time (min/h) and number of steps taken (steps/h) on an hourly basis according to timestamp to generate 24-h data for the periods: PRE, POST, and d 0.

## 6.4 RESULTS

Mean and SD for milk yield and ECM yield were 24.1 ± 4.8 and 26.1 ± 5.0 kg/cow per d, respectively. Mean and SD of precalving BCS and BW was 4.7 ± 0.5 and 548 ± 65 kg, respectively and postcalving BCS and BW was 4.4 ± 0.4 and 485 ± 55 kg, respectively. A brief description of parameters included in the dataset from the 4 experiments used for statistical analyses is provided in Table 6.1. Mean milk yield and standard error of the mean was 24.6 ± 0.99 and 20.5 ± 1.64 kg/cow per d for the HF and HF x J cows, respectively ($P < 0.01$) for the first 35 DIM. Mean ECM yield and standard error of the mean was 26.3 ± 1.08 and 23.4 ± 1.29 kg/cow per d for the HF and HF x J cows, respectively ($P = 0.06$). There was no association between parity or parity by breed and milk yield and ECM yield for the first 35 DIM in the current study.

### *6.4.1 Overall, Group, and Individual Cow Means and Standard Deviation*

Lying time was normally distributed, and the mean and SD across all of the groups for daily lying time was 8.83 ± 2.45 h/d and for daily LB was 8.50 ± 3.75 no./d for the period –21 to +34 d relative to calving. The group means for daily lying time and daily LB varied from 7.94 to 9.69 h/d and 7.43 to 9.71 no./d, respectively. For daily lying time and daily LB, individual cow means varied from 5.77 to 12.6 h/d and 5.02 to 23.1 no./d,

respectively (Figures 6.1a and b). The mean and SD across all of the groups for mean LB duration was 66.3 ± 26.2 min/bout, and the group means varied from 55.4 to 76.0 min/bout, whereas individual cow means varied from 21.4 to 113 min/bout (Figure 6.1c). The mean and SD across all of the groups for daily number of steps taken was 3,805 ± 1,664 steps/d and the group means varied from 3,244 to 4,560 steps/d, whereas individual cow means varied from 1,638 to 6,065 steps/d (Figure 6.1d). The variation among cows differed from group to group; for example, the SD across groups for daily lying time varied from 2.25 to 3.28 h/d, daily LB varied from 2.93 to 7.06 no./d and mean LB duration varied from 22.0 to 32.5 min/bout. Similarly, the SD across groups for daily number of steps taken varied from 1,138 to 2,147 steps/d.

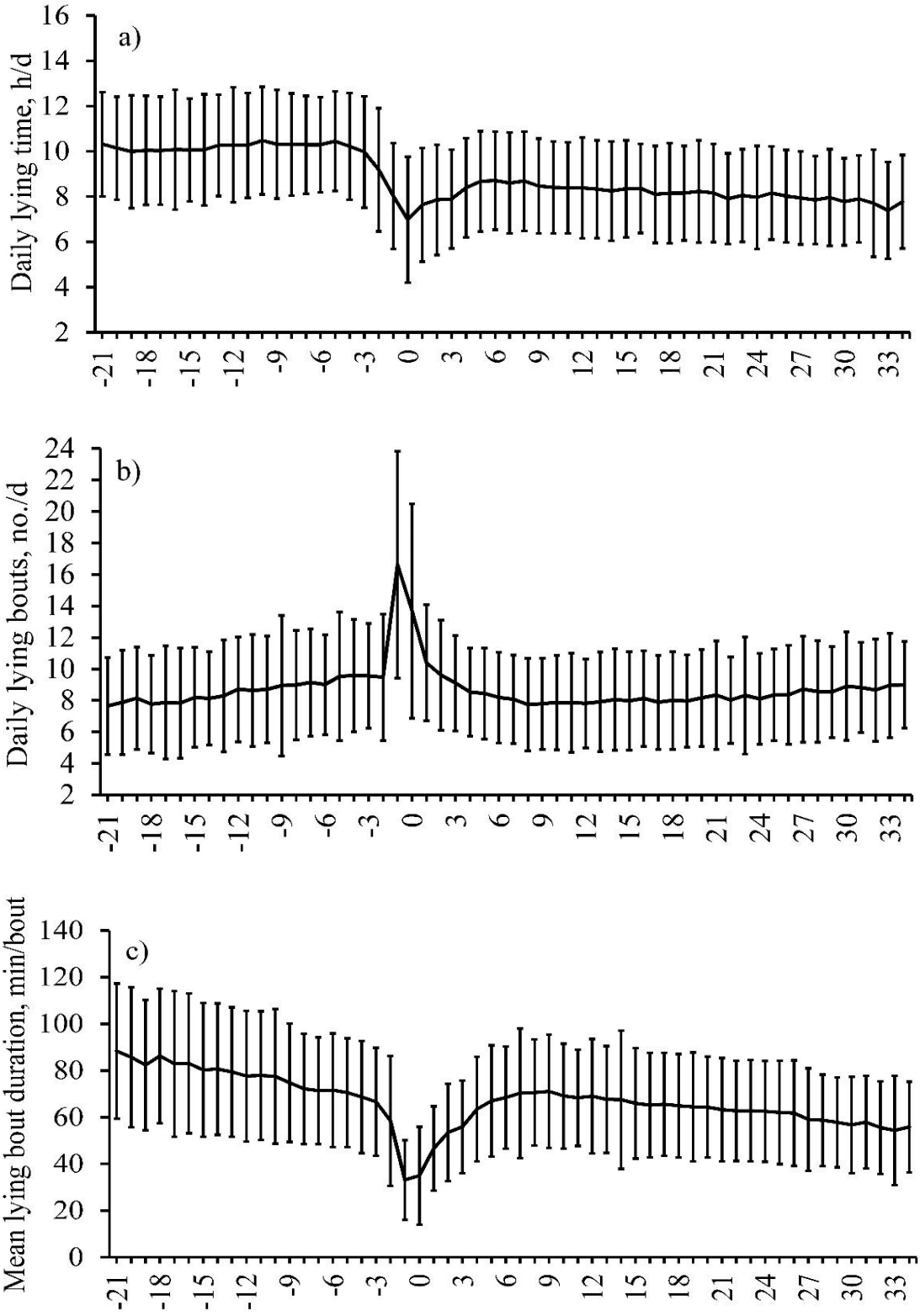


**Figure 6.1. Lying behavior and activity across the transition period in clinically-healthy grazing dairy cows.**

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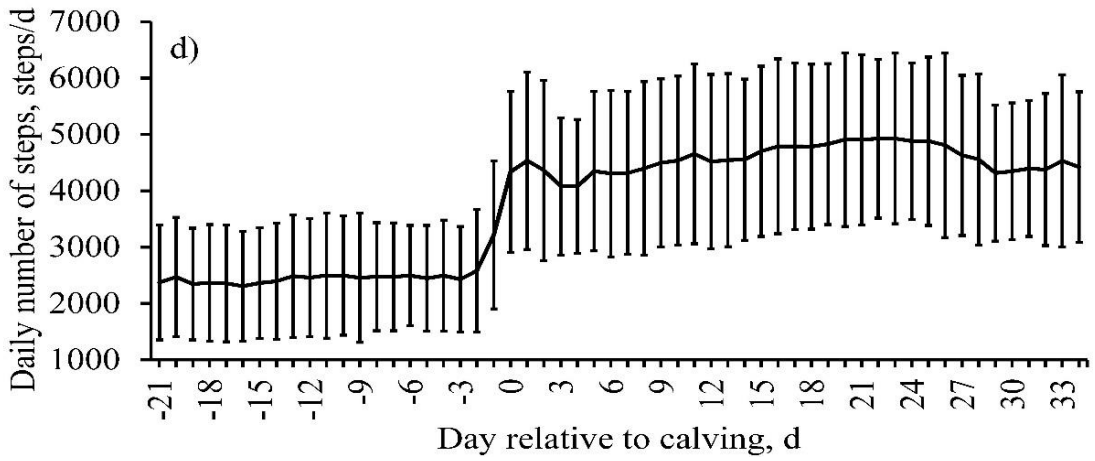


**Figure 6.1 (Continued).** Daily lying time [h/d; (a)], daily lying bouts [(LB) no./d; (b)], mean LB duration [min/bout; (c)], and number of steps taken [steps/d; (d)] during the period –21 to +34 d relative to the day of calving (d 0). Vertical bars represent ± 1 standard deviation of the sample population.

### *6.4.2 Effect of Period on Behavior Parameters*

Precalving, cows spent a greater amount of time lying with longer LB durations than postcalving, although the number of LB was statistically but not biologically different (Table 6.2). On d 0, the time spent lying was lower, LB durations shorter, and number of LB higher compared with PRE and POST periods. On d 0, cows were more active than during the PRE period, as indicated by the greater number of steps taken (Table 6.2). The highest activity levels, as indicated by the number of steps taken, occurred postcalving.

**Table 6.2. Lying behavior and activity during the transition period in clinically-healthy grazing dairy cows.**

Daily lying time (h/d), daily lying bouts (LB; no./d), mean LB duration (min/bout), and number of steps taken (steps/d) for –21 to –3 d (PRE) and 3 to 21 d (POST) relative to the day of calving (d 0) of transition dairy cows.

| Parameter | Period | | | SED[1] | *P*-value |
|---|---|---|---|---|---|
| | PRE | d 0 | POST | | |
| Daily lying time, h/d | 10.3[a] | 7.25[c] | 8.58[b] | 0.14 | <0.001 |
| Daily LB, no./d | 8.15[b] | 12.9[a] | 7.74[c] | 0.21 | <0.001 |
| LB duration, min/bout | 77.3[a] | 39.1[c] | 69.1[b] | 1.62 | <0.001 |
| Daily number of steps, steps/d | 2,289[c] | 4,105[b] | 4,424[a] | 60 | <0.001 |

[a-c]Means with different superscripts are significantly different at the 5% confidence level.
[1]SED = Mean standard error of the difference.

Daily number of steps taken was higher in the parity 2 to 3 cows compared with the parity 6 to 7 and 8+ cows, which were not different from each other during the period –2 to 0 d ($P < 0.05$). The daily number of steps taken was also higher in the parity 2 to 3 cows compared with the parity 8+ cows during the period 1 to 5 d (Figure 6.2). The cBCS and breed had no overall effect on the daily step count. The effects of cBCS, breed, and parity on lying behavior are not reported because these effects were not significant in our study.

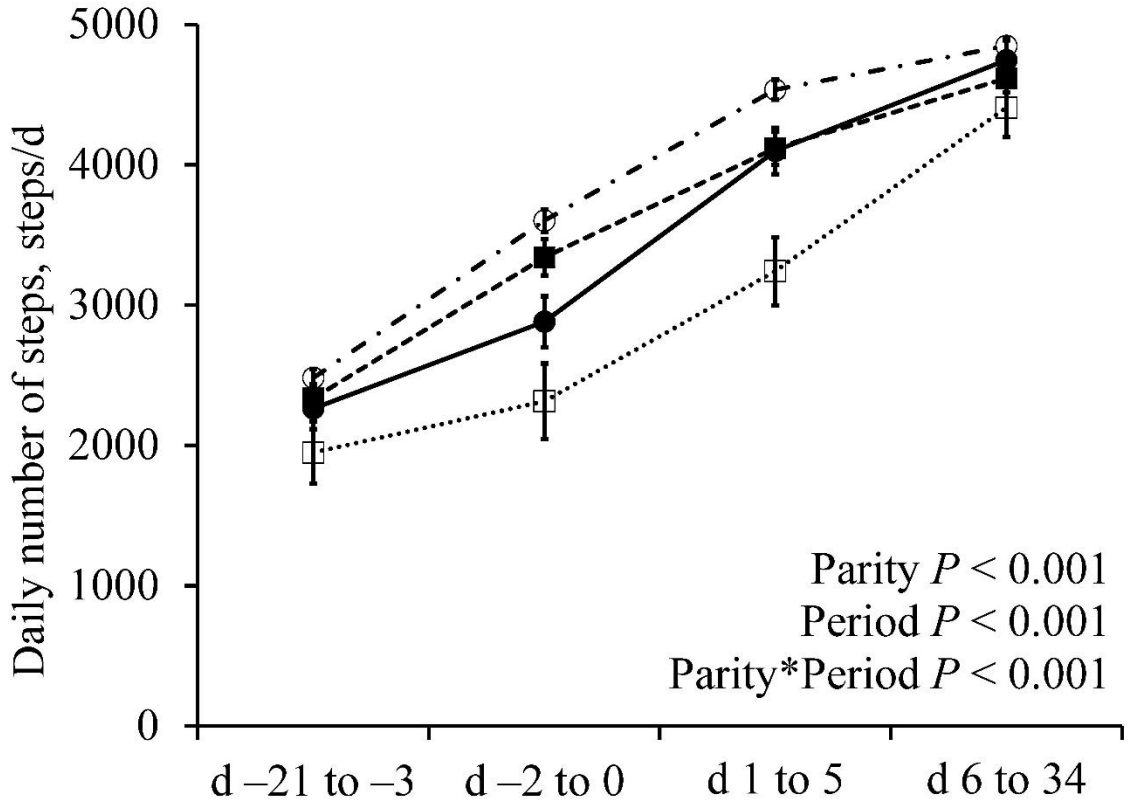


**Figure 6.2. Associations between activity and parity, period, and their interactions during the transition period in grazing dairy cows.**

Interaction between parity and period in mean daily number of steps taken (steps/d) during 4 periods relative to calving (–21 to –3 d, –2 to 0 d, 1 to 5 d, and 6 to 34 d). Parity 2 to 3 (open circles), parity 4 to 5 (filled squares), parity 6 to 7 (filled circles) and parity 8+ (open squares). Vertical bars represent standard error of the mean.

### *6.4.3 Average Changes in Behavior Across Days.*

The multiple-regression models investigated the average change in lying behavior and activity during 4 periods according to the behavior of interest (Table 6.3). Daily lying time was associated with day relative to calving for all periods relative to calving ($P < 0.05$), except –21 to –3 d. Daily lying time declined from 3 d before calving to 0 d where the lowest lying time occurred and then increased from 0 to 5 d (Figure 6.1a; Table

6.3). After 5 d postcalving, a steady decline in daily lying time and LB duration occurred (slope = –0.02 ± 0.01 h/d and –0.34 ± 0.05 min/bout, respectively; $P < 0.05$) (Table 6.3).

The daily LB increased from 2 d before calving (slope = 5.09 ± 0.54 no./d; $P < 0.05$) and was greatest the day before calving (–1 d; Figure 6.1b) with an associated decline in the mean LB duration during this period, which was shortest on the day before calving (Figure 6.1c). Daily LB decreased from –1 to 2 d (slope = –2.30 ± 0.14 no./d; $P < 0.05$) with an associated increase in the mean LB duration during this period.

The number of steps taken increased substantially from –2 until 0 d (slope = 860 ± 47 steps/d), followed by a further increase in the number of steps taken in the days postcalving (2 to 34 d; slope = 37 ± 3 steps/d) (Figure 6.1d and Table 6.3).

**Table 6.3. Multiple-regression models for the associations between lying behavior and activity and days within period relative to calving in grazing dairy cows.**

Regression coefficient (estimate; standard error in parentheses) for parameters influencing daily lying time (h/d), daily lying bouts (LB; no./d), mean LB duration (min/bout), and number of steps taken (steps/d) during 4 periods according to the behavior of interest within the period –21 to +34 d relative to the day of calving (d 0) of transition dairy cows.

| Parameters[1] | Period | | | |
|---|---|---|---|---|
| Daily lying time, h/d | –21 to –3 d | –3 to 0 d | 0 to 5 d | 5 to 34 d |
| Intercept | 6.63 (1.12)* | 5.07 (1.72)* | 6.74 (1.41)* | 7.53 (0.888)* |
| Group | * | * | * | * |
| Day | 0.01 (0.009) | –1.03 (0.067)* | 0.30 (0.036)* | –0.02 (0.005)* |
| Breed: HF[2] | –0.34 (0.129)* | –0.45 (0.194)* | –0.39 (0.162)* | –0.65 (0.107)* |
| Parity: 2–3[3] | –1.18 (0.404)* | –1.39 (0.612)* | –1.73 (0.505)* | 0.57 (0.314) |
| Parity: 4–5[3] | –1.29 (0.399)* | –1.26 (0.606)* | –1.85 (0.502)* | 0.75 (0.311)* |
| Parity: 6–7[3] | –0.82 (0.416)* | –0.54 (0.635) | –1.06 (0.526)* | 0.96 (0.326)* |
| cBCS[4] | 1.11 (0.235)* | 0.69 (0.354) | 0.76 (0.295)* | 0.21 (0.186) |
| PW,[5] $/5 t DM per 10 units | 0.01 (0.009) | –0.01 (0.013) | 0.02 (0.011) | –0.01 (0.007) |

**Table 6.3.** Continued over page.

**Table 6.3 (Continued).** Regression coefficient (estimate; standard error in parentheses) for parameters influencing daily lying time (h/d), daily lying bouts (LB; no./d), mean LB duration (min/bout), and number of steps taken (steps/d) during 4 periods according to the behavior of interest within the period –21 to +34 d relative to the day of calving (d 0) of transition dairy cows.

| Parameters[1] | Period | | | |
|---|---|---|---|---|
| Daily lying time, h/d | –21 to –3 d | –3 to 0 d | 0 to 5 d | 5 to 34 d |
| BrW, [5] $/5 t DM per 10 units | –0.01 (0.019) | –0.04 (0.029) | –0.07 (0.024)* | 0.01 (0.016) |
| | | | | |
| Daily LB, no./d | –21 to –2 d | –2 to –1 d | –1 to 2 d | 2 to 34 d |
| Intercept | 3.18 (1.74) | 15.5 (6.01)* | 6.31 (3.46) | 4.94 (1.07)* |
| Group | * | | * | * |
| Day | 0.13 (0.017)* | 5.09 (0.536)* | –2.30 (0.141)* | –0.01 (0.007) |
| Breed: HF[2] | 0.17 (0.202) | –0.03 (0.664) | –0.47 (0.393) | –0.16 (0.129) |
| Parity: 2–3[3] | –0.12 (0.624) | 0.81 (2.12) | –0.95 (1.23) | 0.94 (0.381)* |
| Parity: 4–5[3] | –1.34 (0.615) | –0.74 (2.10) | –1.53 (1.23) | 0.59 (0.378) |
| Parity: 6–7[3] | –1.01 (0.646) | –0.10 (2.21) | –0.94 (1.29) | 1.00 (0.398)* |

**Table 6.3.** Continued over page.

**Table 6.3 (Continued).** Regression coefficient (estimate; standard error in parentheses) for parameters influencing daily lying time (h/d), daily lying bouts (LB; no./d), mean LB duration (min/bout), and number of steps taken (steps/d) during 4 periods according to the behavior of interest within the period –21 to +34 d relative to the day of calving (d 0) of transition dairy cows.

| Parameters[1] | Period | | | |
|---|---|---|---|---|
| Daily LB, no./d | –21 to –2 d | –2 to –1 d | –1 to 2 d | 2 to 34 d |
| cBCS[4] | 1.73 (0.368)* | 1.37 (1.22) | 1.02 (0.716) | 0.05 (0.224) |
| PW,[5] $/5 t DM per 10 units | 0.02 (0.014) | –0.03 (0.046) | –0.01 (0.027) | –0.02 (0.009) |
| BrW,[5] $/5 t DM per 10 units | 0.08 (0.030)* | 0.17 (0.098) | 0.10 (0.058) | 0.10 (0.019)* |
| | | | | |
| LB duration, min/bout | –21 to –2 d | –2 to –1 d | –1 to 5 d | 5 to 34 d |
| Intercept | 76.1 (12.5)* | –19.66 (22.6) | 52.3 (10.1)* | 76.9 (9.25)* |
| Group | * | | * | * |
| Day | –1.18 (0.100)* | –25.3 (1.69)* | 5.94 (0.282)* | –0.34 (0.049)* |
| Breed: HF[2] | –2.43 (1.44) | –2.87 (2.52) | –1.34 (1.15) | –4.43 (1.11)* |
| Parity: 2–3[3] | 0.33 (4.53) | 2.66 (8.01) | –11.6 (3.62)* | –4.71 (3.29) |

**Table 6.3.** Continued over page.

**Table 6.3 (Continued).** Regression coefficient (estimate; standard error in parentheses) for parameters influencing daily lying time (h/d), daily lying bouts (LB; no./d), mean LB duration (min/bout), and number of steps taken (steps/d) during 4 periods according to the behavior of interest within the period –21 to +34 d relative to the day of calving (d 0) of transition dairy cows.

| Parameters[1] | Period | | | |
|---|---|---|---|---|
| LB duration, min/bout | –21 to –2 d | –2 to –1 d | –1 to 5 d | 5 to 34 d |
| Parity: 4–5[3] | –2.96 (4.46) | 5.51 (7.93) | –9.94 (3.58)* | –2.98 (3.26) |
| Parity: 6–7[3] | 5.95 (4.67) | 5.62 (8.36) | –8.17 (3.78)* | –2.82 (3.44) |
| cBCS[4] | –0.63 (2.63) | 5.07 (4.62) | 4.28 (2.09) | 3.99 (1.93)* |
| PW,[5] $/5 t DM per 10 units | –0.01 (0.100) | 0.06 (0.174) | 0.18 (0.079)* | 0.07 (0.076) |
| BrW,[5] $/5 t DM per 10 units | –0.77 (0.214)* | –0.71 (0.372) | –0.86 (0.169)* | –0.71 (0.164)* |
| | | | | |
| Number of steps, steps/d | –21 to –2 d | –2 to 0 d | 0 to 2 d | 2 to 34 d |
| Intercept | 3,941 (431)* | 4,996 (864)* | 2,922 (1043)* | 594 (498) |
| Group | * | * | * | * |
| Day | 11 (3)* | 860 (47)* | –26 (59) | 37 (3)* |

**Table 6.3.** Continued over page.

**Table 6.3 (Continued).** Regression coefficient (estimate; standard error in parentheses) for parameters influencing daily lying time (h/d), daily lying bouts (LB; no./d), mean LB duration (min/bout), and number of steps taken (steps/d) during 4 periods according to the behavior of interest within the period –21 to +34 d relative to the day of calving (d 0) of transition dairy cows.

| Parameters[1] | Period | | | |
|---|---|---|---|---|
| Number of steps, steps/d | –21 to –2 d | –2 to 0 d | 0 to 2 d | 2 to 34 d |
| Breed: HF[2] | –22 (49) | –4 (97) | –30 (119) | –53 (57) |
| Parity: 2–3[3] | 548 (156)* | 991 (307)* | 1412 (371)* | 650 (168)* |
| Parity: 4–5[3] | 401 (153)* | 775 (304)* | 883 (368)* | 232 (166) |
| Parity: 6–7[3] | 175 (160) | 343 (318) | 886 (386)* | 286 (174) |
| cBCS[4] | –251 (90)* | –199 (177) | 151 (216) | 805 (99)* |
| PW,[5] $/5 t DM per 10 units | 1 (3) | 1 (6) | 6 (8) | 9 (3)* |
| BrW,[5] $/5 t DM per 10 units | 16 (7)* | 38 (14)* | 32 (17) | –7 (8) |

[1]Factors; covariate precalving body condition score (cBCS), Production Worth (PW), and Breeding Worth (BrW).
[2]Breed [Holstein-Friesian (HF) x Jersey = 0] is the reference group for breed effects; *$P < 0.05$; slope is different from reference group for classification variable.
[3]Parity (8+ = 0) is the reference group for parity effects; *$P < 0.05$; slope is different from reference group for classification variable.
[4]Body condition score at –5 wk precalving (on a 1 to 10 scale; Roche et al., 2004).
[5]Genetic merit (New Zealand Animal Evaluation Ltd, Hamilton, New Zealand). Expressed as dollars (NZ$) of net farm income per 5 t of DM relative to a 2000-born genetic base cow for the year 2016 per 10 unit increase.
*$P < 0.05$; slope different from 0 or intercept different from 1.

### *6.4.4 Effect of Period and Time on Behavior Parameters*

The within-day profiles of lying behavior during the PRE, POST, and d 0 periods are presented in Figure 6.3. The mean sunrise and sunset times across the 4 experiments correspond with 0731 h and 1717 h, respectively (Timeanddate.com, 2017). Due to the staggered milking times across the 4 studies, there was no period of zero lying. The greatest increase in the number of steps taken postcalving compared with precalving occurred between 0600 to 0959 h and 1400 to 1759 h, which coincides with milking times (Table 6.4).

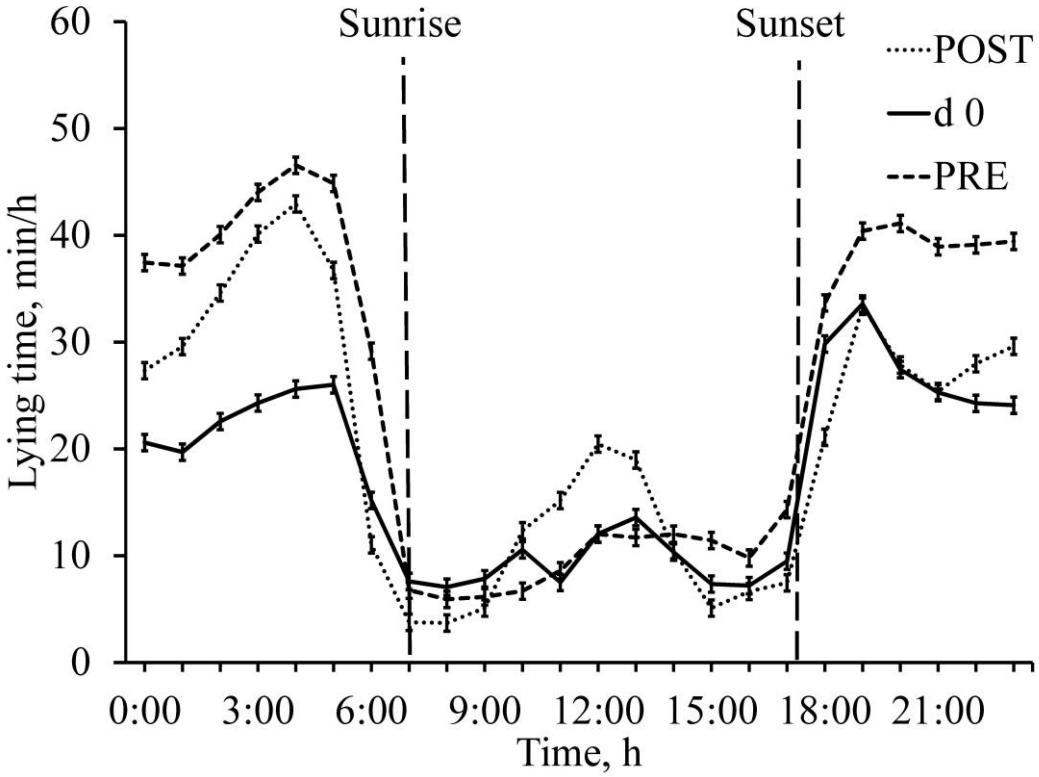


**Figure 6.3. Temporal pattern of lying time during the transition period in grazing dairy cows.**

Temporal pattern of grazing dairy cow lying time (min/h) during the period –3 to –21 d (PRE) and 3 to 21 d (POST) relative to the day of calving (d 0). Vertical bars represent

standard error of the mean. Dashed vertical lines represent sunrise (0731 h) and sunset (1717 h).

A significant period by time interaction was observed for all lying behaviors and activity (Table 6.4). Lying time was reduced across all time intervals during the POST period, except from 1000 to 1359 h where lying time increased compared with the PRE period. Lying time, LB, and LB duration during the day (0600 to 1759 h) in the d 0 period seemed more stable compared with precalving, where differences were either not significant, or changes in behavior were small compared with changes that occurred at night (2200 to 0559 h). For the POST period, the greatest changes in lying time occurred during the day between 0600 and 1359 h (Figure 6.3; Table 6.4) compared with the PRE period. The lying time declined between 0600 and 0959 h (–46.6%) and increased (+91.9%) between 1000 and 1359 h; however, LB duration increased during both time intervals in the POST compared with PRE period. The number of steps taken across all time intervals increased during the POST period, except from 1000 to 1359 h where fewer steps were taken, compared with the PRE period.

**Table 6.4. Lying behavior and activity within day during 3 periods during the transition period in clinically-healthy grazing dairy cows.**

Lying time (min/h), lying bouts (no./h), mean lying bout duration (min/bout), and number of steps taken (steps/h) across 4-hourly time intervals during 3 periods: –21 to –3 d (PRE) and 3 to 21 d (POST) relative to the day of calving (d 0) of transition dairy cows. SED = Mean standard error of the difference.

| Parameter | Period | Time intervals,[1] h | | | | | | SED |
|---|---|---|---|---|---|---|---|---|
| | | 02–05 | 06–09 | 10–13 | 14–17 | 18–21 | 22–01 | |
| Lying time, min/h | PRE | $42.3^{a,x}$ | $10.0^{d,x}$ | $8.39^{e,y}$ | $11.4^{c,x}$ | $38.2^{b,x}$ | $37.4^{b,x}$ | 0.26 |
| | d 0 | $24.1^{b,z}$ | $9.03^{c,x}$ | $10.5^{c,y}$ | $8.34^{c,y}$ | $28.9^{a,y}$ | $22.0^{b,z}$ | 1.05 |
| | POST | $38.5^{a,y}$ | $5.34^{f,y}$ | $16.1^{d,x}$ | $7.07^{e,y}$ | $27.7^{c,y}$ | $28.6^{b,y}$ | 0.24 |
| | SED | 0.67 | 0.67 | 0.67 | 0.67 | 0.67 | 0.67 | |
| Lying bouts, no./h | PRE | $1.05^{d,y}$ | $1.12^{bc,y}$ | $1.13^{ab,x}$ | $1.16^{a}$ | $1.14^{ab,x}$ | $1.09^{c,y}$ | 0.01 |
| | d 0 | $1.40^{a,x}$ | $1.28^{bc,x}$ | $1.19^{cd,x}$ | $1.19^{cd}$ | $1.15^{d,x}$ | $1.32^{ab,x}$ | 0.03 |
| | POST | $1.05^{c,y}$ | $1.15^{a,y}$ | $1.08^{b,y}$ | $1.12^{a}$ | $1.08^{b,y}$ | $1.07^{bc,y}$ | 0.01 |
| | SED | 0.03 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | |
| Lying bout duration, min/bout | PRE | $121^{a,x}$ | $28.4^{f,y}$ | $35.3^{e,y}$ | $51.6^{d}$ | $89.7^{c,x}$ | $103^{b,x}$ | 1.01 |
| | d 0 | $48.5^{ab,z}$ | $27.4^{c,xy}$ | $32.0^{c,y}$ | $41.4^{bc}$ | $59.8^{a,z}$ | $47.4^{b,z}$ | 3.77 |
| | POST | $93.8^{a,y}$ | $36.0^{e,x}$ | $55.2^{c,x}$ | $51.4^{d}$ | $83.4^{b,y}$ | $85.5^{b,y}$ | 0.95 |
| | SED | 2.25 | 2.67 | 2.38 | 2.76 | 2.21 | 2.23 | |
| Number of steps, steps/h | PRE | $68^{d,z}$ | $224^{a,y}$ | $174^{b,y}$ | $140^{c,z}$ | $41^{f,z}$ | $56^{e,z}$ | 2 |
| | d 0 | $105^{c,x}$ | $226^{b,y}$ | $304^{a,x}$ | $307^{a,y}$ | $93^{c,x}$ | $123^{c,x}$ | 8 |
| | POST | $80^{c,y}$ | $420^{a,x}$ | $160^{b,z}$ | $426^{a,x}$ | $62^{d,y}$ | $83^{c,y}$ | 2 |
| | SED | 5 | 5 | 5 | 5 | 5 | 5 | |

[a–f]Means with different superscripts are significantly different at the 5% confidence level within a row.
[x–z]Means with different superscripts are significantly different at the 5% confidence level within a column.
[1]Time intervals include data within each hour specified (i.e., 22–01 covers the period 2200 h to 0159 h).

## 6.5 DISCUSSION

Changes in behavior may be a result of cow, management, or physiological factors. We have quantified the lying behavior and activity of transition dairy cows in a rotational grazing system, including changes to the diurnal profiles for lying behavior and activity. Lying behavior and activity, as measured by number of steps taken, changed substantially during the days immediately before and after calving. Temporal profiles for lying behavior and activity indicate that grazing cows follow a similar pattern of lying behavior and activity throughout the day across the transition period; however, the magnitude of these changes was not constant across the transition period. Variation among individual cow means within groups was greater than the variation among the overall group means for lying behavior and activity. Large individual variation in the behavior of individual cows grouped together is an important consideration. The results of this study provide evidence of the effects of cow, management, and physiological factors on lying behavior and activity of grazing cows.

### *6.5.1 Lying Behavior is Different Under Different Systems*

Adequate daily lying time is regarded as an important metric for the welfare of domesticated animals (Munksgaard and Simonsen, 1996). Changes in daily lying time may be a result of external factors, such as housing system, management, physiological state, or diet (Munksgaard et al., 2005), and may not be indicative of changes to welfare state. Daily lying time and daily LB were 13 to 28% and 30 to 44% less, respectively, in our study of grazed cows than what has been reported for housed cows during the pre- and postcalving periods (Huzzey et al., 2005; Calderon and Cook, 2011; Piñeiro et al., 2019). However, this result appears to be typical for cows at pasture where the daily precalving lying time (10.3 h/d) in our study was similar to the value reported for cows

moved from housing to pasture precalving (10.3 h/d; Rice et al., 2017). The daily postcalving lying time (8.58 h/d) was also within the range of typical values presented for grazing dairy cows (7.50 to 8.50 h/d; Sepúlveda-Varas et al. 2014). The greater daily lying time in housed systems, both pre- and postcalving, could be a result of external factors, such as easier feed accessibility and prehension than the competitive features of grazing (Munksgaard et al., 2005; Sepúlveda-Varas et al., 2014). Greater daily lying time postcalving could also be a result of less time spent walking to and from the milking parlor (Huzzey et al., 2005; Sepúlveda-Varas et al., 2014). Despite the time spent lying by grazing cows being lower compared with housed cows, it is not possible from this study to determine whether this compromises their welfare; however, it probably means the time budgets of cows should be derived within systems if they are to be useful benchmarks for animal welfare.

### *6.5.2 Cow and Farm Factors Affect Lying Behavior and Activity*

Farm-specific factors such as time spent waiting to be milked (Beggs et al., 2018), wintering system (Al-Marashdeh et al., 2019), weather (Chapter 5), and other management factors need to be considered when comparing behavior measurements from different farms or groups of animals. In the current study, the groups were managed similarly across the studies with differences due to studies undertaken across different years, in different locations, with differences in weather and time spent engaged in activities associated with milking. Despite these differences, the variation among the overall group means was less than the range of means among individual cows within the groups, in agreement with the findings of Ito et al. (2009). Several factors have been suggested as drivers of the larger variation among individual cows compared with variation among herds. It was suggested that individual variation is influenced by an

animal's social ranking and variation might be more marked in highly-competitive environments (Phillips and Rind, 2001; Ito et al., 2009). In housed cows, competition for space to lie down in freestalls could limit the lying behavior of some cows (Ito et al., 2009), whereas, in dairy cows on pasture, competition to lie down may be less likely to disrupt normal lying behavior due to higher space availability (Phillips and Rind, 2001). However, a grazing environment might be more conducive to competition for access to feed and this may disrupt normal lying behavior in some cows whereas, in housed cows, competition for feed may be less likely to influence lying behavior where feed is available ad libitum (Phillips and Rind, 2001). Understanding how social ranking, and competition and motivation for access to different resources may disrupt normal lying behavior in some cows and as a potential cause of individual variation in both housed and grazing dairy cows requires further investigation.

Interestingly, the variation among individual cows and farms was greater in the study by Ito et al. (2009), where 43 commercial dairy farms were recruited for the study, compared with the current study. Ito et al. (2009) reported a difference in the range of means for individual cows of 15.3 h/d and 27 no./d for daily lying time and LB, respectively, and 342 min/bout for the difference in the range of mean LB duration among individual housed cows. In the current study, the range was smaller where the difference in the range of means for individual grazing cows for daily lying time, LB, and LB duration was 6.83 h/d, 18.1 no./d, and 91.6 min/bout, respectively. In the study by Ito et al. (2009), cows were housed indoors on a range of bedding materials, and the farms recruited included 2 and 3 times daily milking, as well as once and twice-daily feeding. The greater variation may be due to large variation in management across farms and, in part, due to a larger sample size compared with the current study. The large individual

variation in lying behaviors and daily step counts of grouped cows is an important consideration if benchmarks are established as indicators of welfare, particularly for cows managed under different conditions.

Behaviors expressed may also differ due to cow factors; however, limited studies have investigated both pre- and postcalving lying behavior and activity and the influence of breed, parity, and BCS in grazing dairy cows. Cows in the current study were multiparous and approaching their second to 11th parity. In the current study, increasing parity was associated with lower step counts during the period immediately before and after calving. Duncan and Meyer (2019) reported no effect of parity on step count in prepartum beef cows and heifers, but only reported data for 3 d prepartum. To our knowledge, this is the first study that has reported differences in step count during the transition period due to parity, although this was largely driven by the older cows (parity 6+) in our study. There were no differences in lying behaviors due to parity. Whereas other studies have reported associations between parity and behavior when comparing primiparous with multiparous cows (Calderon and Cook, 2011; Sepúlveda-Varas et al., 2014; Duncan and Meyer, 2019), these results are contrary to the findings of Bewley et al. (2010) where a lack of significance for parity was reported in housed cows. Despite conflicting results, studies should account for parity differences when interpreting behavior data due to differences in social behaviors, milk production, BW, body composition, and DMI between parities that could influence behavioral responses (Phillips and Rind, 2001; Wathes et al., 2007).

In agreement with other studies, in our study, after accounting for other factors in the model, no significant breed (Stone et al., 2017) or BCS (Bewley et al., 2010) effects were observed on daily lying time, daily LB, mean LB duration, and step count during

the transition period; however, studies are limited. Further investigation into potential effects of breed and both pre- and postcalving BCS and changes in BCS on lying behavior and activity across the transition period is warranted.

### *6.5.3 Physiological State Affects Lying Behavior and Activity*

Changes to an animal's physiological state influences their behavior (Munksgaard et al., 2005) and, for a dairy cow, one of the most significant physiological changes that occurs during their life is the change from a nonlactating to a lactating state (Grummer, 1995). On the day of calving, daily lying times were ~3 h less than precalving (–21 to –3 d) and ~1.5 h less than postcalving (3 to 21 d). Cows were lying down more frequently, but for shorter periods and the number of steps taken on the day of calving were almost double that taken by the precalving cow. Daily lying time decreased substantially from –3 to 0 d, and this was accompanied by an increase in the number of LB from –2 d, which peaked at –1 d and remained elevated on the day of calving. These changes in behavior around the day of calving are similar to those reported in housed cows (Huzzey et al., 2005; Calderon and Cook, 2011; Jensen, 2012; Kok et al., 2015) and dairy cows on pasture (Black and Krawczel, 2016; Borchers et al., 2017; Rice et al., 2017). Restlessness and discomfort around the calving event are likely to be responsible for the changes in behavior (i.e., repeated lying down and standing up and pacing; Huzzey et al., 2005; Borchers et al., 2017). The increase in step count may also be due to cows walking in search of a place to calve or seeking isolation from the herd (Duncan and Meyer, 2019). Future research should investigate whether the changes in daily lying time, LB, or number of steps taken around the calving event could be used to predict timing of calving in grazing cows.

Daily lying time declined steadily during the postcalving period until d 34, in agreement with Maselyne et al. (2017). In studies in housed cows, higher energy requirements increasing time spent eating has been attributed to less time lying (Løvendahl and Munksgaard, 2016; Stone et al., 2017) and increasing amounts of milk in the udder leading to discomfort has been attributed as a possible cause for the reduction in lying time (Norring et al., 2012). However, in grazing dairy cows, Tucker et al. (2007a) reported the reduction in lying time was more likely due to a time constraint associated with milking. Udder discomfort and increased feeding time may have been responsible, in part, to a reduction in lying time in the highest-yielding cows in our study; however, lying time was likely constrained by a known management change due to time associated with milking (Tucker et al., 2007a; Norring et al., 2012; Kok et al., 2017).

Postpartum cows spent less time lying (~1.5 h), with no change in the number of LB and took a greater number of steps compared with precalving cows. This is similar to results reported by Huzzey et al. (2005); where an increase in the daily standing time of ~1 h and no change in the number of standing bouts was apparent postcalving and described as “not surprising” due to increased standing time in the milking parlor and time spent walking to and from the milking parlor. In our study, the difference in lying time was only slightly more than the time associated with milking (~40 to 90 min), and, therefore, the lower lying time may have been due to the time constraint associated with milking (Tucker et al., 2007a). This is further supported by the increased step count around morning and afternoon milking (0600 to 0959 and 1400 to 1759 h) and a reduction in lying time in the current study. The lower lying times postcalving with a concomitant increase in the number of steps taken alongside known changes to the management of the lactating cow support that postcalving cows were more active due to increased time spent

walking to and from the milking parlor and standing during the milking routine (Kok et al., 2017). The associations and trade-offs between time associated with milking, lying, feeding, and other activities are poorly understood in grazing dairy cows. Further research in this area is needed to understand the consequences of these associations with lying behavior on welfare and health in grazing dairy cows.

### *6.5.4 Lying Behavior and Activity is Different Within Day*

Patterns in dairy cow behavior within day may be influenced by the calving event and the onset of lactation. On the day of calving, the largest magnitude of change in daily lying time, daily LB, and LB duration occurred at night between 2200 and 0559 h. On average, the LB frequency was higher, but LB duration and lying time were longer, and cows took fewer steps at night on the day of calving compared with the daytime. These quite dramatic behavioral changes on the day of calving, particularly at night, may indicate restlessness due to discomfort; however, they might be part of the normal calving process (von Keyserlingk and Weary, 2007). Edwards (1983) reported that when cows were given a choice to calve indoors or in a 1 ha paddock, cows that calved outdoors did so more often when calving at night. In our study, without exact calving times, it is difficult to determine whether the greater changes in behavior at night occurred due to more cows calving during the night.

Cows appeared to alter their behavior within day during the transition period, where postcalving, greater changes occurred in the middle of the day. Postcalving lying time was lower during five out of six 4 h periods during the day compared with precalving, with one exception: lying time increased during the middle of the day in the lactating cow (1000 to 1359 h). A peak in lying time in the middle of the day has been demonstrated before in housed cows (Schirmann et al., 2012; Kok et al., 2017); however,

to our knowledge, this is the first study to demonstrate this in grazing dairy cows postcalving through the presentation of temporal profiles of lying time. The increased lying time in the middle of the day in the postcalving cow may be related to a greater need for rumination following a large post-milking DMI (Gibb et al., 1999; Sheahan et al., 2013), as rumination is often associated with lying down (Schirmann et al., 2012). DeVries et al. (2003) reported that the pattern of lying in lactating grazing dairy cows is inversed with feeding and Sheahan et al. (2013) presented temporal profiles for grazing behavior that represented the occurrence of major grazing bouts following sunrise and prior to sunset (i.e., crepuscular feeding). This temporal profile for grazing behavior is consistent with the inverse of the temporal profile for lying presented in our study. This, in conjunction with the substantial increase in the number of steps taken across all periods and, in particular, after the morning and afternoon milking, supports the premise that the postcalving cow is more active at the expense of lying time or idling (Dohme-Meier et al., 2014); however, trade-offs between lying, feeding, ruminating, and milking-associated activities require further investigation.

## 6.6 CONCLUSIONS

The results indicate that the changes in dairy cow behavior during the transition period are similar across production systems; however, absolute values are different, are highly variable among individual cows and can be influenced by cow, physiological, and management factors. Daily lying time, daily LB, LB duration, and number of steps taken were substantially altered at the time of the calving event. Postcalving cows took more steps and spent less time lying down compared with precalving cows, and this appeared to be a direct consequence of activity associated with twice daily milking. For this reason, understanding the effects of cow, physiological, and management factors on changes in

behavior and activity, and how grazing dairy cows prioritize certain behaviors is important to take into consideration when using behavior as an indicator of health or welfare. Information available via activity monitors may contribute to the improvement of individual management of transition dairy cows and this research provides a benchmark for typical lying behavior during the transition period in grazing systems.

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## 6.8 SUMMARY

In Chapter 6, I determined the changes in behavior that may be a result of cow and physiological factors and described the between cow and herd-level variation in lying behavior and activity of clinically-healthy grazing dairy cows. I provided evidence of associations between lying behavior and activity and cow factors (e.g., parity) in grazing dairy cows. I described changes in lying behavior and activity in grazing dairy cows during the transition period, including changes to the diurnal profile of lying behavior and activity. Substantial changes in lying behavior and activity occurred during the days immediately before, on the day of, and after calving, but changes were minimal during the far-off period, pre- and postcalving. Within day 24 h profiles for lying behavior and

activity indicated that grazing cows follow a similar pattern of change in their lying behavior and activity throughout the day across the transition period. I identified confounding factors to consider in the analyses in subsequent experimental Chapters and improved my understanding of what constitutes typical lying behavior and activity in clinically-healthy grazing dairy cows to consider before assessing whether behavior could be used as an indicator of health or welfare. In Chapter 7, I described the lying behavior and activity of grazing dairy cows with varying degrees of hypocalcemia compared with cows with normocalcemia to provide evidence that behavioral differences occur before, at the time of, and after disease diagnosis.

# CHAPTER 7. ASSOCIATIONS BETWEEN LYING BEHAVIOR AND ACTIVITY AND HYPOCALCEMIA IN GRAZING COWS DURING THE TRANSITION PERIOD

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## 7.1 ABSTRACT

Hypocalcemia is a common metabolic disorder of transition dairy cows that is considered a gateway disease, increasing the risk of other health disorders and reducing cow performance. Clinical milk fever is associated with long periods of recumbency, and it is plausible that cows experiencing non-paretic hypocalcemia may spend more time lying; hence lying behavior and activity measures may be useful in identifying at-risk cows. The objective of this study was to describe associations among lying behavior and activity measures and among blood calcium (Ca) status at calving during the transition period in grazing dairy cows. Blood was sampled on the day of calving (d 0), and d 1, 2, 3, and 4 postcalving and analyzed for total plasma Ca concentration. Twenty-four, multiparous Holstein-Friesian and Holstein-Friesian x Jersey grazing dairy cows were classified, retrospectively, as clinically-hypocalcemic (CLIN; blood Ca ≤1.4 mmol/L at 1 or more consecutive samplings within 48 h postcalving, but without parturient paresis). These cows were pair-matched (using milk production potential from their estimated breeding value for milk protein, mean body weight at wk –5 and –6 precalving, and, where possible, parity), with 24 cows classified as subclinically-hypocalcemic (SUB; blood Ca >1.4 and <2.0 mmol/L at 2 consecutive samplings within 48 h postcalving), and 24 cows classified as normocalcemic (NORM; blood Ca ≥2.0 mmol/L at 3 consecutive samplings within 72 h postcalving). Lying behavior and activity were monitored using triaxial accelerometers from –21 to +35 d relative to calving. Data were summarized to calculate daily lying time (h/d), daily lying bout number (LB; no./d), mean LB duration (min/bout), and the number of steps taken (steps/d). On d 0, the CLIN group were less active and spent approximately 2.6 h longer lying than the SUB and NORM groups, particularly between 0200 and 1600 h. On d 0, the NORM group had fewer LB (16.3

no./d) than the SUB and CLIN groups (18.2 and 19.2 no./d, respectively). These differences in behavior were no longer detected 2 d postcalving and no further differences were observed. The day before calving, the CLIN group spent 1.4 h longer lying down than the SUB and NORM groups. Further, the relative change in steps from a precalving baseline period (d –14 to –7) until d 0 was positively, linearly associated with blood Ca concentration within 24 h postcalving. Future work should consider daily and temporal changes in behavior in individual cows to determine the potential for these measures to allow early detection of hypocalcemia.

## 7.2 INTRODUCTION

Hypocalcemia usually occurs when a dairy cow is unable to adapt to the increased physiological demand for calcium (**Ca**) at the onset of lactation and maintain eucalcemia (Goff, 2008). Almost all cows experience some degree of hypocalcemia during the first days after calving, as it can take 1 to 2 d for macromineral adaptation to occur (Horst et al., 1994; Goff, 1999). Cows with hypocalcemia are generally identified within the first 12 to 24 h postcalving (Oetzel, 2004; Roche and Berry, 2006; Goff, 2008).

Clinical hypocalcemia is characterized as blood Ca concentrations <1.4 mmol/L (Lindsay and Pethick, 1983; Martín-Tereso and Martens, 2014) and subclinical hypocalcemia is usually characterized as blood Ca <2.0 mmol/L (Oetzel, 2004; Goff, 2008; Reinhardt et al., 2011). These thresholds do not correlate with the appearance of clinical symptoms that accompany the severe form of hypocalcemia known as parturient paresis or milk fever (Horst et al., 1997; Martín-Tereso and Martens, 2014). Cows are stoic animals (Fitzpatrick et al., 2002; Weary et al., 2009); therefore, we expect that cows

experiencing subclinical or clinical hypocalcemia may display more subtle behavioral changes, making them difficult to visually detect.

The prevalence of subclinical hypocalcemia is significant in both housed (range: 25 to 54%; Reinhardt et al., 2011; Ribeiro et al., 2013), and grazing cows (range 30 to 40%; Roche, 2003; Roberts and McDougall, 2019), and is dependent on parity (Horst et al., 1997; Roche and Berry, 2006). Milk fever, itself, has been reported as a risk factor for other metabolic, infectious, and reproductive disorders (Goff, 2008; DeGaris and Lean, 2009; Martinez et al., 2012) and recent work in housed cows indicates that cows with subclinical hypocalcemia within 4 DIM are more likely to experience a disease event [e.g., displaced abomasum (Chapinal et al., 2011), metritis (Neves et al., 2018), and hyperketonemia] or removal from the herd (McArt et al., 2020). The high incidence of subclinical hypocalcemia and potential effects on health, due to the important role that calcium plays in immune function, and smooth and skeletal muscle contractility (Goff, 2008; Murray et al., 2008), support a focus on developing new approaches to identify at-risk cows (Neves et al., 2017). Predicting hypocalcemia and ultimately improving diagnostic and treatment outcomes may be possible by monitoring cow behavior, but the behavioral changes associated with hypocalcemia must first be characterized (Weary et al., 2009; Proudfoot and Huzzey, 2017).

Long lying times and a reduction in activity (i.e., paresis) are common sickness behaviors and are clinical symptoms of milk fever (Hart, 1988). Calcium is required for smooth muscle (e.g., rumen and uterine) and skeletal muscle contractions (Murray et al., 2008), so reduced blood Ca levels can lead to both prolonged labor and restricted movement, particularly transitional behaviors (i.e., standing up and lying down). One study reported altered pre- and postcalving lying behavior (e.g., lying time) in housed

cows diagnosed with subclinical hypocalcemia (blood Ca ≤1.8 mmol/L within 24 h postcalving; Jawor et al., 2012), while another reported no association (blood Ca <2.0 mmol/L within 48 h postcalving; Piñeiro et al., 2019). A lack of association between activity and subclinical hypocalcemia has also been reported (blood Ca <8.55 mg/dL within 72 h postcalving; Liboreiro et al., 2015), but the long time-period examined and the relatively high blood Ca concentration cut-point chosen (equivalent to <2.13 mmol/L) may have reduced some of the resolution. Despite several studies reporting associations between lying behavior, activity, and other behaviors, such as feeding, rumination, and drinking in housed cows diagnosed with hypocalcemia (Jawor et al., 2012; Liboreiro et al., 2015); to our knowledge, lying behavior and activity in periparturient grazing dairy cows classified with varying degrees of hypocalcemia has not been investigated. In addition, there is a lack of consistent information available regarding changes in behavior due to hypocalcemia in both housed and grazing cows (Sepúlveda-Varas et al., 2014) and further studies are warranted.

We hypothesized that grazing cows with subclinical or clinical hypocalcemia spend more time lying down and have reduced activity compared with normocalcemic cows. The objective of the current study was to investigate whether cows classified retrospectively as subclinically- and clinically-hypocalcemic, but without clinical milk fever (i.e., parturient paresis), displayed behavioral differences pre- and postcalving compared with cows classified as normocalcemic.

## 7.3 MATERIALS AND METHODS

### *7.3.1 Animal Management and Experimental Design*

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations in accordance with the New Zealand Animal Welfare Act (Ministry for Primary Industries, 1999). Data for the present study were selected from a dataset of 143 spring-calving, pasture-grazing cows described by Roche et al. (2015; 2017a), which included feeding x BCS treatments. A subset of 72 Holstein-Friesian and Holstein-Friesian x Jersey cows were selected from 2 separate parent experiments. The BCS (Roche et al., 2015) and feed (Roche et al., 2017a) studies were undertaken across 2 separate seasons (2013 and 2014, respectively) and 2 locations (Scott Farm and Lye Farm; Hamilton, New Zealand, both 37°46'S 175°18'E).

Experimental methods for parent experiments are explained in detail by Roche et al. (2015; 2017a). Briefly, however, cows from Roche et al. (2015) were either BCS 4.0 or 5.0 at 1 month before calving (10-point scale, where 1 is emaciated and 10 obese; Roche et al., 2004). Cows within each BCS were then allocated 1 of 3 levels of energy intake during the 3 wk preceding calving [75, 100, or 125% of estimated metabolizable energy (**ME**) requirements], but all cows were managed similarly postcalving (Roche et al., 2015). In a subsequent study, (Roche et al., 2017a) cows were at 1 of 2 BCS at dry-off (approximately 4.25 and 5.0 on a 10-point scale). Following dry-off, cows in both BCS categories were managed to achieve a BCS 5.0 at 1 month before calving. Cows within each 'far-off' feeding level treatment were then allocated to 1 of 3 levels of energy intake during the 3 wk preceding calving (65, 90, or 120% of estimated ME requirements) (Roche et al., 2017a). In both studies, all cows were managed similarly postcalving; cows

were milked twice daily in a rotary parlor and spent ~40 to 90 min/d standing waiting, and walking to and from the milking parlor (~1 to 2.5 km/d walked on tracks).

### *7.3.2 Blood Sampling and Analyses*

Blood was sampled by coccygeal venipuncture weekly from wk 4 pre- to wk 5 postcalving, and additionally on d 0, and d 1, 2, 3, and 4 postcalving in both studies. Blood was collected in evacuated blood tubes containing lithium heparin anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Heparinized samples were placed immediately into iced water and were centrifuged within 30 min of collection at 1,500 x *g* for 12 min at 4°C. Following centrifugation, aspirated plasma was stored at –20°C until assayed. Plasma samples were submitted to Gribbles Veterinary Pathology Ltd. (Hamilton, New Zealand) for analysis. Blood metabolites were assayed using colorimetric techniques at 37°C with a Hitachi Modular P800 analyzer (Roche Diagnostics, Indianapolis, IN). Roche reagent kits were used to measure plasma concentrations of BHB (mmol/L; reduction of $NAD^+$ to NADH during oxidation of D-3-hydroxybutyrate to acetoacetate), Ca (mmol/L; o-cresolphthalein complexone method) and magnesium (**Mg**, mmol/L; xylidyl blue reaction). Plasma non-esterified fatty acids (**NEFA**, mmol/L) concentrations were measured using Wako Chemicals (Osaka, Japan) kit NEFA HR2 measuring oxidative condensation of 3-methyl-N-ethyl-N-β hydroxyethyl aniline with 4-aminoantipyrine. The inter- and intra-assay coefficients of variation for all assays were <5.5% and ≤15%, respectively, as reported in Roche et al. (2015; 2017a).

### *7.3.3 Classification of Calcium Status*

Blood Ca and behavior data were available from 143 cows for analysis; a subset of 72 cows was established based on blood Ca status for statistical analysis. A cow was

classified as being clinically-hypocalcemic (**CLIN**) when blood Ca concentration was ≤1.4 mmol/L at 1 or more consecutive samplings within 48 h postcalving, but parturient paresis was not observed (Lindsay and Pethick, 1983). A cow was classified as subclinically-hypocalcemic (**SUB**) when blood Ca concentrations were >1.4 and <2.0 mmol/L at 2 consecutive samplings within 48 h postcalving (Goff, 2008). Of 143 cows, 36 were identified as CLIN within 48 h postcalving, 26 were identified as SUB, and 44 were identified as normocalcemic (**NORM**; serum Ca concentration ≥2.0 mmol/L at 3 consecutive samplings within 72 h postcalving). The 36 CLIN cows were each pair-matched with a SUB cow and NORM cow using EBV for milk protein, mean BW precalving (wk –5 and –6), and, where possible, parity. Milk protein EBV (data kindly provided by Livestock Improvement Corporation Ltd., Hamilton, New Zealand) was used as a proxy measure for milk protein production potential to pair-match cows as suitable milk component and volume records were unavailable from previous lactations. Most Ca in milk is contained in the casein micelle and, therefore, milk protein production is a good proxy for Ca secretion in milk (Gambra et al., 2013). Parity was grouped as follows: parity 2 and 3 (**parity 2–3**; n = 44) and parity 4 to 7 (**parity 4+**; n = 28). All cows included in the study were multiparous (i.e., approaching their second or greater lactation at the time of calving). A lesser representation of greater parity cows was due to fewer animals fitting the criteria for the SUB or NORM groups. After matching cows based on the criteria outlined above, 24 cows were available for each blood Ca group (24 CLIN cows balanced with 24 NORM cows and 24 SUB cows), and these animals were used for all further analyses. The mean and standard deviation (**SD**) for EBV milk protein, wk –5 to –6 precalving BCS and BW, and the number of cows by parity, breed, and study are presented in Table 7.1.

In Chapter 7, I retrospectively classified cows into groups based on predetermined thresholds for hypocalcemia based on studies from cows in housed systems; however, there is some uncertainty as to whether it is appropriate to apply these thresholds in grazing dairy cows. Therefore, I attempted a principal component analysis to attempt to systematically group cows based on a combination of health markers and I have explained this briefly in Appendix 17.

**Table 7.1. Descriptive data for all cows classified into 1 of 3 blood calcium groups.**

Mean ± standard deviation (SD) of milk protein estimated breeding value (EBV), wk –5 to –6 precalving body weight (BW) and body condition score (BCS), and number of cows (n) by parity, breed, and study for the 3 calcium groups (NORM, SUB, and CLIN)[1].

| Mean ± SD | NORM | SUB | CLIN |
|---|---|---|---|
| Milk protein EBV[2] | 17.7 ± 6.41 | 17.6 ± 6.78 | 16.4 ± 6.93 |
| Wk –5 to –6 precalving | | | |
| BW, kg | 551.8 ± 61.9 | 557.0 ± 45.3 | 559.8 ± 60.8 |
| BCS,[3] 10-point scale | 4.94 ± 0.44 | 4.93 ± 0.41 | 4.71 ± 0.54 |
| n (cows) | | | |
| Parity 2–3[4] | 19 | 10 | 15 |
| Parity 4+[4] | 5 | 14 | 9 |
| Breed (HF)[5] | 17 | 13 | 20 |
| Breed (HF x J)[5] | 7 | 11 | 4 |
| BCS study[6] | 11 | 10 | 15 |
| Feed study[6] | 13 | 14 | 9 |

[1]Cows (n = 24 per group) were classified as having clinical hypocalcemia [CLIN; blood calcium (Ca) ≤1.4 mmol/L within 48 h postcalving but without parturient paresis], subclinical hypocalcemic cows (SUB; blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving), or normocalcemia (NORM; blood Ca ≥2.0 mmol/L within 72 h postcalving).
[2]Milk protein EBV = Estimated Breeding Value for milk protein (DairyNZ, 2018; Johnston et al., 2018).
[3]Body condition score during wk –5 to –6 precalving (on a 1 to 10 scale; Roche et al., 2004).
[4]Parity 2–3 = cows approaching their second or third parity at the time of calving; parity 4+ = cows approaching their fourth, fifth, sixth, or seventh parity at the time of calving.
[5]Breed where HF = Holstein-Friesian and HF x J = HF x Jersey.
[6]Cows were selected from the BCS study as described by Roche et al. (2015) or the feed study as described by Roche et al. (2017a).

### *7.3.4 Milk, BCS, BW, and Breed*

Cows were milked twice daily and individual milk yields were measured at each milking from 1 to 49 DIM using either the Westfalia Surge Metatron Milk Meter (GEA Farm Technologies, Cambridge, New Zealand) or the DeLaval Milk Meter (DeLaval Ltd., Hamilton, New Zealand) for BCS and feed studies, respectively. Milk was sampled once weekly on consecutive afternoon and morning milkings and a composite sample was

analyzed for milk composition by infrared analysis (FT120, Foss Electric, Hillerød, Denmark). Energy-corrected milk yield was calculated as (Nielsen et al., 2009):

kg of ECM = [kg of milk x (383 x fat% + 242 x protein% + 780.8)]/3,140

Body weight was recorded and BCS determined weekly following the morning milking or at approximately 0800 h during the nonlactating period. All BCS assessors were trained and recalibrated at the start of the experiment following the procedures set out in Macdonald and Roche (2011). Animal evaluation data for cow breed, Breeding Worth (**BrW**), and Production Worth (**PW**) were kindly provided by Livestock Improvement Corporation Ltd. (Hamilton, New Zealand). Breeding worth and PW are estimated economic values of a combination of 8 traits (milk fat, protein, milk volume, BW, fertility, SCC, BCS, and residual survival) that are indicators of robustness and production efficiency (DairyNZ, 2018; Johnson et al., 2018). Breeding values are the genetic potential of an animal for the trait of interest and the weighted combination of all 8 EBV traits contribute to the BrW (DairyNZ, 2018). Milk protein EBV was used instead of milk production records due to the experimental cows being involved in studies during previous seasons that may have affected their historical records.

### *7.3.5 Behavioral Data and Editing*

A full description of the behavioral data collection and editing methods are described in Chapter 6. Behavioral data were available for the period –21 d precalving to +35 d postcalving. In brief, each cow was fitted with a triaxial accelerometer (IceTag or IceQube; IceRobotics Ltd., Edinburgh, Scotland) on the lateral side of a hind leg and behavioral data were recorded. Data were downloaded using the IceManager 2010 software (IceRobotics Ltd.) from the on-board memory of the device. Two summary files were generated for each individual cow; 1 file consisted of lying time (s) and number of

steps recorded at 1- and 15-min sampling intervals, for the IceTag and IceQube, respectively and the other file contained lying bouts (**LB**) recorded by day, timestamp (hh:mm:ss), and duration (s). These summary files were used to calculate daily and hourly lying time (h/d), LB (no./d), mean LB duration (min/bout), and number of steps (steps/d) for each cow. A LB is defined as the period between the activity monitor changing from vertical to horizontal and back to vertical. Data excluded from the analysis were data recorded on the day that the accelerometers were removed or fitted to the cows and incorrect accelerometer recordings due to technical errors. In the current study, based on previously determined thresholds for IceRobotics sensors, LB <33 s (Kok et al., 2015) and ≤2 min (Mattachini et al., 2013) were discarded from the raw data recorded by the IceQube and IceTag devices, respectively.

From the output data sets, the sampling dates for each individual cow were assigned an experimental day relative to d 0 (recorded calving date). After visual inspection of the data on a per-cow basis, it was evident that a peak in LB frequency was occurring on d –1 and not on d 0 for some cows. As is common in grazing dairy systems, farm staff collected newborn calves and their dams once daily (Vogels et al., 2013; Lawrence et al., 2017). Consequently, there may be a discrepancy of up to 24 h for the recording of the date of calving. Where we have recorded data that supersedes the day of 'calf collection', we re-assigned the calving day using activity data. Previous studies report that an average of 14 LB/d occurs on the day of calving (Huzzey et al., 2005; Borchers et al., 2017; Rice et al., 2017). In our study, we assumed that ≥14 LB on d –1 was likely associated with a calving event and, therefore, adjusted the calving date to reflect this. Otherwise, it was assumed that recorded calving date was correct.

### *7.3.6 Weather Data*

Daily rainfall (mm; 24-h period) and daily air temperature (°C; recorded at 0900 h) data were retrieved from The National Climate Database (NIWA, 2018) for the duration of the 2 experiments. Data were retrieved from station agent number 26,117 (37.8°S, 175.3°E) for the BCS and feed studies (NIWA, 2018). The distance from the climate station to the study site for the BCS and feed studies is approximately 3 km.

### *7.3.7 Statistical Analyses*

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Results are presented as least squares means (**LSM**) ± standard error of the mean in the text and mean standard error of the difference in tables and figures. Where we have presented LSM for the 3 Ca groups, we have presented group mean effects. The covariance structures selected were compound symmetry or autoregressive based on the lowest Akaike's information criterion. All repeated measures ANOVA models were pairwise comparison-adjusted using Tukey-Kramer. Residuals for cow performance, behavioral, and blood parameters were checked to ensure the assumptions of normality and homogeneity of variance were met. Study and treatment from the parent experiments were concatenated to create a categorical variable, study group. All data were adjusted where appropriate, according to the re-assigned calving day, and these transformed datasets were the basis of subsequent analyses.

Study group (categorical), parity (categorical; 2–3 or 4+), and the difference in days between calving date and the first day in June (**calvingseasonday**) were included in all models described below to adjust for different treatments within the 2 studies, parity differences, and different calving dates.

***Behavioral Parameters.*** Lying behavior and activity data pre- and postcalving were first analyzed to determine mean differences in the group means between the Ca groups for both daily and hourly data. Following this analysis, we further investigated whether the change in behavior from a precalving baseline period to d –1 or d 0 was associated with blood Ca concentrations postcalving. Change in daily and hourly lying time and number of steps taken within cow was included in these subsequent models. Understanding both daily and temporal changes in behavior that precede changes in blood Ca concentrations may provide important information for future work interested in identifying cows at risk of developing hypocalcemia. Due to differences in lying time and steps taken between the 3 Ca groups between 0600 and 1800 h, we further investigated these associations. Specifically, to reflect changes in behavior occurring during the daytime, the hourly data were further summarized to included only data recorded between 0600 and 1800 h and is referred to, herein, as hourly daytime lying time and steps taken. The linear and non-linear associations between change in daily and hourly daytime lying time and number of steps taken precalving (dependent variables) and blood Ca concentrations postcalving (independent variable) were investigated. The separate analyses are described in detail below.

***Differences in Lying Time and Activity Associated with Ca Status.*** Behavior data were summarized for 15 periods: wk –3 and –2 precalving, d –7 to –4 precalving, daily precalving (d –3 to –1), d 0, daily postcalving (d 1 to 3), d 4 to 7 postcalving, and wk 2, 3, 4, and 5 postcalving. Differences in daily lying time, daily number of LB, mean LB duration, and number of steps taken between the 3 Ca groups were analyzed using a repeated measures ANOVA (PROC MIXED) with cow as a random effect, period as a repeated measure, and the fixed effect of Ca status, period, and Ca status x period

interactions. Behavior analyses included fixed effect of daily rainfall (continuous) and mean air temperature at 0900 h (continuous), and interactive effect of rainfall and air temperature as potential explanatory variables.

***Differences in Hourly Behavioral Parameters Within Day Associated with Ca Status.*** Upon examination of the blood Ca profile, we identified d –2 to d +2 relative to calving as the critical time for risk of hypocalcemia, making this period suitable for evaluating within-day associations among behavior and activity parameters and blood Ca status. We were unable to adjust the data according to the exact time of calving; so within each day from d –2 precalving to d 2 postcalving, 24-h behavior data were summarized into 4 h time intervals (i.e., 0200 to 0559 h, 0600 to 0959 h, 1000 to 1359 h, 1400 to 1759 h, 1800 to 2159 h, 2200 to 0159 h). Behavior data were analyzed separately within day for d –2 to d +2 relative to calving using a repeated measures ANOVA (PROC MIXED) to investigate differences in lying time and number of steps taken over a 24-h period due to Ca status. Time interval was included as fixed, hour as a repeated measure, and cow as random effects. On d –2 precalving and d 2 postcalving, lying time or number of steps taken were not different between Ca status within the time periods investigated; therefore, the data are not presented.

***Linear and Nonlinear Associations of Changes in Daily and Hourly Daytime Lying Behavior and Activity Precalving with Blood Ca Postcalving.*** Linear and nonlinear associations of change in daily lying time, daily steps taken, hourly daytime lying time, and hourly daytime steps taken with blood Ca concentrations 24 h postcalving were investigated. Prior to undertaking these analyses, we generated Pearson correlations between summarized behavior variables and blood Ca, to select the variables that were most strongly associated for further analysis.

Blood Ca data were summarized for d 0, d 1, and 2 postcalving, and lying time and activity data were summarized for 4 periods immediately before calving and surrounding the calving event using PROC MEAN procedure on a per-cow basis for all 72 cows. A detailed description of the periods investigated and descriptive data for summarized blood Ca concentrations and behavior and activity data are presented in Appendices 11 and 12 – Supplemental Table 9 and Supplemental Materials and Supplemental Table 10, respectively. All summarized behavior variables and blood Ca formed the dependent and independent variables, respectively. Pearson correlations were generated for summarized behavior variables and blood Ca using PROC CORR and the correlation matrix is presented in Appendix 13 – Supplemental Table 11. The strongest associations were selected for further analysis.

The independent variable selected was the blood Ca concentration within 24 h postcalving. The dependent variables selected were change in daily and hourly daytime lying time and steps taken, calculated as the difference between the behavior on d 0 and mean precalving baseline period (d –14 to –7). Blood Ca concentration was modeled as a continuous measure and behavior variables as continuous effects in 4 separate analyses (2 daily and 2 hourly daytime models). Slopes of the estimated change in each behavior (dependent variable) were investigated using multiple-regression analyses (PROC GLM) with blood Ca concentration within 24 h postcalving as the independent variable. Models included categorical effects of study group, breed, and parity, and continuous effects of calvingseasonday, BrW, PW, milk protein EBV, wk –5 to –6 precalving BW, wk –5 to –6 precalving BCS, and rainfall and air temperature and their interactions as potential explanatory variables. Variables were checked for multicollinearity but were not highly correlated (variance inflation factors ≤10). Nonsignificant variables were eliminated from

the model one at a time where $P > 0.10$ with the exception of study group and calvingseasonday, which were forced in the model even if not significant to adjust for different treatments within the 2 studies and different planned start of calving dates. The final models for all variables investigated included the effect of study group, calvingseasonday, and parity. Both linear and quadratic associations were assessed for the behavior variables. The models that included behavioral parameters without adjusting for other factors are presented in Appendices 14 and 15 – Supplemental Tables 12 and 13.

***Milk, BCS, and BW.*** Weighted means for weekly milk yield were calculated using daily yields on a per-cow basis for wk 1 to 7 postcalving using PROC MEAN procedure. Weighted means for milk yield were used to calculate weekly milk component yields and ECM yield for wk 2 to 6 postcalving. Due to inconsistent records for milk composition data during wk 1 postcalving (colostrum period), these data were excluded from ECM yield analysis. To investigate the associations between milk and ECM, CP and fat yield, milk protein and fat composition, and Ca status, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, week as a repeated measure, and the fixed effect of Ca status, week, and Ca status x week interactions. Breeding Worth and PW as proxies for milk production potential were included in the model as covariates.

To investigate the associations between BCS and BW and Ca status for 6 wk pre- and postcalving, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, week as a repeated measure, and the fixed effect of Ca status, week, and Ca status x week interactions.

***Blood Metabolite and Mineral Markers.*** Blood data for minerals (Ca and Mg) were summarized into six periods [i.e., wk –1 and –2 precalving, d 0, and d 1 and 2 postcalving, d 3 to 7 postcalving and wk 2 postcalving]. Blood data for energy metabolites (NEFA and BHB) were summarized into six periods [i.e., wk –1 and –2 precalving, d 0 to 2 postcalving, and d 3 to 7 postcalving, and then weekly postcalving (wk 2 to 4)].

To investigate the associations between minerals and energy metabolites, and Ca status and period, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, period as a repeated measure, and the fixed effect of the Ca status, period, and Ca status x period interactions. Variables were checked for skewness and normality. Blood BHB was log-transformed for analyses and untransformed LSM, standard error of the mean, and standard error of the difference are presented.

The association between endometrial and protein metabolite measures and Ca status and period were also investigated in addition to the measures presented in Chapter 7; however, these additional analyses were not submitted for publication and are presented in Appendix 16.

## 7.4 RESULTS AND DISCUSSION

### *7.4.1 Differences in Blood Calcium and Magnesium Between Blood Calcium Groups*

The mean and SD for milk protein EBV, wk – 5 to – 6 precalving BCS and BW, and the number of cows by parity, breed, and study are presented in Table 7.1. Descriptive data presented for the 3 blood Ca groups indicate that our classification process was successful in classifying groups with balanced phenotypes. As intended, mean blood Ca concentrations differed ($P < 0.001$) between the 3 blood Ca status groups. The profiles presented in Figure 7.1a indicate that our classification process was successful in

classifying groups with divergent plasma Ca concentrations at calving. There was a Ca status x period interaction ($P < 0.001$); on d 0 and d 1 postcalving, the CLIN group had a lower blood Ca concentration (1.47 ± 0.05 mmol/L) than the SUB group, which had, in turn, a lower blood Ca concentration (1.75 ± 0.04 mmol/L) than the NORM group (2.16 ± 0.05 mmol/L; $P < 0.001$). Mean blood Mg concentration also tended to differ between Ca status groups during the period d –14 precalving to d 14 postcalving (0.74 ± 0.02, 0.71 ± 0.02 and 0.78 ± 0.02 mmol/L, for the NORM, SUB and CLIN groups, respectively; $P = 0.09$); however, there was no Ca status x period interaction for blood Mg ($P = 0.64$; Figure 7.1b).

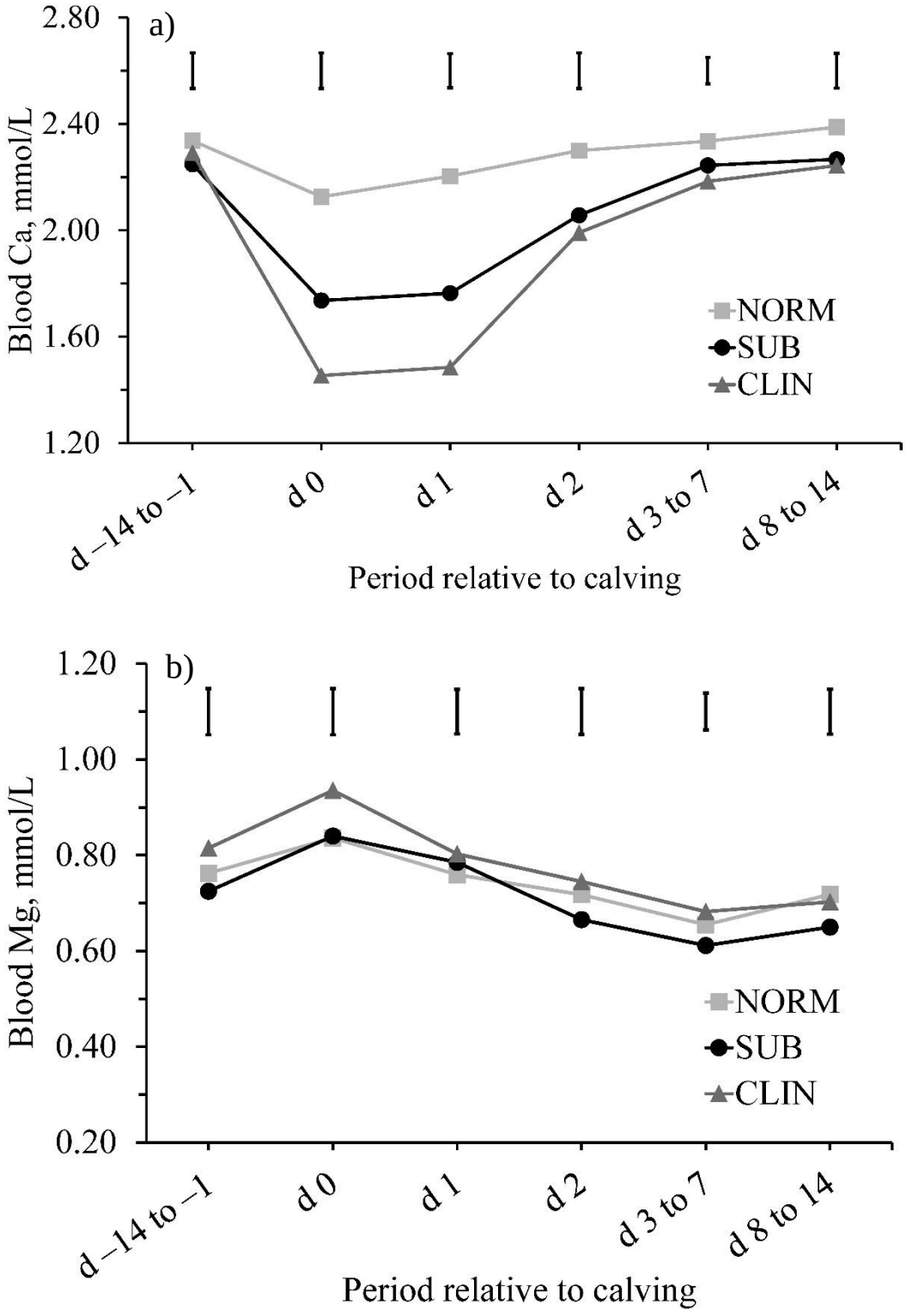


**Figure 7.1. Blood calcium and magnesium concentrations during the transition period in 3 blood calcium groups.**

Blood calcium [Ca; (a)] and magnesium [Mg; (b)] concentrations (mmol/L) during d –14 to –1 precalving and on the day of calving (d 0) and d 1, and 2, d 3 to 7, and d 8 to 14

postcalving for the 3 Ca groups [CLIN (blood Ca ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)]. Error bars represent 2 x mean standard error of the difference.

### *7.4.2 Differences in Lying Time and Activity Associated with Blood Calcium Status*

Quantitative measures of lying behavior and activity may allow early identification of clinical hypocalcemia in grazing dairy cows in time to provide preventative treatment and halt the progression to clinical milk fever, if hypocalcemia is preceded by changes in behavior that occur before calving. To our knowledge, this is the first study to investigate associations between both pre- and postcalving lying behavior and activity and blood Ca status in transition cows grazing pasture.

Daily lying time, daily LB, mean LB duration, and number of steps taken per day over the period –21 to +35 d relative to calving are presented in Figures 7.2a, b, c, and d, respectively. Overall, there was no association between Ca status and lying behavior variables and activity (Table 7.2); however, there were Ca status x period interactions for daily lying time ($P < 0.001$), daily LB ($P < 0.05$), number of steps taken ($P < 0.001$), and there was a trend ($P = 0.09$) for a Ca status x period interaction for LB duration. Herein, we consider 2 distinct types of behavior, referred to as 'sickness behaviors' and 'early indicators of disease'. Sickness behaviors are well established as an evolutionary mechanism to support the body's response to combat disease (Hart, 1988), and are typically observed when the animal displays signs of clinical illness, involving highly-coordinated physiological responses (Dantzer and Kelley, 2007). An expanding body of literature has been able to demonstrate that behavioral changes can occur well

before disease diagnosis; these have been ascribed as early indicators of disease (Proudfoot and Huzzey, 2017). Sickness behaviors are, therefore, deemed to be behaviors occurring from the day of calving onwards, whereas behaviors observed before parturition will be referred to as early indicators of disease.

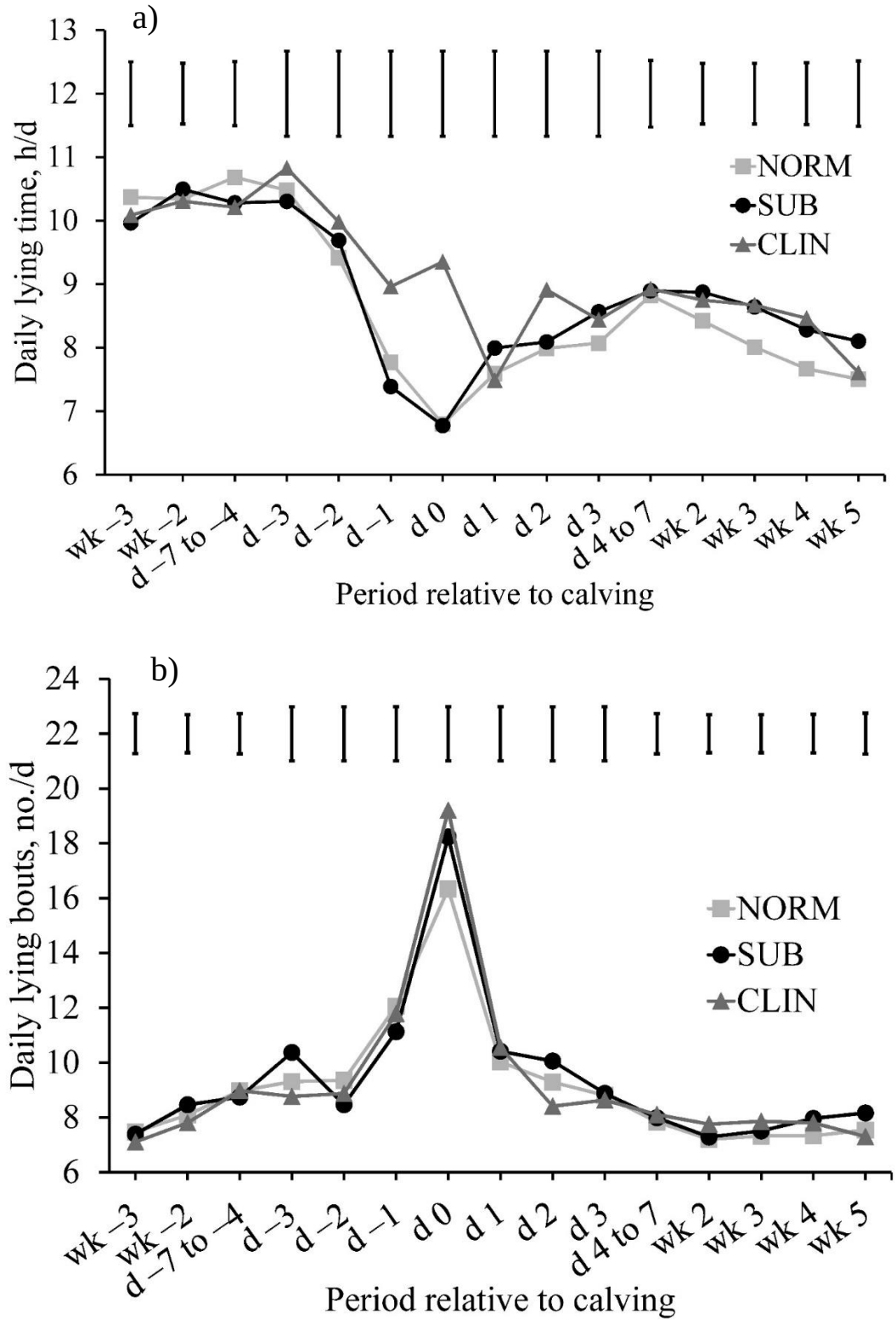


**Figure 7.2. Lying behavior and activity during the transition period in 3 blood calcium groups.**

Figure and figure legend continued over page.

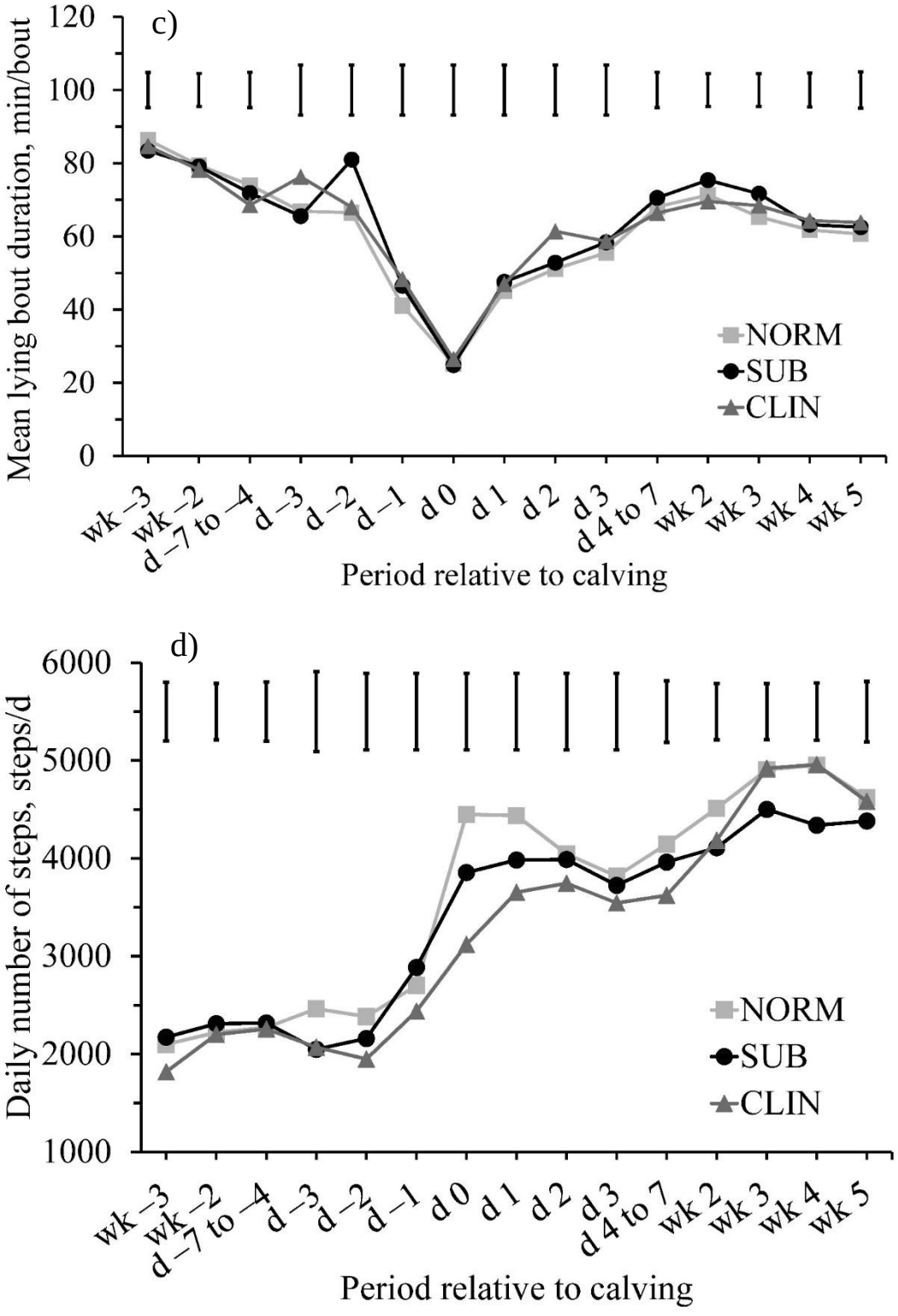


**Figure 7.2 (Continued).** Daily lying time [(a); h/d], lying bouts [LB (b); no./d], mean LB duration [(c); min/bout], and number of steps [(d); steps/d] during the period –21 to +34 d relative to the day of calving (d 0) in the 3 calcium (Ca) groups [CLIN (blood Ca ≤1.4

mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)]. Error bars represent 2 x mean standard error of the difference.

### *7.4.3 Lying Time and Activity as Sickness Behaviors*

On the day of calving, cows in the CLIN group were less active (3,118 ± 274 steps/d; $P < 0.05$) and spent ~2.6 h longer lying (9.4 ± 0.47 h/d; $P < 0.001$) than cows in the SUB and NORM groups, which were not different from each other (3,853 ± 266 vs. 4,448 ± 285 steps/d and 6.8 ± 0.46 vs. 6.8 ± 0.49 h/d, respectively) (Figures 7.2a and d). The longer total daily lying time on d 0 in the CLIN group was predominantly driven by their greater number of LB (Figure 7.2b) as the mean LB duration did not differ between groups (Figure 7.2c). Cows in the CLIN group remained less active the day after calving than cows in the NORM group (3,652 ± 274 vs. 4,436 ± 285 steps/d; $P < 0.05$); however, both groups were not different from the SUB group (3982 ± 266 steps/d).

There is evidence that sick cows generally spend more time lying (Kaufman et al., 2016; Barragan et al., 2018). In our study, few differences were recorded between the SUB and NORM groups; however, the differences we recorded in the CLIN group are consistent with the findings of Jawor et al. (2012) who reported that housed cows experiencing hypocalcemia (blood Ca ≤1.8 mmol/L within 24 h of calving) spent more time lying (by 2.7 h on the day after calving), when compared with normocalcemic cows (blood Ca >1.8 mmol/L within 24 h of calving). Sepúlveda-Varas et al. (2014) defined changes in lying behavior postcalving in grazing dairy cows; however, subclinical hypocalcemia was defined as blood Ca ≤2.0 mmol/L between 3 and 22 d postcalving,

therein potentially failing to detect most hypocalcemic cows. In addition, cows were grouped according to the presence of 1 or more health events (i.e., retained placenta, metritis, mastitis, hypocalcemia, and subclinical ketosis), making it difficult to confidently understand differences in behavior specifically due to hypocalcemia. Our observed increase in lying time in the CLIN group may reflect an energy-conserving behavior associated with hypocalcemia (Johnson, 2002; Jawor et al., 2012), but is more likely a result of compromised movement due to the importance of Ca for skeletal muscle contraction (Murray et al., 2008; Jawor et al., 2012).

Few studies that have investigated the association between activity and blood Ca concentrations and measurements are often different. Nevertheless, there is a consistent theme that activity declines in cows experiencing hypocalcemia, although the extent of the decline depends on the extent of the hypocalcemia. For example, Liboreiro et al. (2015) reported no associations between steps taken pre- and postcalving, possibly due to the relatively high blood Ca threshold used to define hypocalcemia (blood Ca <2.14 mmol/L within 72 h postcalving). Other studies, however, have reported reduced activity, such as self-grooming (Fogsgaard et al., 2012) and reduced number of steps taken have been reported due to other diseases (Edwards and Tozer, 2004; Liboreiro et al., 2015; Barragan et al., 2018). Collectively, the literature indicates that ‘sick’ cows either walk less, perform other activities fewer times each day, and lie for longer.

**Table 7.2. Behavior and performance measures for 3 blood calcium groups.**

Mean lying behavior, activity, and cow performance measures for the 3 blood calcium (Ca) groups [24 cows per group; CLIN (blood Ca ≤1.4 mmol within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)].

| Parameter | NORM | SUB | CLIN | SED[1] | *P*-value |
|---|---|---|---|---|---|
| Lying time,[2] h/d | 8.66 | 8.82 | 9.13 | 0.45 | 0.59 |
| Lying bouts (LB),[2] no./d | 9.13 | 9.40 | 9.26 | 0.65 | 0.91 |
| LB duration,[2] min/bout | 61.2 | 63.6 | 63.3 | 4.11 | 0.82 |
| Steps taken,[2] steps/d | 3,601 | 3,381 | 3,270 | 272 | 0.50 |
| Milk yield,[3] kg/d | 26.4 | 26.8 | 26.6 | 1.00 | 0.90 |
| ECM yield,[4] kg/d | 28.4 | 28.5 | 28.7 | 0.91 | 0.93 |
| CP yield,[4] kg/d | 0.93 | 0.93 | 0.94 | 0.02 | 0.93 |
| Fat yield,[4] kg/d | 1.15 | 1.19 | 1.21 | 0.03 | 0.36 |
| Milk protein,[4] % | 3.47 | 3.42 | 3.46 | 0.07 | 0.61 |
| Milk fat,[4] % | 4.45 | 4.41 | 4.46 | 0.15 | 0.91 |
| BW,[5] kg | 523 | 541 | 542 | 18.1 | 0.51 |
| BCS,[5,6] 10-point scale | 4.59 | 4.63 | 4.64 | 0.07 | 0.78 |

[a–b]Means with different superscripts are significantly different at the 5% confidence level.
[1]SED = mean standard error of the difference.
[2]Behavior summarized for the period –21 to +35 d relative to calving.
[3]Milk yield during the first 7 wk of lactation.
[4]Energy-corrected milk (ECM), crude protein (CP) and fat yield, and milk fat and protein % during wk 2 to 6 of lactation.
[5]Mean body weight (BW) and body condition score (BCS) for the period 6 wk pre- and postcalving.
[6]Body condition score (10-point scale, where 1 is emaciated and 10 is obese; Roche et al., 2004) during the 6 wk pre- and postcalving period.

There has been limited research to determine the effects of cow health on daily walking activity, particularly in grazing cows, where cows can be required to walk considerable distances to and from the milking parlor and to spend time grazing pasture to meet their nutrient requirements (Beggs et al., 2018). Grazing is an energy-expensive activity (Kaufmann et al., 2011), requires optimal skeletal muscle function, and affects

the time available for other activities (Chapter 6). The magnitude of differences in behavior between cows experiencing varying degrees of hypocalcemia may, therefore, differ in grazing vs. indoor systems. Whether the associations between behavior and health are more obvious in grazing systems warrants further investigation; however, our data provide confidence that lying and walking behavior are affected by peripartum blood Ca concentration.

### *7.4.4 Lying Bouts as Sickness Behaviors*

To our knowledge, LB on d 0 have not been reported for cows experiencing hypocalcemia. We observed that cows in the NORM group had fewer LB (16.3 ± 0.72 no./d) on d 0 than cows in the SUB (18.2 ± 0.68 no./d; $P < 0.05$) and CLIN groups (19.2 ± 0.69 no./d; $P < 0.01$) (Figure 7.2b), highlighting a potential effect of blood Ca status on this peripartum characteristic. In clinically-healthy cows, LB increase substantially on the day of calving in both housed and grazing cows (Huzzey et al., 2005: Chapter 6), and the increase in transitioning between lying and standing positions has been hypothesized to result from discomfort associated with calving; while number of LB are reduced in ketotic compared with nonketotic cows. Itle et al. (2015) speculated that ketotic cows might be less willing to engage in energetically-expensive behaviors; therefore, transitioning less frequently between lying and standing positions (Susenbeth et al., 2004). In contrast, in our study, cows experiencing clinical and subclinical hypocalcemia had more LB on d 0 than normocalcemic cows. While it is difficult to explain this increase in LB, Proudfoot et al. (2009a) reported that cows with dystocia had a greater number of LB than cows with eutocia and hypothesized that this might be indicative of greater pain. Calcium is important for smooth muscle function; hence, low blood Ca concentrations may prolong labor due to weak contractions (Murray et al., 2008; Jawor et al., 2012) and represent a

risk factor for dystocia (Correa et al., 1993; López Bernal, 2003). While no cows in our study experienced clinical dystocia and we do not have the data to support, it is plausible that SUB and CLIN cows in our study experienced more painful or prolonged labor without clinical dystocia, contributing to their increased number of LB on the day of calving.

In our study, cows were either subclinically or clinically-hypocalcemic, but without paresis, and changes in lying behavior and activity were relatively short lived, with no difference evident by d 3 postcalving. In other studies, where cows only experienced subclinical disease, changes in behavior were also short lived or small (Jawor et al., 2012; Sepúlveda-Varas et al., 2014; Liboreiro et al., 2015). It should be noted that, in our study, absolute values for daily lying time in the 3 blood Ca groups fell within 1 h of the range of postcalving lying times (range: 7.5 to 8.5 h/d) reported as 'normal' in literature (Sepúlveda-Varas et al., 2014). The transient behavioral difference observed in our study and the lack of differences in blood Ca concentrations between the 3 blood Ca groups by 3 d postcalving reflects animals' self-curing and restoring blood Ca to sufficient concentrations to overcome the debilitating effects of hypocalcemia. While the severity of the disease may, in part, influence whether differences in behavior are evident after disease diagnosis (Sepúlveda-Varas et al., 2014), our results indicate that while observed mean differences for the behavior measures reported are relatively small, lying and walking behavior are associated with peripartum blood Ca status.

### *7.4.5 Lying Behavior and Activity Within Day Associated with Blood Calcium Status*

Mean sunrise and sunset times in both studies were 0725 and 1720 h, respectively (Timeanddate.com, 2017). Maximal lying time and minimal steps taken coincided with periods of darkness (i.e., between 1800 and 0600 h), irrespective of Ca status (Tables 7.3

and 7.4, respectively); this outcome is in agreement with other studies undertaken in grazing dairy cows (O'Connell et al., 1989; Sheahan et al., 2011). In our study, the difference between day and night activity was less for cows in the CLIN group and, therefore, the association between blood mineral status and behavior was not diurnally homogenous. On d –1 precalving, the CLIN group tended to spend more time lying per hour ($P = 0.10$), which was due, in part, to a Ca status x time interval interaction ($P < 0.05$) (Table 7.3). On the day of calving, a Ca status association with lying time ($P < 0.01$) indicates that overall, cows in the CLIN group spent more time lying (24.5 ± 1.59 min/h) than cows in the NORM and SUB groups (17.5 ± 1.89 vs. 18.2 ± 1.62 min/h, respectively; $P < 0.05$). Temporal lying behavior and activity data indicate a Ca status x time interval interaction ($P < 0.001$ and 0.01, respectively; Tables 7.3 and 7.4), where during the early morning (between 0200 and 0600 h) and daytime (between 1000 and 1400 h), the CLIN group spent more time lying down ($P < 0.05$) and took fewer steps (between 1400 and 1800 h; $P < 0.01$) than the NORM and SUB groups (Tables 7.3 and 7.4). In our study, differences in lying time and activity for cows experiencing hypocalcemia were more prominent at certain times of the day and during certain periods relative to disease diagnosis, which is consistent with other studies investigating behavior associated with other diseases (Huzzey et al., 2007; Itle et al., 2015). Grazing dairy cows display behavioral synchrony (O'Connell et al., 1989), where they perform similar behavioral activities at the same time. Therefore, identifying cows that are lying down while most of the herd are standing grazing may be an alternative and novel approach to studying behavior in grazing dairy cows to detect cows at risk of, or with, a disease.

Similar to the daily lying time and activity data presented, the differences in diurnal profiles were short-lived. We detected differences within day between Ca groups

on the day before and the day of calving; however, no further differences were evident at 1 to 2 d postcalving. Prospective studies are needed to determine whether lying behavior and activity on the day of calving for individual cows could alert producers to a cow experiencing hypocalcemia.

**Table 7.3. Lying time within day during the day before, the day of, and the day after calving in 3 blood calcium groups.**

Mean lying time (min/h) across 4 h time intervals on the day before calving (d –1 precalving), the day of calving (d 0) and the day after calving (d 1 postcalving) in the 3 calcium (Ca) groups [CLIN (blood Ca ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)].

| Parameter | | Time interval,[1] h | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Lying time, min/h | | 02–05 | 06–09 | 10–13 | 14–17 | 18–21 | 22–01 | *P*-value |
| d –1 precalving | NORM | 41.4$^{a,y}$ | 8.03$^{d}$ | 6.30$^{d}$ | 7.99$^{d}$ | 28.4$^{c,x}$ | 27.9$^{b}$ | <0.001 |
| | SUB | 40.5$^{a,y}$ | 7.40$^{c}$ | 3.63$^{c}$ | 6.26$^{c}$ | 21.2$^{b,y}$ | 31.3$^{b}$ | <0.001 |
| | CLIN | 48.4$^{a,x}$ | 8.73$^{c}$ | 8.03$^{c}$ | 7.39$^{c}$ | 29.5$^{b,x}$ | 32.3$^{b}$ | <0.001 |
| | *P*-value | <0.01 | 0.89 | 0.29 | 0.82 | <0.01 | 0.26 | |
| d 0 | NORM | 26.6$^{a,y}$ | 8.95$^{c,y}$ | 9.32$^{c,y}$ | 4.85$^{c,x}$ | 28.6$^{b}$ | 26.8$^{b}$ | <0.001 |
| | SUB | 25.3$^{a,y}$ | 11.4$^{b,x}$ | 12.6$^{b,y}$ | 7.24$^{c,y}$ | 28.1$^{a}$ | 24.4$^{a}$ | <0.001 |
| | CLIN | 39.7$^{a,x}$ | 16.3$^{b,x}$ | 18.9$^{b,x}$ | 16.6$^{b,y}$ | 28.3$^{a}$ | 27.1$^{a}$ | <0.001 |
| | *P*-value | <0.01 | 0.02 | 0.01 | <0.01 | 0.99 | 0.63 | |
| d 1 postcalving | NORM | 37.8 | 4.39 | 14.9 | 2.79 | 29.1 | 25.5 | <0.001 |
| | SUB | 39.4 | 5.90 | 19.3 | 6.05 | 27.8 | 23.8 | <0.001 |
| | CLIN | 38.4 | 5.45 | 17.8 | 6.90 | 30.1 | 26.0 | <0.001 |
| | *P*-value | 0.85 | 0.87 | 0.33 | 0.35 | 0.71 | 0.71 | |

[a–d]Means with different superscripts are significantly different at the 5% confidence level within a row (over time).
[x–y]Means with difference superscripts are significantly different at the 5% confidence level within a column (across groups).
[1]Time intervals include data within each hour specified (i.e., 02–05 covers the period 0200 to 0559 h).

**Table 7.4. Activity within day during the day before, the day of, and the day after calving in 3 blood calcium groups.**

Mean number of steps (steps/h) across 4 h time intervals on the day before calving (d –1 precalving), the day of calving (d 0), and the day after calving (d 1 postcalving) in the 3 calcium (Ca) groups [CLIN (blood Ca ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)].

| Parameter | | Time interval,[1] h | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Steps taken, steps/h | | 02–05 | 06–09 | 10–13 | 14–17 | 18–21 | 22–01 | *P*-value |
| d –1 precalving | NORM | $14^{d}$ | $195^{a}$ | $176^{ab}$ | $142^{b}$ | $72^{c}$ | $54^{cd}$ | <0.001 |
| | SUB | $5^{c}$ | $180^{a}$ | $181^{a}$ | $174^{a}$ | $71^{b}$ | $41^{bc}$ | <0.001 |
| | CLIN | $15^{b}$ | $191^{a}$ | $163^{a}$ | $165^{a}$ | $55^{b}$ | $47^{b}$ | <0.001 |
| | *P*-value | 0.86 | 0.76 | 0.68 | 0.31 | 0.64 | 0.81 | |
| d 0 | NORM | $43^{c}$ | $216^{b}$ | $276^{a,x}$ | $317^{a,x}$ | $85^{c}$ | $87^{c}$ | <0.001 |
| | SUB | $71^{d}$ | $155^{c}$ | $229^{b,xy}$ | $286^{a,x}$ | $54^{d}$ | $91^{d}$ | <0.001 |
| | CLIN | $39^{b}$ | $181^{a}$ | $200^{a,y}$ | $207^{a,y}$ | $65^{b}$ | $68^{b}$ | <0.001 |
| | *P*-value | 0.48 | 0.12 | 0.04 | <0.01 | 0.56 | 0.69 | |
| d 1 postcalving | NORM | $21^{e}$ | $300^{b,x}$ | $174^{c,x}$ | $350^{a}$ | $41^{de}$ | $77^{d}$ | <0.001 |
| | SUB | $21^{c}$ | $345^{a,x}$ | $101^{b,y}$ | $323^{a}$ | $51^{c}$ | $89^{b}$ | <0.001 |
| | CLIN | $31^{e}$ | $237^{b,y}$ | $176^{c,x}$ | $303^{a}$ | $74^{d}$ | $83^{d}$ | <0.001 |
| | *P*-value | 0.91 | <0.01 | <0.01 | 0.22 | 0.45 | 0.90 | |

[a–e]Means with different superscripts are significantly different at the 5% confidence level within a row (over time).
[x–y]Means with difference superscripts are significantly different at the 5% confidence level within a column (across groups).
[1]Time intervals include data within each hour specified (i.e., 02–05 covers the period 0200 to 0559 h).

### *7.4.6 Lying Time and Activity as Early Indicators of Disease*

Behavioral changes as early indicators of disease have been documented in transition cows; however, research in grazing dairy cows is limited. On the day before calving, cows in the CLIN group spent 1.4 h longer lying down (9.0 ± 0.47 h/d) than cows

in the SUB group (7.4 ± 0.46 h/d), which did not differ from cows in the NORM group (7.8 ± 0.49 h/d); however, there were no evident differences in lying behavior prior to this (Figure 7.2a). This result contrasts with the study by Jawor et al. (2012), where cows experiencing subclinical hypocalcemia stood for 2.6 h longer than normocalcemic cows during the 24-h period before parturition. The subclinically-hypocalcemic cows in their study produced, on average, 6 kg/d more milk during wk 2 to 4; therefore, these authors speculated that the increase in standing time might be attributed to increased udder fill, which may have made lying down more uncomfortable. In contrast, we hypothesize that the increase in lying time in the CLIN group, in our study, may be attributed to weak skeletal muscle contractility, which could reduce the desire to stand (Murray et al., 2008). It is not possible to determine whether other stressors or modification in lying behavior predisposed cows to hypocalcemia, or whether increased time spent lying down was indicative of a change in behavior caused by hypocalcemia (Proudfoot and Huzzey, 2017). Nevertheless, our data indicate a change in behavior preceding the hypocalcemic event, which suggests that lying and activity measures could be early indicators of this disease.

### *7.4.7 Modeling Early Indicators of Disease Within Cow*

Due to large cow-to-cow variation (Chapter 6), a review of behavior and health of transition cows by Proudfoot and Huzzey (2017) recommended that future work focuses on studying within-cow changes, as these are likely to be more sensitive for detecting changes in behavior due to ill health (Ito et al., 2009). Relative changes in behavior within cow, for example, are the measure of choice for estrus detection (Silper et al., 2015a, b). To further improve our understanding of within-cow changes in behavior over time, we investigated linear and nonlinear associations between relative change in

behavior [from a baseline period (either –21 or –14 to –7 d precalving) to d –1 or d 0] and blood Ca concentrations within 24 h postcalving. Correlations for the aforementioned periods investigated are presented in Appendix 13 – Supplemental Table 11. During the preliminary stages of our analysis, we also investigated the associations between mean daily lying time and number of steps taken on d 0 and blood Ca concentrations within 24 h postcalving (data not presented). However, we detected the strongest relationships for models investigating the change in daily and hourly daytime lying behavior and activity relative to d 0; therefore, we presented these models. Several studies indicate that changes in lying behavior (e.g., lying time and LB) may be disease dependent with increased (Sepúlveda-Varas et al., 2014) and decreased lying behavior (Itle et al., 2015), or both reported due to disease (Jawor et al., 2012).

***Linear and Nonlinear Associations.*** In our study, the change in lying time from a baseline period (d –14 to –7 precalving) to d 0 had negative linear and nonlinear associations with blood Ca concentration within 24 h postcalving ($P < 0.02$). The final model, which also included calvingseasonday and parity explained 39% of the variation (Table 7.5). In addition, the change in hourly lying time during the day (between 0600 and 1800 h) from a baseline period (d –14 to –7 precalving) until d 0 had a negative linear association with blood Ca concentration within 24 h postcalving ($P < 0.01$) and the final model explained 41% of the variation (Table 7.6). A relative 5 min increase in mean lying time per h during the day on d 0 compared with the baseline period was associated with a decrease in blood Ca concentration within 24 h postcalving of 0.09 mmol/L (Table 7.6). Change in daily steps taken from a baseline period (d –14 to –7 precalving) to d 0 was also associated with blood Ca concentration within 24 h postcalving ($P < 0.05$) and the final model explained 36% of the variation (Table 7.5). A relative increase of 1000 steps/d

on d 0 compared with the baseline period was associated with an increase in blood Ca concentration of 0.07 mmol/L within 24 h postcalving. Parity was an important associated factor in these models, which is unsurprising, considering the well-known association of hypocalcemia with increasing age (Horst et al., 1997).

Our results indicate that behavioral measures, such as relative change in daily and hourly daytime lying time and activity compared with a baseline period before calving, the time of day the behavior was recorded, and the magnitude of change within-cow at calving, may provide useful information for the early identification of hypocalcemia. Whether these changes in behavior are caused by hypocalcemia, or predispose cows to develop hypocalcemia is not known (Proudfoot and Huzzey, 2017). Understanding the drivers of the behavioral changes identified in our study has the potential to improve our understanding of cause and effect relationships for transition cow disease and improve the identification and prevention of hypocalcemia on farm. Future work should consider a prediction modelling or machine-based learning approach to explore the potential to detect hypocalcemia and other transition-cow diseases in individual cows.

**Table 7.5. Linear and nonlinear associations between changes in daily lying behavior and activity precalving and blood calcium concentrations within 24 h postcalving.**

Regression coefficient [estimate and standard error (SE)] for change (Δ) in daily lying time (h/d) and Δ in daily number of steps taken (steps/d) from a baseline period (d –14 to –7 precalving) until the day of calving (d 0) and associations with blood calcium (Ca) concentration (mmol/L) within 24 h postcalving adjusted for study group, calving season day (calvingseasonday), and parity.

| Parameter[1] | | | 95% Confidence Limits | | |
|---|---|---|---|---|---|
| Blood Ca, mmol/L (24 h postcalving) | Estimate | SE | Lower | Upper | *P*-value |
| Δ Daily lying time model | | | | | |
| Intercept | 1.55 | 0.27 | 1.00 | 2.09 | <0.001 |
| Calvingseasonday[2] | 0.005 | 0.005 | –0.004 | 0.014 | 0.297 |
| Parity: 2–3[3] | 0.35 | 0.13 | 0.08 | 0.62 | 0.012 |
| Parity: 4+ (Reference Group)[3] | 0.00 | - | - | - | - |
| Linear (Δ Daily lying time), h/d | –0.08 | 0.03 | –0.14 | –0.02 | 0.006 |
| Quadratic (Δ Daily lying time), h/d | –0.008 | 0.004 | –0.016 | 0.0002 | 0.015 |
| R-squared | 0.39 | | | | |
| Δ Daily steps model | | | | | |
| Intercept | 1.50 | 0.28 | 0.94 | 2.06 | <0.001 |
| Calvingseasonday[2] | 0.006 | 0.005 | –0.004 | 0.015 | 0.237 |
| Parity: 2–3[3] | 0.35 | 0.14 | 0.07 | 0.63 | 0.017 |
| Parity: 4+ (Reference Group)[3] | 0.00 | - | - | - | - |

**Table 7.5.** Continued over page.

**Table 7.5 (Continued).** Regression coefficient [estimate and standard error (SE)] for change (Δ) in daily lying time (h/d) and Δ in daily number of steps taken (steps/d) from a baseline period (d –14 to –7 precalving) until the day of calving (d 0) and associations with blood calcium (Ca) concentration (mmol/L) within 24 h postcalving adjusted for study group, calving season day (calvingseasonday), and parity.

| Parameter[1] | | | 95% Confidence Limits | | |
|---|---|---|---|---|---|
| Blood Ca, mmol/L (24 h postcalving) | Estimate | SE | Lower | Upper | *P*-value |
| Δ Daily steps per 1000 units,[4] steps/d | 0.07 | 0.03 | 0.40E-2 | 0.13 | 0.038 |
| R-squared | 0.36 | | | | |

[1]Estimates for study group are not included to avoid cluttering the table. In total, 12 study groups were included in the analysis to investigate associations after adjusting for study group (treatment within-study differences).
[2]Calvingseasonday = difference between calving date and 1st June in days within the herd.
[3]Parity 2–3 = cows approaching their second or third parity at the time of calving; parity 4+ = cows approaching their fourth, fifth, sixth, or seventh parity at the time of calving. Parity 4+ is the reference group for parity effects; $P < 0.05$; slope is different from reference group for classification variable.
[4]Steps taken per 1000 unit increase. E = 10 to the power of.

**Table 7.6. Linear and nonlinear associations between changes in hourly daytime lying behavior and activity precalving and blood calcium concentrations within 24 h postcalving.**

Regression coefficient [estimate and standard error (SE)] for change in (Δ) hourly daytime lying time (min/h) and Δ hourly daytime number of steps taken (steps/h) (between 0600 and 1800 h) from a baseline period (d –14 to –7 precalving) until the day of calving (d 0) and associations with blood calcium (Ca) concentration mmol/L) within 24 h postcalving adjusted for study group, calving season day (calvingseasonday), and parity.

| Parameter[1] | | | 95% Confidence Limits | | |
|---|---|---|---|---|---|
| Blood Ca, mmol/L (24 h postcalving) | Estimate | SE | Lower | Upper | *P*-value |
| Δ Hourly daytime lying model | | | | | |
| Intercept | 1.60 | 0.29 | 1.02 | 2.18 | <0.001 |
| Calvingseasonday[2] | 0.006 | 0.005 | –0.003 | 0.02 | 0.179 |
| Parity: 2–3[3] | 0.38 | 0.13 | 0.12 | 0.64 | 0.005 |
| Parity: 4+ (Reference Group)[3] | 0.00 | - | - | - | - |
| Δ Hourly day lying time, min/h | –0.02 | 0.006 | –0.03 | –0.006 | 0.003 |
| R-squared | 0.41 | | | | |
| Δ Hourly daytime steps model | | | | | |
| Intercept | 1.51 | 0.30 | 0.91 | 2.11 | <0.001 |
| Calvingseasonday[2] | 0.005 | 0.005 | –0.005 | 0.02 | 0.306 |
| Parity: 2–3[3] | 0.38 | 0.14 | 0.10 | 0.66 | 0.008 |
| Parity: 4+ (Reference Group)[3] | 0.00 | - | - | - | - |
| Δ Hourly daytime steps,[4] steps/h | 0.001 | 0.0005 | 0.18E-4 | 0.002 | <0.05 |

**Table 7.6.** Continued over page.

**Table 7.6 (Continued).** Regression coefficient [estimate and standard error (SE)] for change in (Δ) hourly daytime lying time (min/h) and Δ hourly daytime number of steps taken (steps/h) (between 0600 and 1800 h) from a baseline period (d –14 to –7 precalving) until the day of calving (d 0) and associations with blood calcium (Ca) concentration (mmol/L) within 24 h postcalving adjusted for study group, calving season day (calvingseasonday), and parity.

| Parameter[1] | | | 95% Confidence Limits | | |
|---|---|---|---|---|---|
| Blood Ca, mmol/L (24 h postcalving) | Estimate | SE | Lower | Upper | *P*-value |
| R-squared | 0.35 | | | | |

[1]Estimates for study group are not included to avoid cluttering the table. In total, 12 study groups were included in the analysis to investigate associations after adjusting for study group (treatment within-study differences).
[2]Calvingseasonday = difference between calving date and 1st June in days within the herd.
[3]Parity 2–3 = cows approaching their second or third parity at the time of calving; parity 4+ = cows approaching their fourth, fifth, sixth, or seventh parity at the time of calving. Parity 4+ is the reference group for parity effects; $P < 0.05$; slope is different from reference group for classification variable.
[4]E = 10 to the power of.

### *7.4.8 Differences in Metabolic Indices and Milk Production Between Blood Calcium Groups*

Associations between subclinical and clinical hypocalcemia (without milk fever) and milk production vary between studies (Jawor et al., 2012; Ribeiro et al., 2013; Neves et al., 2018). In our study, average milk yield for the first 7 wk of lactation did not differ between blood Ca status groups (Table 7.2); however, there was a tendency ($P = 0.06$) for a Ca status x week interaction (Figure 7.3). Milk yield was lowest in the CLIN group with the SUB group intermediate at wk 1 postcalving, but, subsequently, increased at a faster rate in the CLIN and SUB groups than in the NORM group to wk 7. Further, there were no associations between Ca status and ECM, CP yield, fat yield, milk fat%, or protein% (Table 7.2), nor any Ca status x week interactions for ECM yield and milk fat% ($P = 0.67$ and 0.84, respectively; Figure 7.4a) during wk 2 to 6 of lactation. A tendency

for a Ca status x week interaction for CP yield ($P = 0.07$) was driven by temporal changes. There was a Ca status x week interaction for milk protein% ($P < 0.01$), where the CLIN group had greater milk protein% at wk 2 in milk than the SUB and NORM groups, which were not different from each other ($P = 0.99$), but there were no further differences between the groups beyond 2 wk in milk (Figure 7.4b). Body condition score and BW profiles during the pre- and postcalving are presented in Figures 7.5a and b. There were no BCS or BW associations with Ca status (Table 7.2), nor with Ca status x week interactions ($P = 0.23$ and 0.98, respectively).

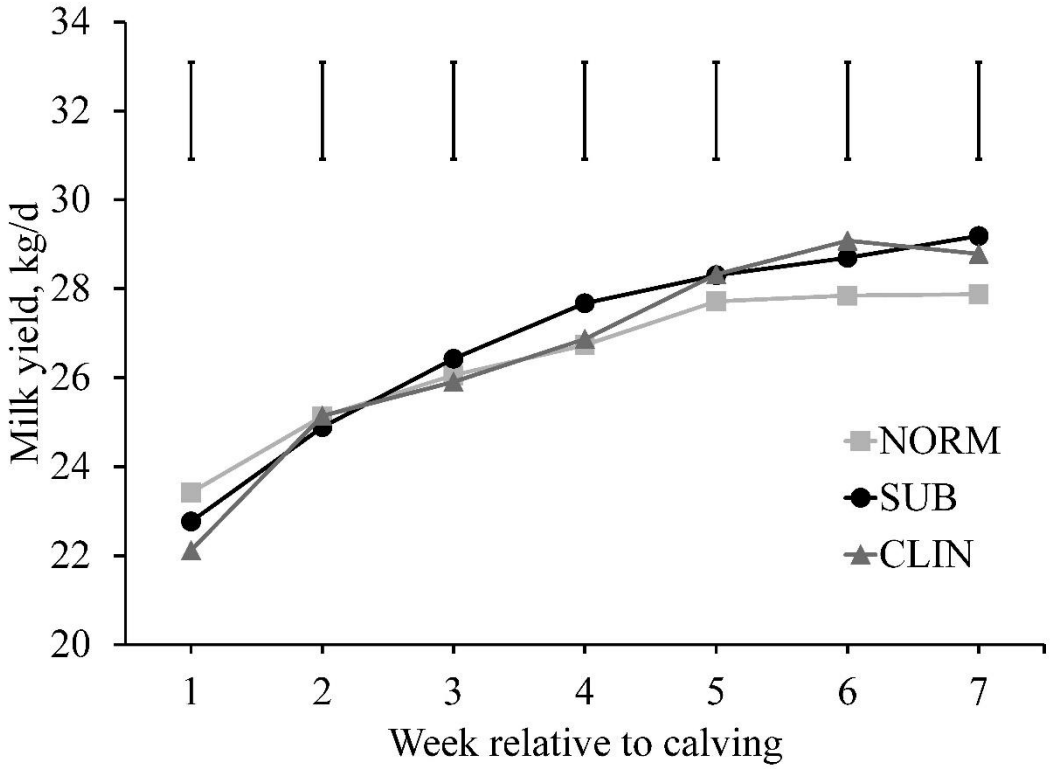


**Figure 7.3. Milk yield during early lactation in 3 blood calcium groups.**

Milk yield (kg/d) during the first 7 wk of lactation for the 3 calcium (Ca) groups [CLIN (blood Ca ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)]. Error bars represent 2 x mean standard error of the difference.

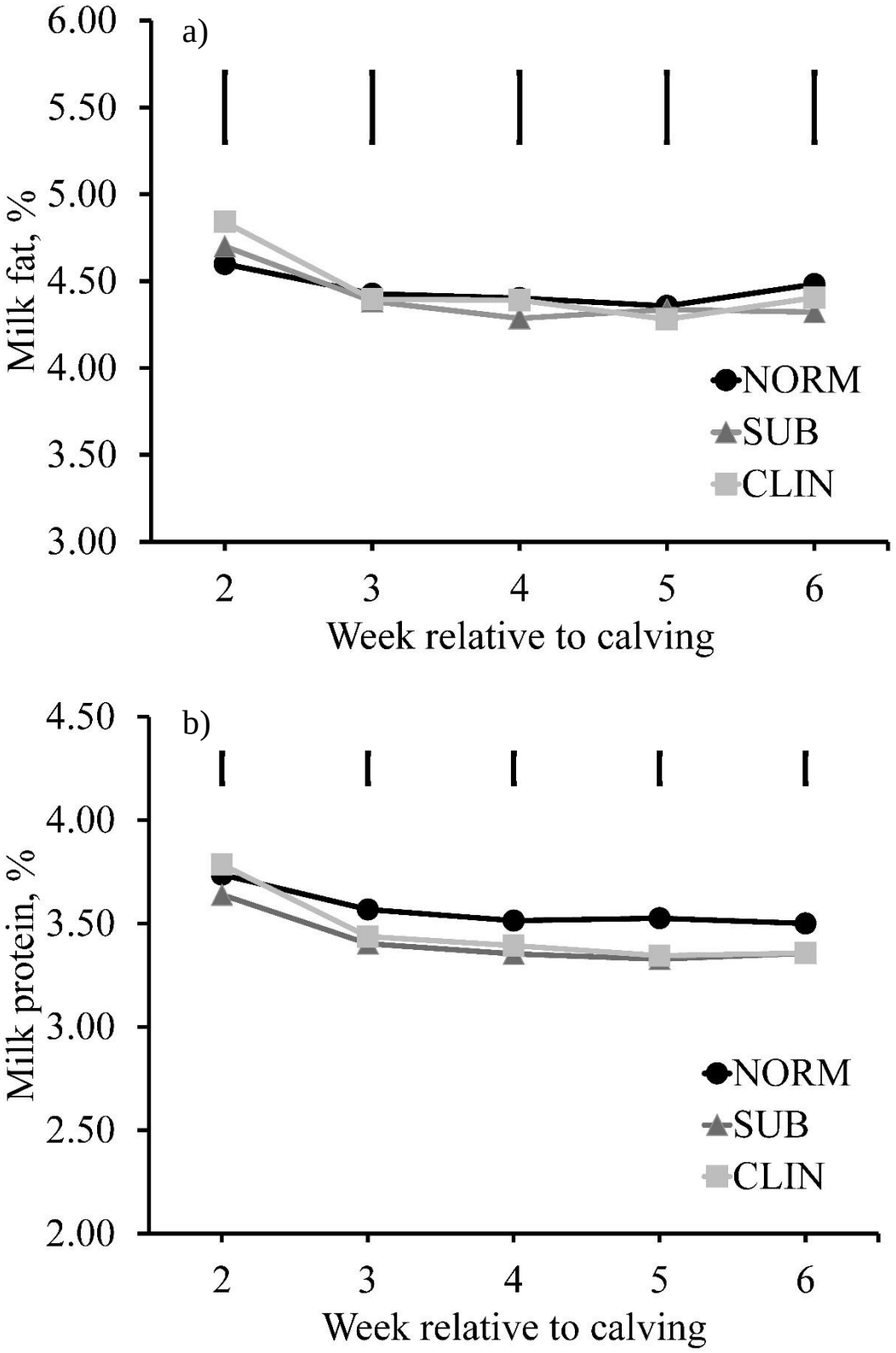


**Figure 7.4. Milk composition during early lactation in 3 blood calcium groups.**

Milk fat [(a); %] and protein [(b); %] during wk 2 to 6 of lactation for the 3 calcium (Ca) groups [CLIN (blood Ca ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and

<2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)]. Error bars represent 2 x mean standard error of the difference.

Similarly, associations among hypocalcemia status and metabolic indices are inconsistent in the published literature. In our study, blood NEFA and BHB were not different between the 3 Ca groups ($P$ = 0.46 and 0.14, respectively). There was a Ca status x period interaction for blood NEFA concentrations ($P$ < 0.05) driven by temporal changes (Figure 7.6a); however, there were no differences between the 3 Ca groups within each period. Blood NEFA concentrations increased to wk +2 before declining to wk +4 (Figure 7.6a). Further, there was no Ca status x period association on blood BHB concentration ($P$ = 0.16; Figure 7.6b). Contrary to our findings, increased blood NEFA (Reinhardt et al., 2011; Martinez et al., 2012; Ribeiro et al., 2013) and BHB concentrations (Martinez et al., 2012) have been associated previously with hypocalcemia. Although, in our study, greater blood NEFA concentrations indicate that cows were in negative energy balance commonly experienced during early lactation; however, their blood BHB concentrations were well below the >1.0 to 1.4 mmol/L thresholds typically used to indicate hyperketonemia (Oetzel, 2004).

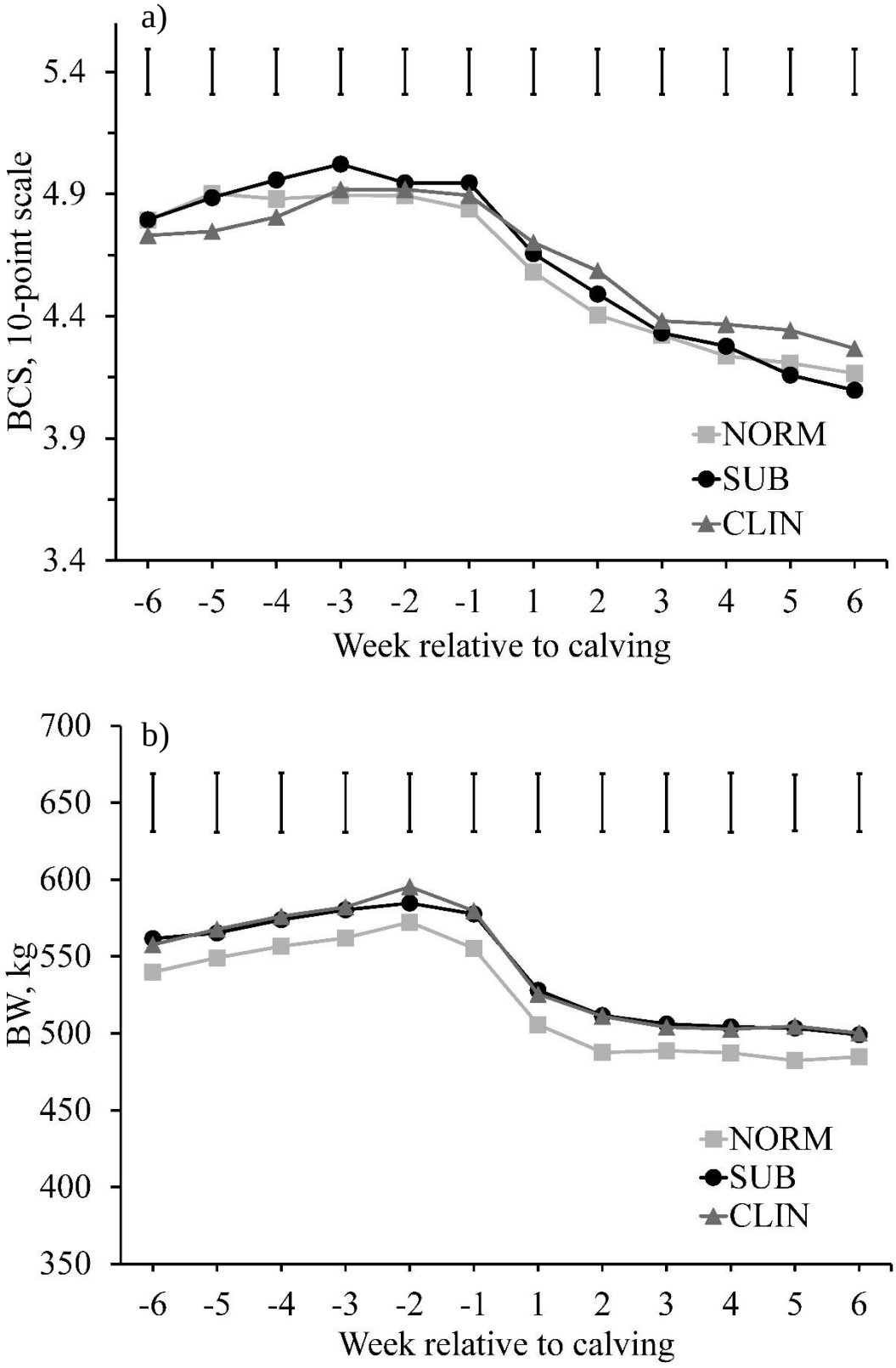


**Figure 7.5. Body condition score and body weight pre- and postcalving for 3 blood calcium groups.**

Figure legend continued over page.

**Figure 7.5 (Continued).** Mean body condition score [BCS (a)] (10-point scale, where 1 is emaciated and 10 is obese; Roche et al., 2004) and body weight [BW (b); kg] during the 6 wk pre- and postcalving for 3 calcium (Ca) groups [CLIN (blood Ca ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)]. Error bars represent 2 x mean standard error of the difference.

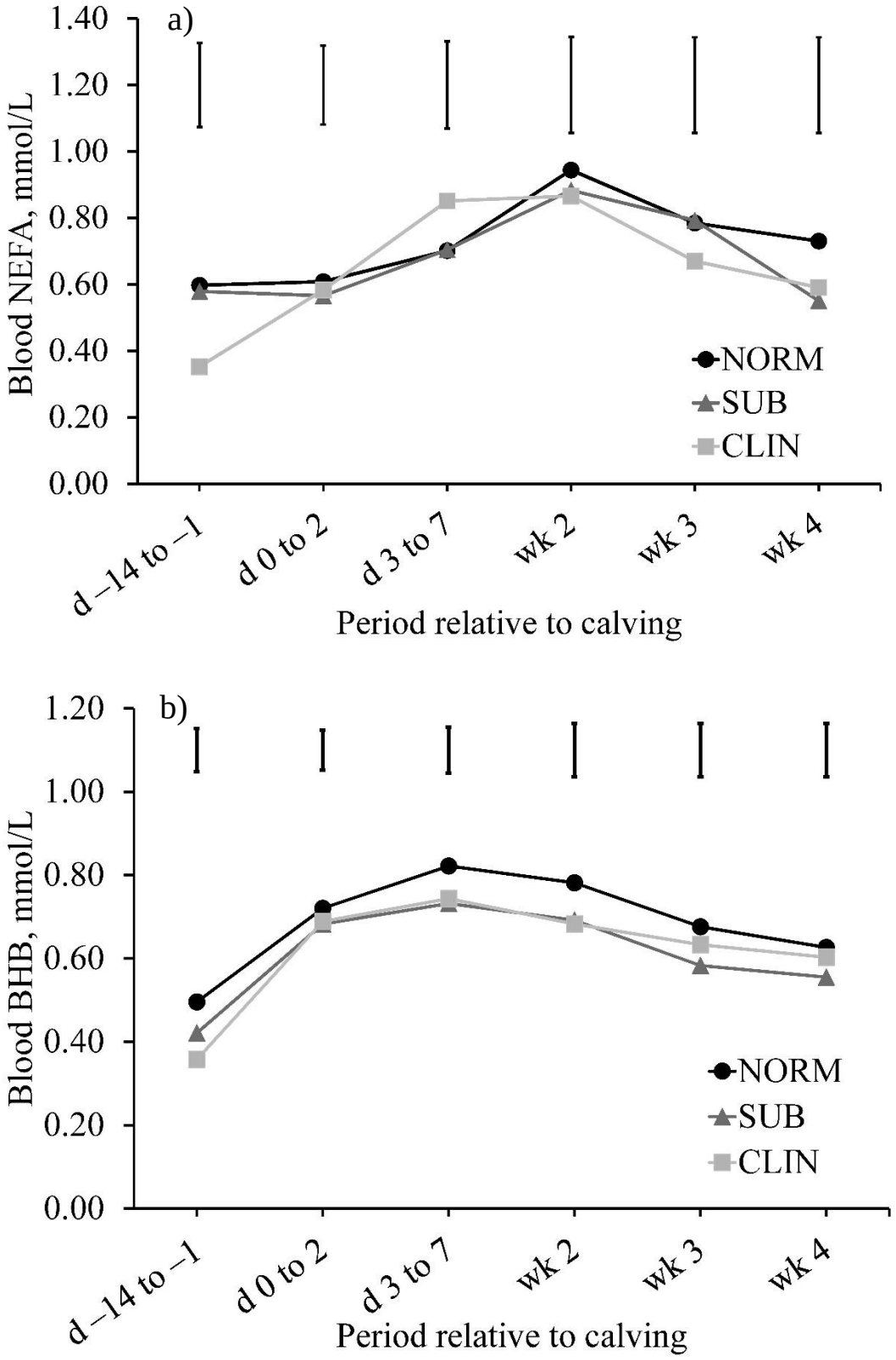


**Figure 7.6. Blood metabolite concentrations during the transition period in 3 blood calcium groups.**

Figure legend continued over page.

**Figure 7.6 (Continued).** Blood non-esterified fatty acid [NEFA; (a)] and β-hydroxybutyrate [BHB; (b)] concentrations (mmol/L) during wk –1 and – 2 precalving, d 0 to 2 postcalving, d 3 to 7 postcalving, and wk 2 to 4 postcalving for the 3 calcium (Ca) groups [CLIN (blood Ca ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)]. Error bars represent 2 x mean standard error of the difference.

In our study, there were no associations between hypocalcemia and milk production, metabolic indices, BCS, and BW measures; however, cows within each group were balanced according to genetic potential for milk protein yield, BW during wk –5 to –6 precalving, and parity, to try to isolate any casual relationship between blood Ca status and cow behavior (Jawor et al., 2012). Our results, therefore, do not support a casual link between peripartum blood Ca status and milk production in non-paretic cows. Despite the lack of association between hypocalcemia and metabolic health in our study, cows with subclinical hypocalcemia are more at risk of being removed from the herd or experiencing another disease event (Chapinal et al., 2011; McArt et al., 2020). Our dataset was not large enough to evaluate this association, but if true, easily applied technology for identifying subclinically affected cows could be used to help better manage cow health and improve cow longevity, both important attributes in cow welfare.

## 7.5 CONCLUSIONS

We have characterized the behavioral differences before, at the time of, and after calving in groups of grazing dairy cows classified with varying degrees of hypocalcemia. Groups of cows experiencing clinical hypocalcemia without paresis were less active on the day of calving, spent more time lying, and had more LB compared with cows classified as subclinically-hypocalcemic or normocalcemic. Cows in the clinically-hypocalcemic group also spent more time lying down during the hours of darkness on the day before calving. Changes in behavior were short-lived as differences were no longer present by 2 d postcalving. Future work should consider behavioral asynchrony determined through temporal changes as well as overall changes in behavior to detect cows with a disease. Behavioral data after disease detection may also be useful for monitoring the treatment or recovery, or both, in cows experiencing a health challenge on farm. Behavioral changes also occurred before calving, where relative change in steps taken from a 2 wk baseline before calving had a positive linear association with blood Ca concentration around the time of calving. Our results indicate that hypocalcemic cows, on average, exhibit behavioral characteristics different to normocalcemic cows, but further research is required to determine whether these differences have predictive potential at the individual cow level.

## 7.6 ACKNOWLEDGEMENTS

This work was funded by New Zealand dairy farmers through DairyNZ Inc. (project number RD1405) and by the Ministry of Business, Innovation, and Employment (contract numbers DRCX1302 and DRCX1201). S. Hendriks is also supported by the Colin Holmes Dairy Scholarship and the Ministry for Primary Industries Postgraduate Scholarship.

## 7.7 SUMMARY

In Chapter 7, I described differences in lying behavior and activity in groups of grazing dairy cows retrospectively classified as either clinically-hypocalcemic (without paresis), subclinically-hypocalcemic, or normocalcemic at calving. Cows in the clinically-hypocalcemic group spent more time lying and had reduced activity compared with the normocalcemic and subclinically-hypocalcemic groups during the day before and the day of calving. Furthermore, clinically- and subclinically-hypocalcemic cows transitioned between lying and standing more frequently around the time of calving. I provided evidence that changes in lying behavior and activity before and at the time of calving in grazing dairy cows could be valuable measures for identifying cows at risk of hypocalcemia; however, lying behavior and activity could also be predictors of other diseases and I explored this in Chapter 8.

# CHAPTER 8. CHANGES IN LYING BEHAVIOR AND ACTIVITY DURING THE TRANSITION PERIOD COULD INDICATE HYPERKETONEMIA RISK IN GRAZING DAIRY COWS

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## 8.1 ABSTRACT

Lying behavior and activity-monitoring technologies may provide a practical solution for predicting and diagnosing metabolic disease in grazing dairy cows during the transition period. During early lactation, most dairy cows experience negative energy balance (NEB); however, failure to cope with NEB can place cows at greater risk of developing metabolic diseases, such as subclinical ketosis. Our objective was to, retrospectively, characterize the lying behavior and activity of grazing dairy cows grouped according to energy status during the transition period. Blood was sampled on the day of calving (d 0), daily for 1 to 4 d postcalving and then weekly from wk 2 to 5 postcalving for analysis of plasma non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB). Two hundred and forty four multiparous Holstein-Friesian and Holstein-Friesian x Jersey grazing dairy cows were classified into 1 of 3 energy status groups, based on blood NEFA and BHB during the first 2 wk postcalving. A cow was classified as having low NEFA and low BHB (Lo–Lo; n = 78) when both blood NEFA was <1.0 mmol/L and blood BHB was ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving. A cow was classified as having high NEFA and low BHB (Hi–Lo; n = 134) when both blood NEFA was ≥1.0 mmol/L and blood BHB was ≤1.0 mmol/L during 1 or more samplings during the first 2 wk postcalving. A cow was classified as having high NEFA and high BHB (Hi–Hi; n = 32) when blood NEFA was ≥1.0 mmol/L and blood BHB was ≥1.2 mmol/L during 1 or more samplings during the first 2 wk postcalving. Accelerometers (IceTag or IceQube devices; IceRobotics Ltd., Edinburgh, Scotland) monitored daily lying time (h/d), daily lying bouts (LB; no./d), mean LB duration (min/bout), and the number of steps taken (steps/d) during the transition period (–21 to +35 d relative to calving). Changes in lying behavior and activity occurred earlier

than the mean day that cows were classified Hi–Hi, which was 4.9 ± 3.32 d postcalving (mean ± standard deviation). Up to 3 wk preceding calving, cows in the Hi–Hi group were more active, had fewer daily LB, and spent less time lying down than those in the Lo–Lo group. In addition, cows had lower odds of being classified Hi–Hi postcalving when their increase in daily steps taken on the day after calving was greater relative to a baseline period 2 wk before calving. Prospective studies are required to determine if lying behavior and activity monitoring technologies could allow the prediction, diagnosis, and monitoring of metabolic disease at an individual cow level in grazing dairy cows.

## 8.2 INTRODUCTION

Following parturition, all cows experience some degree of negative energy balance (**NEB**), whereby they mobilize body tissue to support the demands of lactation (Nielsen and Ingvartsen, 2004); however, the inability of cows to successfully adapt to this physiological state can result in the development of metabolic disorders such as clinical ketosis (**CK**) and subclinical ketosis (**SCK**) (Herdt, 2000; Nielsen and Ingvartsen, 2004). Subclinical ketosis is commonly diagnosed as blood BHB concentrations ≥1.2 or 1.4 mmol/L; these are reported cut-points, above which BHB concentrations have adverse associations with production, reproduction, and health outcomes in housed systems (LeBlanc et al., 2005; Duffield et al., 2009).

The incidence of SCK in housed systems ranges from 11 to 37% in Europe (Suthar et al., 2013) and 40 to 60% in North America (Duffield, 2000; McArt et al., 2012). In contrast, the incidence of CK (e.g., blood BHB concentrations ≥3.0 mmol/L) is much lower (2 to 15%; Duffield, 2000; McArt et al., 2012). In pasture-based systems, such as those in New Zealand, incidence of SCK of 68% has been reported during the first 5 wk

postcalving (Compton et al., 2015); however, the authors noted considerable variation between herds in the incidence, the timing, and degree of peak prevalence. Differences in the timing and frequency of diagnosis methodologies between studies makes it difficult to compare the epidemiology of hyperketonemia (**HYK**) and SCK. Nevertheless, its high reported incidence and prevalence across dairy systems internationally, support the premise that timely prediction and treatment of HYK may be advantageous.

Elevated blood BHB is the gold standard measure for diagnosing SCK (Duffield et al., 2009) and increased blood non-esterified fatty acids (**NEFA**) are a reliable measure of NEB and can predict cows at risk of developing transition-cow disease (LeBlanc, 2010; Ospina et al., 2010; Rodríguez-Jimenez et al., 2018). However, routine blood testing programs to predict and monitor SCK are often impractical, especially in pasture-grazed systems where large numbers of cows calve within a seasonally-concentrated timeframe. Other detection methods such as those using devices to remotely and continuously monitor behavior, or inline milk sensors to predict and diagnose SCK or excessive NEB are, therefore, of increasing interest to researchers and producers and are practical options for use in commercial pasture-grazing systems (Borchers et al., 2016).

Research using quantitative measures of behavior to explore associations among lying and standing behavior and activity and SCK (Kaufman et al., 2016; Rodríguez-Jimenez et al., 2018) and CK has been undertaken in housed cows (Edwards and Tozer, 2004; Itle et al., 2015). Cows experiencing SCK have reduced activity and spend more time lying down before (Kaufman et al., 2016; Piñeiro et al., 2019) and at the time of SCK diagnosis (Kaufman et al., 2016; Rodríguez-Jimenez et al., 2018). The availability of advanced and robust technologies that can accurately measure lying behavior and activity makes them a practical option for use in commercial pasture-grazing systems

(Borchers et al., 2016) and may provide a solution for predicting and diagnosing SCK. To our knowledge, lying behavior and activity in grazing dairy cows classified according to both elevated NEFA and BHB in blood postcalving has not been characterized. Grazing is an energy-expensive activity (Kaufmann et al., 2011) and grazing cows are required to walk considerable distances to be milked and to meet their nutrient needs (Aharoni et al., 2013). Therefore, we hypothesized that notable increases in lying time and reductions in activity would occur prior to the classification of grazing cows according to elevated NEFA and BHB in blood postcalving. Our objective was to investigate whether cows retrospectively classified according to elevated NEFA with or without elevated BHB in blood postcalving displayed behavioral differences before, at the time of, and after diagnosis, when compared with cows classified with lower NEFA and BHB in blood postcalving.

## 8.3 MATERIALS AND METHODS

### *8.3.1 Animal Handling, Experimental Design, and Management*

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations in accordance with the New Zealand Animal Welfare Act (Ministry for Primary Industries, 1999). Data for the present study were selected from a dataset of 310 cows described in Chapter 6. A subset of 244 multiparous Holstein-Friesian and Holstein-Friesian x Jersey cows were selected from 3 individual parent experiments [BCS, feed, and zeolite studies described in Roche et al. (2015; 2017a; 2018), respectively, with additional information provided in Crookenden et al. (2020) for the zeolite study]. The BCS, feed, and zeolite studies were undertaken in each of 3 years (2013, 2014, and 2016, respectively) and 2 locations [Scott Farm; BCS study, and Lye Farm; feed and zeolite studies (both Hamilton, New Zealand, 37°46’S 175°18’E)].

Briefly, cows from Roche et al. (2015) (BCS study) were managed to be BCS 4.0 or 5.0 at 1 month before calving (10-point scale, where 1 is emaciated and 10 obese; Roche et al., 2004) and then, within each BCS category, cows were allocated 1 of 3 levels of ME intake during the 3 wk preceding calving (75, 100, or 125% of estimated ME requirements; Roche et al., 2015). Cows from Roche et al. (2017a; feed study) were managed to be in 1 of 2 BCS categories at dry-off (approximately 4.25 and 5.0 on a 10-point scale). Following dry-off, cows in both BCS categories were managed to achieve a BCS 5.0 at 1 month before calving. Cows within each 'far-off' feeding level treatment were then allocated to 1 of 3 levels of ME intake during the 3 wk preceding calving (65, 90, or 120% of estimated ME requirements) (Roche et al., 2017a). Cows from Roche et al. (2018; zeolite study) were allocated to 1 of 2 treatment groups (Control and Zeolite) during the precalving period. Cows were BCS 5.0 at the start of the experimental period (~3 wk precalving). Treatment cows received 500 g/cow per d Zeolite A (80% sodium aluminosilicate, synthetic embedded in starch; Optimate MF+, Blue Pacific Minerals, New Zealand) and supplementation ceased at the first signs of calving (Crookenden et al., 2020).

During the experimental period, nonlactating and lactating cows were offered a mixture of fresh perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture daily. Different-sized grazing areas (range: 23 to 60 $m^2$/cow) were allocated to cows depending on their treatment ME allocation (Roche et al., 2009). Cows were rotationally grazed as described by Roche et al. (2005) and managed as a typical commercial herd of grazing cows under a spring-calving system. During the experimental period, nonlactating cows received pasture silage in the feed study and maize silage in the zeolite study as supplementary feeds. Lactating cows received pasture silage in all

studies and maize silage in the BCS study as supplementary feeds. During the postcalving period, cows were milked twice daily in a rotary parlor. Total time spent standing being milked and walking to and from the milking parlor on tracks ranged from ~40 to 90 min/d.

### *8.3.2 Blood Sampling and Analyses*

The blood sampling protocols and analyses are described in detail in the studies mentioned above. Briefly, blood was sampled on d 0, and d 1, 2, 3, and 4 postcalving in the BCS, feed, and zeolite studies. Blood was also sampled weekly, for 4 wk pre- until 5 wk postcalving in the BCS and feed studies and periodically pre- and postcalving [mean sampling times were d –19, –14, and –7 precalving and d 7, 14, 21, and 28 postcalving with a mean standard deviation (**SD**) of ±1.9 d] in the zeolite study. Blood was sampled by coccygeal venipuncture into evacuated blood tubes containing lithium heparin anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Heparinized samples were placed immediately into iced water and centrifuged within 30 min of collection at 1,500 x *g* for 12 min at 4°C. Following centrifugation, aspirated plasma was stored at –20°C until assayed for metabolite analyses. Full details of the metabolite analyses are described in Roche et al. (2015), Crookenden et al. (2020), and otherwise in detail below.

Plasma samples were analyzed for metabolites by Gribbles Veterinary Pathology Ltd. (Hamilton, New Zealand). Blood metabolites were assayed using colorimetric techniques at 37°C with a Hitachi Modular P800 analyzer (Roche Diagnostics, Indianapolis, IN). Roche reagent kits were used in the BCS and feed studies and the Randox kit was used in the zeolite study to measure plasma concentrations of BHB (mmol/L; reduction of NAD+ to NADH during oxidation of D-3-hydroxybutyrate to acetoacetate). Plasma NEFA concentrations (mmol/L) were measured using Wako

Chemicals (Osaka, Japan) kit NEFA HR2 measuring oxidative condensation of 3-methyl-N-ethyl-N-β hydroxyethyl aniline with 4-aminoantipyrine in the BCS and feed studies and using the acyl CoA synthetase, acyl-CoA oxidase (ACS-ACOD) colorimetric method using the Wako NEFA C kit in the zeolite study. The inter- and intra-assay coefficients of variation for all assays were <5.5% and ≤15%, as reported in Roche et al. (2015; 2017a) and Crookenden et al. (2020).

### *8.3.3 Classification of Energy Status*

Increased blood NEFA (≥1.0 mmol/L) is a reliable measure of NEB and can predict cows at risk of developing transition cow disease such as SCK and metritis (LeBlanc, 2010; Ospina et al., 2010; Rodríguez-Jimenez et al., 2018). Elevated blood BHB (≥1.2 mmol/L) is the gold standard measure for diagnosing SCK (Duffield et al., 2009) based on scientifically supported concentrations at or above which cows are at greater risk of reduced cow performance and ill health in housed (Chapinal et al., 2011) and grazing cows (Compton et al., 2015). Although, in grazing cows due to considerable variation between herds in the incidence, the timing, and degree of peak prevalence of SCK, it is inconclusive whether blood BHB (≥1.2 mmol/L) alone is suitable for diagnosing SCK in grazing cows (Compton et al., 2015; Phyn et al., 2017). Both blood NEFA and BHB can indicate whether the cow's adaptation to early lactation NEB has been successful or not (LeBlanc, 2010; McArt et al., 2013; Abdelli et al., 2017), and therefore, in our study, cows were classified according to blood NEFA and BHB status during the first 2 wk postcalving, and retrospectively, allocated to 1 of 3 groups.

Of 310 cows available from the dataset, 17 cows were removed prior to classification due to missing blood metabolite records during wk 1 or 2 postcalving. A total of 293 cows were available for selection and none of the cows were recorded as

exhibiting any symptoms of clinical disease or lameness during the experimental periods of the 3 parent experiments. A cow was classified as having low NEFA and low BHB (**Lo–Lo**; n = 78) when both blood NEFA was <1.0 mmol/L and BHB was ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving. A cow was classified as having high NEFA and low BHB (**Hi–Lo**; n = 134) when both blood NEFA was ≥1.0 mmol/L and blood BHB was ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving. A cow was classified as having high NEFA and high BHB (**Hi–Hi**; n = 32) when both blood NEFA was ≥1.0 mmol/L and blood BHB was ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving. To complete the factorial, cows were classified as having low NEFA and high BHB (**Lo–Hi**) when blood NEFA was <1.0 mmol/L and blood BHB was ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving; however, due to a low number of subjects (n = 6), this group was deemed insufficient for meaningful statistical analysis and was excluded. In addition, 34 cows were not allocated to a group due to their blood BHB concentrations falling in between the thresholds set for classification (e.g., blood BHB was >1.0 and <1.2 mmol/L). A subset of 244 cows were included in the final dataset for further statistical analysis. All cows included in the study were multiparous (i.e., approaching their second or greater parity at the time of calving), and parity was grouped as follows: parity 2 to 3 (**parity 2–3**; n = 158), and parity 4 to 9 (**parity 4+**; n = 86) (Table 8.1). The mean parity ± SD across the 3 energy status groups was 3.21 ± 0.41, 3.40 ± 0.49 and 3.50 ± 0.51 for the Lo–Lo, Hi–Lo, and Hi–Hi groups, respectively.

**Table 8.1. Descriptive data for all cows classified into 1 of 3 energy status groups.**
Number of cows (n) by parity, breed, and study for the 3 energy status groups (Lo–Lo, Hi–Lo, Hi–Hi)[1].

| n (cows) | Lo–Lo (n = 78) | Hi–Lo (n = 134) | Hi–Hi (n = 32) |
|---|---|---|---|
| Parity 2–3[2] | 62 | 80 | 16 |
| Parity 4+[2] | 16 | 54 | 16 |
| Breed (HF)[3] | 53 | 105 | 25 |
| Breed (HF x J)[3] | 25 | 29 | 7 |
| BCS study[4] | 40 | 59 | 9 |
| Feed study[4] | 37 | 47 | 12 |
| Zeolite study[4] | 1 | 28 | 11 |

[1]A cow was classified as having low NEFA and low BHB concentrations (Lo–Lo) when both blood NEFA was <1.0 mmol/L and blood BHB was ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving. A cow was classified as having high NEFA and low BHB (Hi–Lo) when blood NEFA was ≥1.0 mmol/L and blood BHB was ≤1.0 mmol/L during 1 or more samplings during the first 2 wk postcalving. A cow was classified as having high NEFA and high BHB (Hi–Hi) when blood NEFA was ≥1.0 mmol/L and blood BHB was ≥1.2 mmol/L during 1 or more samplings during the first 2 wk postcalving.
[2]Parity 2–3 = cows approaching their second or third parity at the time of calving; parity 4+ = cows approaching their fourth, fifth, sixth, seventh, eighth, or ninth parity at the time of calving.
[4]Breed where HF = Holstein-Friesian and HF x J = HF x Jersey.
[4]Cows were selected from the BCS study as described by Roche et al. (2015), the feed study as described by Roche et al. (2017a), or the zeolite study as described by Roche et al. (2018).

### *8.3.4 Milk, BCS, BW, and Breed*

Cows were milked twice daily and milk yield was measured daily from 1 to 35 DIM. Milk was sampled weekly on consecutive afternoon and morning milkings, and a composite sample was analyzed for milk composition by infrared analysis (FT120, Foss Electric, Hillerød, Denmark). Energy-corrected milk yield was calculated as (Nielsen et al., 2009):

kg of ECM = [kg of milk x (383 x fat% + 242 x protein% + 780.8)]/3,140

Body weight was recorded and BCS (scale of 1 to 10, where 1 is emaciated and 10 obese; Roche et al., 2004) was determined weekly following the morning milking or

at approximately 0800 h during the nonlactating period. All BCS assessors were trained and recalibrated at the start of the experiment following the procedures set out in Macdonald and Roche (2011). Animal evaluation data for cow breed, Breeding Worth (**BrW**), and Production Worth (**PW**) were provided by Livestock Improvement Corporation Ltd. (Hamilton, New Zealand). Breeding Worth and PW are estimated economic values of a combination of 8 traits (milk fat, protein, and milk volume, BW, fertility, SCC, BCS, and residual survival) that are indicators of robustness and production efficiency (DairyNZ, 2018; Johnson et al., 2018). Breeding values are the genetic potential of an animal for the trait of interest and the combination of all 8 EBV traits contribute to the BrW (DairyNZ, 2018).

Protein metabolites, liver enzymes, proinflammatory cytokines, inflammatory markers, and liver TAG associated with energy status were analyzed in addition to the measures presented in Chapter 8; however, these additional analyses were not submitted for publication and, therefore, are presented in Appendix 18.

### *8.3.5 Behavioral Data and Editing*

A full description of the behavioral data collection and editing methods are described in Chapter 6. Behavioral data were available for analysis for the period –21 d precalving to +35 d postcalving, relative to the day of calving (**d 0**). In brief, a triaxial accelerometer (IceTag or IceQube; IceRobotics Ltd., Edinburgh, Scotland) was fitted to each cow on the lateral side of a hind leg and behavioral data were recorded. Data were downloaded using the IceManager 2010 software (IceRobotics Ltd.) from the on-board memory of the device. Two summary files were generated for each individual cow; 1 file consisted of lying time (s) and number of steps recorded at 1- and 15-min sampling intervals, for the IceTag and IceQube, respectively, and the other file contained lying

bouts (**LB**) by date, timestamp (hh:mm:ss), and duration (s). These summary files were used to calculate daily and hourly lying time (h/d), LB (no./d), mean LB duration (min/d), and number of steps (steps/d) for each individual cow. A LB is defined as the period between the accelerometer changing from vertical to horizontal and back to vertical. Data excluded from the analysis included data recorded on the day that accelerometers were removed from, or fitted to the cows, and incorrect recordings due to technical errors.

From the output data sets, the sampling dates for each individual cow were assigned an experimental day relative to d 0 based on the recorded calving date. Farm staff collected newborn calves and their dams for first milking once daily. Consequently, there can be a discrepancy of up to 24 h for recording the date of calving. Therefore, lying behavior and activity were adjusted, where appropriate, using activity data to re-assign calving day [i.e., we assumed that ≥14 LB on d –1 was likely associated with a calving event (Huzzey et al., 2005; Borchers et al., 2017)]. Otherwise, it was assumed that recorded calving date was correct. The methodology used to adjust for the discrepancy in assignment of calving day in our study has not been validated, and this is a limitation of our study. All data were adjusted, where appropriate, according to re-assigned calving day, and these transformed datasets were the basis of subsequent analyses.

#### *8.3.6 Weather*

Daily rainfall (mm; 24-h period) and daily air temperature (°C; recorded at 0900 h) data were retrieved from The National Climate Database (NIWA, 2018) for the duration of the 3 experiments. Data were retrieved from station agent number 26,117 (37.8°S, 175.3°E) for all 3 studies (NIWA, 2018). The distance from the climate station to the study site for the 3 studies is ~3 km.

### *8.3.7 Statistical Analyses*

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Results are presented as least squares means (**LSM**) ± standard error of the mean in the text and mean standard error of the difference (**SED**) in tables and figures. Where we have presented LSM for the 3 energy status groups, we have presented group mean effects. The covariance structures selected were compound symmetry or autoregressive based on the lowest Akaike information criterion. Study and treatment from the parent experiments were concatenated to create a categorical variable study group.

Study group (categorical) and calving season day (**calvingseasonday**) within the herd (difference in days between calving date and the first day in June) were included to adjust for different treatments and different calving dates within the 3 studies in all models described below. Due to the greater risk of SCK in older pasture-grazed cows, all models were adjusted for parity (categorical; parity 2–3 or parity 4+) (Compton et al., 2015). All repeated measures ANOVA models were pairwise comparison-adjusted using Tukey-Kramer.

***Blood Metabolite Markers.*** The sampling dates for blood data were assigned an experimental day relative to d 0 based on the re-assigned calving day. Blood data for energy metabolites (NEFA and BHB) were summarized into 6 periods [i.e., d –14 to –1 precalving, d 0 to 2 postcalving, d 3 to 7 postcalving, and then weekly postcalving (wk 2 to 4)]. To investigate the associations between metabolites, and energy status and period, a repeated measures ANOVA was undertaken using PROC MIXED, with cow as a random effect, period as a repeated measure, and the fixed effect of energy status, period, and energy status x period interactions. Variables were checked for skewness and to meet the assumption of normal distribution. Log-transformation was used to normalize blood

BHB for the analysis and untransformed LSM, standard error of the mean, and SED are presented.

***Behavioral Parameters.*** In our study, based on previously determined thresholds for IceRobotics sensors, LB <33 s (Kok et al., 2015) and ≤2 min (Mattachini et al., 2013) were discarded from the raw data recorded by the IceQube and IceTag devices, respectively. Behavior data were summarized for 15 periods: wk –3 and –2 precalving, d –7 to –4 precalving, daily precalving (d –3 to –1), d 0, daily postcalving (d 1 to 3), d 4 to 7 postcalving, and wk 2, 3, 4, and 5 postcalving. Differences in daily lying time, daily number of LB, mean LB duration, and number of steps taken between the 3 energy status groups were analyzed using a repeated measures ANOVA (PROC MIXED) with cow as a random effect, period as a repeated measure, and the fixed effect of energy status, period, and energy status x period interactions. Behavior analyses included fixed effect of daily rainfall (continuous) and mean air temperature at 0900 h (continuous), and their interactions as potential explanatory variables. Energy status, parity, and energy status x parity interactions were also investigated for all models described above; however, no interactive energy status x parity effects were detected for daily lying time ($P$ = 0.99), daily LB ($P$ = 0.96), mean LB duration ($P$ = 0.88), or number of steps taken ($P$ = 0.44).

***Diurnal Behavioral Parameters.*** To further understand differences between the 3 energy status groups and the effect of time of day on lying and stepping behavior before the mean day of classification according to high blood NEFA and BHB (mean ± SD; 4.9 ± 3.3 d; range: 0 to 14 d), 24-h behavior data were analyzed during the 2 wk before calving (wk –2 to –1 precalving). We summarized the 24-h behavior data records during this 2-wk precalving period due to differences in the daily lying time and stepping behavior identified between the 3 energy status groups during this time. The 24-h behavior data

were summarized by hour, where 0000 h was equivalent to the period from midnight until 0059 h (1200 h = 1200 to 0059 h, 0100 h = 0100 to 0159 h, and so on). Behavior data were analyzed using a repeated measures ANOVA (PROC MIXED) to investigate the effect of energy status and time of day on lying time and number of steps taken over a 24-h period during the 2-wk precalving period, with cow as a random effect, hour as a repeated measure, and the fixed effect of energy status, hour, and energy status x hour interactions.

***Logistic Regression.*** The effects of lying behavior and other cow factors on the presence or absence of both high blood NEFA concentrations (≥1.0 mmol/L) and blood BHB (≥1.2 mmol/L) was investigated using the GLIMMIX procedure [(distribution = binomial and link = logit) as performed by Kaufman et al. (2016)]. This was done where Hi–Hi and Lo–Lo cows were compared to investigate the association between behavior and cow performance variables with the presence or absence of Hi–Hi using firstly, univariable, and subsequently, multivariable logistic regression models. Behavior variables assessed included mean daily lying time, daily LB, LB duration, and number of steps taken during the weeks before the mean day of classification according to high blood NEFA and BHB postcalving (3 periods: wk –2 precalving, wk –1 precalving, and 5 d postcalving) and change in daily lying time and steps taken (calculated as the daily lying time and steps taken on d 1 postcalving minus mean daily lying time and steps taken during wk –2 precalving). Cow performance variables assessed included parity (continuous), precalving BCS and BW (mean BCS and BW during wk –6 and –5 precalving), change in BCS and BW precalving (calculated as the mean BCS and BW during wk –2 and –1 precalving minus the mean BCS and BW during wk –6 to –5 precalving), PW, BrW, and calvingseasonday. Variables with $P < 0.20$ were checked

using the CORR procedure in SAS for strong correlations between the explanatory variables. Due to the lying behavior and step variables exhibiting multicollinearity ($P < 0.05$), multivariable logistic regression models were constructed separately for each week and each different behavior. Body condition score and BW measures also exhibited multicollinearity ($P < 0.05$) and were included in the model one at a time. The behavior and performance variables with the lowest *P*-value and most relevant to the producer were retained for the multivariable model. Manual backward elimination of variables with $P < 0.10$ was then used to construct a complete logistic model, and those variables retained in the final multivariable models are presented. Due to the known effects of parity and season on SCK (Tveit et al., 1992; Compton et al., 2015), parity, and calvingseasonday were forced into the final models, and study group was included as a random effect.

***Milk, BCS, and BW***. Weighted means for weekly milk yield were calculated using daily yields on a per-cow basis for wk 1 to 7 postcalving using PROC MEAN. Weighted means for milk yield were used to calculate weekly milk component yields and ECM yield for wk 2 to 6 postcalving. Due to a lack of records for milk composition from 141 cows during wk 1 postcalving (colostrum period), these data were excluded from ECM yield and milk protein and fat composition analysis. To investigate the associations between milk and ECM yield, and milk protein and fat composition, and energy status, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, week as a repeated measure, and the fixed effect of energy status, week, and energy status x week interactions. Covariates BrW and PW were included in the model as proxies for milk production potential.

Body condition score and BW were summarized into 2 periods pre- (–4 to –1 wk) and postcalving (1 to 6 wk). A repeated measures ANOVA was undertaken using PROC

MIXED, with cow as a random effect, week as a repeated measure, and the fixed effect of energy status to investigate the differences in BCS and BW (4 wk pre- and 6 wk postcalving) between the 3 energy status groups pre- and postcalving. An additional analysis was undertaken to investigate the associations between BCS and BW, and energy status and week. A repeated measures ANOVA was performed using PROC MIXED, with cow as a random effect, week as a repeated measure, and the fixed effect of energy status, week, and energy status x week interactions. Mean wk –5 to –6 precalving BCS and BW were included as covariates in the models investigating BCS and BW, respectively.

Estimated DMI was back-calculated from the energy requirements of the cows, and additional analyses were undertaken to investigate the associations between estimated DMI and energy status for 4 wk pre- and 6 wk postcalving. The energy status and energy status x week interactions for estimated DMI and additional analyses for milk yield and composition are presented as Supplemental Materials and Supplemental Figures 3, 4a, b, and c (Appendix 18). In addition to the measures presented in Chapter 8, endometrial measures, protein metabolites, liver enzymes, proinflammatory cytokines, inflammatory markers, and liver triacylglyceride (**TAG**) associated with energy status were analyzed; however, these additional analyses were not submitted for publication and are presented in Appendix 18.

## 8.4 RESULTS AND DISCUSSION

Wearable technologies that can accurately measure lying behavior and activity may provide a non-invasive and practical method for the prediction and diagnosis of SCK or NEB in grazing dairy cows during early lactation. Successful prediction of SCK or

NEB may allow producers to treat and manage animals to reduce metabolic disease risk during the transition period. To our knowledge, this is the first study to characterize the lying behavior and activity before, at the time of, and after calving in groups of grazing cows classified according to elevated NEFA and BHB (Hi–Hi) and elevated NEFA without elevated BHB (Hi–Lo) compared with cows classified according to lower levels of NEFA and BHB (Lo–Lo) in blood postcalving.

### *8.4.1 Energy Balance, BCS, and BW*

Mean blood NEFA and BHB concentrations differed ($P < 0.001$) between the 3 energy status groups, with energy status x period interactions ($P < 0.001$) detected for both metabolites, as presented in Figures 8.1a and b, respectively. During d –14 to –1 precalving, the Lo–Lo group had greater ($P < 0.05$) blood NEFA concentrations than the Hi–Lo group (0.56 ± 0.03 vs. 0.46 ± 0.02 mmol/L, respectively), but both groups were not different ($P \geq 0.58$) from the Hi–Hi group (0.51 ± 0.05 mmol/L). During d 0 to 2 and d 3 to 7 postcalving, blood NEFA concentrations were greatest ($P < 0.01$) in the Hi–Hi group (0.85 ± 0.04 and 1.14 ± 0.05 mmol/L, respectively) followed by the Hi–Lo group (0.68 ± 0.02 and 0.91 ± 0.02 mmol/L, respectively), which, in turn, had greater ($P < 0.001$) blood NEFA concentrations than the Lo–Lo group (0.46 ± 0.03 and 0.56 ± 0.03 mmol/L, respectively). During wk 2 postcalving, blood NEFA concentrations in the Hi–Hi and Hi–Lo groups were not different ($P = 0.36$) from each other (1.06 ± 0.06 and 0.97 ± 0.03 mmol/L, respectively), but, both groups had higher ($P < 0.001$) blood NEFA concentrations than the Lo–Lo group (0.69 ± 0.04 mmol/L). While there were no significant differences ($P \geq 0.15$) in blood NEFA concentrations between energy status groups during wk 3 postcalving, a difference ($P < 0.05$) between the Lo–Lo and Hi–Lo groups occurred in wk 4 postcalving (0.54 ± 0.04 and 0.68 ± 0.03 mmol/L, respectively);

however, neither group were different ($P \geq 0.32$) from the Hi–Hi group, which were intermediate (0.58 ± 0.06 mmol/L). Overall, the NEFA concentrations recorded in our study indicate that the treatment group classification based on high and low NEFA concentrations during the first 2 wk postcalving was successful.

Further, during the period d –14 to –1 precalving, blood BHB concentrations were greatest ($P < 0.001$) in the Hi–Hi group (0.59 ± 0.02 mmol/L) compared with the Hi–Lo and Lo–Lo groups (0.45 ± 0.01 and 0.43 ± 0.02 mmol/L), which tended to be different from each other ($P = 0.10$). Similarly, during d 0 to 2 postcalving, the Hi–Hi group had greater ($P < 0.001$) blood BHB concentrations (0.85 ± 0.02 mmol/L) than the Hi–Lo (0.66 ± 0.01 mmol/L) and Lo–Lo groups (0.63 ± 0.02 mmol/L), which were not different from each other ($P = 0.28$). Between d 3 to 7 and wk 2 postcalving, blood BHB concentrations were greatest ($P < 0.001$) in the Hi–Hi group (0.97 ± 0.02 and 0.80 ± 0.03 mmol/L, respectively), and the Hi–Lo group (0.68 ± 0.01 and 0.65 ± 0.02 mmol/L, respectively) had greater ($P < 0.05$) blood BHB concentrations than the Lo–Lo group (0.62 ± 0.02 and 0.58 ± 0.02 mmol/L, respectively). These differences between energy status groups were reduced by wk 3 and 4 postcalving (Figure 8.1b). Again, these differences indicate that our classification of cows into high and low BHB concentrations during the first 2 wk postcalving was successful.

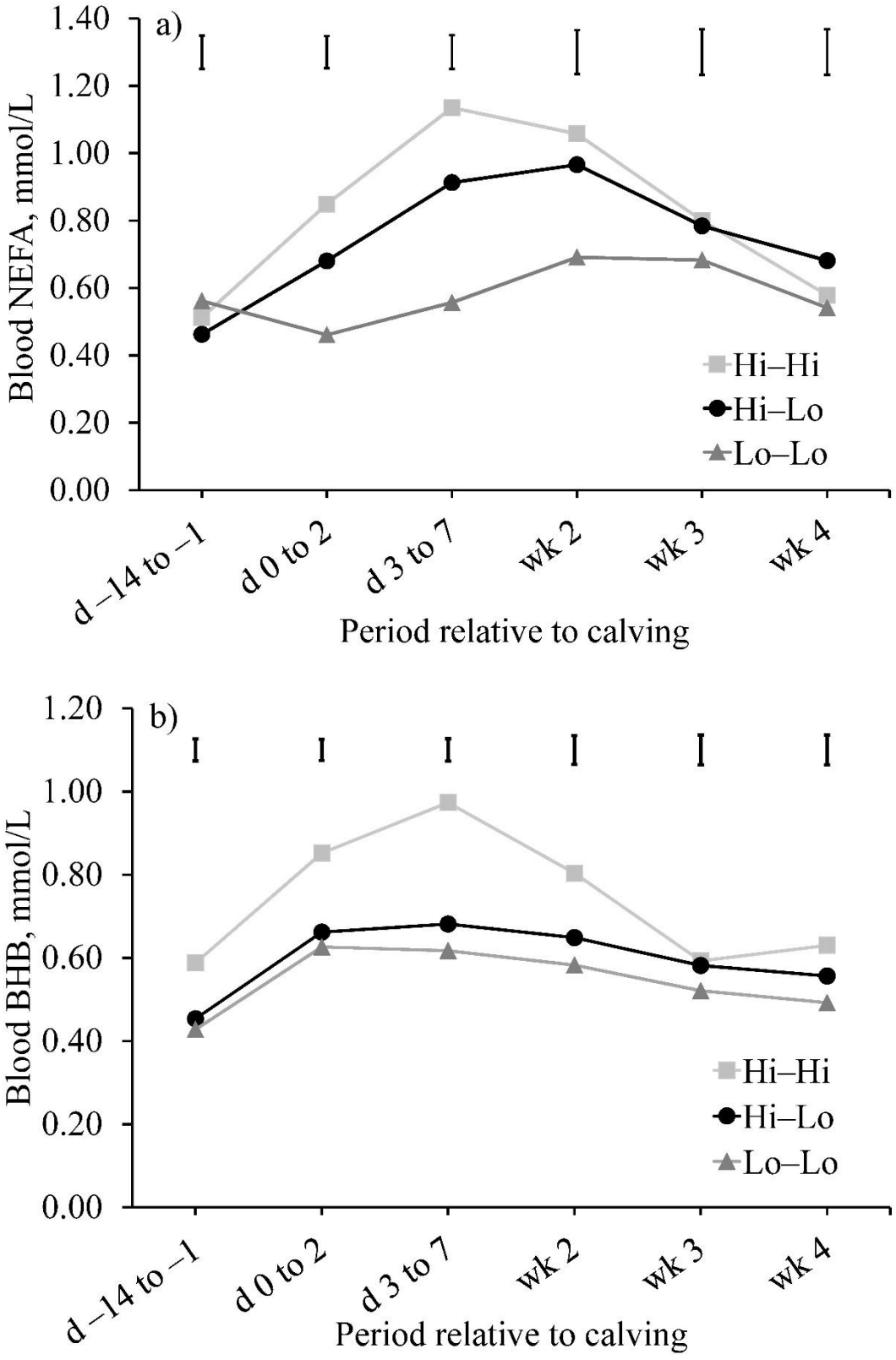


**Figure 8.1. Blood metabolite concentrations during the transition period in 3 energy status groups.**

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**Figure 8.1 (Continued).** Blood non-esterified fatty acid [NEFA; (a)] and β-hydroxybutyrate [BHB; (b)] concentrations (mmol/L) during wk –1 and – 2 precalving, d 0 to 2 postcalving, d 3 to 7 postcalving, and wk 2 to 4 postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

The postcalving metabolic profiles (NEFA and BHB) are mirrored in the energy status x week interactions ($P < 0.001$) for BCS and BW; the profiles during the 4 wk pre- and 6 wk postcalving are presented in Figures 8.2a and b, respectively. Precalving mean BCS and BW were not significantly different between the energy status groups, however, postcalving mean BCS and BW were lowest, on average, in the Hi–Hi group compared with the Lo–Lo and Hi–Lo groups (Table 8.2). During wk 2 to 6 postcalving, cows in the Hi–Hi group lost more BCS than cows in the Lo–Lo ($P < 0.01$) and Hi–Lo groups ($P < 0.05$), and during wk 1 to 6 postcalving, cows in the Hi–Hi group lost more BW than cows in the Lo–Lo ($P < 0.01$) and Hi–Lo ($P < 0.05$) groups. Associations between mean milk yield and composition during the first 2 months of lactation and energy status are presented in Table 8.2, while energy status x week interactions are presented in Supplemental Materials and Supplemental Figures 4a, b, and c (Appendix 18). Briefly, ECM yield during the first 6 wk postcalving was not different in the Hi–Hi and Lo–Lo groups, although, overall, ECM yield was 1.3 kg/d greater in the Hi–Lo group (Table 8.2); there was no energy status x week interaction on ECM yield ($P = 0.13$).

The combination of both high blood NEFA and BHB concentrations in the Hi–Hi group may explain their higher mean NEFA concentrations during d 0 to 7 compared with the Hi–Lo group classified using the same high NEFA threshold, but a lower BHB threshold. Combined with their lower BCS and BW postcalving, our results indicate that the Hi–Hi group were experiencing a greater degree of body fat mobilization and liver oxidation of fatty acids was less effective; consequently, blood NEFA and BHB were elevated reflecting a more severe NEB early postpartum than the Hi–Lo and Lo–Lo groups. During severe NEB, large amounts of circulating NEFA are taken up by the liver. When the liver is unable to oxidize all presented NEFA completely, this leads to hepatic lipidosis, incomplete fatty acid oxidation, and the production of ketone bodies (i.e., BHB, acetoacetate, and acetone), which are then elevated in blood (LeBlanc, 2010). Hepatic lipidosis in the Hi–Hi cows is supported by their high liver TAG content (Supplemental Materials and Supplemental Figure 8).

Cows in the Hi–Lo group were also experiencing NEB and likely also experiencing increased body fat mobilization relative to cows in the Lo–Lo group, as indicated by their increased blood NEFA concentrations during the first 2 wk postcalving; however, the lack of difference in BCS and BW during this time and relatively small increase in blood BHB compared with the Lo–Lo group indicates that these animals were less likely to be maladapted and the liver was better able to oxidize NEFA compared with the Hi–Hi group (Herdt, 2000). Therefore, our data support that cows in the Hi–Lo group were nonketotic and at a relatively less severe state of NEB.

Blood BHB concentrations ≥1.2 mmol/L are often used to diagnose SCK in housed cows (Duffield et al., 2009); however, in grazing dairy cows, a higher proportion of forage in the diet (e.g., pasture or pasture silage), leads to more ruminal butyrate

production, and subsequently, greater basal concentrations of blood BHB than cows eating a higher proportion of starch (e.g., grains or TMR; Roche et al., 2010). Therefore, elevated blood BHB does not always indicate SCK and impaired performance in grazing dairy cows (Phyn et al., 2017). As a result, it is currently unclear whether blood BHB ≥1.2 mmol/L is an appropriate threshold for diagnosing SCK in grazing dairy cows and despite our data being indicative of SCK in the Hi–Hi group, we will describe these cows in our study as HYK rather subclinically ketotic.

**Table 8.2. Behavior and cow performance parameters for the 3 energy status groups.**

Behavior and cow performance measures for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving)].

| Parameter | Lo–Lo | Hi–Lo | Hi–Hi | SED[1] | *P*-value |
|---|---|---|---|---|---|
| Daily lying time, h/d | 8.85 | 8.59 | 8.45 | 0.26 | 0.35 |
| Daily LB, no./d | 9.93[a] | 9.24[ab] | 8.71[b] | 0.41 | <0.05 |
| Mean LB duration, min/bout | 59.3 | 61.1 | 64.4 | 2.55 | 0.25 |
| Steps taken, steps/d | 3603 | 3586 | 3740 | 128 | 0.50 |
| Milk yield,[2] kg/d | 24.7[b] | 26.2[a] | 25.4[a] | 0.58 | <0.01 |
| ECM yield,[3] kg/d | 26.9[b] | 28.2[a] | 26.9[b] | 0.55 | <0.01 |
| Milk fat,[3] % | 4.48 | 4.48 | 4.37 | 0.08 | 0.41 |
| Milk protein,[3] % | 3.60[a] | 3.48[b] | 3.45[b] | 0.03 | <0.001 |
| BCS,[4] 10-point scale | | | | | |
| Precalving | 4.82 | 4.88 | 4.86 | 0.03 | 0.11 |
| Postcalving | 4.38[a] | 4.32[a] | 4.17[b] | 0.04 | <0.001 |
| BW,[4] kg | | | | | |
| Precalving | 559 | 557 | 558 | 2.58 | 0.54 |
| Postcalving | 495[a] | 487[b] | 473[c] | 3.50 | <0.001 |

[a–c]Means with different superscripts are significantly different at the 5% confidence level.
[1]SED = mean standard error of the difference.
[2]Milk yield during the first 7 wk of lactation.
[3]Energy-corrected milk (ECM) yield, milk fat and protein % during wk 2 to 6 in milk.
[4]Body weight (BW) and body condition score [(BCS) 10-point scale, where 1 is emaciated and 10 is obese; Roche et al., 2004] during the 4 wk pre- and 6 wk postcalving period.

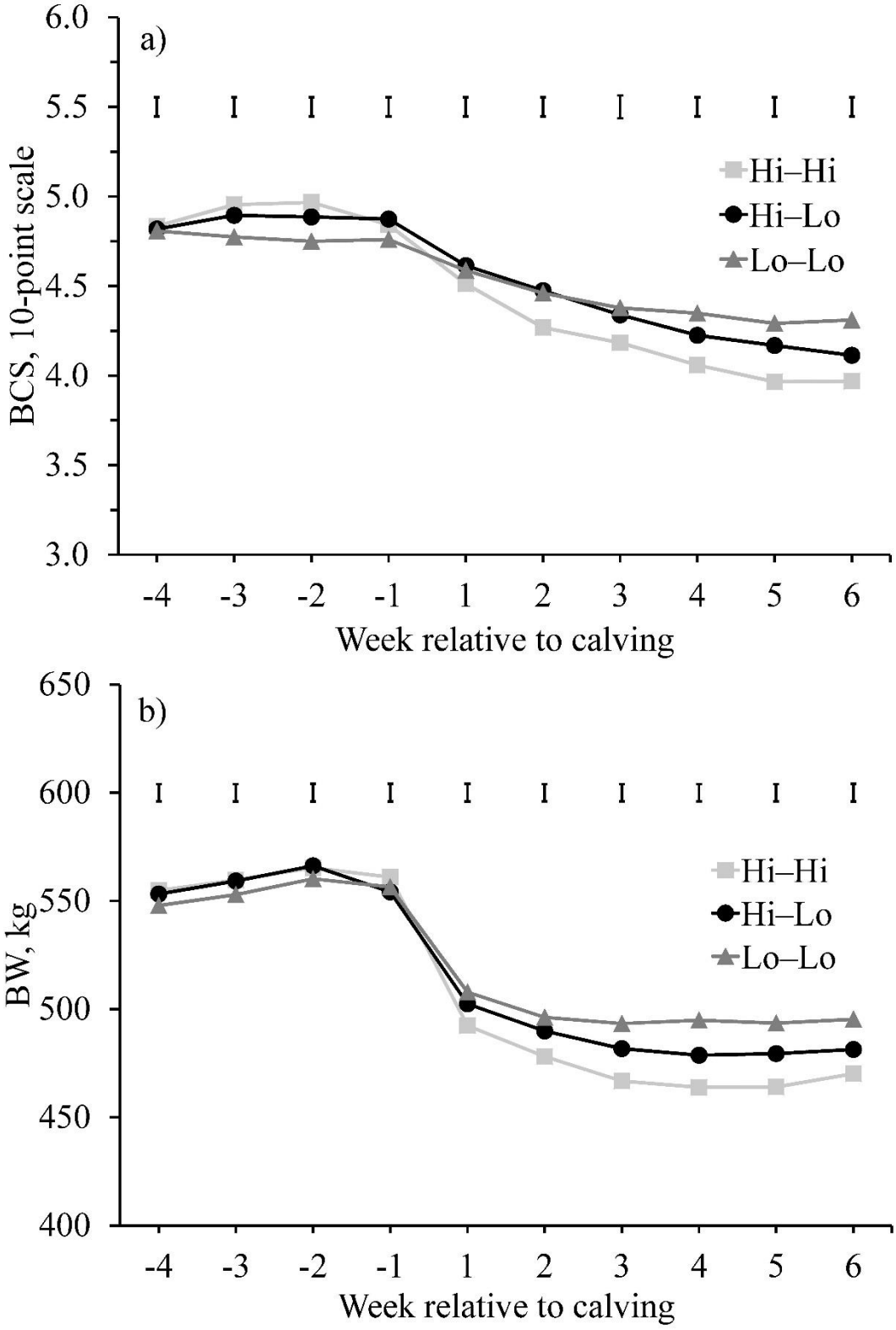


**Figure 8.2. Body condition score and body weight pre- and postcalving for the 3 energy status groups.**

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**Figure 8.2 (Continued).** Mean body condition score [BCS (a); units] (10-point scale, where 1 is emaciated and 10 is obese; Roche et al., 2004) and body weight [BW (b); kg] during the 4 wk pre- and 6 wk postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

### *8.4.2 Differences in Lying Time and Activity Associated with Energy Status*

We examined differences in lying behaviors and activity during the transition period (–21 to +35 d relative to calving) between cows retrospectively classified into 3 different energy status groups postcalving. Although there were no overall differences in daily lying time, mean LB duration, and number of steps taken between energy status groups, the Hi–Hi group had a lower number of daily LB than the Lo–Lo group (Table 8.3); however, both groups were not different from the Hi–Lo group. There were also energy status x period interactions ($P < 0.001$) for daily lying time, daily LB number, mean LB duration, and number of steps taken per day during the transition period and early lactation (Figures 8.3a, b, c, and d, respectively). The behavioral differences occurring at particular times during this period allow us to make inferences about behavior surrounding disease diagnosis.

There are 2 distinct types of behavior surrounding disease diagnosis; these are often referred to as ‘sickness behaviors’ or ‘early indicators of disease’ (Proudfoot and Huzzey, 2017). Sickness behaviors are behavioral changes that occur at the time of and

after disease diagnosis and are a physiological response to disease (Hart, 1988; Dantzer and Kelley, 2007). By comparison, early indicators of disease are behavioral changes that occur before the onset of disease (Proudfoot and Huzzey, 2017). In our study, it is difficult to distinguish, with certainty, between postcalving behaviors that truly reflect sickness and those that are early indications of disease. This is due to infrequent blood sampling during the first 2 wk postcalving and associated differences in the timing of classification of individual cows as Hi–Hi. However, in our study, the mean (± SD) day cows were classified as Hi–Hi was 4.9 ± 3.32 d postcalving; consistent with other studies monitoring the prevalence of SCK in housed (McArt et al., 2012) and grazing systems (Compton et al., 2014; Bonfatti et al., 2019) that reported that peak prevalence of SCK (blood BHB ≥1.2 mmol/L) occurs within the first 5 to 12 DIM. Therefore, for this study, behavioral changes occurring before 5 d postcalving will be referred to as early indicators of disease, and behavioral changes occurring on or after day 5 will be referred to as sickness behaviors and discussed in the following sections.

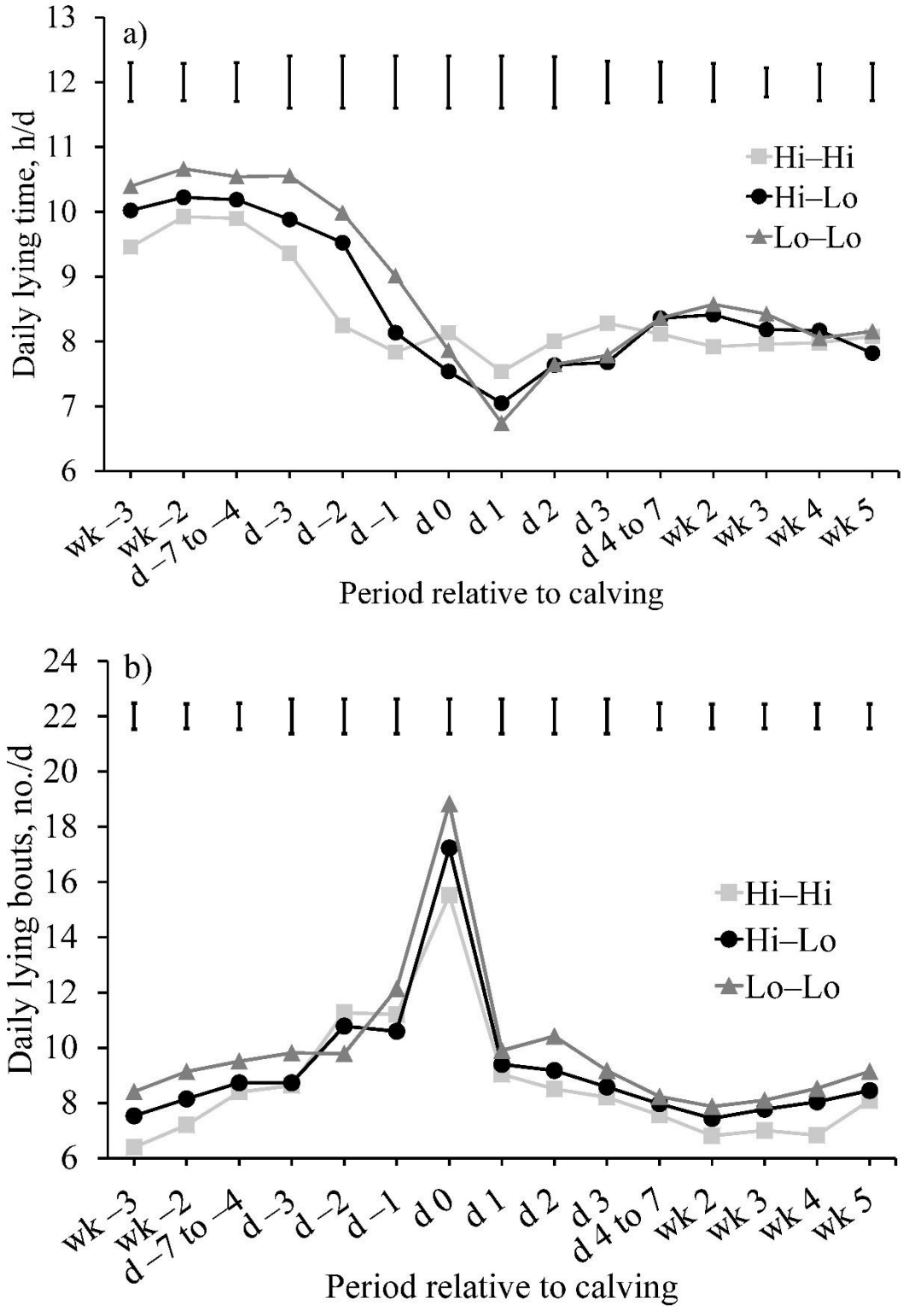


**Figure 8.3. Daily lying behavior and activity during the transition period for the 3 energy status groups.**

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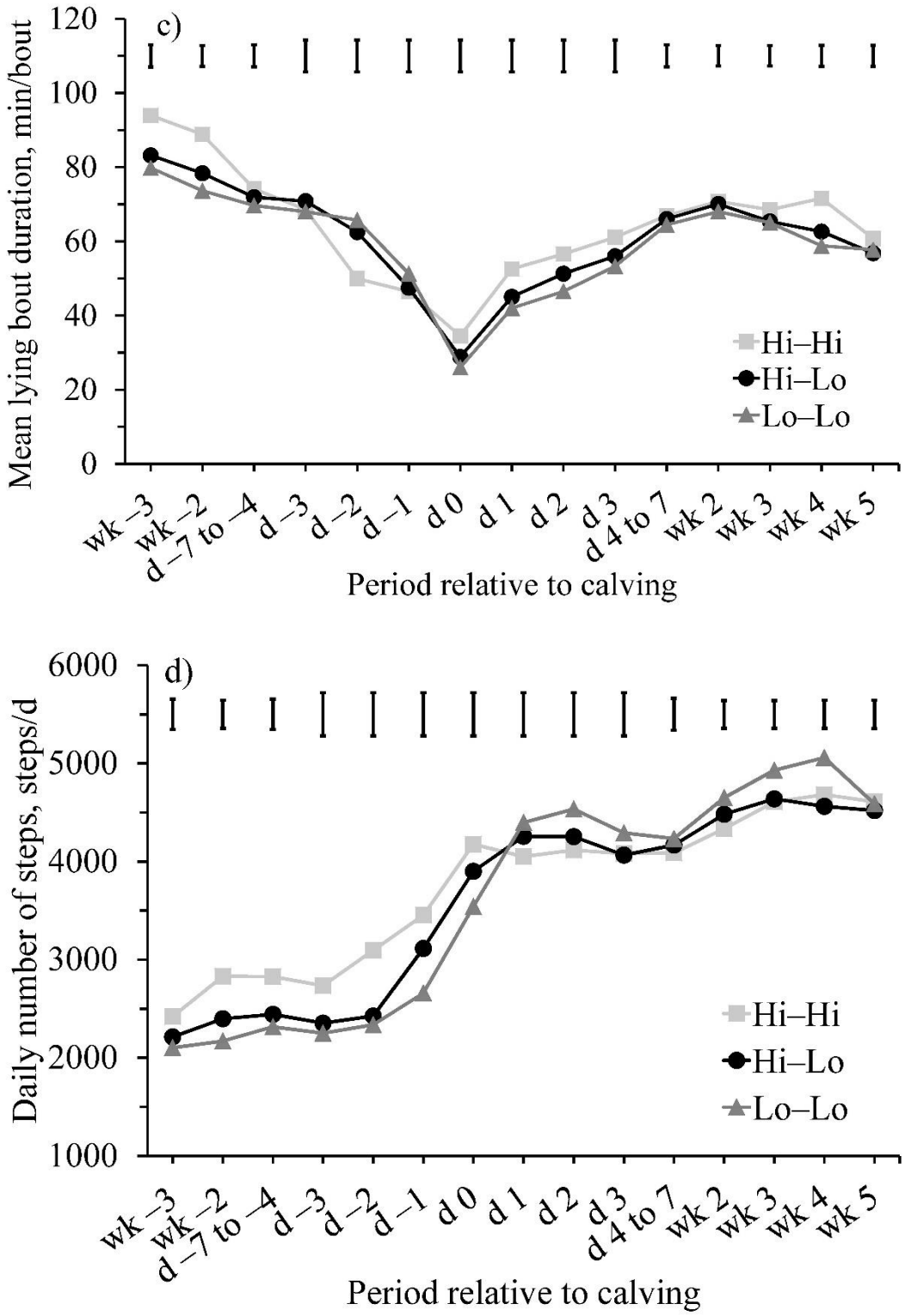


**Figure 8.3 (Continued). Daily lying behavior and activity during the transition period in 3 energy status groups.**

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**Figure 8.3 (Continued).** Daily lying time (a; h/d), lying bouts [LB (b); no./d], mean LB duration (c; min/bout), and number of steps (d; steps/d) during the period –21 to +35 d relative to the day of calving (d 0) for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x standard error of the difference.

***Lying Time, LB, and Activity as Sickness Behaviors.*** In our study, we did not detect any differences ($P \geq 0.12$) in daily lying time from d 0 to 35 postcalving between grazing cows differing in energy status during the first 2 wk postcalving (Figure 8.3a). Nevertheless, the Hi–Hi cows experiencing more severe NEB with HYK did engage in what appears to be energy-conserving behaviors by having fewer, yet longer, LB and reduced activity postcalving (Figures 3b, c, and d). Others have reported that cows experiencing SCK engage in other energy-conserving behaviors such as reduced step activity (Edwards and Tozer, 2004; Liboreiro et al., 2015); whereas, Kaufman et al. (2016) reported that housed cows with SCK (blood BHB ≥1.2 mmol/L within 3 wk postcalving) spent more time lying in wk 3 and 4 postcalving than healthy cows (blood BHB <1.2 mmol/L and no other health conditions within 3 wk postcalving). Cows experiencing SCK tend to be in a more severe state of NEB (Nielsen and Ingvartsen, 2004); therefore, it is, perhaps, unsurprising that cows in the Hi–Hi group would engage in less energetically-expensive behaviors in an attempt to conserve energy or due to feeling unwell despite not exhibiting clinical symptoms.

Lying bouts are an energy-expensive activity (Susenbeth et al., 2004) due to the effort required for an animal to change from a standing to a lying position and vice versa (Itle et al., 2015). A tendency for an energy status association with daily LB during wk 2 and 3 postcalving ($P = 0.08$ and $P = 0.10$, respectively) reflected fewer LB (~1 no./d) by cows in the Hi–Hi group than their peers in the Lo–Lo group, with the Hi–Lo group intermediate and not different between both groups ($P \geq 0.21$). During wk 4 postcalving, cows in the Hi–Hi group had fewer but longer LB (6.83 ± 0.42 no./d and 71.6 ± 2.65 min/bout, respectively) than cows in the Hi–Lo [8.04 ± 0.21 no./d; ($P < 0.05$) and 62.6 ± 1.31 min/bout, respectively; ($P < 0.01$)] and Lo–Lo groups [8.53 ± 0.30 no./d; ($P < 0.01$) and 58.8 ± 1.88 min/bout, respectively; ($P < 0.001$)], which were not different ($P \geq 0.21$) from each other (Figure 8.3b). The lower number of daily LB in the Hi–Hi group for up to 3 wk after the mean day of Hi–Hi classification (Figure 8.3b), may be due, at least in part, to the animal's unwillingness to engage in this behavior due to lack of energy or feeling unwell. In contrast, Rodríguez-Jimenez et al. (2018) reported that housed cows experiencing HYK (blood BHB ≥1.4 mmol/L within 15 DIM) tended to have more LB during the first 15 DIM than healthy cows (blood BHB <1.4 mmol/L within 15 DIM). In their study, however, behavioral differences were observed within the period that coincided with the timing of blood sampling and disease diagnosis; therefore, it cannot be determined with certainty that their reported increase in LB is a sickness behavior caused by HYK, or an early indicator of disease, or a possible predisposing behavior in housed cows. Our results demonstrate that differences in LB number and duration may be useful to aid diagnosis of cows experiencing NEB with HYK in a grazing system.

We also report that step activity may be another useful measure in grazing cows as it differed following energy status diagnosis in early lactation. During wk 3 and 4

postcalving, cows in the Hi–Hi group tended ($P = 0.12$ and $P = 0.06$, respectively) to take fewer steps (4,607 ± 133 steps/d and 4,681 ± 134 steps/d, respectively) than the Lo–Lo group (4,930 ± 93 and 5,059 ± 94 steps/d, respectively), and while the Hi–Hi group were not different ($P \geq 0.70$) from the Hi–Lo group (4639 ± 64 and 4562 ± 66 steps/d, respectively), there was a difference ($P < 0.05$) between the Lo–Lo and Hi–Lo groups (Figure 8.3d). Reductions in activity after disease diagnosis have been associated with SCK in housed systems (Liboreiro et al., 2015; Stangaferro et al., 2016a). Similarly, Liboreiro et al. (2015) investigated the activity of cows experiencing SCK within 3 wk postcalving (blood BHB >1.0 mmol/L) and reported a reduction in activity among cows diagnosed with SCK from d 7 to 17, except on d 14 postcalving. Reduced activity in the Hi–Hi group may reflect an energy-conserving behavior due to lower energy availability (Proudfoot and Huzzey, 2017).

To our knowledge, our study is the first to report decreased LB and activity up to 3 wk after the mean day (4.9 d postcalving) cows were classified according to elevated NEFA and BHB in blood postcalving (Hi–Hi). While were unable to determine a casual link between behaviour postcalving and NEB and HYK in our study, our results indicate that in early lactation, grazing cows in the Hi–Hi group behaved differently to their non-HYK herdmates (Hi–Lo and Lo–Lo groups), which could have implications for management and genetic programs. Prospective studies are required to investigate whether these differences translate and have diagnostic and monitoring potential at the individual-cow level.

***Use of Lying Time, LB, and Activity as Early Indicators of Disease.*** Cows alter their behavior during the transition period and this has been well-documented in housed systems (Huzzey et al., 2005; Calderon and Cook, 2011), and, recently, in cows kept

outdoors and fed TMR (Black and Krawczel, 2016; Rice et al., 2017), and in cows grazing pasture (Chapter 6). These behavioral changes are thought to be caused by a combination of increased physiological, metabolic, and nutritional demands as the cow changes from a pregnant to lactating state (Grummer, 1995; Drackley, 1999), and management related changes associated with milk harvesting (Chapter 6; Huzzey et al., 2005; Sepúlveda-Varas et al., 2014). Furthermore, particularly in grazing cows, the postcalving cow is more active at the expense of lying time (Chapter 6) and idling (Dohme-Meier et al., 2014). Due to these known behavioral differences during the transition period, behavioral changes before classification of cows as Hi–Hi (4.9 d postcalving) will be discussed separately for the periods pre- and postcalving in the following sections.

***Potential Early Indicators of Disease Before the Day of Calving.*** Relatively little is known about the associations between activity and SCK more than 5 d before SCK diagnosis; however, our results indicate that greater step activity precalving may indicate risk of metabolic disease during early lactation. During wk –2, d –4 to –7 and on d –2 precalving, cows in the Hi–Hi group were more active and took, on average, 569 more steps/d than cows in the Lo–Lo ($P < 0.01$) and Hi–Lo groups ($P < 0.05$), which were not different from each other (Figure 8.3d). On d –1 precalving, cows in the Hi–Hi group were more active ($P < 0.01$) than the Lo–Lo group (3453 ± 210 vs. 2658 ± 139 steps/d), but were not different ($P = 0.31$) from the Hi–Lo group (3114 ± 102 steps/d). In contrast to our results, Liboreiro et al. (2015) did not detect any associations between activity (–21 to –1 d precalving) and SCK, which may reflect the conservative definition and the timing of sampling used to classify cows as SCK (blood BHB cutpoint >1.0 mmol/L within 3 wk postcalving). While greater activity precalving was associated with cows classified as Hi–Hi postcalving in our study, a single predictor variable such as activity,

may not be suitable to predict metabolic disease, particularly where there is substantial individual variation (Rutten et al., 2013) and at-risk animals cannot always be distinguished from those not at risk (Stangaferro et al., 2016a).

In this regard, precalving daily lying time and LB number in combination with activity may provide a better predictor of energy status postcalving. Cows in our study that were classified as Hi–Hi spent less time lying than cows classified as Lo–Lo and had fewer daily LB than those classified as Lo–Lo and Hi–Lo cows during the weeks before calving (Figures 8.3a and b). Cows in the Hi–Hi group spent less time lying during wk –3 and –2 precalving (9.46 ± 0.28 ($P < 0.05$) and 9.93 ± 0.27 h/d ($P = 0.07$), respectively) and had fewer LB (6.79 ± 0.43 and 7.20 ± 0.42 no./d (both $P < 0.001$), respectively) than the Lo–Lo group [10.4 ± 0.20 and 10.7 ± 0.19 h/d ($P < 0.07$), respectively, and 8.41 ± 0.31 and 9.13 ± 0.29 no./d, respectively]. During wk –3 and –2 precalving, the Hi–Lo group were intermediate between the Lo–Lo ($P < 0.05$) and Hi–Hi groups ($P \leq 0.10$) for number of LB (7.35 ± 0.21 and 8.14 ± 0.20 no./d, respectively); however, the Hi–Lo group spent a similar amount of time lying ($P \geq 0.13$) each day (10.0 ± 0.14 and 10.2 ± 0.13 h/d, respectively) compared with the Lo–Lo and Hi–Hi groups.

The most variation in lying time between energy status groups occurred in the 3 d before calving, while daily LB were not different between groups in the 3 d before the day of calving (Figure 8.3b). The Hi–Hi group spent less time ($P < 0.05$) lying down than the Lo–Lo group during d –3 (9.36 ± 0.39 vs. 10.6 ± 0.26 h/d; $P < 0.05$), –2 (8.25 ± 0.39 vs. 9.98 ± 0.26 h/d; $P < 0.001$), and –1 (7.83 ± 0.39 vs. 9.01 ± 0.26 h/d; $P < 0.05$), but were only different ($P < 0.01$) from the Hi–Lo group on d –2 (9.52 ± 0.19 h/d), and the Hi–Lo group were not different ($P = 0.32$) from the Lo–Lo group on that day (9.98 ± 0.26 h/d). Further, LB duration was only different on d –2, where the Hi–Hi group

had shorter bouts (both $P < 0.05$) of lying (49.9 ± 4.09 min/bout) than the Hi–Lo and Lo–Lo groups [62.5 ± 2.00 and 65.7 ± 2.72 min/bout, respectively], which were not different from each other ($P = 0.60$). Although, the Hi–Lo group spent less time ($P < 0.05$) lying down on d –1 (8.14 ± 0.19 h/d) and tended to spend less time ($P = 0.09$) lying down on d –3 (9.88 ± 0.19 h/d) than the Lo–Lo group (9.01 ± 0.26 and 10.6 ± 0.26 h/d, respectively).

Daily LB increased in all 3 groups as the cows approached calving and were lowest ($P < 0.01$) in the Hi–Lo group (10.6 ± 0.29 no./d) on the day before calving compared with the Lo–Lo group (12.1 ± 0.40 no./d); the Hi–Hi group were intermediate (11.2 ± 0.60 no./d); however, were not different from both groups ($P \geq 0.41$). There were no further significant differences in daily LB number between groups during the lead-in to calving (Figure 8.3b), despite the large decrease in total daily lying time in Hi–Hi cows in the 3 d precalving (Figure 8.3a). Similarly, Itle et al. (2015) reported longer standing times (i.e., less time spent lying down) during the week before calving in housed cows diagnosed with CK (blood BHB >3.0 mmol/L at 3 consecutive samplings during 3 wk postcalving). While cows in our study had fewer daily LB before Hi–Hi classification, there are no reported associations between daily LB number before disease diagnosis and SCK in housed cows (Kaufman et al., 2016; Rodríguez-Jimenez et al., 2018).

Single short lived, point in time differences between the Hi–Hi and Hi–Lo groups in lying time occurred within 3 d precalving (1 d duration), but larger more prolonged differences in daily LB and activity occurred within 3 wk precalving. The lack of material differences immediately precalving between the Hi–Hi and Hi–Lo groups in time spent lying, the number of LB, and activity precalving (Figures 8.3a, b, and c) means it would be difficult to distinguish between cows likely to develop HYK during NEB (i.e., high

NEFA and high BHB) postcalving and cows likely to have less severe NEB without HYK (i.e., high NEFA and low BHB) postcalving by focusing on individual behaviors within 3 d precalving and absolute values alone. However, monitoring a combination of behavior changes (e.g., daily lying time, LB, and activity) across time, while accounting for individual variation, may be useful to detect HYK (Jawor et al., 2012). Future work should consider integrating multiple behavior variables within cow to predict disease.

Several other authors have reported reductions in activity (Itle et al., 2015), feeding behavior (e.g., fewer visits to or less time spent at the feed bunk), and DMI before diagnosis of SCK and CK (Rodríguez-Jimenez et al., 2018; Sahar et al., 2020). Authors have hypothesized that this is a consequence of social rank or competition for access to feed or lying space in housed systems (Huzzey et al., 2006; Itle et al., 2015). Whereas, Sahar et al. (2020) reported that cows that spent more time eating and engaging in a greater number of agonistic interactions during the precalving period were more likely to remain healthy compared with cows that developed HYK, metritis or both postcalving. Such behavior is likely, representative of more socially-dominant cows. In our study, the Hi–Hi and Hi–Lo groups had lower (~1 kg DM; $P < 0.05$) estimated DMI between wk –3 to –1 precalving (Appendix 18 – Supplemental Materials and Supplemental Figure 3), were more active, spent less time lying, and had fewer daily LB. These behavioral differences have led us to a different hypothesis. Greater activity and less lying, if they are a consequence of social rank (Itle et al., 2015), leading to greater distress (Munksgaard and Simonsen, 1996), may put cows at greater risk for HYK. When grazing, dominant cows have higher pasture biting rates than subordinate cows (Phillips and Rind, 2002). Bite rate is negatively associated with time required to meet a target DMI (Cosgrove and Edwards, 2007); therefore, it is plausible that lower-ranking cows may need to spend

more time grazing and searching for feed to meet their energy requirements (Ungerfeld et al., 2014; O'Driscoll et al., 2019). If true, the stress associated with a social subordinate ranking, which is reflected in the greater activity, less time lying, and fewer LB, may put cows at greater risk of metabolic diseases like HYK; however, it is not known whether cows experience such competitive interactions for access to feed in a grazing environment, where there is greater space availability (Phillips and Rind, 2001). Future research should consider the cause and effect associations between lying, activity, feeding, and social behavior and HYK and NEB, and how social hierarchical placement might influence the risk for metabolic disorders.

***Diurnal Behavior as Early Indicators of Disease Before Calving.*** We also detected that grazing cows classified as Hi–Hi 2 wk postpartum had altered patterns of diurnal activity and lying characteristics in the 2 wk before calving. The within-day profiles of lying time and activity during 2 wk precalving indicate that there were energy status x hour interactions for hourly lying time and number of steps taken (Figures 8.4a and b, respectively). Differences in precalving behavior between postcalving energy status groups were evident within distinct periods of the day and the range of mean values (range = minimum and maximum LSM and standard error of the mean) within the hours specified are reported below. Lying time was lowest ($P < 0.001$) and activity was greatest ($P < 0.05$) in the Hi–Hi group (range = 21.6 ± 1.20 to 34.6 ± 1.20 min/h and 78 ± 8 to 225 ± 8 steps/h, respectively) during the night and early morning (between 0100 and 0700 h) compared with the Hi–Lo group (range = 26.7 ± 0.56 to 44.0 ± 0.57 min/h and 40 ± 3 to 110 ± 3 steps/h, respectively). Further, cows in the Hi–Lo group spent less time lying ($P < 0.001$) and took more steps ($P < 0.01$) than the Lo–Lo group (range = 31.8 ± 0.78 to 52.2 ± 0.78 min/h and 9 ± 5 to 65 ± 5 steps/h, respectively). The opposite was evident

during the middle of the day to late afternoon (between 1200 to 1800 h); cows in the Hi–Hi group spent more time ($P < 0.001$) lying down (range = 13.8 ± 1.17 to 18.0 ± 1.18 min/h) than the Lo–Lo group (range = 6.80 ± 0.76 to 12.8 ± 0.76 min/h), and took fewer steps ($P < 0.01$) between 1200 and 1700 h than cows in the Lo–Lo group (range = 107 ± 7 to 138 ± 8 vs. 144 ± 5 to 191 ± 5 steps/h, respectively). Between 1200 and 1400 h, and 1500 and 1600 h, the Hi–Hi group spent more time ($P < 0.05$) lying down (range = 16.0 ± 1.17 to 16.3 ± 1.18 min/h) than the Hi–Lo group (range = 8.72 ± 0.76 to 9.36 ± 0.76 min/h), and took fewer ($P < 0.05$) steps between 1300 and 1400 h (138 ± 8 vs. 160 ± 3 steps/h). The marked differences in the level of diurnal behavioral activities in the Hi–Hi group compared with the Hi–Lo and Lo–Lo groups are supported by Itle et al. (2015) who reported that across all hours of the day, freestall housed cows diagnosed with CK postcalving spent more time standing in the week before calving than nonketotic cows. However, consistent differences between the 3 groups were not observed across all time points within day, indicating that associations between precalving behavior and postcalving energy status are dependent upon time of day in grazing cows. Our findings may reflect behavioral differences between systems, as cows housed indoors express less synchronization of behavior than cows kept on pasture (e.g., it is less common for the majority of the herd to engage in the same behaviors at the same time; O'Connell et al., 1989; Fregonesi et al., 2007).

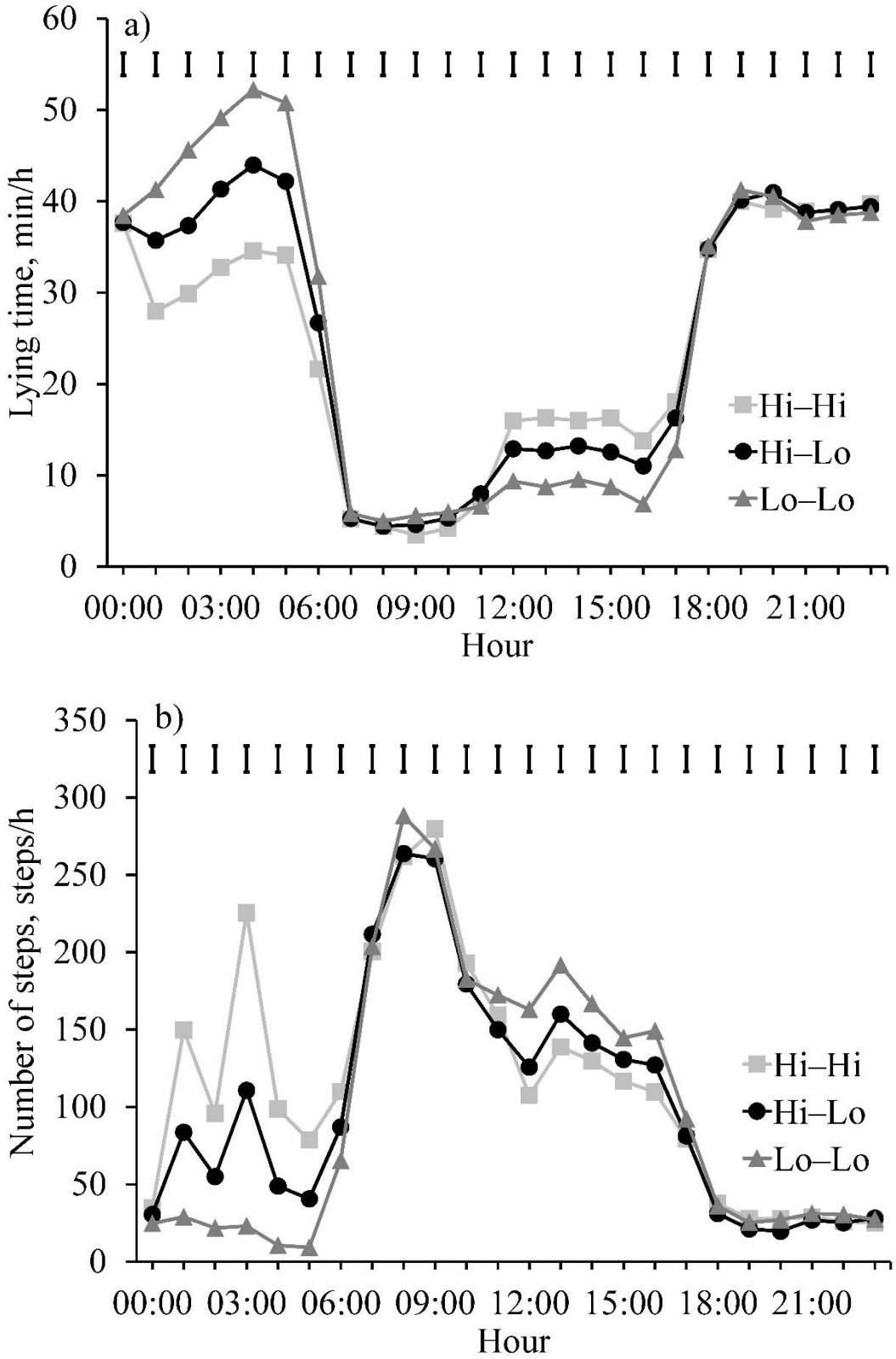


**Figure 8.4. Temporal profiles for lying time and number of steps taken 2 wk precalving in the 3 energy status groups.**

Figure legend continued over page.

**Figure 8.4 (Continued).** Mean lying time (min/h) and number of steps taken (steps/h) across hourly time intervals during –2 to –1 wk precalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference. Time intervals include data within each hour specified (i.e., 0100 h covers the period 0100 to 0159 h).

We cannot determine the cause of precalving behavioral differences within day between the 3 energy status groups from our study and they may simply reflect a pre-existing subclinical condition(s) that increases the risk of HYK and NEB postcalving; however, aversion-type behaviors are also a possible explanation where Hi–Hi cows were less likely to engage in the same behaviors as the rest of the herd to avoid competitive interactions for feed or lying space (Huzzey et al., 2006; Itle et al., 2015; Sahar et al., 2020). Irrespective of the cause of this change in behavior, our findings indicate that grazing dairy cows classified according to elevated NEFA and BHB (Hi–Hi) perform different behavioral activities at specific times of the day than their herd mates classified according to elevated NEFA without elevated BHB (Hi–Lo) compared with cows classified according to lower levels of NEFA and BHB (Lo–Lo) in blood postcalving. Altered diurnal behavior patterns could provide early indications of disease that cannot be understood from total daily behavior measures alone. Few studies report diurnal behavior patterns when investigating associations between behavior and disease; however, many devices allow raw data to be downloaded with a timestamp (Bonk et al.,

2013) and, therefore, time of day can typically be determined. Future research should consider reporting behavioral data for both daily and within-day associations due to the additional information these data can provide.

***Early Indicators of Disease on the Day of and After Calving.*** Differences in lying and activity behaviors at calving and during the early postpartum period were evident in cows that were subsequently more metabolically stressed. In our study, cows in the Hi–Hi group took more steps ($P < 0.05$) than the Lo–Lo group (4175 ± 210 vs. 3540 ± 139 steps/d, respectively) on the day of calving, with the Hi–Lo group intermediate (3901 ± 102 steps/d; $P = 0.09$), but not different from the Hi–Hi group (Figure 8.3d). In contrast, Edwards and Tozer (2004) reported lower activity up to 5 d postcalving in cows diagnosed with CK (mean day of diagnosis ± SD = 10 ± 8.2 d postcalving).

Although there were no differences in total daily lying time between groups on the day of calving (Figure 8.3a), the Hi–Hi group had fewer LB ($P < 0.05$) than the Hi–Lo group (15.5 ± 0.60 vs. 17.2 ± 0.30 no./d); both groups had fewer LB ($P < 0.05$) than the Lo–Lo group (18.8 ± 0.40 no./d) (Figure 8.3b). Consistent with our results, Itle et al. (2015) reported that cows diagnosed with CK had fewer standing bouts; however, there were no further differences in daily standing bouts during wk 1 postcalving in their study. Whereas, in our study, on d 2 postcalving, cows in the Hi–Hi and Hi–Lo groups had, on average, 1.9 and 1.3 less LB/d ($P < 0.05$), respectively, than the Lo–Lo group (Figure 8.3b). Other than this transient difference in daily LB in the days immediately postcalving (d 1 to 3), we did not detect any further differences in daily lying time, LB, or activity between the 3 energy status groups.

Typically, cows have more LB on the day of calving (Huzzey et al., 2005) and take more steps (Chapter 6), and this is thought to be associated with discomfort around

the time of calving (Huzzey et al., 2005). The reduction in LB and increase in steps taken on the day of calving in the Hi–Hi cows may reflect an early manifestation of metabolic stress in the cows diagnosed with HYK during NEB postcalving, or it may be due to a calving related issue. It cannot be determined from our study, however, why the cows classified according to elevated NEFA and BHB in blood postcalving did not display longer lying times (Rodríguez-Jimenez et al., 2018; Piñeiro et al., 2019) and reduced activity (Edwards and Tozer, 2004) reported by others and whether the behavior exhibited at calving was directly associated with HYK or a secondary issue. Interestingly, Stangaferro et al. (2016a) reported individual differences in cow behavior where some cows had reduced activity 5 d before clinical diagnosis, but for others cows their activity did not change before being diagnosed with ketosis. Therefore, as proposed by Proudfoot and Huzzey (2017), future work should focus on understanding individual differences in cow behavior due to disease risk to improve the predictive potential of behavior measures. Care should be taken when interpreting the findings reported during early lactation in our study due to the variation in the day of diagnosis (range: 0 to 14) and, therefore, the potential to mask some effects.

### *8.4.3 Early Indicators of Disease to Predict Disease Risk*

Univariable logistic regression identified that the mean number of steps taken during wk –2 and wk –1 precalving was not associated with an increased risk of being classified with high blood NEFA and BHB postcalving (Hi–Hi); but the multivariable logistic model, including relative change in daily steps taken between wk –2 precalving and d 1 postcalving within cow, was associated with decreased odds of being classified Hi–Hi (Table 8.3). Weak associations between mean values for lying and standing time (Rodríguez-Jimenez et al., 2018) and activity before disease diagnosis and blood BHB

postcalving have been reported (Edwards and Tozer, 2004). Therefore, in agreement with our results, within-cow changes in behavior over time are an alternative approach that allows the deviations from normal behavior within cow to be related to disease risk (Proudfoot and Huzzey, 2017). In our study, a relative increase in daily steps taken of 1131 steps/d on d 1 postcalving compared with wk –2 precalving decreased the odds of being classified Hi–Hi by 55%, when compared with cows classified Lo–Lo. We also investigated if lying behaviors were predictive of cows classified Hi–Hi. We report that mean daily LB during wk –2 precalving was associated with decreased odds of being classified Hi–Hi (Table 8.4). Overall, a cow engaging in 4.27 more LB per day during wk –2 precalving is 70% less likely to be classified Hi–Hi compared with Lo–Lo. In other words, a larger increase in steps taken on the day after calving from a baseline period 2 wk precalving and greater daily LB 2 wk precalving was associated with a better health outcome in grazing cows.

In both models, change in BCS (difference between BCS during wk –1 and –2 precalving and wk –5 and –6 precalving) was retained in the final models and a +0.27 point change in BCS was associated with increased odds of being classified Hi–Hi postcalving (Tables 8.3 and 8.4) in agreement with others (Duffield, 2000; Vanholder et al., 2015).

**Table 8.3. Logistic regression models for change in daily number of steps taken and other factors associated with hyperketonemia.**

Logistic regression model for change in (Δ) daily steps taken (steps/d) and other factors associated with elevated NEFA and BHB postcalving (Hi–Hi; n = 32) relative to healthy animals (Lo–Lo; n = 78). SD = standard deviation.

| Variable | Mean (SD) | Odds ratio (95% CI)[1] | *P*-value |
|---|---|---|---|
| Parity | 2.84 (1.52) | 2.39 (1.20 to 4.73) | 0.014 |
| Δ BCS,[2] 10-point scale | 0.09 (0.27) | 1.89 (0.91 to 3.94) | 0.089 |
| Δ daily steps taken,[3] steps/d | 1,935 (1,131) | 0.45 (0.23 to 0.89) | 0.022 |
| Calvingseasonday[4] | 43.1 (9.84) | 0.48 (0.24 to 0.94) | 0.032 |

[1]Adjusted odds–ratio and 95% confidence interval (CI) for 1 SD increase in each variable in the model.
[2]Δ BCS = change in BCS (calculated as the difference between the mean BCS during wk –1 and –2 precalving and mean BCS during wk –5 and –6 precalving (10-point scale: Roche et al., 2004).
[3]Δ daily steps taken = change in daily steps taken (calculated as the differences between the daily steps taken on d 1 postcalving and the daily steps taken during wk –2 precalving).
[4]Calvingseasonday = difference in days between calving date and the first day of June within the herd.

We also investigated if daily lying time was predictive of cows classified Hi–Hi; however, when we included relative change in daily lying time in a multivariable logistic model, it was not retained in the final model. Kaufman et al. (2016) modeled 2 logistic regressions; the first model compared SCK cows with cows that had no SCK or other health problem, and the second model compared SCK cows with 1 or more other health problem to cows that had no SCK or other health problem. In the first model, lying time was not retained in the final model; however, in the second model, Kaufman et al. (2016) reported that an increase in daily lying time during wk 1 postcalving by 2.2 h/d increased the odds of a cow being diagnosed with SCK and 1 or more other health problem by 1.80 ($CI_{95}$ = 1.00 to 3.39; $P$ = 0.05); therefore, in agreement with our findings, they concluded that differences in lying time were not large enough to be associated with increased odds of SCK alone. Our results indicate, however, that the use of within-cow changes in stepping behavior and mean daily LB precalving may be suitable measures.

**Table 8.4. Logistic regression models for mean daily lying bouts and other factors associated with hyperketonemia.**

Logistic regression model for daily lying bouts (LB; no./d) and other factors associated with elevated NEFA and BHB postcalving (Hi–Hi; n = 32) relative to healthy animals (Lo–Lo; n = 78). SD = standard deviation.

| Variable | Mean (SD) | Odds ratio (95% CI)[1] | *P*-value |
|---|---|---|---|
| Parity | 2.84 (1.52) | 2.62 (1.32 to 5.20) | 0.006 |
| Δ BCS,[2] 10-point scale | 0.09 (0.27) | 1.94 (0.90 to 4.20) | 0.090 |
| Daily LB (wk –2),[3] no./d | 8.94 (4.27) | 0.30 (0.10 to 0.93) | 0.038 |
| Calvingseasonday[4] | 43.1 (9.84) | 0.58 (0.29 to 1.15) | 0.115 |

[1]Adjusted odds–ratio and 95% confidence interval (CI) for 1 SD increase in each variable in the model.
[2]Δ BCS = change in BCS (calculated as the difference between the mean BCS during wk –1 and –2 precalving and mean BCS during wk –5 and –6 precalving (10-point scale; Roche et al., 2004).
[3]Mean daily LB during wk –2 precalving.
[4]Calvingseasonday = difference in days between calving date and the first day of June within the herd.

Goldhawk et al. (2009) reported that reductions in mean feeding behavior (e.g., fewer visits to the feeder and reduced feeding time) and Kaufman et al. (2016) reported that reductions in mean daily lying time, increased the risk of developing SCK. Consistent with their findings, our results indicate that overall, cows that are less active (e.g., taking fewer steps) and those engaging in less energetically-expensive behaviors (e.g., fewer daily LB) before disease diagnosis, appear to be more at risk of developing HYK.

Few studies (reviewed by Rutten et al., 2013) have investigated the ability for behavior to predict metabolic disease, which supports the importance of our study and future research. Nevertheless, the information currently available in literature indicates that single behavioral predictors, in particular, absolute values, are unable to sufficiently predict metabolic disease with a level of sensitivity and specificity that is accurate enough for on-farm use (Edwards and Tozer, 2004; Rutten et al., 2013). In our study, we also

investigated if lying behaviors were predictive of cows classified according to elevated blood NEFA and BHB postcalving (Hi–Hi; n = 32) relative to animals classified according to elevated blood NEFA without elevated BHB postcalving (Hi–Lo; n = 134); however, according to the multivariable models, after backward stepwise elimination, change in lying time, change in steps taken, and daily LB during wk –2 precalving were all removed from the final models indicating that these factors were not associated with the odds of being diagnosed Hi–Hi relative to Hi–Lo. Our findings indicate that it may be difficult to distinguish between cows that are at increased risk of NEB with HYK and those that experience NEB without HYK postcalving using a single behavior variable alongside easily obtainable on-farm data (e.g., parity, BCS, and BW). Whether multiple mean behavior variables, health markers (e.g., additional tests), or easily obtainable on-farm measures in combination have the potential to improve the potential for detecting metabolic disease and differing levels of severity requires further research (Stangaferro et al., 2016a; Rodríguez-Jimenez et al., 2018). Future work should consider focusing on within-cow changes in behavior and using the approach of combining multiple behavior and other variables when undertaking research interested in predicting disease.

## 8.5 CONCLUSIONS

Cows identified as experiencing NEB with HYK after calving were more active, spent less time lying, and had fewer LB during the 2 wk before calving than cows with lower blood NEFA and BHB postcalving. In addition, multivariable logistic regressions indicated that a greater number of LB per day during the 2 wk before calving and a greater increase in number of steps on the day after calving compared with the 2-wk precalving period, were associated with lower odds of subsequently developing NEB with HYK. Cows classified according to elevated NEFA and BHB in blood postcalving also

displayed differences in their diurnal behavioral activities at specific times of the day. Our results, therefore, indicate that lying behavior and activity during the transition period have potential use in identifying cows at increased risk of being diagnosed with NEB with HYK postcalving under grazing conditions. Prospective studies are needed to investigate whether daily as well as within-day differences in behaviour translate at the individual level and have potential use in idenitying cows at increased risk of developing SCK postcalving under grazing conditions.

## 8.6 ACKNOWLEDGEMENTS

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## 8.7 SUMMARY

In Chapter 8, I investigated whether cows retrospectively classified according to elevated NEFA and BHB (Hi–Hi) and elevated NEFA without elevated BHB (Hi–Lo) displayed behavioral differences before, at the time of, and after diagnosis, when compared with cows classified according to lower levels of NEFA and BHB (Lo–Lo) in blood postcalving. Changes in behavior occurred before the classification of cows as Hi–Hi, whereby Hi–Hi cows took more steps, spent less time lying, and had fewer LB bouts up to 2 wk before calving. I provided evidence that cows at risk of developing HYK with NEB behaved differently than non-HYK cows and that lying behavior and activity could

be valuable measures to identify grazing dairy cows at greater risk of experiencing maladaptation to NEB during early lactation.

# CHAPTER 9. GENERAL DISCUSSION

Wearable technologies such as accelerometers are potential tools to improve the prediction, management, and treatment of diseases in dairy cows. I investigated whether there were associations between animal behavior (derived from accelerometers) and subclinical metabolic diseases in grazing dairy cows and whether behavior measured using wearable technologies could differentiate between cows that remained healthy and those that subsequently developed a health condition.

## 9.1 OVERVIEW

Specifically, I completed a technical review of the studies that have validated accelerometer-derived lying behavior to determine factors that should be considered when using accelerometer devices for research purposes (Chapter 3). From this review, I identified a lack of consensus regarding both the appropriate use of accelerometers and the editing methodologies for derived data. I then investigated the within-device variability in unedited and edited data derived from the 2 accelerometer devices, IceTag and IceQube (IceRobotics Ltd., Edinburgh, Scotland), used in my collated retrospective database of lying behaviors and activity in transition dairy cows grazing pasture (Chapter 4). This process provided the rationale for the editing criteria applied to the original data under investigation in subsequent chapters. Furthermore, I investigated the associations between lying behavior and activity and wet and cold weather (Chapter 5), which allowed me to account for inclement weather as a confounding variable in subsequent analyses. Using the refined methologies developed in previous chapters, I then described ‘normal’ behaviors for time spent lying, lying bouts (**LB**), and activity in clinically-healthy grazing dairy cows during the transition period (Chapter 6), which

provided a basis for evaluating deviations from 'normal' and whether these behavioral changes are associated with subclinical metabolic disease. In Chapters 7 and 8, I investigated whether differences in between and within-day profiles of lying behavior and activity were associated with postpartum blood calcium and metabolic/energy status, respectively, consistent with an increased risk of metabolic disease.

## 9.2 NOVELTY

Several subject areas I explored in this thesis involved novel work that contributes to the progression of accelerometer usage in dairy cows. Information regarding the appropriate use of behavior-monitoring technologies in grazing dairy cows is limited (Ledgerwood et al., 2010). Therefore, I undertook a technical review of the studies that had validated accelerometer-derived lying behavior (Chapter 3) before determining an appropriate methodology for editing and interpreting my behavior data (Chapter 4); this was the first review to provide a detailed overview and summary of validation studies published to date (Chapter 3). I concluded that several methodological issues surrounding the appropriate use of accelerometers still exist due to limited robust validation studies, despite the plethora of research groups using accelerometers. Nevertheless, after testing various editing criteria from previous studies on my collated dataset, I believe that I was still able to develop suitable editing thresholds (Chapter 4) to use in my subsequent analyses. I recommend that future studies to validate editing criteria against gold standard measures (e.g., video or visual observations) will further progress the accuracy of lying behavior data derived from accelerometers.

My analyses also indicated that inclement weather conditions affect behavior profiles and should be accounted for when investigating behavior data (Chapter 5). I hypothesized that lying behavior and activity in grazing dairy cows could be influenced

by prevailing weather because animals kept outside are exposed to all types of weather conditions while acquiring and consuming food, lying, yarding, and walking to and from the milking parlor (Tucker et al., 2007b). In particular, grazing dairy cows calve during late winter and early spring when inclement weather conditions including cold temperatures, heavy rain, and strong winds are common. I, therefore, investigated the associations between lying behavior and activity and several weather variables (Chapter 5). To my knowledge, this work was the first to investigate inclement weather as a potential confounding variable when studying pre- and postcalving lying behavior and activity in dairy cows grazing outside on pasture. I determined that decreased air temperature and increased rainfall were associated with a decline in daily lying time, number of LB, and LB duration during both pre- and postcalving periods. I concluded that behavioral studies should consider some environmental variables as covariates in statistical analyses to adjust for environmental factors that might cause additional variation in the dataset outside of the scope of the research question.

In my next study (Chapter 6), I determined specific cow factors that influenced lying behavior and activity of grazing cows. My analyses indicated that the breeds and BCS within the ranges available in my dataset had little or no effect on lying behavior and activity; however, parity and physiological state (i.e., pre-, peri-, or postcalving) can alter behavior. My results indicated that parity was associated with fewer steps taken during the period immediately before and after calving, although there were no differences in lying behaviors due to parity. A lack of association between daily lying time and breed (Stone et al., 2017), parity (Calderon and Cook, 2011), and BCS (Bewley et al., 2010), and between activity and parity (Duncan and Meyer, 2019) have been reported. In contrast, others have reported increases in daily lying time with parity

(Piñeiro et al., 2019) and decreases in daily lying time with BCS (Matthews et al., 2012). Differences due to breed, parity, and BCS reported in our study with cows grazed outside on pasture and in housed cows are inconsistent, but despite conflicting results, I concluded that due to the influence of parity on other cow factors (e.g., social behavior, milk production, BW, body composition, and DMI), behavioral studies should consider cow variables as covariates in statistical analyses that might cause additional variation in the dataset. Further investigation into the cow factors that could influence behavior is warranted, particularly in grazing cows.

I further characterized the variation in lying behavior and activity and reported the range of mean values for lying behavior and activity within and between seventeen groups of grazing cows from my collated database (Chapter 6). My assessment of the variation in lying behavior and activity within and between groups of grazing cows indicated that the range of mean values for lying behavior and activity was greater among individual cows within groups relative to less variation among groups means, in agreement with the study by Ito et al. (2009) in housed cows. Despite environmental and management related differences between the groups in my study, I concluded that the large variation in individual lying behaviors and activity in grazing cows is an important consideration if benchmarks are established as indicators of welfare.

My characterization of 'normal' lying behavior and activity in transition dairy cows in a rotational grazing system also indicated some key differences relative to typical patterns in housed cows (Chapter 6). Grazing dairy cows are typically kept in larger groups and expend more energy through activity to acquire feed and walking long distances to and from the milking parlor (Kaufmann et al., 2011; Beggs et al., 2018); I hypothesized, therefore, that behavioral differences during the transition from late

gestation to early lactation would be more apparent in grazing cows than previously reported in housed cows. To my knowledge, the daily and within-day profiles of lying behavior and activity that I defined in Chapter 6 were the first to be published in clinically-healthy grazing dairy cows across the transition period (pre-, peri-, and postcalving). The daily and within-day profiles were consistent with what has been reported in housed cows (Huzzey et al., 2005; Calderon and Cook, 2011; Schirmann et al., 2012); however, the daily mean values indicate that grazing dairy cows are more active and spend less time lying each day, particularly postcalving. My results indicated that the time budgets of cows are influenced by the system under which they are kept. Importantly, these results imply that benchmarks to define 'normal' behavior (e.g., for animal welfare regulations or to indicate ill health) should be defined within farming systems rather than translated across systems.

My analyses in Chapters 5 and 6 contributed to understanding the effects of cow, physiological, management, and environmental factors on the 'normal' patterns of lying behavior and activity in clinically-healthy grazing dairy cows during the transition period. I determined which factors are most important to consider when using behavior as an indicator of health or welfare. These outcomes from Chapters 5 and 6 were then used in Chapters 7 and 8 to investigate associations among the profiles of between and within-day lying behavior and activity and risk of important metabolic diseases. To my knowledge, these relationships have not been previously investigated in grazing dairy cows during the transition period. Changes to lying (or standing) behavior and activity have been associated with clinical or subclinical illness in housed cows during this period (Edwards and Tozer, 2004; Itle et al., 2015; Kaufman et al., 2016). Grazing cows, however, are, sometimes, required to walk long distances to and from the milking parlor and to spend

time grazing pasture to meet their energy requirements (Beggs et al., 2018); this affects the time available for other activities, like lying. Consequently, I expected that any differences in behavior between cows diagnosed with subclinical metabolic disease postcalving and those defined as clinically healthy may vary under a grazing system relative to relationships previously reported in housed cows. Therefore, I investigated the associations between daily and within-day lying behaviors and activity and hypocalcemia (Chapter 7) and negative energy balance (**NEB**; defined as high blood NEFA with or without hyperketonemia (**HYK**; Chapter 8) in grazing dairy cows. My results indicated that differences in lying behavior and activity were associated with cows at risk of developing subclinical metabolic disease postcalving.

I determined that differences in lying behavior and activity were evident several weeks before the diagnosis of HYK; these behaviors were ascribed as 'early indicators of disease' (Chapter 8). In my study, cows experiencing NEB with HYK during the first 2 wk postcalving spent less time lying and were more active during the 3 weeks preceding calving; this has not been previously reported in housed or grazing cows. Further, cows diagnosed with clinical hypocalcemica (without paresis) postcalving spent more time lying down the day before calving which could also be an early indication of disease, however, this difference was short lived (Chapter 7). Nevertheless, these are novel and exciting findings, as they could mean that the detection of behavioral changes prior to the onset of subclinical disease to identify at-risk cows is possible, which may assist farmers in earlier intervention and an improved health outcome for the cow (Proudfoot and Huzzey, 2017).

I also ascribed behavior occurring at the time of and after disease diagnosis as 'sickness behavior'. My results indicate that cows experiencing hypocalcemia spend

more time lying (Jawor et al., 2012) and are less active at the time of disease diagnosis although differences are short lived with no further differences observed 2 d postcalving (Chapter 7). Further, my results indicate that cows experiencing HYK have fewer and longer LB at the time of and after disease diagnosis and from 3 wk postcalving activity is reduced (Chapter 8). These differences in these lying behaviors and activity were evident up to 4 wk postcalving (Liboreiro et al., 2015). Collectively, my results indicate that metabolic diseases could be detected in cows not displaying overt clinical symptoms by monitoring lying behaviors and activity using accelerometer devices.

It is important that researchers consider within-cow changes in behavior to better understand the associations among behavior and disease (Proudfoot and Huzzey, 2017). I, therefore, investigated the associations among relative within-cow changes in behavior and the occurrence of either hypocalcemia or NEB with HYK and identified that within-cow changes in behavior could be used to identify disease. To my knowledge, my analyses are the first to identify that within-cow changes in lying behavior and activity could be used to identify cows at risk of developing hypocalcemia and HYK. A relative increase in the number of steps taken from a baseline period 2 wk before calving until the day of calving was positively associated with blood Ca concentrations within 24 h postcalving, whereas, an increase in lying time per h during the daytime (between 0600 to 1800h) on the day of calving relative to a baseline period 2 wk before calving was associated with increased risk of hypocalcemia (lower blood Ca) (Chapter 7). Further, cows that increased their activity by increasing the number of steps taken from 2 wk before calving (baseline) until the day of calving and engaging in a greater number of mean daily LB during to the 2 wk before calving had decreased odds of being diagnosed with NEB with HYK within the first 2 wk postcalving. I concluded that grazing dairy

cows engaging in more active behaviors during the 2 wk preceding calving were less likely to experience a poor health outcome postcalving and that measures of changes in behavior within-cow should be the focus of future studies due to the weak associations between mean values for lying time and activity and disease (Rutten et al., 2013).

Furthermore, my analyses in Chapters 7 and 8 support that identifying differences in within-day behavior profiles could be used as an alternative approach to predict and detect metabolic disease. Many commercially-available activity monitors provide relatively high-resolution hourly data on cow behavior. I hypothesized that physiological changes associated with disease would alter within-day patterns of behavior, particularly because of the tendency for grazing cows to exhibit greater behavioral and herd synchrony (i.e., cows engage in the same behaviors at the same time) than housed cows (O'Connell et al., 1989). I, therefore, investigated associations between within-day lying behavior and activity during the transition period and hypocalcemia postcalving (Chapter 7) or NEB with or without HYK (Chapter 8) in grazing dairy cows to establish whether within-day differences in lying behavior and activity were associated with cows at risk of developing metabolic disease. My results indicated that cows experiencing hypocalcemia or HYK and NEB postcalving exhibit different behavioral activities at specific times of the day before disease diagnosis when compared with clinically-healthy cows. Cows diagnosed as clinically-hypocalcemic postcalving spent more time lying down in the morning and early afternoon (0200 to 1400 h) and took fewer steps in the afternoon (1400 to 1800 h) on the day before calving compared with their clinically-healthy herdmates (Chapter 7). Cows diagnosed with NEB with HYK during the first 2 wk postcalving spent more time lying and took fewer steps in the middle of the day and early afternoon (1200 to 1700 h) during the 2 wk before calving compared with their clinically-healthy

herdmates (Chapter 8). To my knowledge, no one has previously associated temporal behavioral changes with hypocalcemia, and even though temporal changes in behavior have been associated with clinical ketosis in housed cows (Itle et al., 2015), my results provide confidence that those relationships are also evident in grazing dairy cows experiencing NEB with HYK. Future work should consider reporting within-day patterns of behavior as a potential indicator of disease in both housed and grazing dairy cows.

Early identification of cows with health disorders using wearable behavior-monitoring technologies presents a multitude of opportunities. My work indicates that behavioral differences exist between clinically-healthy grazing dairy cows and those with subclinical metabolic disease; but further research is needed to understand behavior and disease relationships, particularly in grazing cows, before the potential for implementation and adoption of behavior monitors on farms will be realized. Other factors specific to New Zealand grazing systems that could create challenges for the adoption of these technologies on farms are briefly discussed in Appendix 19.

Future research should increase our understanding of the potential interpretation and use of the data from behavior-monitoring technologies to implement treatment plans, or management changes, or to monitor disease recovery. The increased interest in these technologies and the potential benefits to the farmers and animals, if implemented successfully, supports that more work is warranted in this area.

## 9.3 LIMITATIONS OF THE EXPERIMENTAL CHAPTERS

The limitations of the experimental chapters in this thesis are also explained below. As identified and discussed in Chapters 3 and 4, a robust methodology for editing original data from IceQube and IceTag devices has not been determined for grazing dairy

cows. Due to time constraints and limited resources, it was not possible for me to undertake a validation study before using the collated data and, therefore, we chose editing criteria based on the available literature from validation studies undertaken in freestall housed cows and following visual inspection of my data under 3 different editing criteria (Chapter 4). While we are confident that our chosen editing criteria produced biologically sound outcomes, a validation study to determine the optimal editing criteria for the use of IceQube and IceTag devices in grazing dairy cows would strengthen the accuracy of lying behavior data derived from accelerometer devices. This information could improve the use of this technology in both research and commercial herds and should be considered in future research endeavors.

In Chapters 7 and 8, due to once-daily calf collection in these studies that may cause a discrepancy of up to 24 h in the recording of calving date, I chose to re-assign calving day using the behavior data as an indicator of actual calving day. A substantial increase in LB in cows on the day of calving has been reported in literature (Huzzey et al., 2005; Borchers et al., 2017; Rice et al., 2017) and I used this knowledge to adjust the data to improve the accuracy of calving day. I acknowledge that a limitation of my work is the lack of validation surrounding the methodology I used to re-assign calving day and, therefore, I cannot determine with certainity how this adjustment influenced the study outcomes. A discrepancy in the assignment of calving day could have profound effects on the interpretation of my results, particularly if effects are short lived or significant effects are masked due to incorrect adjustements. My work highlights the need to improve the recording of calving day in experiments undertaken in grazing cows. Future work should focus on developing algorithms to either, retrospectively assign calving day, or

predict calving using behavior data, which would have applications in research and commercially.

Due to the retrospective nature of the database collated for this thesis, the analyses in Chapters 5 to 8 were limited by the data available. The use of lying behavior and activity using wearable technologies has the potential for use as tools to predict, manage, and treat disease in grazing dairy cows; however, large-scale prospective studies are required. In Chapters 7 and 8, cows were retrospectively classified according to blood markers indicating hypocalcemia and NEB with or without HYK, respectively. This created challenges associated with the timing of sampling and the size of the dataset being fixed. To minimize this limitation, a range of scientifically-supported thresholds (from previously published studies) were investigated for both diseases, to determine the potential sample size that could be obtained from the database; a final decision was made according to a sufficient sample size of 'case' animals being selected under a justifiable classification protocol. However, causality between behavior and both diseases could not be determined due to association analyses based upon retrospective classification of cows for disease risk. It would be valuable to undertake larger prospective studies to test the validity of the associations between behavior and these metabolic diseases. In future work, researchers should carefully consider the addition of other explanatory variables (e.g., social hierarchy, DMI, parity, BCS, BW, and milk production) in the experimental design and the possibility to combine behavior and other performance variables in the analysis to improve our understanding of cause and effect relationships using a multifactorial approach.

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# APPENDICES

## APPENDIX 1. INVITED KEYNOTE SPEAKER (ABSTRACT)

**The use of precision technologies to predict calving and ill health in transition dairy cows**

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Presented at *Australia and New Zealand College of Veterinary Scientists Science Week: Cattle Chapter*, Gold Coast, Australia on 4 July 2019 and Abstract Published in *Proceedings of the New Zealand and Australia College of Veterinary Scientists: Cattle Chapter*, 2019. Page 21.

### *Appendix 1.1. Precision Technologies to Predict Calving and Ill Health*

Precision technologies that measure cow behavior and activity are of growing interest to dairy farmers, with a range of wearable devices already on the market. Identifying sick animals has traditionally relied on visual observation of abnormal behaviors (e.g., reduced appetite, restlessness, or depression), which is subjective, time-consuming, and a skilled task. In addition, the chance of veterinary care and success of management interventions in treating or preventing disease relies on early and correct identification of the health disorder. Wearable technologies that enable continuous monitoring of activity offer a more proactive approach to animal health care on dairy farms. Alerts to abnormal or altered behaviors that are provided by these technologies can indicate early-stage illnesses or heightened risk of disease development. Several studies have investigated the associations between various health challenges, including dystocia, clinical ketosis, lameness, mastitis, endometritis, and subclinical hypocalcemia. The use of these technologies could change the way that illnesses are identified in individual dairy cows and may lead to improved animal health and welfare through more effective treatment and disease management.

# APPENDIX 2. SUPPLEMENTAL MATERIALS AND SUPPLEMENTAL TABLE 1

Validation studies for a range of accelerometers available for both commercial and research purposes are limited. Due to limited validation studies, it is difficult for researchers to determine a suitable method for editing, and interpreting data from accelerometers. Many published studies using accelerometers also lack detailed reporting in their methodology, which makes it difficult to replicate studies, undertake comparisons across studies, or to understand the limitations of the technology (Supplemental Table 1).

The IceTag (IceRobotics Ltd., Edinburgh, Scotland) was specifically developed for research purposes (IceRobotics Ltd., 2017) and is the most validated accelerometer. To our knowledge, in total, 5 studies have validated the IceTag device (Munksgaard et al., 2006; McGowan et al., 2007; Mattachini et al., 2013; Rutter et al., 2014; Nielsen et al., 2018); however, many of the validation studies have limited application due to limited dataset size (McGowan et al., 2007), lack of specific reporting of editing methodology (Rutter et al., 2014), and lack of validation of both lying time and lying bout (**LB**) behavior (Munksgaard et al., 2006; Nielsen et al., 2018). A description of the editing criteria applied to the behavior data across 6 studies published between 2010 to 2014 where IceTag devices were used are presented in Supplemental Table 1. Two of the studies failed to report whether the data were edited prior to interpretation (Telezhenko et al., 2012; Kokin et al., 2014). It would appear that the study by Kokin et al. (2014), where LB in the range of 10 to 656 per d was reported, did not apply editing criteria to the data. For an individual cow to transition between lying and standing positions 656 times in an experimental day is unlikely to be biologically plausible and demonstrates the danger of using accelerometers inappropriately. Bewley et al. (2010) and Gibbons et al.

(2012) provided supporting reference to validation studies; however, as previously mentioned, the validation studies referenced have limitations. The study referenced by both studies was that by O'Driscoll et al. (2008), which was a study validating the Tinytag® Explorer device (Gemini Dataloggers Ltd., Chichester, UK) which operate using a mercury tilt switch and, therefore, function differently from IceTag devices. These studies and that of Telezhenko et al. (2012), however, reported lying behavior that corresponds with values commonly reported in literature; therefore, the authors may have edited the data, or the number of false LB in the data was minimal, but these authors did not report this.

The range of methods used across studies supports that, in some cases, the factors that should be considered when using accelerometers are poorly understood. It is important to consider the optimum settings for the accelerometers before applying them in the field and that where LB records are being reported, at a minimum a suitable accelerometer, sampling interval, and LB criterion are used and reported in the methodology. Future studies using accelerometers should report accurate and detailed methods to support the development of best-practice standards for the application and use of accelerometers in both the research and commercial space (Anderson et al., 2013; Brown et al., 2013).

**Supplemental Table 1. Editing criteria and validation studies referenced where IceTag devices were used in research undertaken in housed cows.**

Description of studies where IceTag devices (1-min sampling interval[1]; 1-s sampling frequency; IceRobotics Ltd., Edinburgh, Scotland) were used in housed dairy cows during lactation, where the interest was in lying time and lying bouts. The editing criteria used and the validation study referenced is also presented.

| Source | Editing criteria[2] | Validation study referenced |
|---|---|---|
| Endres and Barberg (2007) | Lying bouts <2 min removed | Recommendation of the manufacturer |
| Bewley et al. (2010) | Lying bouts calculated using per minute percentages of lying or standing. If the within-minute lying percentage ≥50% than cow defined as lying for that minute. | Munksgaard et al. (2006); McGowan et al. (2007); O'Driscoll et al. (2008) |
| Blackie et al. (2011) | Lying bouts calculated as the sum of consecutive minutes where the within-minute lying percentage >90% (i.e., the cow had been lying down for 54 of previous 60 s) | - |
| Gibbons et al. (2012) | - | Munksgaard et al. (2006) |
| Telezhenko et al. (2012) | - | - |
| Kokin et al. (2014) | - | - |

[1]Sampling interval describes time between samples of the device and within each sampling interval is a summary of all registered counts of data recorded at a predetermined sampling frequency.
[2]Editing criteria describes the editing of the original data derived from the device as described in the experimental methodology of the manuscript.

**Supplemental Table 2. Validation studies undertaken to determine the accuracy of lying behavior derived from accelerometer devices used in research.**

Description of validation studies undertaken to determine the accuracy of various commercially-available activity monitors typically used in research for estimating lying time and lying bouts in housed and grazing dairy cows.

| Source; data collection period | n (cow)[1] | Subject[2] | System[3] | Device, direction; attachment[4] | Behavior validated[5] | Gold standard[6] |
|---|---|---|---|---|---|---|
| Müller and Schrader (2003); 5 d | 12 | L HF | FS; TMR | Actiwatch OD; HL | LB and LT | Video |
| Munksgaard et al. (2006); not specified | 12 | | | IceTag 3D; HL | LT | Direct |
| McGowan et al. (2007); 3 d (~9.3 h total) | 15 | D Primi Multi H HF J | P PA; G | IceTag 3D; HL | LB and LT | Direct |
| Ledgerwood et al. (2010; Exp. 1); 1.5 d | 12 | L HF J | FS LS Y; TMR | HOBO 3D; HL | LB and LT | Video |
| Ledgerwood et al. (2010; Exp. 2); 1.5 d | 12 | D Primi Multi H–F | DB I Y; TMR | HOBO 3D; HL | LB and LT | Video |
| Felton et al. (2012); 6 d | 19 | L Multi H | I P S TS; TMR | AfiAct 3D; HL | LB and LT | Video |
| Siegford et al. (2012); Elischer et al. (2013); 15 d | 15 | L Primi Multi H | FS P; TMR | ACT 3D; NC IceQube 3D: HL | LT | Direct |
| Mattachini et al. (2013); 1 d | 5 | L Multi HF | LS; TMR | HOBO 3D; HL | LB and LT | Video |
| Mattachini et al. (2013); 1 d | 12 | L Multi HF | LS; TMR | IceTag 2D; HL | LB and LT | Video |
| Rutter et al. (2014); 8 h | 16 | L HF | LS P | IceTag 3D | LB and LT | Direct |

**Supplemental Table 2.** Continued over page.

**Supplemental Table 2 (Continued).** Description of validation studies undertaken to determine the accuracy of various commercially-available activity monitors typically used in research for estimating lying time and lying bouts in housed and grazing dairy cows.

| Source; data collection period | n (cow)[1] | Subject[2] | System[3] | Device, direction; attachment[4] | Behavior validated[5] | Gold standard[6] |
|---|---|---|---|---|---|---|
| Kok et al. (2015); 6 d | 28 | L Multi | C FS; TMR | IceQube 3D; HL | LB | Logger |
| Borchers et al. (2016); 8 d (4 h/d) | 48 | L Primi Multi H | C FS Y; TMR | AfiAct 3D, HOBO 3D, IceQube 3D; HL<br>TAC 3D; FL | LT | Direct |
| Charlton et al. (2017); 2 h | 48 | L HF | P | IceQube 3D; FL HL | LB and LT | Direct |
| Henriksen and Munksgaard (2019); 2 h | 40 | D Primi Multi H J | DB FS LS SL; PMR | AfiTagII 3D; HL | LB and LT | Direct |
| Nielsen et al. (2018); 19.8 h | 6 | L Primi Multi HF | FS | IceTag 3D; HL | LT | Video |
| Nielsen et al. (2018); 29.5 h | 10 | L Primi Multi HF | FS | CowScout 3D; FL | LT | Video |

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[1]n (cow) describes the number of subjects that were enrolled in the study.
[2]Subject describes the physiological state, parity, and breed of the subjects, respectively. If either of these 3 phenotypes is not specified, then it was not described in the paper. Physiological state; D = dry, L = lactating; Parity; Primi = primiparous, Multi = multiparous; Breed; H = Holstein, HF = Holstein-Friesian, J = Jersey.
[3]System describes the management system used. Housing and underfoot conditions; C = concrete underfoot, DB = deep-bedded underfoot, FS = freestall barn, I = individually-housed, LS = loose-housed, P = pasture underfoot, PA = pasture access, S = sand underfoot, SL = slatted floor underfoot, TS = tie-stall, Y = yard access. Feeding; G = grazing, PMR = partial mixed ration, TMR = total mixed ration. If any of these systems is not specified, then it was not described in the paper.
[4]Device, direction; attachment describes the name of the activity monitor tested, the directions in which signals are received, and where the device was attached to the animal. Device: Actiwatch = The Actiwatch® Activity Monitoring System (Cambridge Neurotechnology, Cambridgeshire, UK), AfiAct = AfiMilk® Pedometer Plus (ceased manufacture) and AfiTagII = AfiTag® II Pedometer (Afimilk, Kibbutz, Afikim, Israel), ACT = Automatic Milking System Activity Monitor (Lely, Maassluis, The Netherlands), HOBO = HOBO Pendant G Acceleration Data Logger (Onset Computer Corporation, Pocasset, MA), IceTag 3D = IceTag 3D Activity Monitor, IceTag 2D = IceTag 2.004 Activity Monitor (ceased manufacture 2008) and IceQube = IceQube Activity Monitor (IceRobotics Ltd., Edinburgh, Scotland), CowScout = CowScout Leg Sensor (GEA Farm Technologies, Bönen, Germany), TAC = The Track a Cow (ENGS, Rosh Pina, Israel). Direction; 2D = two-dimensional, 3D = three-dimensional, OD = omnidirectional. Attachment; FL = front leg, HL = hind leg, NC = neck-worn collar. If the attachment is not specified, then it was not described in the paper.
[5]Behavior validated describes the lying behaviors validated in the study. LB = lying bouts, LT = lying time.
[6]Gold standard describes the type of observation or device that the device being tested was compared with. Direct = behavior recorded by an observer, Logger = behavior recorded by a logger of the same type on the opposite leg of the test device, Video = behavior recorded using video monitoring and transcribed retrospectively.

**Supplemental Table 3. Summary of reported measures of agreement between accelerometer-derived lying time and gold standard measures for accelerometers validated in dairy cows.**

Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying time in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Müller and Schrader (2003) | Actiwatch | 10 min | <1 min | $r_s = 0.65^{***}$ | - | - |
| Munksgaard et al. (2006) | IceTag 3D | 1 min | - | $r_p = 0.99^{x}$ | - | - |
| McGowan et al. (2007) | IceTag 3D | 1 min | - | - | - | - |
| Ledgerwood et al. (2010; Exp. 1) | HOBO | 6 s | - | $R^2 > 0.99$ | 99.3 | 99.8 |
| | HOBO | 30 s | - | $R^2 > 0.99$ | 99.3 | 99.7 |
| | HOBO | 1 min | - | $R^2 > 0.99$ | 99.3 | 99.8 |
| | HOBO | 5 min | - | $R^2 > 0.98$ | 99.3 | 99.8 |
| | HOBO | 6 s | ≤6 s | $R^2 > 0.99$ | 99.8 | 99.9 |
| | HOBO | 30 s | ≤30 s | $R^2 > 0.99$ | 99.8 | 99.9 |
| | HOBO | 1 min | ≤1 min | $R^2 > 0.99$ | 99.7 | 99.9 |
| | HOBO | 5 min | ≤5 min | $R^2 > 0.98$ | 98.8 | 99.5 |
| | HOBO | 6 s | ≤12 s | $R^2 > 0.99$ | 99.9 | 100 |
| | HOBO | 30 s | ≤1 min | $R^2 > 0.99$ | 99.7 | 99.9 |

**Supplemental Table 3.** Continued over page.

**Supplemental Table 3 (Continued).** Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying time in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Ledgerwood et al. (2010; Exp. 1)· | HOBO | 1 min | ≤2 min | $R^2 > 0.99$ | 99.6 | 99.9 |
| | HOBO | 5 min | ≤10 min | $R^2 > 0.98^*$ | 97.1 | 98.9 |
| | HOBO | 6 s | ≤36 s | $R^2 > 0.99$ | 99.8 | 99.9 |
| | HOBO | 30 s | ≤3 min | $R^2 > 0.99$ | 99.5 | 99.8 |
| | HOBO | 1 min | ≤6 min | $R^2 > 0.99$ | 98.9 | 99.5 |
| | HOBO | 5 min | ≤30 min | $R^2 \leq 0.92$ | 91.4 | 95.5 |
| Ledgerwood et al. (2010; Exp. 2) | HOBO | 6 s | - | $R^2 > 0.99^*$ | 97.6 | 99.4 |
| | HOBO | 30 s | - | $R^2 > 0.99^*$ | 97.6 | 99.5 |
| | HOBO | 1 min | - | $R^2 > 0.99^*$ | 97.4 | 99.4 |
| | HOBO | 5 min | - | $R^2 > 0.98^*$ | 96.2 | 99.0 |
| | HOBO | 6 s | ≤6 s | $R^2 > 0.99$ | 99.2 | 99.8 |
| | HOBO | 30 s | ≤30 s | $R^2 > 0.99$ | 99.4 | 99.9 |
| | HOBO | 1 min | ≤1 min | $R^2 > 0.99$ | 99.3 | 99.8 |
| | HOBO | 5 min | ≤5 min | $R^2 > 0.98$ | 97.1 | 99.3 |

**Supplemental Table 3.** Continued over page.

**Supplemental Table 3 (Continued).** Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying time in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Ledgerwood et al. (2010; Exp. 2) | HOBO | 6 s | ≤12 s | $R^2 > 0.99$ | 99.4 | 99.9 |
| | HOBO | 30 s | ≤1 min | $R^2 > 0.99$ | 99.4 | 99.9 |
| | HOBO | 1 min | ≤2 min | $R^2 > 0.99$ | 99.0 | 99.8 |
| | HOBO | 5 min | ≤10 min | $R^2 > 0.98^*$ | 95.3 | 98.9 |
| | HOBO | 6 s | ≤36 s | $R^2 > 0.99$ | 99.5 | 99.9 |
| | HOBO | 30 s | ≤3 min | $R^2 > 0.99$ | 98.8 | 99.7 |
| | HOBO | 1 min | ≤6 min | $R^2 > 0.99$ | 97.9 | 99.6 |
| | HOBO | 5 min | ≤30 min | $R^2 > 0.85$ | 82.3 | 95.5 |
| Felton et al. (2012) | AfiAct | 1 min | - | $\rho_c = 0.89$; $r_p = 0.91^*$ | - | - |
| Siegford et al. (2012); Elischer et al. (2013) | IceQube | 15 min | - | $r_p = 0.97^{***}$; $R^2 = 0.85$ | - | - |
| | ACT | 2 h | - | $r_p = –0.57$ ** | - | - |
| Mattachini et al. (2013) | HOBO | 1 min | ≤2 min | - | 99.0 | >99.0 |
| | IceTag 2D | 1 s | ≤25 s | - | 99.7 | 100 |

**Supplemental Table 3.** Continued over page.

**Supplemental Table 3 (Continued).** Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying time in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Mattachini et al. (2013) | IceTag 2D | 1 min | ≤2 min | - | 99.7 | 100 |
| | IceTag 2D | 1 min | ≤2 min | R2 = 1.00 | - | - |
| | IceTag 2D | 2 min | ≤2 min | R2 = 1.00 | - | - |
| | IceTag 2D | 3 min | - | R2 > 0.99 | - | - |
| | IceTag 2D | 4 min | - | R2 > 0.99 | - | - |
| | IceTag 2D | 5 min | - | R2 > 0.99 | - | - |
| | IceTag 2D | 10 min | - | R2 > 0.99 | - | - |
| | IceTag 2D | 15 min | - | R2 = 0.98 | - | - |
| | IceTag 2D | 30 min | - | $R^2 = 0.96^{*}$ | - | - |
| | IceTag 2D | 60 min | - | $R^2 = 0.84^{*}$ | - | - |
| Rutter et al., 2014 | IceTag 3D | 5 min | - | - | - | - |
| Borchers et al., 2016 | IceQube | 15 min | - | $\rho_c > 0.99$; $r_p > 0.99^{**}$ | - | - |
| | HOBO | 15 min | - | $\rho_c = 0.81 – 0.87$; $r_p = 0.83 – 0.87^{**}$ | - | - |

**Supplemental Table 3.** Continued over page.

**Supplemental Table 3 (Continued).** Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying time in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Borchers et al. (2016) | HOBO | 60 min | - | $\rho_c = 0.92$; $r_p = 0.93^{**}$ | - | - |
| | TAC | 1 min | - | $\rho_c > 0.99$; $r_p = 0.99^{**}$ | - | - |
| | AfiAct | 1 min | - | $\rho_c > 0.99$; $r_p = 0.99^{**}$ | - | - |
| Charlton et al. (2017) | IceQube | 1 s | - | $R^2 = 0.99^{x}$ | - | - |
| Henriksen and Munksgaard (2019) | AfiTagII | 15 min | <3 min | $r_p = 0.98$ | - | - |
| Nielsen et al. (2018) | IceTag 3D | 15 min | - | $\rho_c > 0.99$ | - | - |
| | CowScout | 15 min | - | $\rho_c > 0.99$ | - | - |

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[1]Device: Actiwatch = The Actiwatch® Activity Monitoring System (Cambridge Neurotechnology, Cambridgeshire, UK), AfiAct = AfiAct® Pedometer Plus (ceased manufacture) and AfiTagII = AfiTag® II Pedometer (Afimilk, Kibbutz, Afikim, Israel), ACT = Automatic Milking System Activity Monitor (Lely, Maassluis, The Netherlands), HOBO = HOBO Pendant G Acceleration Data Logger (Onset Computer Corporation, Pocasset, MA), IceTag 3D = IceTag 3D Activity Monitor, IceTag 2D = IceTag 2.004 Activity Monitor (ceased manufacture 2008) and IceQube = IceQube Activity Monitor (IceRobotics Ltd., Edinburgh, Scotland), CowScout = CowScout Leg Sensor (GEA Farm Technologies, Bönen, Germany), TAC = The Track a Cow (ENGS, Rosh Pina, Israel).
[2]Sampling interval describes time between samples of the device and within each sampling interval is a summary of all registered counts of data recorded at a predetermined sampling frequency.
[3]Lying bout (LB) criterion describes the minimum LB duration that is considered a true bout of lying and LB ≤ the LB criterion are removed from the data to discard false LB before interpretation. If no LB criterion is presented, the study did not apply a LB criterion to the data.
[4]Reliability is described by the following agreement statistics: $\rho_c$ = concordance correlation coefficient, $R^2$ = coefficient of determination ([x]Statistical significance were not reported. [*]Slope and intercept were significantly different ($P < 0.05$) from 1 and 0, respectively. [**]Slope and intercept were significantly different ($P < 0.01$) from 1 and 0, respectively. If no superscript is presented, the slope and intercept were not different from 1 and 0), $r_p$ = Pearson correlation coefficient ([*]$P < 0.05$, [**]$P < 0.01$; [***]$P < 0.001$), $r_s$ = Spearman rank correlation ([*]$P < 0.05$, [**]$P < 0.01$; [***]$P < 0.001$). If no reliability measures are presented, the study did not present reliability measures.
[5]Se = sensitivity.
[6]Sp = specificity.

**Supplemental Table 4. Summary of reported measures of agreement between accelerometer-derived lying bouts and gold standards for accelerometers validated in dairy cows.**

Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying bouts in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Müller and Schrader (2003) | Actiwatch | 10 min | <1 min | $r_p = 0.80^{**}$ | - | - |
| McGowan et al. (2007) | IceTag 3D | 1 min | - | - | - | - |
| Ledgerwood et al. (2010; Exp. 1) | HOBO | 6 s | - | $R^2 < 0.25$ | 99.3 | 99.8 |
| | HOBO | 30 s | - | $R^2 < 0.60$ | 99.3 | 99.7 |
| | HOBO | 1 min | - | $R^2 < 0.70$ | 99.3 | 99.8 |
| | HOBO | 5 min | - | $R^2 \geq 0.92$ | 99.3 | 99.8 |
| | HOBO | 6 s | ≤6 s | $R^2 < 0.80$ | 99.8 | 99.9 |
| | HOBO | 30 s | ≤30 s | $R^2 \geq 0.93$ | 99.8 | 99.9 |
| | HOBO | 1 min | ≤1 min | $R^2 \geq 0.93$ | 99.7 | 99.9 |
| | HOBO | 5 min | ≤5 min | $R^2 > 0.90$ | 98.8 | 99.5 |
| | HOBO | 6 s | ≤12 s | $R^2 < 0.80$ | 99.9 | 100 |
| | HOBO | 30 s | ≤1 min | $R^2 \geq 0.93$ | 99.7 | 99.9 |

**Supplemental Table 4.** Continued over page.

**Supplemental Table 4 (Continued).** Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying bouts in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Ledgerwood et al. (2010; Exp. 1) | HOBO | 1 min | ≤2 min | $R^2 \geq 0.93$ | 99.6 | 99.9 |
| | HOBO | 5 min | ≤10 min | $R^2 > 0.90$ | 97.1 | 98.9 |
| | HOBO | 6 s | ≤36 s | $R^2 \geq 0.97$ | 99.8 | 99.9 |
| | HOBO | 30 s | ≤3 min | $R^2 > 0.90$ | 99.5 | 99.8 |
| | HOBO | 1 min | ≤6 min | $R^2 > 0.90$ | 98.9 | 99.5 |
| | HOBO | 5 min | ≤30 min | $R^2 > 0.90^*$ | 91.4 | 95.5 |
| Ledgerwood et al. (2010; Exp. 2) | HOBO | 6 s | - | $R^2 < 0.30$ | 97.6 | 99.4 |
| | HOBO | 30 s | - | $R^2 < 0.50$ | 97.6 | 99.5 |
| | HOBO | 1 min | - | $R^2 < 0.60$ | 97.4 | 99.4 |
| | HOBO | 5 min | - | $R^2 \geq 0.92^*$ | 96.2 | 99.0 |
| | HOBO | 6 s | ≤6 s | $R^2 < 0.60$ | 99.2 | 99.8 |
| | HOBO | 30 s | ≤30 s | $R^2 \geq 0.93$ | 99.4 | 99.9 |
| | HOBO | 1 min | ≤1 min | $R^2 \geq 0.93^*$ | 99.3 | 99.8 |
| | HOBO | 5 min | ≤5 min | $R^2 < 0.70^*$ | 97.1 | 99.3 |

**Supplemental Table 4.** Continued over page.

**Supplemental Table 4 (Continued).** Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying bouts in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Ledgerwood et al. (2010; Exp. 2) | HOBO | 6 s | ≤12 s | $R^2 < 0.80$ | 99.4 | 99.9 |
| | HOBO | 30 s | ≤1 min | $R^2 \geq 0.93$ | 99.4 | 99.9 |
| | HOBO | 1 min | ≤2 min | $R^2 \geq 0.93^*$ | 99.0 | 99.8 |
| | HOBO | 5 min | ≤10 min | $R^2 < 0.60^*$ | 95.3 | 98.9 |
| | HOBO | 6 s | ≤36 s | $R^2 \geq 0.97$ | 99.5 | 99.9 |
| | HOBO | 30 s | ≤3 min | $R^2 < 0.90$* | 98.8 | 99.7 |
| | HOBO | 1 min | ≤6 min | $R^2 < 0.80$* | 97.9 | 99.6 |
| | HOBO | 5 min | ≤30 min | $R^2 < 0.10$* | 82.3 | 95.5 |
| Felton et al. (2012) | AfiAct | 1 min | - | $\rho_c = 0.94$; $r_p = 0.94$* | - | - |
| Mattachini et al. (2013) | IceTag 2D | 1 min | ≤2 min | $R^2 = 0.98$ | - | - |
| | IceTag 2D | 2 min | ≤2 min | $R^2 = 0.92$ | - | - |
| | IceTag 2D | 3 min | - | $R^2 = 0.89$ | - | - |
| | IceTag 2D | 4 min | - | $R^2 = 0.83$ | - | - |
| | IceTag 2D | 5 min | - | $R^2 = 0.82$ | - | - |

**Supplemental Table 4.** Continued over page.

**Supplemental Table 4 (Continued).** Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying bouts in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Mattachini et al. (2013) | IceTag 2D | 10 min | - | $R^2 = 0.72$ | - | - |
| | IceTag 2D | 15 min | - | $R^2 = 0.59$ | - | - |
| | IceTag 2D | 30 min | - | $R^2 = 0.36$ | - | - |
| | IceTag 2D | 60 min | - | $R^2 = 0.05$* | - | - |
| Rutter et al. (2014) | IceTag 3D | 5 min | - | - | - | - |
| Kok et al. (2015) | IceQube | 1 s | ≤1 s | Acc = 0.96 | 100 | 0.00 |
| | IceQube | 1 s | ≤4 s | Acc = 0.96 | 100 | 14.1 |
| | IceQube | 1 s | ≤33 s | Acc = 0.99 | 99.3 | 97.7 |
| | IceQube | 1 s | ≤55 s | Acc = 0.99 | 98.9 | 100 |
| | IceQube | 1 s | ≤90 s | Acc = 0.99 | 98.4 | 100 |
| | IceQube | 1 s | <148 s | Acc = 0.98 | 97.5 | 100 |
| | IceQube | 1 s | ≤245 s | Acc = 0.97 | 96.7 | 100 |

**Supplemental Table 4.** Continued over page.

**Supplemental Table 4 (Continued).** Summary of reported measures of agreement for a range of accelerometers validated since 2003 for lying bouts in dairy cows.

| Source | Device[1] | Sampling interval[2] | LB criterion[3] | Reliability[4] | Se[5] | Sp[6] |
|---|---|---|---|---|---|---|
| Charlton et al. (2017) | IceQube | 1 s | - | $R^2 = 0.76^{***}$ | - | - |
| Henriksen and Munksgaard (2019) | AfiTagII | 1 min | <3 min | $R^2 = 0.97^{**}$ | - | - |

Text under table on next page.

Text under table from previous page.

[1]Device: Actiwatch = The Actiwatch® Activity Monitoring System (Cambridge Neurotechnology, Cambridgeshire, UK), AfiAct = AfiAct® Pedometer Plus (ceased manufacture) and AfiTagII = AfiTag® II Pedometer (Afimilk, Kibbutz, Afikim, Israel), ACT = Automatic Milking System Activity Monitor (Lely, Maassluis, The Netherlands), HOBO = HOBO Pendant G Acceleration Data Logger (Onset Computer Corporation, Pocasset, MA), IceTag 3D = IceTag 3D Activity Monitor, IceTag 2D = IceTag 2.004 Activity Monitor (ceased manufacture 2008) and IceQube = IceQube Activity Monitor (IceRobotics Ltd., Edinburgh, Scotland), CowScout = CowScout Leg Sensor (GEA Farm Technologies, Bönen, Germany), TAC = The Track a Cow (ENGS, Rosh Pina, Israel).
[2]Sampling interval describes time between samples of the device and within each sampling interval is a summary of all registered counts of data recorded at a predetermined sampling frequency.
[3]Lying bout (LB) criterion describes the minimum LB duration that is considered a true bout of lying and LB ≤ the LB criterion are removed from the data to discard false LB before interpretation. If no LB criterion is presented, the study did not apply a LB criterion to the data.
[4]Reliability is described by the following agreement statistics: $\rho_c$ = concordance correlation coefficient, $R^2$ = coefficient of determination ([x]Statistical significance were not reported. [*]Slope and intercept were significantly different ($P < 0.05$) from 1 and 0, respectively. [**]Slope and intercept were significantly different ($P < 0.01$) from 1 and 0, respectively. If no superscript is presented, the slope and intercept were not different from 1 and 0), $r_p$ = Pearson correlation coefficient ([*]$P < 0.05$, [**]$P < 0.01$; [***]$P < 0.001$), $r_s$ = Spearman rank correlation ([*]$P < 0.05$, [**]$P < 0.01$; [***]$P < 0.001$). If no reliability measures are presented, the study did not present reliability measures.
[5]Se = sensitivity.
[6]Sp = specificity.

# APPENDIX 6. SUPPLEMENTAL TABLE 5

**Supplemental Table 5. Reported editing criteria applied to IceTag devices in studies undertaken in grazing cows or cows kept on pasture and fed total mixed rations.**

Description of editing criteria applied to IceTag 3D devices (IceRobotics Ltd., Edinburgh, Scotland) used in studies where cattle were grazing pasture, kept on pasture, or had access to pasture where the interest was in measuring both lying time and lying bouts (LB) published from 2010 to 2019. The source, subject and system description, LB criterion, and validation study referenced are described in the table. TMR = total mixed ration.

| Source | Subjects; system description[1] | LB criterion[2] | Validation study referenced |
|---|---|---|---|
| Umstatter et al. (2015) | Dry Aberdeen Angus x Limousin and Charolais cows; Grazing pasture | ≤4 min | Tolkamp et al. (2010) |
| Black and Krawczel (2016) | Dry Holstein cows; Indoor deep-bedded or sand freestalls or pasture | ≤2 min | Endres and Barberg (2007) |
| Rice et al. (2017) | Dry pregnant Holstein cows; Fed TMR and moved to pasture 1 wk precalving | <2 min | Munksgaard et al. (2006); Bewley et al. (2010) |
| Black and Krawczel (2019) | Dry Holstein and Holstein-Jersey cross cows; Freestall barn fed TMR with pasture access | <2 min | Endres and Barberg (2007) |

[1]Subjects and system description describes the physiological state and breed of the subjects and the management system the cows were kept under.

[2]Lying bout (LB) criterion describes the minimum LB duration that is considered a true bout of lying and LB ≤ the LB criterion are removed from the data to discard false LB before interpretation.

# APPENDIX 7. SUPPLEMENTAL TABLE 6

**Supplemental Table 6. Description of the 4 parent experiments that were included in the collated database.**

Description of the 4 parent experiments [nonsteroidal anti-inflammatory drugs (NSAID), body condition score (BCS), feed, and zeolite studies] including the number of cows (n), location, range of calving dates, and reference of experimental methods pertaining to each study.

| Study | n (cows) | Location[1] | Calving dates | Reference |
|---|---|---|---|---|
| NSAID | 24 | Whareroa Farm, Hawera, New Zealand | Jul 13 – Aug 10, 2012 | Meier et al. (2014) |
| BCS | 136 | Scott Farm, Hamilton, New Zealand | Jul 24 – Aug 6, 2013 | Roche et al. (2015) |
| Feed | 108 | Lye Farm, Hamilton, New Zealand | Jul 1 – Aug 4, 2014 | Roche et al. (2017a) |
| Zeolite | 42 | Lye Farm, Hamilton, New Zealand | Jun 30 – Jul 20, 2016 | (Roche et al., 2018; J. R. Roche, unpublished data[2]) |

[1]Whareroa Farm, Hawera, New Zealand (39.6°S, 174.3°E); Scott Farm: Hamilton, New Zealand (37°46'S 175°18'E); Lye Farm: Hamilton, New Zealand (37°46'S 175°18'E).

[2]In brief, multiparous Holstein-Friesian cows were randomly allocated to 1 of 2 treatment groups: untreated control (n = 25) and a treatment group (n = 25) receiving 500 g/cow per d Zeolite A (80% sodium aluminosilicate, synthetic embedded in starch; Optimate MF+, Blue Pacific Minerals, New Zealand). Treated cows were individually supplemented prepartum with 2–3 kg DM/cow per d maize silage mixed with the zeolite supplement. Control cows individually received the same daily allowance of maize silage precalving, but without the zeolite supplement. Maize silage was fed once daily precalving before cows received a common fresh allocation of pasture. Zeolite supplementation ceased at the first signs of calving, and all cows were managed similarly on a common pasture-based diet postcalving. All cows were grazing ryegrass/white clover pasture with a fresh allocation of pasture at least once daily.

# APPENDIX 8. SUPPLEMENTAL TABLE 7

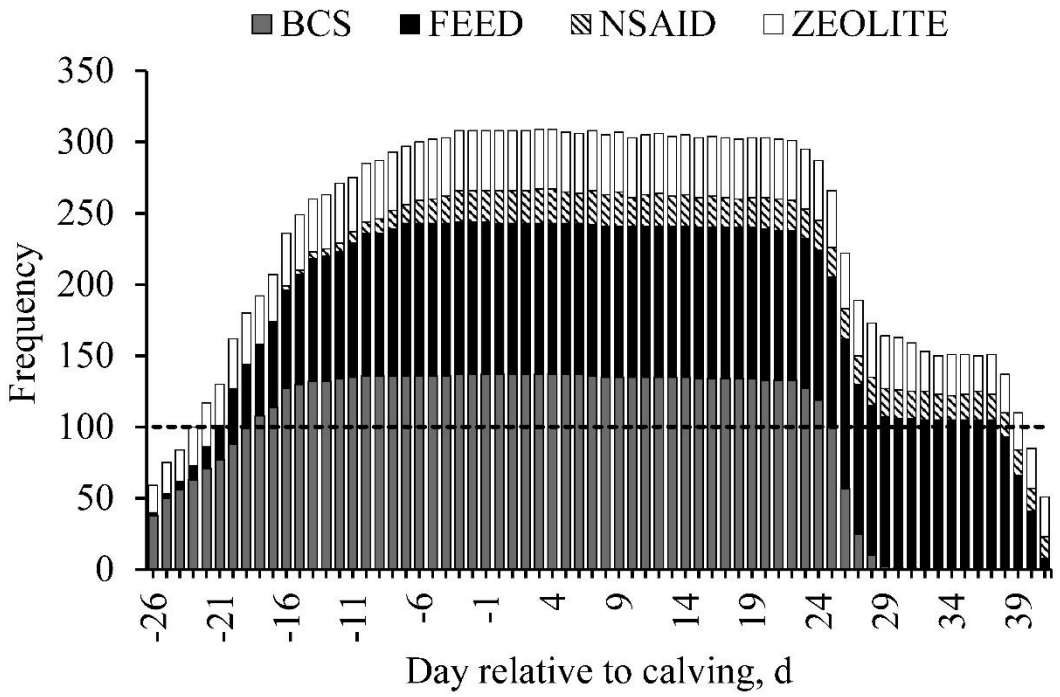
**Supplemental Table 7. Reason for removal of cows from the collated behavior database.**

Number of cows (n) removed from the collated behavior database, cow ID, and reason for removal of cows for the body condition score (BCS), feed, nonsteroidal anti-inflammatory drugs (NSAID), and zeolite studies. Device errors are where cows were removed due to incomplete data, inaccessible files, or where the device fell off during the experimental period and data could not be retrieved.

| Reason for removal | n | Cow ID |
|---|---|---|
| BCS Study | | |
| Device errors | 0 | |
| Sickness/Mortality[1] | 2 | 6008, 9657 |
| Feed Study | | |
| Device errors | 12 | 149, 157, 161, 168, 175, 4001, 5110, 6050, 6057, 7109, 7677, 8841, 8846, 9127, 9165, 9249, 9264, 9809 |
| Sickness/Mortality[1] | 3 | 5052, 7110, 7224 |
| NSAID Study | | |
| Device errors | 39 | 4, 10, 12, 15, 21, 37, 40, 44, 46, 51, 306, 307, 309, 319, 331, 335, 370, 371, 588, 695, 700, 862, 939, 1259, 1321, 1348, 1351, 1362, 1364, 1378, 1416, 1455, 1513, 1561, 1596, 1600, 1601, 1609, 1666 |
| Sickness/Mortality[1] | 0 | |
| Zeolite Study | | |
| Device errors | 8 | 139, 1128, 1186, 2131, 2132, 2143, 2673, 8871 |
| Sickness/Mortality[1] | 5 | 631, 1134, 3110, 3664, 8623 |
| Total removed | 69 | |

[1]Sickness/mortality refers to cows removed due to clinical disease (e.g., infection, dystocia, mastitis, or milk fever) or cows requiring veterinary intervention.

## APPENDIX 9. SUPPLEMENTAL FIGURE 1




**Supplemental Figure 1. The frequency distribution of the number of cows with behavior records across the 4 parent experiments.**

The frequency distribution of the number of cows with behavior records in the combined behavior database for each of the 4 studies [body condition score (BCS), feed, nonsteroidal anti-inflammatory drugs (NSAID), and zeolite studies] for the period –26 to +41 d relative to the day of calving (d 0).

# APPENDIX 10. SUPPLEMENTAL MATERIALS AND SUPPLEMENTAL TABLE 8

### *Appendix 10.1 Removing False Lying Bouts from Raw Data Recorded by Accelerometer Devices*

A suitable threshold for the minimum duration of a lying bout (**LB**) record should be specified before LB data are analyzed to discard false records from the original data caused by minor movements due to shifts in position, grooming, or grazing (O'Driscoll et al., 2008; Kok et al., 2015). In many studies, however, thresholds are not used or thresholds applied are not suitable for the device of interest (Arachchige et al., 2013; Kok et al., 2015). It is important in studies under grazing conditions that erroneous bouts are removed from the raw data due to the overestimation of LB that occurs in cows at pasture (Rutter et al., 2014). In our study, based on previously determined thresholds for IceRobotics sensors (IceRobotics Ltd., Edinburgh, Scotland), LB <33 s and ≤2 min were discarded from the raw data recorded by the IceQube (Kok et al., 2015) and IceTag devices, respectively (Mattachini et al., 2013). The descriptive data for the unedited and edited data are presented in Supplemental Table 8.

**Supplemental Table 8. Descriptive data for the unedited and edited data for IceQube and IceTag devices during the transition period.**

Number of records (n), mean, standard deviation (SD) of the daily lying bouts (LB; no./d) and LB duration (min/bout) for the period –21 to +34 d relative to calving (d 0).

| | IceQube | | | IceTag | | |
|---|---|---|---|---|---|---|
| Unedited data | n | mean | SD | n | mean | SD |
| LB, no/d | 63,851 | 8.80 | 3.63 | 2,285,025 | 305 | 293 |
| LB duration, min/bout | 63,851 | 58.2 | 51.3 | 2,285,025 | 1.76 | 12.9 |
| Edited data | LB <33 s removed | | | LB ≤2 min removed | | |
| LB, no./d | 62,607 | 8.02 | 3.02 | 65,462 | 8.74 | 4.06 |
| LB duration, min/bout | 62,607 | 63.8 | 50.3 | 65,462 | 56.3 | 52.5 |

# APPENDIX 11. SUPPLEMENTAL TABLE 9

Blood calcium data were summarized for 3 periods postcalving including the day of calving (d 0), and d 1 and 2 postcalving on a per-cow basis. The overall number of records, means, standard deviation, minimum, and maximum values are presented in Supplemental Table 9.

**Supplemental Table 9. Blood calcium concentration parameters for all cows.**
Number of records (n), mean, standard deviation (SD), minimum, and maximum values for blood calcium (Ca) concentrations (mmol/L) on the day of calving (d 0), and d 1 and 2 postcalving.

| Parameter | n | mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Blood Ca, mmol/L | | | | | |
| d 0 | 67 | 2.12 | 0.30 | 1.19 | 3.02 |
| d 1 postcalving | 72 | 1.81 | 0.37 | 0.95 | 2.52 |
| d 2 postcalving | 67 | 1.79 | 0.34 | 1.14 | 2.36 |

# APPENDIX 12. SUPPLEMENTAL MATERIAL AND SUPPLEMENTAL TABLE 10

Change in daily lying time and daily steps taken and change in hourly daytime (between 0600 and 1800 h) lying time and hourly daytime steps taken were calculated as the difference between the behavior on the day before calving (d –1) or day of calving (d 0) and 2 baseline periods (d –21 to –7 or d –14 to –7 precalving). To summarize the 4 baseline periods for each behavior, the mean daily and hourly daytime lying time and steps taken were summarized using PROC MEAN in SAS on a per-cow basis for all 72 cows. Mean daily and hourly daytime lying time and steps taken on d –1 or d 0 were subtracted from the mean daily and hourly daytime lying and steps taken during d –21 to –7 or d –14 to –7 precalving on a per-cow basis. The overall number of records, means, standard deviation, minimum, and maximum values for changes in daily and hourly daytime lying time and steps taken are presented in Supplemental Table 10.

**Supplemental Table 10. Change in daily and hourly daytime lying behavior and activity parameters calculated for all cows.**

Number of records (n), mean, standard deviation (SD), minimum, and maximum values for change in (Δ) daily lying time (h/d), Δ daily steps taken (steps/d), Δ hourly daytime lying time (min/h), and Δ hourly daytime steps taken (steps/h).

| Parameter[1] | n | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Δ Daily lying time, h/d | | | | | |
| d –14/–7 precalving to d 0 | 72 | –2.68 | 2.84 | –9.84 | 8.30 |
| d –21/–7 precalving to d 0 | 72 | –2.61 | 2.89 | –9.51 | 9.53 |
| d –14/–7 precalving to d –1 | 72 | –2.38 | 2.19 | –7.26 | 2.00 |
| d –21/–7 precalving to d –1 | 72 | –2.31 | 2.19 | –6.41 | 2.92 |
| Δ Daily steps taken, steps/d | | | | | |
| d –14/–7 precalving to d 0 | 72 | 1,572 | 1,367 | –1,871 | 5,247 |
| d –21/–7 precalving to d 0 | 72 | 1,621 | 1,348 | –1,703 | 5,037 |
| d –14/–7 precalving to d –1 | 72 | 2,657 | 1,085 | 2.38 | 5,810 |
| d –21/–7 precalving to d –1 | 72 | 2,657 | 1,085 | 2.66 | 5,810 |
| Δ Hourly daytime lying time, min/h[2] | | | | | |
| d –14/–7 precalving to d 0 | 72 | 3.01 | 7.90 | –13.5 | 31.7 |
| d –21/–7 precalving to d 0 | 72 | 2.66 | 8.01 | –13.4 | 32.0 |
| d –14/–7 precalving to d –1 | 72 | –1.32 | 4.44 | –14.5 | 10.5 |
| d –21/–7 precalving to d –1 | 72 | –1.67 | 4.53 | –14.5 | 10.4 |
| Δ Hourly daytime steps taken, steps/h[2] | | | | | |
| d –14/–7 precalving to d 0 | 72 | 70 | 82 | –144 | 287 |
| d –21/–7 precalving to d 0 | 72 | 72 | 81 | –136 | 272 |
| d –14/–7 precalving to d –1 | 72 | 9 | 69 | –143 | 204 |
| d –21/–7 precalving to d –1 | 72 | 10 | 69 | –171 | 207 |

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[1]d 0 = day of calving; d –1 = the day before calving.
[2]Daytime = behavior recorded between 0600 and 1800 h.

# APPENDIX 13. SUPPLEMENTAL TABLE 11

**Supplemental Table 11. Correlation matrix for the associations between change in lying behavior and activity precalving and blood calcium concentrations postcalving.**

Best Pearson correlation coefficients between change in (Δ) daily lying time (h/d), Δ daily steps taken (steps/d), Δ hourly daytime (behavior recorded between 0600 and 1800 h) lying time (min/h), and Δ hourly daytime steps taken (steps/h) and postcalving blood calcium (Ca) concentrations (mmol/L) at 3 different times: day of calving (d 0), d 1 and 2 postcalving. Associations significant at the 10% level are marked with an asterisk*.

| Parameter[1] | Blood Ca, mmol/L | | |
|---|---|---|---|
| | d 0 | d 1 | d 2 |
| Δ Daily lying time, h/d | | | |
| d –14/–7 precalving to d 0 | –0.35* | –0.22* | –0.33* |
| d –21/–7 precalving to d 0 | –0.37* | –0.23* | –0.34* |
| d –14/–7 precalving to d –1 | –0.01 | –0.25* | –0.27* |
| d –21/–7 precalving to d –1 | –0.04 | –0.27* | –0.29* |
| Δ Daily steps, steps/d | | | |
| d –14/–7 precalving to d 0 | 0.38* | 0.25* | 0.38* |
| d –21/–7 precalving to d 0 | 0.36* | 0.23* | 0.37* |
| d –14/–7 precalving to d –1 | 0.05 | 0.05 | 0.08 |
| d –21/–7 precalving to d –1 | 0.05 | 0.05 | 0.08 |
| Δ Hourly daytime lying time, min/h | | | |
| d –14/–7 precalving to d 0 | –0.41* | –0.31* | –0.43* |
| d –21/–7 precalving to d 0 | –0.40* | –0.31* | –0.43* |

**Supplemental Table 11.** Continued over page.

**Supplemental Table 11 (Continued).** Best Pearson correlation coefficients between change in (Δ) daily lying time (h/d), Δ daily steps taken (steps/d), Δ hourly daytime lying time (min/h), and Δ hourly daytime steps taken (steps/h) and blood calcium (Ca) concentrations (mmol/L) at 3 different times: day of calving (d 0), d 1 and 2 postcalving. Associations significant at the 10% level are marked with an asterisk*.

| Parameter[1] | Blood Ca, mmol/L | | |
|---|---|---|---|
| | d 0 | d 1 | d 2 |
| Δ Hourly daytime lying time, min/h | | | |
| d –14/–7 precalving to d –1 | –0.10 | 0.01 | –0.05 |
| d –21/–7 precalving to d –1 | –0.09 | 0.01 | –0.06 |
| Δ Hourly daytime steps, steps/h | | | |
| d –14/–7 precalving to d 0 | 0.36* | 0.25* | 0.43* |
| d –21/–7 precalving to d 0 | 0.37* | 0.24* | 0.42 |
| d –14/–7 precalving to d –1 | 0.02 | –0.03 | 0.12 |
| d –21/–7 precalving to d –1 | 0.02 | –0.05 | 0.10 |

[1]d 0 = day of calving; d –1 = the day before calving.
[2]Daytime = behavior recorded between 0600 and 1800 h.

# APPENDIX 14. SUPPLEMENTAL TABLE 12

**Supplemental Table 12. Linear and nonlinear associations between change in daily lying behavior and activity precalving and blood calcium concentrations 24 h postcalving.**

Regression coefficient [estimate and standard error (SE)] for linear and nonlinear associations between change in (Δ) daily lying time (h/d) and Δ daily steps taken (steps/d) from a baseline period (d –14 to –7 precalving) until the day of calving (d 0) with blood calcium (Ca) concentrations (mmol/L) within 24 h postcalving.

| Parameter | | | 95% Confidence Limits | | |
|---|---|---|---|---|---|
| Blood Ca, mmol/L (24 h postcalving) | Estimate | SE | Lower | Upper | *P*-value |
| Δ Daily lying time models | | | | | |
| Intercept | 1.65 | 0.06 | 1.53 | 1.78 | <0.001 |
| Linear (Δ Daily lying time), h/d | –0.05 | 0.02 | –0.08 | –0.02 | 0.004 |
| R-squared | 0.12 | | | | |
| Intercept | 1.64 | 0.06 | 1.53 | 1.76 | <0.001 |
| Linear (Δ Daily lying time), h/d | –0.09 | 0.03 | –0.14 | –0.04 | 0.001 |
| Quadratic (Δ Daily lying time), h/d | –0.008 | 0.004 | –0.016 | –0.0003 | 0.048 |
| R-squared | 0.18 | | | | |
| Δ Daily steps models | | | | | |
| Intercept | 1.63 | 0.06 | 1.50 | 1.75 | <0.001 |
| Linear (Δ Daily steps per 1000 units),[1] steps/d | 0.10 | 0.03 | 0.04 | 0.16 | 0.002 |

**Supplemental Table 12.** Continued over page.

**Supplemental Table 12 (Continued).** Regression coefficient [estimate and standard error (SE)] for linear and nonlinear associations between change in (Δ) daily lying time (h/d) and Δ daily steps taken (steps/d) from a baseline period (d –14 to –7 precalving) until the day of calving (d 0) with blood calcium (Ca) concentrations (mmol/L) within 24 h postcalving.

| Parameter | | | 95% Confidence Limits | | |
|---|---|---|---|---|---|
| Blood Ca, mmol/L (24 h postcalving) | Estimate | SE | Lower | Upper | *P*-value |
| Δ Daily steps models | | | | | |
| R-squared | 0.14 | | | | |
| Intercept | 1.60 | 0.07 | 1.46 | 1.74 | <0.001 |
| Linear (Δ Daily steps per 1000 units),[1] steps/d | 0.16 | 0.07 | 0.02 | 0.29 | 0.021 |
| Quadratic (Δ Daily steps per 1000 units),[1,2] steps/d | –0.16E-4 | 0.20E-4 | –0.46E-4 | –0.15E-4 | 0.314 |
| R-squared | 0.16 | | | | |

[1]Steps taken per 1000 unit increase.
[2]E = 10 to the power of.

# APPENDIX 15. SUPPLEMENTAL TABLE 13

**Supplemental Table 13. Linear and nonlinear associations between changes in hourly daytime lying behavior and activity precalving and blood calcium concentrations 24 h postcalving.**

Regression coefficient [estimate and standard error (SE)] for linear and nonlinear associations between change in (Δ) hourly daytime (behavior recorded between 0600 and 1800 h) lying time (min/h) and Δ hourly daytime steps taken (steps/h) from a baseline period (d –14 to –7 precalving) until the day of calving (d 0) with blood calcium (Ca) concentrations (mmol/L) within 24 h postcalving.

| Parameter | | | 95% Confidence Limits | | |
|---|---|---|---|---|---|
| Blood Ca, mmol/L (24 h postcalving) | Estimate | SE | Lower | Upper | *P*-value |
| Δ Hourly daytime lying model | | | | | |
| Intercept | 1.83 | 0.04 | 1.76 | 1.91 | <0.001 |
| Linear (Δ Hourly daytime lying time), min/h | –0.02 | 0.005 | –0.03 | –0.009 | <0.001 |
| R-squared | 0.17 | | | | |
| Δ Hourly daytime steps model | | | | | |
| Intercept | 1.68 | 0.06 | 1.50 | 1.75 | <0.001 |
| Linear (Δ Hourly daytime steps),[1] steps/h | 0.002 | 0.005 | 0.5E-3 | 0.002 | 0.002 |
| R-squared | 0.13 | | | | |

[1]E = 10 to the power of.

# APPENDIX 16. SUPPLEMENTAL MATERIALS (CHAPTER 7)

Endometrial and protein metabolite measures associated with blood calcium (**Ca**) status were analyzed in addition to the measures presented in Chapter 7; however, these additional analyses were not submitted for publication and are presented below. Cows were retrospectively classified into 1 of 3 blood Ca groups and this is explained in detail in Chapter 7 (Section 7.3.3). Briefly, cows were classified as clinically-hypocalcemic (without paresis; **CLIN**) when blood Ca was ≤1.4 mmol/L within 48 h postcalving, as subclinically-hypocalcemic (**SUB**) when blood Ca was >1.4 and <2.0 mmol/L within 48 h postcalving and normocalcemic (**NORM**) when blood Ca was ≥2.0 mmol/L within 72 h postcalving.

### *Appendix 16.1 Additional Methodology*

***Blood Sampling and Analyses.*** Blood was sampled by coccygeal venipuncture weekly, from wk 4 pre- to wk 5 postcalving, and additionally on d 0, and d 1, 2, 3, and 4 postcalving in both studies. Blood was collected in evacuated blood tubes containing lithium heparin anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Heparinized samples were placed immediately into iced water following collection and were centrifuged within 30 min of collection at 1,500 x *g* for 12 min at 4°C. Following centrifugation, aspirated plasma was stored at –20°C until assayed. Plasma samples were submitted to Gribbles Veterinary Pathology Ltd. (Hamilton, New Zealand) for analysis. Blood metabolites were assayed using colorimetric techniques at 37°C with a Hitachi Modular P800 analyzer (Roche Diagnostics, Indianapolis, IN). Roche reagent kits were used to measure plasma concentrations of albumin (**ALB**; g/L; bromocresol green reaction at pH 4.1), Ca (mmol/L; o-cresolphthalein complexone method), magnesium (Mg) (mmol/L; xylidyl blue reaction), and total protein (**TP**, g/L; biuret method). Plasma

globulin (**GLO**; g/L) was calculated at the difference between TP and ALB. The inter- and intra-assay coefficients of variation for all assays were <5.5% and ≤15%, as are reported in Roche et al. (2015) and (2017a).

***Endometrial Cytology and Metricheck Sampling.*** Uterine endometrial cytology samples were collected at 10 to 16 d postcalving and 31 to 37 d postcalving as described by Meier et al. (2014). Briefly, a stylet with a cytology brush attached was used to collect a sample from the uterine wall. The brush was rolled onto a microscope slide and its contents air-dried. The dry slides were stained using Diff-Quik (Dade Behring, Newark, DE) and a veterinary pathologist (IVABS, Massey University, Palmerston North, New Zealand) determined the proportion of polymorphonucleated cells (**PMNC**, %) on the swab. Areas of each slide that contained small clusters of epithelial cells (5 to 20 per cluster) were preferentially selected and all identifiable nucleated cells counted. Approximately 200 nucleated cells per slide were enumerated, with PMNC distinguished from non-PMNC, to allow the proportions of nucleated cells that were PMNC to be calculated.

On the completion of endometrial sampling, vaginal content was sampled using a Metricheck device (Simcro Tech Ltd., Hamilton, New Zealand); this device consists of a 40-mm-diameter hemisphere of silicon attached to a 500-mm-long stainless steel rod. The vaginal content was scored (0 being no sample, 1 being clear mucus, and 5 being purulent discharge; McDougall et al., 2007).

### *Appendix 16.2 Statistical Analyses*

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Results are presented as least squares means (**LSM**) ± standard error of the mean in the text and mean standard error of the difference in tables and figures. The covariance

structures selected were compound symmetry or autoregressive based on the lowest Akaike's information criterion. Study and treatment from the parent experiments were concatenated to create a categorical variable, study group. All data were adjusted where appropriate, according to the re-assigned calving day (as described in Chapter 7: Section 7.3.5), and these transformed datasets were the basis of subsequent analyses.

Study group (categorical), parity (categorical; parity 2–3 or parity 4+), and the difference in days between calving date and the first day in June (**calvingseasonday**) were included to adjust for different treatments within the 2 studies, parity differences, and different calving dates in all models described below.

***Blood Metabolite and Mineral Markers.*** Blood data for protein metabolites [TP, ALB, GLO, albumin:globulin ratio (**AGR**)] were summarized into 6 periods [i.e., wk –1 and –2 precalving, d 0 to 2 postcalving, d 3 to 7 postcalving, and then weekly postcalving (wk 2 to 4)].

To investigate the associations between protein metabolites, and Ca status and period, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, and the fixed effect of the Ca status, period, and Ca status x period interactions. Variables were checked for skewness and to meet the assumption of normal distribution. Log-transformation was used to normalize blood AST and for the analysis, and untransformed LSM, standard error of the mean, and mean standard error of the difference are presented.

***Endometrial Cytology and Metricheck.*** Metricheck scores and PMNC were available for 2 periods postcalving: 10 to 16 d and 31 to 37 d postcalving. To investigate the associations between metricheck score and PMNC and energy status, a PROC

MIXED ANOVA was undertaken with the fixed effect of Ca status, group, and parity as fixed effects.

### *Appendix 16.3 Results and Discussion*

***Endometritis.*** Metricheck score at 10 to 16 d postcalving and PMNC (%) at 10 to 16 d and 31 to 37 d postcalving were not different between the 3 blood Ca groups (Supplemental Table 14); however, at 31 to 37 d postcalving, 20% of the cows in the CLIN group had a metricheck score ≥2, but no cows in the SUB or NORM groups had metricheck scores ≥2. Metricheck score >1 has been associated with reduced reproductive performance and endometritis, which is an infection of the endometrium without signs of systemic illness and detected through visualization of purulent material in the vagina (McDougall et al., 2007). Overall, the CLIN group had greater ($P < 0.05$) metricheck scores than cows in the SUB and NORM groups, which were not different (Supplemental Table 14). This could indicate cows experiencing hypocalcemia are at increased risk for intrauterine infection (Whiteford and Sheldon, 2005). Hypocalcemia reduces smooth muscle and has been associated with myometrial contractility (Murray et al., 2008) and, therefore, may affect uterine involution (Heppelmann et al., 2015), which has been reported to be negatively associated with reproductive performance (LeBlanc et al., 2002). Interestingly, evidence exists to suggest an association between low ALB and AGR pre- and postcalving and endometritis (Burke et al., 2010).

**Supplemental Table 14. Endometrial and cytology results for 3 blood calcium groups at 2 sampling points postcalving.**

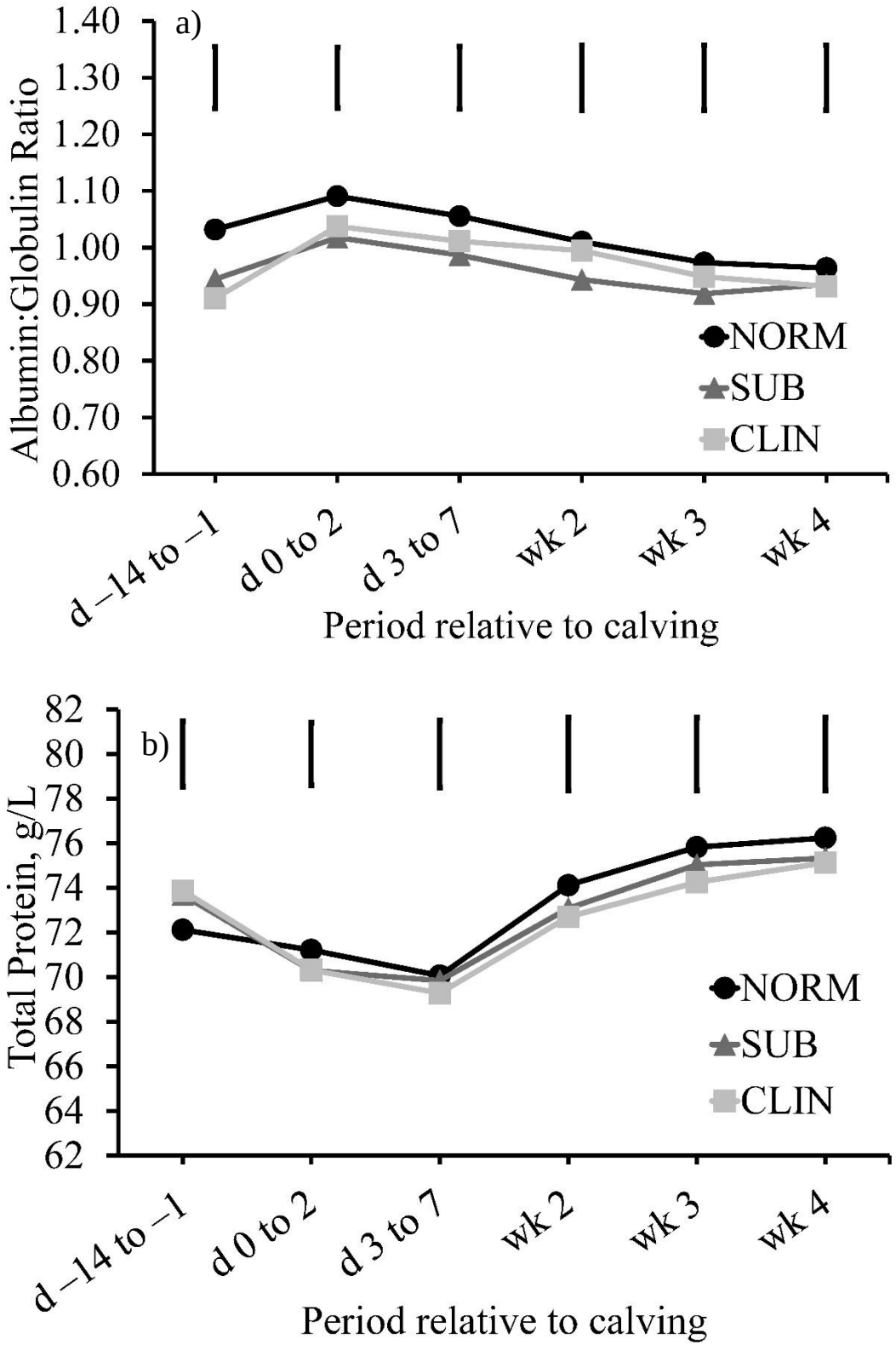
Overall mean polymorphonucleated cells (PMNC, %) and metricheck score differences between the 3 calcium (Ca) groups [CLIN (blood Ca ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca ≥2.0 mmol/L within 72 h postcalving)].

| Parameter | NORM | SUB | CLIN | SED[1] | *P*-value |
|---|---|---|---|---|---|
| 10 to 16 d postcalving | | | | | |
| PMNC, % | 27.5 | 36.0 | 42.7 | 10.7 | 0.41 |
| Metricheck score | 1.25 | 1.71 | 1.69 | 0.34 | 0.35 |
| 31 to 37 d postcalving | | | | | |
| PMNC, % | 13.1 | 10.6 | 16.2 | 6.17 | 0.65 |
| Metricheck score | 0.98[b] | 0.89[b] | 1.41[a] | 0.19 | <0.01 |

[a–b]Means with different superscripts are significantly different at the 5% confidence level.
[1]SED = mean standard error of the difference.

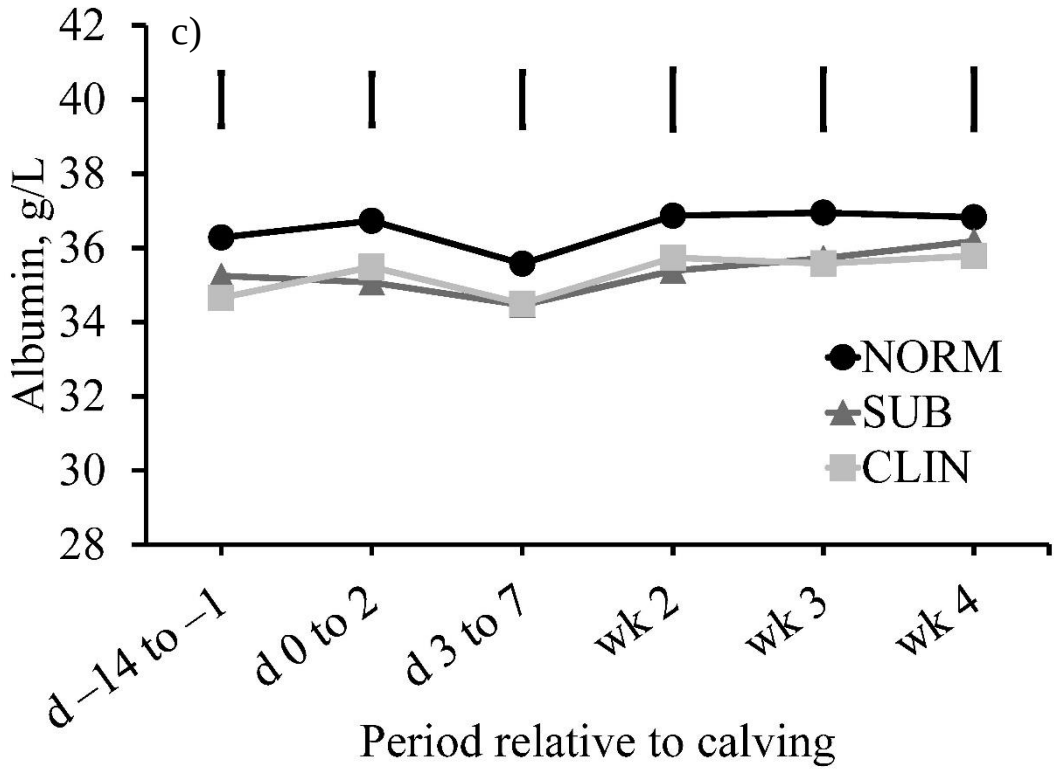
***Protein Metabolites.*** Overall there was no association between Ca status and AGR (*P* = 0.46), GLO (*P* = 0.83), and TP (*P* = 0.88); There was no interaction of Ca status x period on TP (*P* = 0.20), and ALB concentration (*P* = 0.72) (Supplemental Figures 2b and c). In our study, blood ALB concentration tended to be associated with Ca status (*P* = 0.11), where blood ALB tended to be elevated in the NORM group compared with the CLIN group (36.5 ± 0.50 vs. 35.3 ± 0.42 g/L; *P* = 0.06). There was an interaction of Ca status x period for blood AGR (*P* < 0.05), and a tendency for a Ca status x period interaction on GLO concentration (*P* = 0.12) (Supplemental Figures 2a and d). During the period from –14 to –1 d precalving, cows in the NORM group had greater AGR (1.09 ± 0.04 vs. 0.91 ± 0.04; *P* < 0.05) than cows in the CLIN group because of lower (*P* < 0.05) blood GLO concentrations in the NORM group compared with the CLIN group

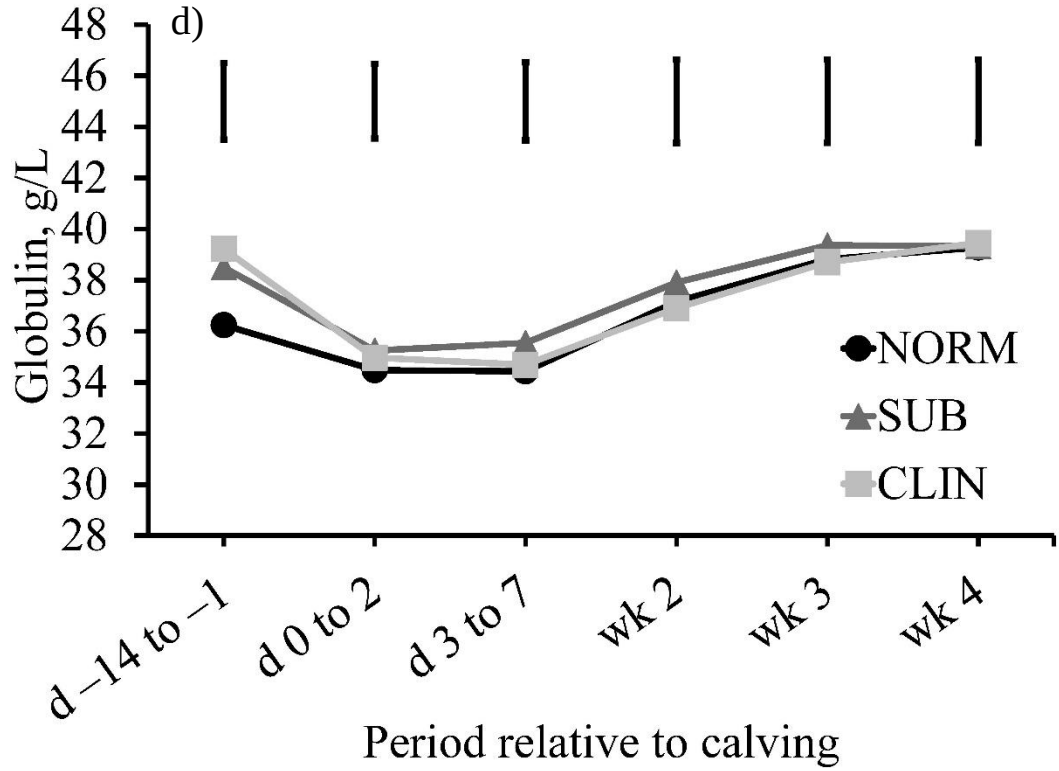
(36.2 ± 1.23 vs. 39.2 ± 1.07 g/L; $P < 0.01$); however, these differences were short lived and further differences were not present postcalving. The decrease in ALB and AGR may be a secondary effect to impaired liver function (Bertoni et al., 2008) and this may play a role in predisposing cows to hypocalcemia; however, further research is needed to understand these complex associations.




**Supplemental Figure 2. Protein metabolite concentrations during the transition period in 3 energy status groups.**

Figure and figure legend continued over page.




**Supplemental Figure 2 (Continued).** Albumin to globulin ratio (a), total protein [g/L; (b)], albumin [g/L; (c)], and globulin [g/L; (d)] during d –14 to –1 precalving, d 0 to 2

postcalving, d 3 to 7 postcalving, and wk 2 to 4 postcalving for 3 calcium (Ca) groups [CLIN (blood Ca concentration ≤1.4 mmol/L within 48 h postcalving); SUB (blood Ca concentration >1.4 and <2.0 mmol/L within 48 h postcalving); NORM (blood Ca concentration ≥2.0 mmol/L within 72 h postcalving)]. Error bars represent 2 x mean standard error of the difference.

# APPENDIX 17: STATISTICAL APPROACHES CONSIDERED

Predetermined thresholds to categorize cows based on a single variable (i.e., blood mineral and metabolic markers) are typically based on studies from cows in housed systems and there is some uncertainty as to whether it is appropriate to apply these thresholds in grazing dairy cows. A key objective of this thesis was to understand the associations between behavior and transition-cow disease. To achieve this, I initially attempted a principal component analysis (**PCA**) that would allow the use of multiple variables (i.e., blood minerals, metabolites, and immune markers) from the database to simplify complex interactions between these variables across multiple time points during the transition period. A PCA is a dimension reduction technique that allows simple and interpretable factors to be obtained by transforming multiple original parameters into a new set of linear combinations (principal components; **PC**) that represent the data's variance (Budaev, 2010; Tremblay et al., 2018). The PCA separated the blood minerals and metabolites into separate PC where parameters were clustered according to blood mineral and metabolite markers indicating varying degrees of hypocalcemia and hyperketonemia, rather than complex disease states where an animal has 1 or more conditions. Unfortunately, I was unable to simplify the complex interactions expected, which may be, in part, related to the size of the dataset. The likelihood that complex associations between variables could allow animals to be clustered into groups based on their disease state (e.g., diseased vs. non-diseased) would increase with a larger dataset (Tremblay et al., 2018; Xu et al., 2019). The PCA was deemed an inappropriate technique to analyze the data in this thesis and instead, predetermined thresholds were used to differentiate subclinically ill and non-diseased animals for specific metabolic diseases; however, PCA is a rudimentary form of machine-based learning, which, could be an

appropriate approach to use in future work and is already being used in research to predict calving (Borchers et al., 2017), cow performance (Shahinfar et al., 2014; Dolecheck et al., 2015), or disease events (Xu et al., 2019) using large datasets.

# APPENDIX 18. SUPPLEMENTAL MATERIALS (CHAPTER 8)

Estimated DMI, protein metabolites, liver enzymes, proinflammatory cytokines, inflammatory markers, and liver triacylglyceride (**TAG**) associated with energy status were analyzed in addition to the measures presented in Chapter 8; however, these additional analyses were not submitted for publication and are presented below. Cows were retrospectively classified into 1 of 3 energy status groups and this is explained in detail in Chapter 8 (Section 8.3.3). Briefly, cows were classified as having low blood non-esterified fatty acids (**NEFA**) and low BHB (**Lo–Lo**) when both blood NEFA was <1.0 mmol/L and blood BHB was ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving), cows were classified as high NEFA and low BHB (**Hi–Lo**) when both blood NEFA was ≥1.0 mmol/L and blood BHB was ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving, and cows were classified as high NEFA and high BHB (**Hi–Hi**) when both blood NEFA was ≥1.0 mmol/L and blood BHB was ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving.

### *Appendix 18.1 Additional Methodology*

***Estimated DMI.*** Weekly DMI was estimated by back-calculation from the energy requirements of the cows (Holmes and Davey, 1981; Nicol and Brookes, 2007), using the BW of each cow, calculated BW change of each cow per week, pregnancy and activity requirements, the measured milk yield postcalving and milk composition, and the estimated metabolizable (**ME**) content of pasture for the period –4 to –1 wk precalving and 1 to 6 wk postcalving.

Maintenance requirements were calculated on a daily basis using weekly mean BW during the pre- and postcalving period. Maintenance calculations are similar for dry

(Holmes and Davey, 1981) and lactating cows (Nicol and Brookes, 2007). Maintenance requirements were calculated as:

$$Maintenance\ (Dry)(MJ/d) = 0.55\ MJ \text{ x BW}^{0.75}\ (kg)$$

$$Maintenance\ (Lactating)(MJ/d) = 0.56\ MJ \text{ x BW}^{0.75}\ (kg)$$

As recommended by Nicol and Brookes (2007), 5% per MJ ME was added(subtracted) from the maintenance requirements for the days that the diet was below(above) 11.0 MJ ME/kg DM.

Activity requirements associated with grazing and additional costs of walking were calculated on a daily basis using an approximate horizontal distance walked per day of 2 km pre- and 4 km postcalving (Nicol and Brookes, 2007). Activity requirement was added to the total maintenance requirement and was calculated as:

$$Activity\ (MJ/d) = 0.0037\ MJ \text{ x BW } (kg) \text{ x horizontal distance walked } (km)$$

Pregnancy requirements were calculated based on the stage of pregnancy and calf birth weight (**CBW**). Calf collection occurred daily and calf birth weight measures were determined by weighing the newborn calf immediately after calf collection and within 24 h of birth. Pregnancy requirements were calculated as (NRC, 2001):

$$Pregnancy\ (MJ/d) = \frac{[0.00318t - 0.0352 \text{ x } (\text{CBW}/45)]}{(k_g)\ x\ 0.239\ Mcal/MJ}$$

where *t* is the number of days pregnant between 190 and 280 d (longer gestation periods result in no change in energy requirements) and *CBW* is the calf birth weight. This pregnancy requirement equation includes the efficiency of energy use for the gravid uterus ($k_g$ = 0.14) (NRC, 2001). For the calculation of the number of days pregnant, a set gestation length of 280 d was used. The value for $k_g$ assumes that the diet contains more

than 11.0 MJ ME/kg DM (Holmes and Davey, 1981), therefore, as recommended by Nicol and Brookes (2007), 5% was added(subtracted) per MJ below(above) 11.0 MJ ME/kg DM.

Energy requirements for BW change were initially calculated on a daily basis for individual cows. Mean BW change per day was calculated for the pre- and postcalving periods using the weekly regressed BW records within the pre- (wk –4 to –1) and postcalving periods (wk 1 to 6) and the difference in BW over time. The BW change (kg/d) was calculated as:

$$BW\ change\ (kg/d) = \frac{\text{Weekly BW}\ (Final) - \text{Weekly BW}\ (Initial)}{\text{Time}\ (days)}$$

Following the calculation of BW change, it was noted that the daily change in BW for individual cows ranged from –11.3 to +15.9 kg/d pre- and postcalving, which according to Roche et al. (2006), is not possible when feeding an adequate pasture-based diet. Body weight measurements can be inaccurate when measured over short periods due to differences in gut fill, and in this experiment, further error would have been introduced due to differences in the conceptus weight for individual cows (Thomson and Barnes, 1993). Therefore, weekly BW records were instead regressed over time to predict mean daily BW change for individual cows for the pre- (wk –4 to –1) and postcalving (wk 1 to 6) periods and these were used to calculate the energy required(spared) due to changes in BW.

In the weeks before calving, to calculate BW change a conceptus-free BW as used. Conceptus weight was calculated as follows:

$$Conceptus\ weight\ (kg) = (18 + ((t - 190)\ \text{x}\ 0.665))\ \text{x}\ (\text{CBW}/45)$$

where *t* is the number of days pregnant, and *CBW* is the calf birth weight (kg) for individual cows (NRC, 2001). The data used to generate the mean BW change was calculated using the conceptus-free weights and ranged from –1.61 to +1.03 kg/d pre- and –0.61 to +0.25 kg/d postcalving.

When cows are mobilizing fat and protein in body tissues to supply energy, the dietary ME spared is 30.0 MJ/kg loss in dry cows and 28.0 MJ/kg loss in lactating cows. Dietary ME spared due to BW loss was calculated as (Nicols and Brookes, 2007):

$$BW\ loss\ (Dry)\ (MJ/d) = 30.0\ MJ \text{ x BW loss } (kg/d)$$

$$BW\ loss\ (Lactating)\ (MJ/d) = 28.0\ MJ \text{ x BW loss } (kg/d)$$

This BW loss equation includes the efficiency of use of body tissue mobilized for the synthesis of milk. As recommended by Nicol and Brookes (2007) 8% was added(subtracted) per MJ ME below(above) 11.0 MJ ME/kg DM.

When cows are synthesizing body tissue, dietary ME is utilized to gain BW and the net efficiency with which ME is utilized above maintenance for the synthesis of body tissue differs in dry and lactating cows. Body weight gain was calculated as (Nicol and Brookes, 2007):

$$BW\ gain\ (Dry)(MJ/d) = \frac{48.0\ MJ \text{ x BW gain } (kg/d)}{k_{GD}}$$

$$BW\ gain\ (Lactating)(MJ/d) = \frac{38.0\ MJ \text{ x BW gain } (kg/d)}{k_{GL}}$$

where *k* is the efficiency of energy use for the synthesis of body tissue in dry and lactating cows ($k_{GD}$ = 0.55 and $k_{GL}$ = 0.65, respectively) (Holmes and Davey, 1981). The value for $k_{GD}$ and $k_{GL}$ assumes that the diet contains more than 11.0 MJ ME/kg DM (Holmes and Davey, 1981), therefore, as recommended by Nicol and Brookes (2007) 12% and 5% was

added(subtracted) per MJ ME for diets below(above) 11.0 MJ ME/kg DM for dry and lactating cows, respectively.

Milk energy requirement was calculated on a daily basis, using daily records of milk yield and weighted weekly mean milk composition. Due to a lack of records for milk composition during colostrogenesis (wk 1 postcalving), the milk composition values from wk 2 to 6 were used to calculate milk energy requirements during early lactation. Milk energy requirement is the ME required for milk production based on milk yield, milk fat, crude protein (**CP**), and lactose composition. Milk energy requirement was calculated as (NRC, 2001):

$$Milk\ energy\ (MJ/d) = \frac{0.0929 \text{ x Fat \%} + 0.0547 \text{ x CP \%} + 0.0395 \text{ x Lactose \% x milk yield } (kg)}{k_L \text{ x } 0.239\ Mcal/MJ}$$

where $k_L$ is the efficiency of energy use for the synthesis of milk ($k_L$ = 0.65) (Holmes and Davey, 1981). As recommended by Nicol and Brookes (2007), 8% was added(subtracted) per MJ below(above) 11.0 MJ ME/kg DM.

Total energy requirements per day were then calculated as the sum of energy required for maintenance, activity, BW change, pregnancy (precalving only), and milk (postcalving only) for individual cows. Several studies have confirmed that the current energy estimates are considerably lower than required in both dry (Holmes and Grainger, 1982; Mandok et al., 2012) and lactating grazing cows (Yan et al., 1997; Bruinenberg et al., 2002). Studies have reported energy estimates ranging from 10 to 41% less than predicted requirements (NRC, 2001); therefore, we adjusted our energy estimates by +25% to reflect a value that falls within the mid-range of predicted requirements reported in the international literature (NRC, 2001).

Dry matter intake was estimated for each cow using the total energy requirement per day, divided by the estimated combined ME concentration of the pasture and supplementary feeds. Pasture was sampled before grazing, in addition to samples of supplementary feeds offered, and these were bulked weekly, dried at 60°C for 72 h, ground to pass through a 2.0-mm sieve (Christy Lab Mill: Christy Turner Ltd., Suffolk, UK), and analyzed by wet chemistry (Ankom Technology method 3; Dairy One, Ithaca, NY) to determine ME content.

Dry matter intake was calculated as:

$$DMI\ (kg\ DM/d) = \frac{\text{total energy requirements } (MJ/d)}{\text{ME content of pasture and supplements } (MJ/kg\ DM)}$$

***Milk Yield and Composition.*** Full details of the measurement of milk yield and analyses for milk composition are described in Chapter 8 (8.3.4 Milk, BCS, BW, and Breed).

***Blood Sampling and Analyses.*** Full details of the blood sampling protocols and metabolite and inflammatory analyses are described in Roche et al. (2015), Crookenden et al. (2020), in Chapter 8, and otherwise in detail below. After the collection of blood samples, aspirated plasma was stored at –20°C and –80°C until assayed for metabolite and inflammatory analyses, respectively. Plasma samples were analyzed by Gribbles Veterinary Pathology Ltd. (Hamilton, New Zealand). Blood metabolites were assayed using colorimetric techniques at 37°C with a Hitachi Modular P800 analyzer (Roche Diagnostics, Indianapolis, IN). Roche reagent kits were used to measure plasma concentrations of albumin (**ALB**, g/L; bromocresol green reaction at pH 4.1), calcium (**Ca**, mmol/L; o-cresolphthalein complexone method (BCS and feed studies) and 5-nitro-5´-methyl-(1,2-bis(o-aminophenoxy)ethan-N,N,N´,N´-tetraacetic acid (NM-BAPTA)

method (zeolite study)], magnesium (**Mg**, mmol/L; xylidyl blue reaction), total protein (**TP**, g/L; biuret method), glutamate dehydrogenase (**GDH**, U/L; catalyzing activity of NADH-dependent conversion of α-ketoglutarate to glutamate), and aspartate aminotransferase (**AST**, U/L; catalyzing activity of transamination of L-aspartate to oxaloacetate). Glutamate dehydrogenase was measured in the feed and zeolite studies only. Plasma globulin (**GLO**, g/L) was calculated as the difference between TP and ALB.

Plasma IL-6 (pg/ml), IL-1β (pg/ml), haptoglobin (**Hp**, mg/ml), cholesterol (m*M*), total antioxidant capacity (**TAC**; mM), and reactive oxygen species (**ROS**, μM) were analyzed for a subset of blood samples collected on d 0 to 3, wk 1 and wk 4 postcalving in all 3 studies (n = 131 cows). Commercially-available bovine ELISA kits were used to analyze plasma concentrations of IL-6 (GenWay Biotech Inc., San Diego, CA), IL-1β (Pierce, Thermo Scientific, Rockford, IL), and Hp (LifeDiagnostics Inc., West Chester, PA). Commercially-available fluorimetric kits were used to analyze cholesterol (Cayman Chemical Company, Ann Arbor, MI), TAC (Cayman Chemical Company) and ROS [STA-342, Cell Biolabs Inc, San Diego, CA (feed and zeolite studies) and Biotek Instruments, Winooski, VT (BCS study)]. The inter- and intra-assay coefficients of variation for all assays were <5.5% and ≤15% and are reported in Roche et al. (2015; 2017a) and Crookenden et al. (2020).

***Liver Tissue Sampling and Analyses.*** The liver sampling protocol and analysis are described in detail in Roche et al. (2015) and Crookenden et al. (2016) for the BCS study, and in detail, for the zeolite study below. Liver samples were collected by biopsy during wk 1, 2, and 4 postcalving in the BCS (n = 78) and on d 1, 7, and 14 postcalving from a subset of cows in the zeolite study (n = 17). Briefly, after shaving and disinfecting an area in the region of the 11th intercostal space, the area was anesthetized with 7 mL of

2% lignocaine (Lopaine 2%, lignocaine hydrochloride 20 mg/mL, Ethical Agents, South Auckland, New Zealand) and an incision made through the skin in the right 11th intercostal space at the level of the greater trochanter. A 12-gauge x 20-cm biopsy needle was passed into the liver and 1 g (wet weight) of liver tissue was collected, snap-frozen in liquid nitrogen, and stored at –80°C.

Liver TAG (% of wet weight) assays were undertaken by Gribbles Veterinary Pathology Ltd. In the BCS study, liver TAG content was analyzed using a modified procedure provided in the Wako LabAssay TM Triglyceride Kit (290–63701, Wako Chemicals USA Inc., Richmond, VA) and is outlined, in detail, in Roche et al. (2015). Briefly, in the zeolite study, approximately 30 mg of liver tissue was added to 1 ml of 20% potassium hydroxide in water, vortexed, and left to digest overnight at room temperature. The digested samples were vortexed and 0.5 ml of 10% sulphuric acid in water was added to neutralize the solution followed by 0.5 ml of 1% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propane-sulfonate) in water. The samples were vortexed and centrifuged at 3,000 rpm for 15 min. The supernatant was collected and liver TAG content was analyzed using the standard procedure provided in the Roche Kit (no. 1192142881, Roche Diagnostics).

***Endometrial Cytology and Metricheck Sampling.*** Uterine endometrial cytology samples were collected during 2 sampling periods: 11 to 17 d and 31 to 38 d postcalving as described by Meier et al. (2014). Samples were collected from cows in the BCS and feed studies only at 11 to 17 d and from cows in all 3 studies at 31 to 38 d postcalving. Briefly, a sample from the uterine wall was collected using a stylet with a cytology brush attached. The contents of the brush were rolled onto a microscope slide and air-dried. The dry slides were stained using Diff-Quik (Dade Behring, Newark, DE) and a veterinary

pathologist [BCS and feed studies (IVABS, Massey University, Palmerston North, New Zealand) and zeolite studies (Gribbles Veterinary Pathology Ltd.)] determined the proportion of polymorphonucleated cells (**PMNC**, %) in the swab. Areas of each slide that contained small clusters of epithelial cells (5 to 20 per cluster) were preferentially selected and all identifiable nucleated cells counted. Approximately 200 nucleated cells per slide were enumerated, with PMNC distinguished from non-PMNC, to allow the proportions of nucleated cells that were PMNC to be calculated.

On the completion of endometrial sampling, vaginal content was sampled using a Metricheck device (Simcro Tech Ltd., Hamilton, New Zealand); this device consists of a 40-mm-diameter hemisphere of silicon attached to a 500-mm-long stainless steel rod. The vaginal content was scored (0 being no sample, 1 being clear mucus, and 5 being purulent pus; McDougall et al., 2007).

### *Appendix 18.2 Statistical Analyses*

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Results are presented as least square means (**LSM**) ± standard error of the mean in the text and mean standard error of the difference in tables and figures. The covariance structures selected were compound symmetry or autoregressive based on the lowest Akaike's information criterion. Study and treatment from the parent experiments were concatenated to create a categorical variable study group. All data were adjusted where appropriate, according to re-assigned calving day, and these transformed datasets were the basis of subsequent analyses.

Study group (categorical) and calving season day within the herd (difference in days between calving date and the first day in June) were included to adjust for different treatments and different calving dates within the 3 studies in all models described below.

Due to the greater risk of SCK in older pasture-grazed cows, all models were adjusted for parity (categorical; 2–3 or 4+) (Compton et al., 2015) and pairwise comparison-adjusted using Tukey-Kramer. All blood protein markers, liver enzymes, proinflammatory cytokines, and inflammatory markers were checked for skewness and to meet the assumption of normal distribution. Untransformed LSM, standard error of the mean, and standard error of the difference are presented for all analyses undertaken on log-transformed data.

***Estimated DMI.*** Estimated DMI was summarized into 2 periods: –4 to –1 wk precalving and 1 to 6 wk postcalving. To investigate the associations between estimated DMI and energy status and week and their interactions for 4 wk pre- and 6 wk postcalving, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, week as a repeated measure, and the fixed effect of energy status, week, and energy status x week interactions. Mean wk –6 to –5 precalving BW was included as a covariate.

***Milk Yield and Composition.*** Weighted means for weekly milk yield for wk 1 to 7 postcalving and ECM yield for wk 2 to 6 postcalving were calculated as outlined in Chapter 8 (8.3.7 Statistical Analyses). To investigate the associations between milk and ECM yield, and milk protein and fat composition, and energy status, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, week as a repeated measure, and the fixed effect of energy status, week, and energy status x week interactions. Covariates BrW and PW were included in the model as proxies for milk production potential.

***Blood Protein Markers and Liver Enzymes.*** Blood data for protein metabolites [TP, ALB, GLO, albumin:globulin ratio (**AGR**)] and liver enzymes (AST and GDH) were summarized into 6 periods [i.e., d –14 to –1 precalving, d 0 to 2 postcalving, d 3 to 7

postcalving, and then weekly postcalving (wk 2 to 4)]. Records for GDH were only available for analysis from a subset of 136 cows [Hi–Hi (n = 23); Hi–Lo (n = 75); Lo–Lo (n = 38)] from the feed and zeolite studies. Due to lack of blood Ca and Mg records in the zeolite study cows, blood data were summarized into 4 periods (i.e., d – 14 to –1 precalving, d 0 to 2 postcalving, d 3 to 7 postcalving, and d 8 to 14 postcalving). To investigate the associations between energy and protein metabolites, and energy status and period, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, period as a repeated measure, and the fixed effect of the energy status, period, and energy status x period interactions. Log-transformation was used to normalize blood AST and GDH.

***Proinflammatory Cytokines and Inflammatory Markers.*** A subset of 131 cows [Hi–Hi (n = 21); Hi–Lo (n = 76); Lo–Lo (n = 34)] were selected for analysis of proinflammatory cytokines (IL-1β and IL-6) and inflammatory markers (Hp, cholesterol, TAC, and ROS). Data were summarized into 3 periods postcalving (i.e., d 0 to 6, d 7 to 15, and d 22 to 28 postcalving). To investigate the associations between proinflammatory cytokines and inflammatory markers and energy status and period, a repeated measures ANOVA was performed using PROC MIXED with cow as a random effect, period as a repeated measure, and the fixed effect of the energy status, period, and energy status x period interactions. Log-transformation was used to normalize Il-1β, IL-6, and Hp.

***Liver TAG.*** A subset of 95 cows [Hi–Hi (n = 10); Hi–Lo (n = 55); Lo–Lo (n = 29)] were selected for analysis of liver TAG from the BCS and zeolite studies and were summarized into 3 period postcalving (i.e., d 0 to 6, d 7 to 15, and d 22 to 28 postcalving). To investigate the associations between liver TAG and energy status and period, a repeated measures ANOVA was performed using PROC MIXED with cow as a random

effect, period as a repeated measure, and the fixed effect of the energy status, period, and energy status x period interactions.

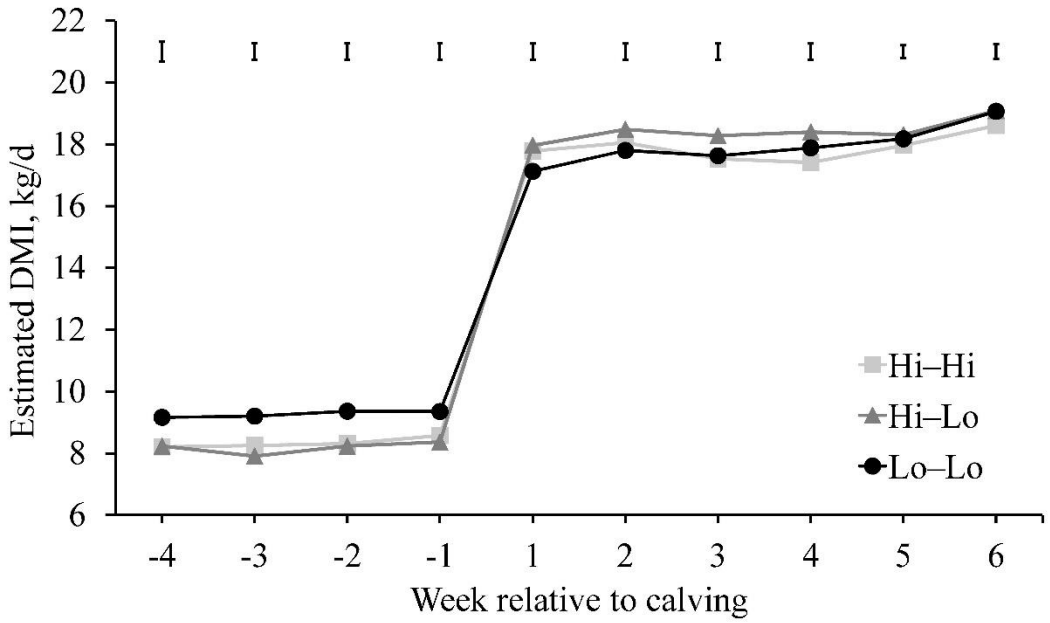
***Endometrial Cytology and Metricheck.*** Metricheck scores and PMNC were available for 11 to 17 d and 31 to 38 d postcalving. To investigate the associations between metricheck score and PMNC and energy status, an ANOVA was undertaken using PROC MIXED with the fixed effect of energy status, study group, and parity as fixed effects.

### *Appendix 18.3 Results and Discussion*

***Estimated DMI.*** Precalving estimated DMI (ME requirement in parentheses; MJ/d) was 8.15 ± 0.37 (101), 8.52 ± 0.18 (105), and 9.12 ± 0.26 (113) kg/d in the Hi–Hi, Hi–Lo, and Lo–Lo groups, respectively ($P = 0.07$). Postcalving estimated DMI was 18.0 ± 0.24 (220), 18.3 ± 0.11 (223), and 17.9 ± 0.17 (218) kg/d in the Hi–Hi, Hi–Lo, and Lo–Lo groups, respectively ($P = 0.14$). There was an energy status x week interaction ($P < 0.001$) for estimated DMI and the range of mean values (range = minimum and maximum LSM and standard error of the mean) within the weeks specified are reported below. During wk –4 to –1 precalving, Hi–Hi (range = 8.21 ± 0.30 to 8.58 ± 0.25 kg/d) and Hi–Lo groups (range = 7.91 ± 0.12 to 8.36 ± 0.12 kg/d) were not different ($P \geq 0.440$ from each other, but had lower ($P < 0.05$) estimated DMI (range = 9.16 ± 0.20 to 9.36 ± 0.17 kg/d) than the Lo–Lo group (Supplemental Figure 3). These results indicate that cows with elevated blood NEFA with or without elevated BHB postcalving have a reduced feed intake before they calve.

Several studies have reported reductions in feeding behavior (e.g., fewer visits to the feed bunk and less time spent feeding) and DMI after the diagnosis of SCK in housed

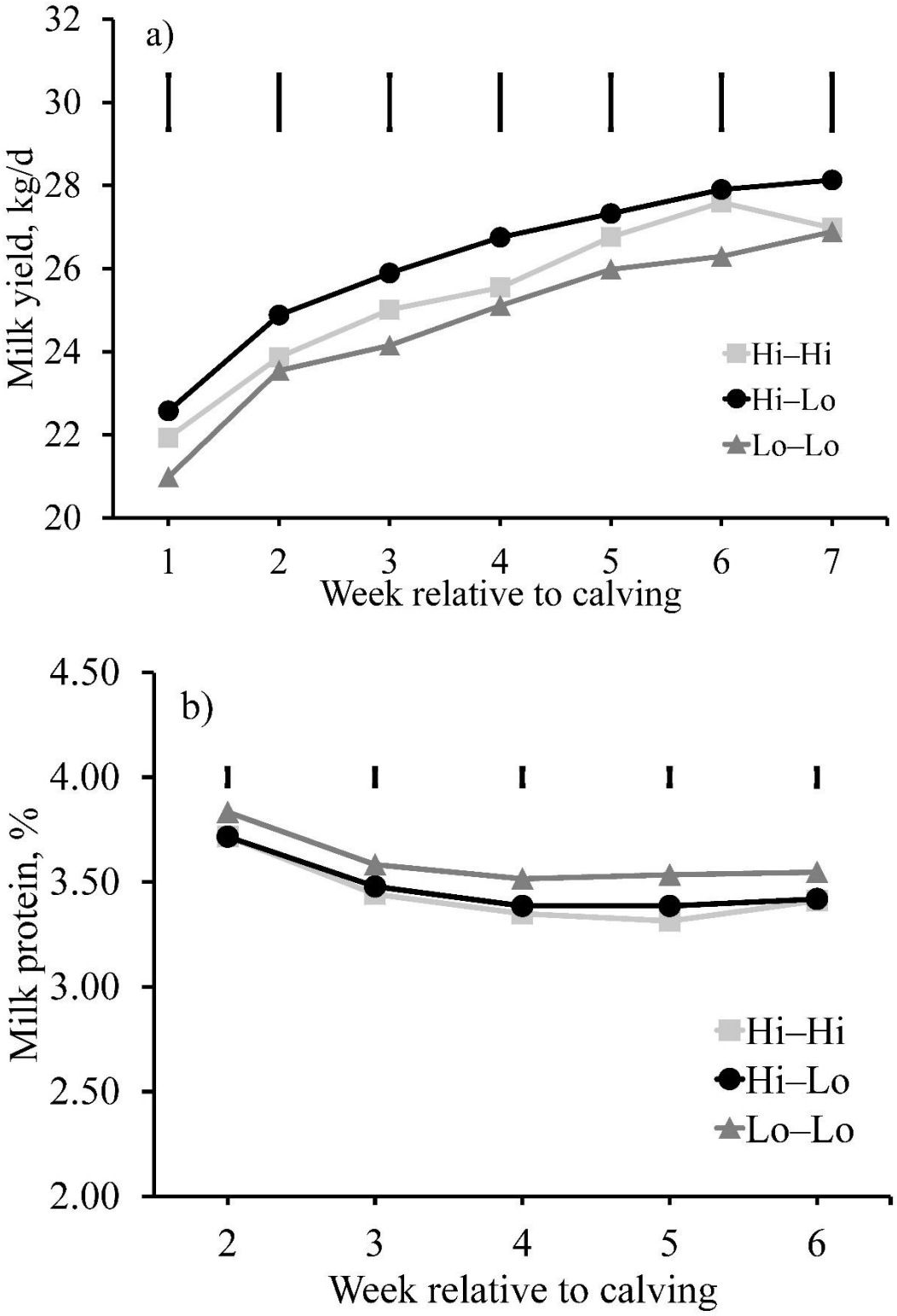
cows (Goldhawk et al., 2009; Rodríguez-Jimenez et al., 2018); in our study, estimated DMI in grazed cows was statistically different between the 3 energy status groups but was not necessarily biologically significant (Supplemental Figure 3). During wk 3 and 4 postcalving, cows in the Hi–Hi group had, on average, 0.7 kg/d lower ($P < 0.01$) estimated DMI (17.5 ± 0.24 and 17.4 ± 0.24 kg/d, respectively) than the Hi–Lo group (18.3 ± 0.12 vs. 18.4 ± 0.12 kg/d, respectively), but were not different ($P \geq 0.26$) from the Lo–Lo group (17.6 ± 0.17 and 17.9 ± 0.17 kg/d, respectively). During wk 2 and 3 postcalving, the Hi–Hi group had reduced activity and because walking is an energetically-expensive activity that is important in grazing cows to meet their nutrient needs (Aharoni et al., 2013), we expected larger differences in estimated DMI. In our study, estimated DMI was calculated based on a fixed ME value and back-calculation from ME requirements (Nicol and Brookes, 2007); therefore, it is difficult to determine with certainty whether the reduced activity was associated with grazing behavior and, therefore, this warrants further investigation.




**Supplemental Figure 3. Estimated dry matter intake during the transition period in 3 energy status groups.**

Estimated dry matter intake (DMI; kg/d) during the 4 wk pre- and 6 wk postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

***Milk Yield and Composition***. Studies investigating the associations between SCK and milk production in grazing cows are limited (Compton et al., 2015). Milk yield during the first 7 wk postcalving and milk protein% and fat% profiles during the first 6 wk postcalving are presented in Supplemental Figures 4a, b, and c. Milk protein% during the first 6 wk postcalving was lower, on average, in the Hi–Hi and Hi–Lo groups than the Lo–Lo group (Table 8.2), and there was no significant ($P = 0.18$) energy status x week interaction (Supplemental Figure 4b). Energy status was not associated with mean milk fat% during early lactation (Table 8.2), but there was an energy status x week interaction ($P < 0.01$) on milk fat% (Supplemental Figure 4c). During wk 5 postcalving, the Hi–Hi group had a lower ($P < 0.05$) milk fat% than the Lo–Lo group (4.08 ± 0.11% vs. 4.42 ± 0.08%) and tended ($P = 0.10$) to have a lower milk fat% than the Hi–Lo group (4.33 ± 0.05%), which were not different ($P = 0.65$) from the Lo–Lo group. This short-lived difference is unlikely to be biologically significant and disagrees with studies reporting associations between SCK and higher milk fat%; however, consistent with our results, associations between SCK and lower milk protein% are well supported (Duffield, 2000; Abuajamieh et al., 2016; Bonfatti et al., 2019).




**Supplemental Figure 4. Milk yield during early lactation in 3 energy status groups.**

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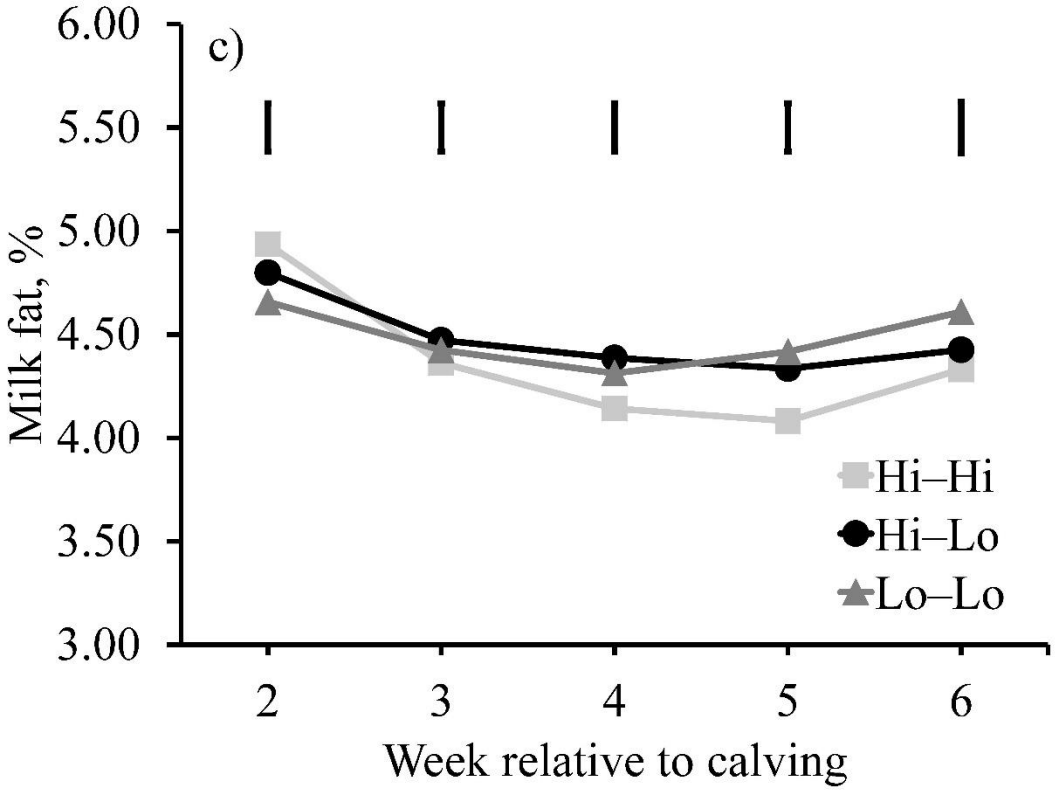



**Supplemental Figure 4 (Continued). Milk yield during the first 7 weeks of lactation in 3 energy status groups.**

Milk yield [kg/d; (a)], milk protein [%; (b)], and milk fat [%; (c)] during the first 7 wk of lactation for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

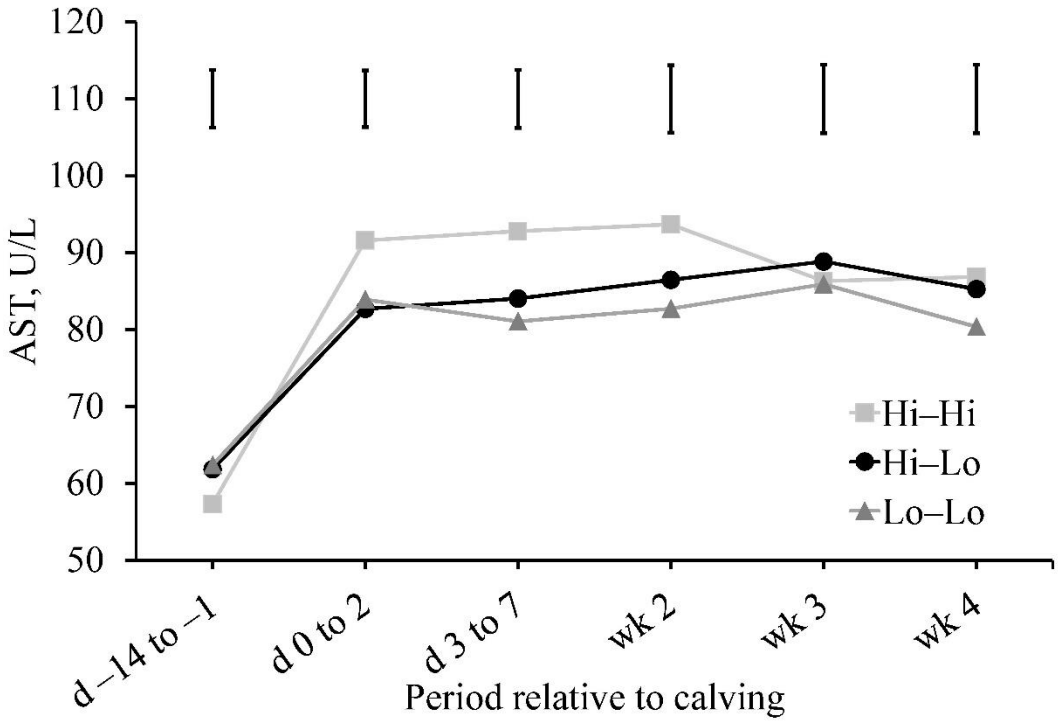
There was an overall effect of energy status ($P < 0.01$), and an energy status x week interaction ($P < 0.05$) on milk yield during the first 7 wk postcalving (Table 8.2 and Supplemental Figure 4a, respectively); Mean ECM yield during the first 6 wk postcalving were not significantly different between the Hi–Hi and Lo–Lo groups, but both were 1.3 kg/d greater than the Hi–Lo group (Table 8.2). The energy status x week interaction on ECM yield was not significant ($P = 0.13$). This result is consistent with two studies undertaken in grazing dairy cows where associations between SCK (blood BHB ≥1.2 mmol/L) and milksolids production (Compton et al., 2015) and milk yield (Bonfatti et al., 2019) were investigated, and they reported comparable milk production in SCK and non-SCK cows. Others have reported lower milk yields and DMI in housed dairy cows at similar blood BHB concentrations (McArt et al., 2012; Abuajamieh et al., 2016); however, in our study, the estimated DMI postcalving was also not different between the Lo–Lo and Hi–Hi groups (Supplemental Figure 3). Housed cows are typically high producing (Kolver and Muller, 1998) and the competitive environment for access to resources may exacerbate the SCK condition in these animals (Itle et al., 2015), which may explain the milk production discrepancies reported in grazing and housed cows. Further research is needed to understand SCK in grazing cows and associations with milk production, DMI, and the severity of SCK.

Liver function, inflammatory markers, infectious disease, and blood minerals were associated with energy status. Evidence suggests that SCK is not simply the result of excessive adipose mobilization, as indicated by a poor association between circulating NEFA and BHB (McCarthy et al., 2015), but is often associated with impaired liver function as dairy cows often undergo an inflammatory condition during the transition period (Bertoni et al., 2008; Trevisi et al., 2012). Due to the importance of the liver to

metabolize the surge of NEFA during the transition period, these conditions can considerably exacerbate SCK (Bertoni et al., 2008). Therefore, it is conceivable that the increased synthesis and production of inflammatory biomarkers in the liver can diminish its functional capacity and render this organ unable to metabolize all presented NEFA, and, consequently, ketone levels are elevated (Trevisi et al., 2012; Rodríguez-Jiminez et al., 2018).

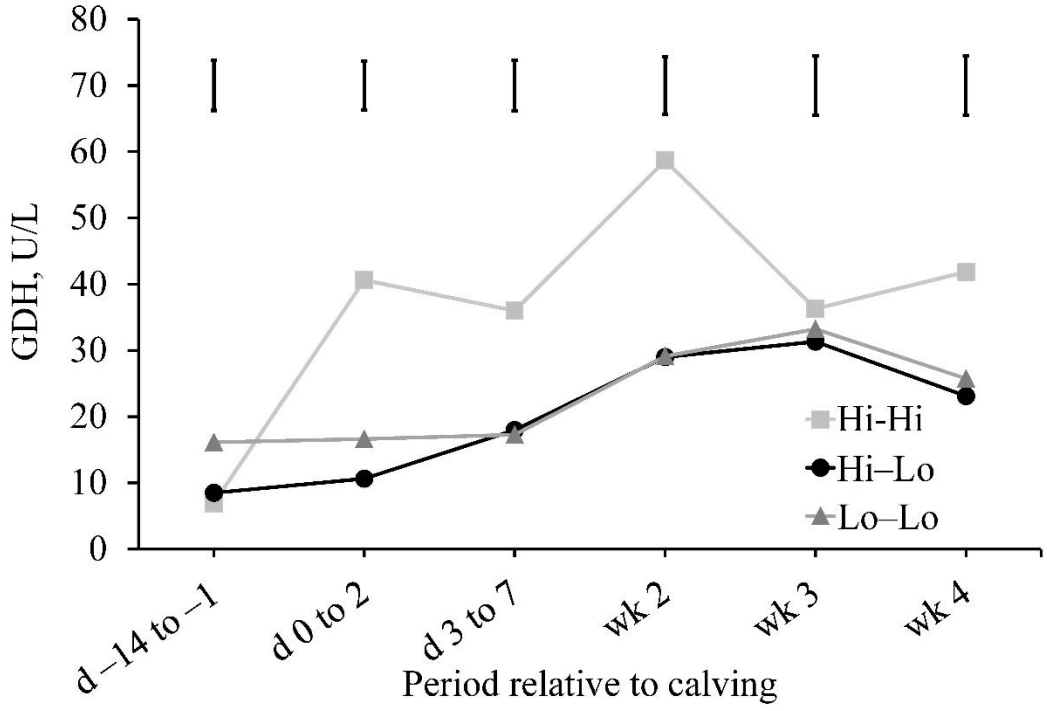
***Liver Function.*** The pathophysiology of SCK remains unclear and it is of particular interest to determine why some cows are ostensibly susceptible or predisposed to SCK (Abujamieh et al., 2016). We used concentrations of TP, ALB, cholesterol, AST, GDH, and liver TAG as indicators of liver function (Bertoni and Trevisi, 2013). There was no overall association of energy status on albumin:globulin ratio (**AGR**) ($P = 0.80$), TP ($P = 0.15$), ALB ($P = 0.33$), GLO ($P = 0.53$), cholesterol ($P = 0.39$), AST ($P = 0.15$), and GDH concentrations ($P = 0.09$). An energy status x period interaction ($P < 0.001$) on AST and GDH was present (Supplemental Figures 5 and 6). During 3 to 7 d and wk 2 postcalving, the Hi–Hi group had greater ($P < 0.01$) blood AST concentrations (92.8 ± 3.50 and 93.7 ± 4.06 U/L, respectively) than the Lo–Lo group (81.1 ± 2.58 and 82.7 ± 2.94 U/L, respectively), but were not different ($P \geq 0.26$) from the Hi–Lo group (84.0 ± 1.74 and 86.5 ± 2.00 U/L, respectively). During 3 to 7 d and wk 2 postcalving, cows in the Hi–Hi group had greater ($P < 0.05$) blood GDH concentrations (36.0 ± 8.21 and 58.7 ± 8.34 U/L, respectively) than the Lo–Lo group (17.3 ± 6.34 and 29.1 ± 6.34 U/L, respectively). During 3 to 7 d postcalving, the Hi–Hi group tended ($P = 0.10$) to have greater blood GDH concentrations than the Hi–Lo group (18.0 ± 4.66 U/L), and, during wk 2 postcalving, had greater ($P < 0.05$) GDH concentrations than the Hi–Lo

group (29.0 ± 4.66 U/L), but there was no difference ($P \geq 0.35$) between the Lo–Lo and Hi–Lo groups during both periods.




**Supplemental Figure 5. Aspartate aminotransferase concentrations during the transition period in 3 energy status groups.**

Aspartate aminotransferase (AST; U/L) during d –14 to –1 precalving, d 0 to 2 postcalving, d 3 to 7 postcalving, and wk 2 to 4 postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L during 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L during 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

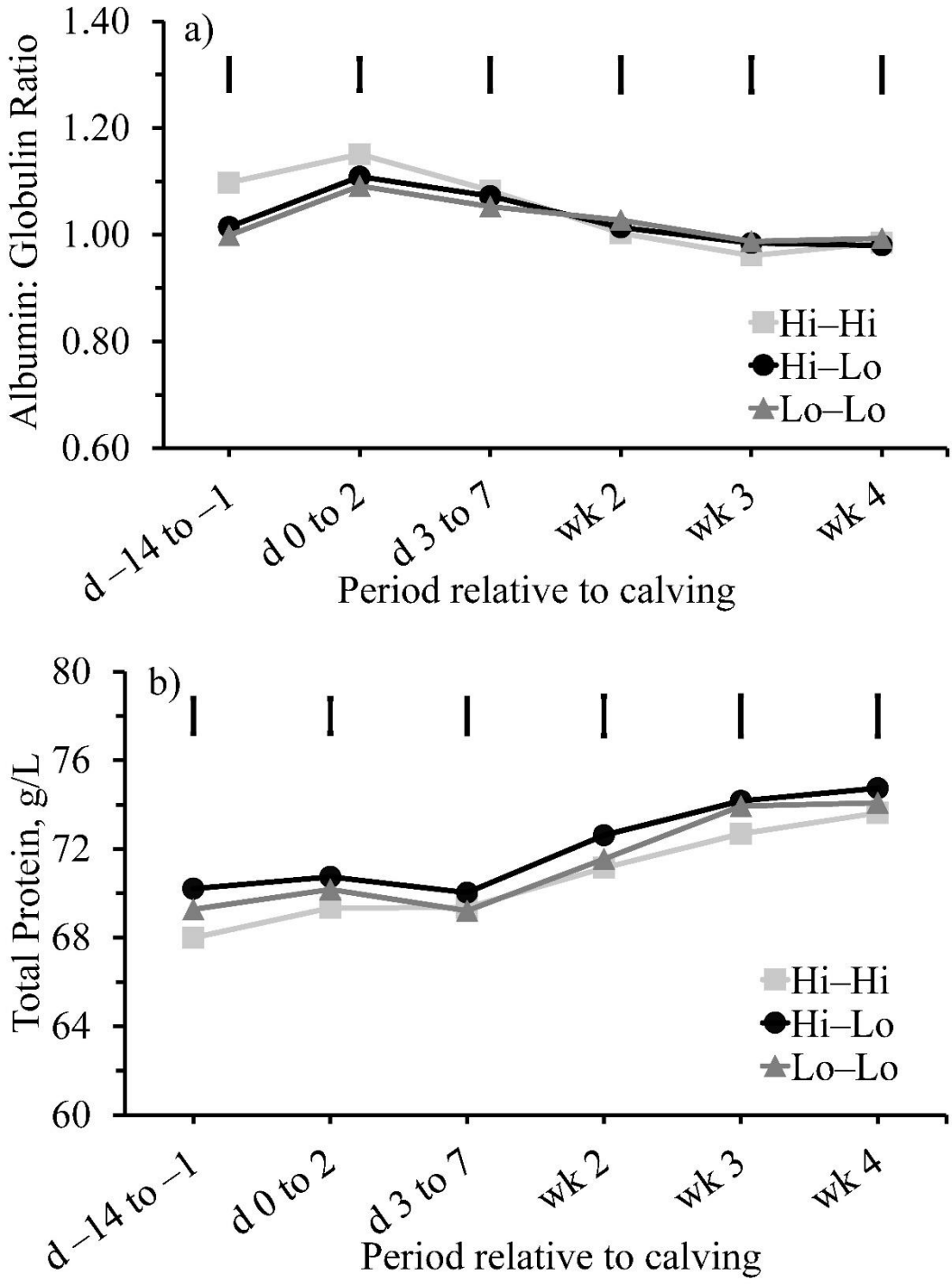



**Supplemental Figure 6. Glutamate dehydrogenase concentrations during the transition period in 3 energy status groups.**

Glutamate dehydrogenase (GDH; U/L) during d –14 to –1 precalving, d 0 to 2 postcalving, d 3 to 7 postcalving and wk 2 to 4 postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L during 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L during 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

There was no interactive energy status x period association on blood TP ($P = 0.44$) or cholesterol concentration ($P = 0.21$); an interaction of energy status x period was present for blood AGR ($P < 0.001$), ALB ($P < 0.001$), and GLO concentrations ($P < 0.01$) (Supplemental Figures 7a, b, c, and d). During –14 to –1 d precalving, the Hi–Hi group

had greater ($P = 0.01$) AGR (1.10 ± 0.03) than the Hi–Lo and Lo–Lo groups (1.01 ± 0.01 and 1.00 ± 0.02, respectively) (Supplemental Figure 7a). This was due, in part, to lower ($P < 0.05$) blood GLO concentrations in the Hi–Hi group (34.2 ± 0.78 g/L) compared with the Hi–Lo group (36.4 ± 0.38 g/L), and a tendency ($P = 0.08$) for lower blood GLO concentrations than the Lo–Lo group (36.3 ± 0.56 g/L), which were not different ($P = 0.99$) from the Hi–Lo group. During d 3 to 7 postcalving, blood ALB concentrations were not different ($P \geq 0.29$) in both the Lo–Lo and Hi–Lo groups compared with the Hi–Hi group (35.8 ± 0.36 g/L), which were intermediate; however, cows in the Lo–Lo group had lower ($P < 0.05$) blood ALB concentrations than the Hi–Lo group (35.1 ± 0.26 vs. 35.9 ± 0.18 g/L) (Supplemental Figure 7c). During wk 3 postcalving, the Hi–Hi group had lower ($P < 0.01$) blood ALB concentrations (35.2 ± 0.41 g/L) than Lo–Lo and Hi–Lo groups (36.6 ± 0.29 and 36.5 ± 0.20 g/L, respectively). Low blood ALB (Bertoni et al., 2008; Bertoni and Trevisi, 2013) alongside elevated AST and GDH in the Hi–Hi group postcalving, supports that cows with elevated blood NEFA and BHB postcalving, in our study, were likely to be under greater stress and experiencing more severe liver dysfunction and, possibly, hepatic tissue damage than cows with low BHB with or without elevated NEFA (Bertoni et al., 2008).




**Supplemental Figure 7. Blood protein metabolite concentrations during the transition period for the 3 energy status groups.**

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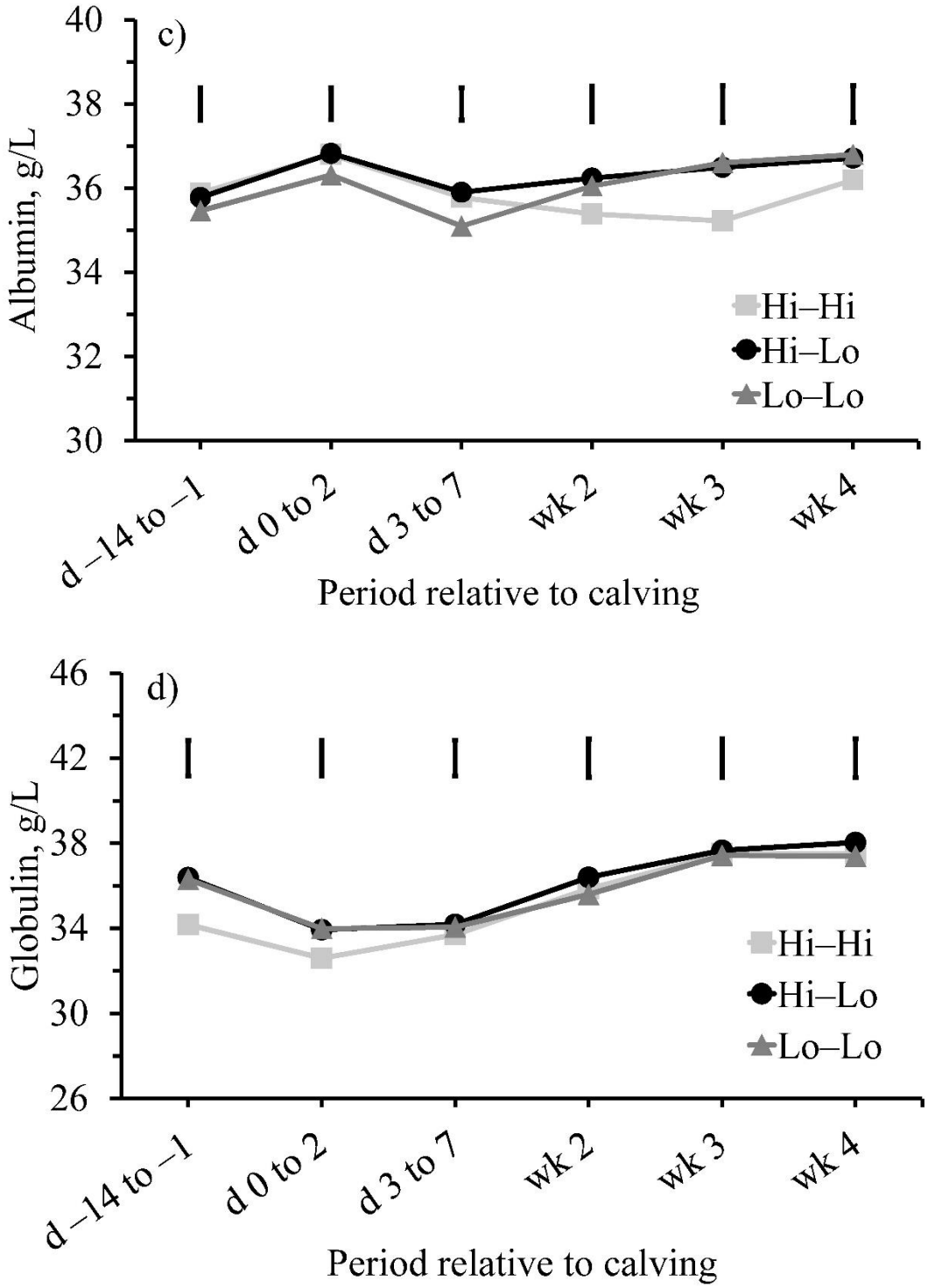
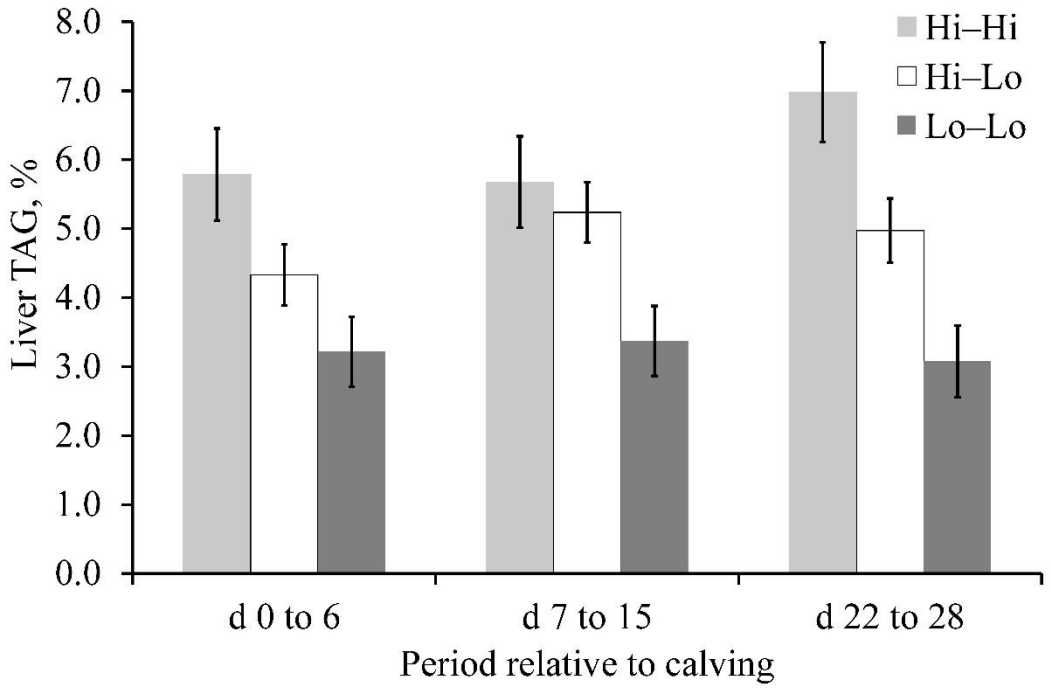



**Supplemental Figure 7 (Continued).** Blood albumin to globulin ratio (a), total protein [g/L; (b)], albumin [g/L; (c)], and globulin [g/L; (d)] during d –14 to –1 precalving, d 0 to 2 postcalving, d 3 to 7 postcalving, and wk 2 to 4 postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive

samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L during 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L during 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

Postcalving energy status was associated with liver TAG concentrations during early lactation ($P < 0.01$); the Hi–Hi (6.15 ± 0.86 %) and Hi–Lo groups (4.85 ± 0.53 %), which were not different ($P = 0.17$) from each other, had the highest liver TAG ($P < 0.01$) compared with the Lo–Lo group (3.22 ± 0.60 %) (Supplemental Figure 8). There was also an energy status x period interaction ($P < 0.01$) for liver TAG, which indicated that the differences between energy status groups were greatest at wk 3 to 4 postcalving. Between 0 to 6 d postcalving, cows in the Hi–Hi group had greater ($P < 0.01$) liver TAG (5.79 ± 0.90 %) than the Lo–Lo group (3.08 ± 0.62%); both were not different (both $P = 0.15$) from the Hi–Lo group (4.33 ± 0.54%) (Supplemental Figure 8). Between 7 to 15 d postcalving, liver TAG was greater ($P < 0.05$) in the Hi–Hi and Hi–Lo groups (5.68 ± 0.90% and 5.24 ± 0.54%, respectively) relative to the Lo–Lo group (3.37 ± 0.61%). Liver TAG between 22 to 28 d postcalving was greater ($P < 0.05$) in the Hi–Hi group (6.98 ± 0.94%) than in the Hi–Lo group (4.97 ± 0.55%), which, in turn, was greater ($P < 0.01$) than in the Lo–Lo group (3.08 ± 0.62%). The elevated liver TAG postcalving indicates potential hepatic lipidosis (fat accumulation in the liver) in the Hi–Hi group, which impairs normal liver function and may exacerbate HYK (Herdt, 2000; McArt et al., 2013).

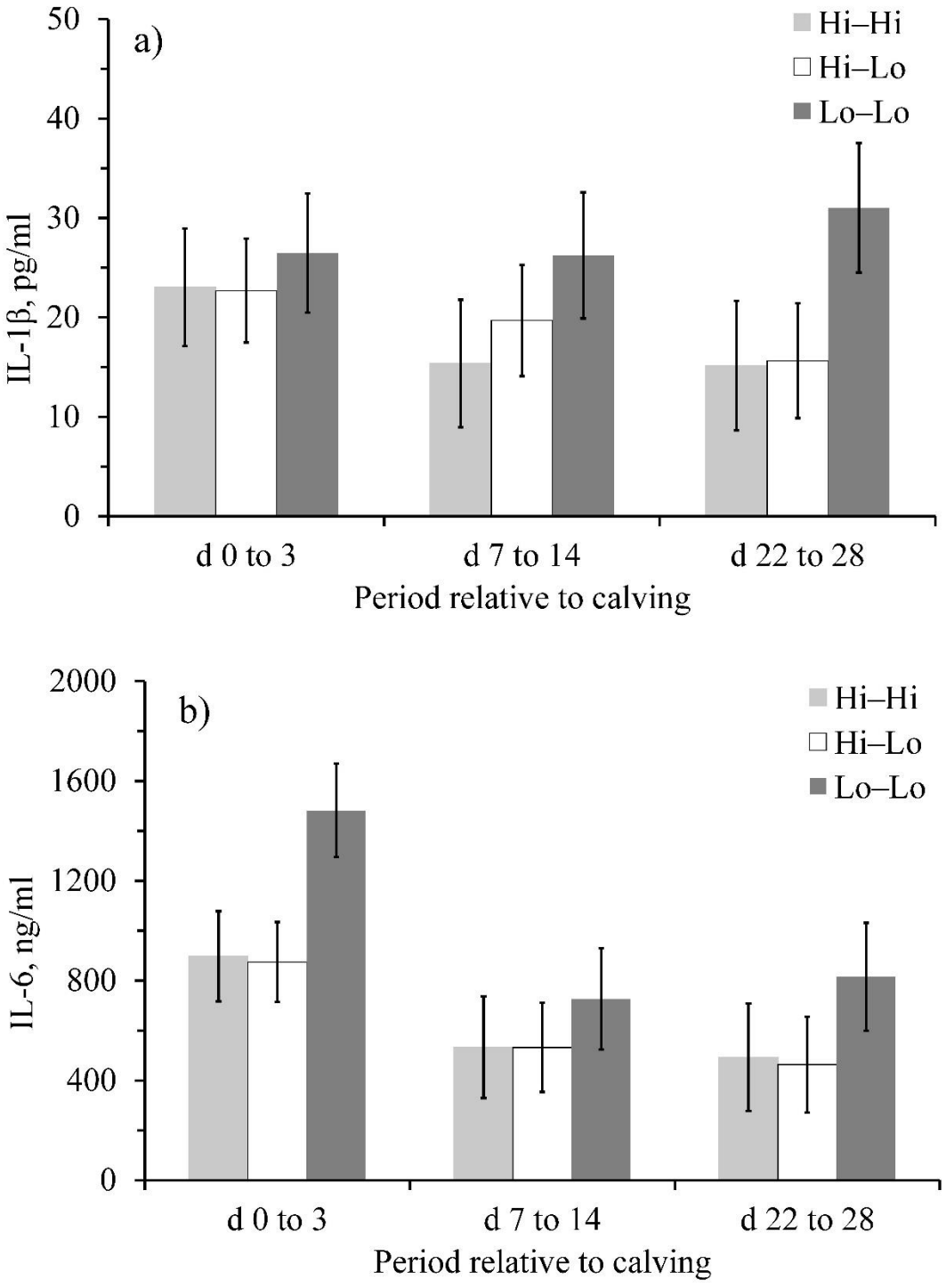



**Supplemental Figure 8. Liver triacylglyceride concentrations during the transition period in 3 energy status groups.**

Liver triacylglyceride (TAG; %) during d 0 to 6, d 7 to 15, and d 22 to 28 postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.2 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L during 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L during 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

***Inflammatory Markers and Metabolic Stress.*** Blood TAC and ROS are indicators of metabolic stress; however, in our study, there was no association of energy status, or energy status x period interaction on TAC ($P = 0.60$ and $P = 0.90$, respectively) and ROS ($P = 0.81$ and $P = 0.38$, respectively). Proinflammatory cytokines (IL-1β and IL-6) and Hp are indicators of inflammatory state; therefore, if diminishing liver function

is associated with greater inflammatory state of the hyperketonemic animal, proinflammatory cytokines may be elevated (Trevisi et al., 2012; Supplemental Figures 9a and b). There was no association of energy status, or energy status x period interaction on Hp ($P$ = 0.34 and $P$ = 0.95, respectively). There was no energy status x period interaction ($P$ = 0.19) on IL-1β; however, overall, IL-1β tended ($P$ = 0.09) to be associated with energy status, where the Hi–Hi group were not different ($P \geq 0.14$) from both Lo–Lo and Hi–Lo groups (1.16 ± 0.04 pg/ml), but the Hi–Lo group tended ($P$ = 0.09) to have lower IL-1β concentrations (1.17 ± 0.02 pg/ml) than the Lo–Lo group (1.26 ± 0.04 pg/ml) (Supplemental Figure 9a). There was no overall association of energy status on IL-6 ($P$ = 0.26), but an energy status x period interaction ($P < 0.05$) was present (Supplemental Figure 9b). During 0 to 3 d postcalving, the Hi–Hi group had lower ($P < 0.01$) IL-6 concentrations (898 ± 141 pg/ml) and the Hi–Lo group tended ($P$ = 0.08) to have lower IL-6 concentrations (875 ± 78 pg/ml) than the Lo–Lo group (1482 ± 146 pg/ml). Elevated IL-6 is thought to play a major role in the inflammatory response; it increases markedly in cows with induced ketosis postcalving (Loor et al., 2007) and elevated IL-6 concentrations (>300 pg/ml) can indicate impaired liver function and a greater state of inflammation (Trevisi et al., 2012). Intriguingly, the Lo–Lo group had the highest IL-6 concentrations immediately postcalving; however, inflammation postcalving substantially increases energy requirements (Esposito et al., 2014), and, therefore, it is plausible that elevated blood NEFA postcalving (with or without elevated blood BHB) limited the animal's ability to upregulate the production and secretion of IL-6 (Sheldon et al., 2018). Despite this, in our study, all groups had IL-6 concentrations surpassing 300 pg/mL, which indicates all cows were experiencing an inflammatory condition but to a lesser or greater degree.




**Supplemental Figure 9. Proinflammatory cytokine concentrations during the transition period in 3 energy status groups.**

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**Supplemental Figure 9 (Continued).** Interleukin-1β [IL-1β, pg/ml; (a)] and IL-6 [pg/ml; (b)] during d 0 to 3, d 7 to 14, and d 22 to 28 postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L during 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L during 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

Metabolic stress is associated with increased risk of infectious disease (Suriyasathaporn et al., 2000; Compton et al., 2015) and this may be due to an impaired inflammatory response. We used metricheck scores and PMNC for 2 periods: 11 to 17 d and 31 to 38 d postcalving as measures of infectious disease (Supplemental Table 15). The Hi–Hi group had higher ($P < 0.05$) metricheck scores at 11 to 17 d postcalving and higher PMNC ($P < 0.01$) at 31 to 38 d postcalving than Lo–Lo and Hi–Hi groups. At 11 to 17 d postcalving, the proportion of cows within each group that had a metricheck score of ≥2 was 38%, 30%, and 23% in the Hi–Hi, Hi–Lo, and Lo–Lo groups, respectively. At 31 to 38 d postcalving, the proportion of cows within each group that had a metricheck score of ≥2 was 19%, 9.0%, and 6.4% in the Hi–Hi, Hi–Lo, and Lo–Lo groups, respectively. Overall, in our study, the Hi–Hi group had greater metricheck scores, in particular, more cows with scores ≥2, and greater PMNC, which may indicate an impaired inflammatory response in cows experiencing elevated blood NEFA and BHB postcalving.

**Supplemental Table 15. Endometrial and cytology results for the 3 energy status groups at 2 sampling points postcalving.**

Overall mean polymorphonucleated cells (PMNC, %) and metricheck score differences at 11 to 17 d and 31 to 38 d postcalving between the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA concentration ≥1.0 mmol/L and blood BHB concentrations ≤1.0 mmol/L during 1x or more samplings the first 2 wk postcalving); Hi–Hi (blood NEFA concentration ≥1.0 mmol/L and blood BHB concentration ≥1.2 mmol/L during 1x or more samplings the first 2 wk postcalving)].

| Parameter | Lo–Lo | Hi–Lo | Hi–Hi | SED[1] | *P*-value |
|---|---|---|---|---|---|
| 11 to 17 d postcalving | | | | | |
| PMNC, % | 38.0 | 38.0 | 48.2 | 6.79 | 0.42 |
| Metricheck score[2] | 1.25[b] | 1.51[b] | 2.06[a] | 0.22 | <0.01 |
| 31 to 38 d postcalving | | | | | |
| PMNC, % | 7.74[b] | 9.03[b] | 19.8[a] | 2.99 | <0.001 |
| Metricheck score[2] | 1.07 | 1.11 | 1.34 | 0.19 | 0.09 |

[a–b]Means with different superscripts are significantly different at the 5% confidence level.
[1]SED = mean standard error of the difference.
[2]Metricheck score (0 being no sample, 1 being clear mucus, and 5 being purulent pus; McDougall et al., 2007).

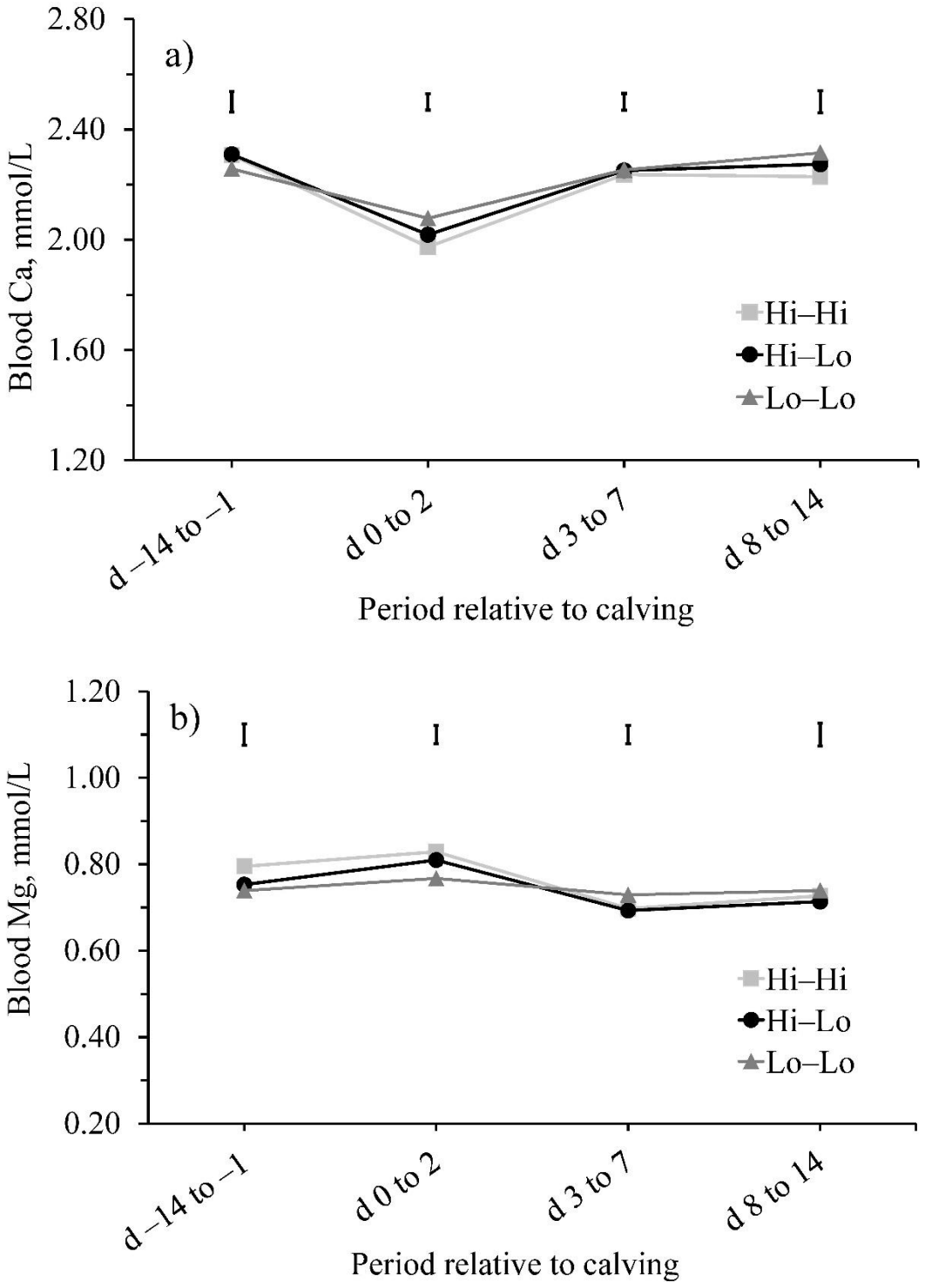
Animals require >1 kg of glucose to supply the activated immune system in the first 12 h after in-vivo challenge with lipopolysaccharide from gram-negative bacteria (Kvidera et al., 2017). Some evidence indicates that depriving the endometrial tissue of glucose can impair the secretion of IL-1β and IL-6 and the inflammatory response to pathogens (Sheldon et al., 2018), and while we cannot determine with certainty from our study whether elevated NEFA and BHB postcalving impaired the immune response (Kvidera et al., 2017), it is possible that, the Hi–Hi group were unable to supply sufficient energy to elicit a satisfactory immune response to resist infection (Medzhitov, 2008;

Sheldon et al., 2018), as supported by lower IL-6 and IL-1β concentrations in these cows. Further work is needed to understand the associations between SCK, inflammation, the immune response, and infectious disease.

***Blood Minerals.*** Blood Ca and Mg were analyzed as indicators of calcium homeostasis. Overall there was no association of energy status on blood Ca ($P = 0.37$) or Mg ($P = 0.53$); however, an energy status x period interaction ($P < 0.01$) was present for blood Ca and Mg concentrations (Supplemental Figures 10a and b). During 0 to 2 d postcalving, blood Ca and Mg concentrations were lower ($P < 0.05$) in the Hi–Hi (1.97 ± 0.03 and 0.83 ± 0.02 mmol/L, respectively) and Hi–Lo groups (2.02 ± 0.01 and 0.81 ± 0.01 mmol/L, respectively) than the Lo–Lo group (2.08 ± 0.02 and 0.77 ± 0.01 mmol/L, respectively). There were no differences ($P \geq 0.19$) between the Hi–Lo and Hi–Hi groups, and no further sustained differences between the energy status groups from 3 d postcalving.

Studies supporting that blood BHB concentrations ≥1.2 mmol/L postcalving is associated with a greater risk of negative outcomes for health and performance have predominantly been undertaken in housed systems. In agreement with our results, a cut-point of 1.2 mmol/L for the definition of SCK in pasture-based dairy herds in New Zealand (Compton et al., 2015) is feasible, based on the associations between blood BHB ≥1.2 mmol/L and inflammation and infectious disease in grazing dairy cows reported in our study, despite no association with milk production; however, larger prospective studies are needed to improve our understanding of the associations between SCK and NEB and cow performance in grazing systems. The physiological alterations in inflammatory and liver function in the cows experiencing high blood NEFA and BHB concentrations 2 wk postcalving, in our study, indicate that the health of the Hi–Hi cows

was compromised. The blood BHB thresholds used to define SCK may not be appropriate for grazing dairy cows as they are derived from studies undertaken in housed cows (Compton et al., 2014; Phyn et al., 2017). The cut-points for BHB concentrations used for the definition of SCK may need to be revised in the future when more data become available (Compton et al., 2014; Compton et al., 2015). In the future, monitoring behavioral changes may allow these metabolic and immune-compromised animals to be identified, and subsequently, a management change or intervention could be implemented, allowing a better health outcome.




**Supplemental Figure 10. Blood mineral concentrations during the transition period in 3 energy status groups.**

Figure legend continued over page.

**Supplemental Figure 10 (Continued).** Blood calcium [Ca; (a)] and magneisum [Mg; (b)] concentrations (mmol/L) during d –14 to –1 precalving, d 0 to 2 postcalving, d 3 to

7 postcalving, and d 8 to 15 postcalving for the 3 energy status groups [Lo–Lo (blood NEFA <1.0 mmol/L and blood BHB ≤1.0 mmol/L at 2 consecutive samplings during the first 2 wk postcalving); Hi–Lo (blood NEFA ≥1.0 mmol/L and blood BHB ≤1.0 mmol/L at 1 or more samplings during the first 2 wk postcalving); Hi–Hi (blood NEFA ≥1.0 mmol/L and blood BHB ≥1.2 mmol/L at 1 or more samplings during the first 2 wk postcalving)]. Error bars represent 2 x mean standard error of the difference.

# APPENDIX 19: TECHNOLOGY ADOPTION BARRIERS

### *Appendix 19.1 Challenges Due to Lack of Infrastructure In Grazing Dairy Cows*

Farms in New Zealand and Australia are predominantly pasture based where few cows are housed indoors (Beggs et al., 2018). Pasture is the predominant feed in these systems (Roche et al., 2017b); therefore, the land area is a key resource for pasture-based dairy farms. The average herd size in New Zealand and Australia has continued to increase (Dairy Australia, 2019; LIC and DairyNZ, 2019) and farms occupy large land areas to support their stock. Therefore, it is not uncommon for cows to be placed in paddocks which may be considerable distances (up to 4 km) from the milking parlor (Beggs et al., 2018) and during the dry period cows may be grazed off-farm (Edwards et al., 2014). The ratio of caretakers to animals in large herds is reduced and when cows are grazing in paddocks far away from the milking parlor or off farm, this further decreases the opportunity for staff to closely monitor individual cows (Stafford and Gregory, 2008). This creates a unique opportunity for the use of precision technologies that allow the individual monitoring of animals but also creates a unique challenge due to the infrastructure (connectivity) required to allow automated data capture from monitoring technologies (Gargiulo et al., 2018).

Wireless transceivers require cows to regularly pass within close proximity of the transceiver (range 5 to 1000+ m) to allow automatic data download (Richeson et al., 2018); therefore, in housed systems, the proximity of cows to the ‘technology hub’ removes some of the complexities of implementing precision technologies (Gargiulo et al., 2018). In grazing systems, however, animals may be spread out and outside of the antenna range, which creates problems with automated data capture (Petersson-Wolfe et al., 2017). Portable wireless transceivers may provide a solution for some of the

connectivity issues experienced on farms where the animals are not always located close to a central location, however, requires a large capital investment ($2,500 to $4,000 per transceiver; B. T. Dela Rue, DairyNZ, Hamilton, New Zealand, personal communication) and portable units would require regular shifting by farm staff. Alternatively, the use of devices with cellular data retrieval provides another solution; however, many rural properties in New Zealand do not have cellular coverage available (Federated Farmers of New Zealand, 2017). Resolving these connectivity issues in New Zealand grazing systems may allow the use of data download technologies to be more practical for many farmers.

# APPENDIX 20. DRC 16 FORMS

The ‘Statements of Contribution’ to Doctoral thesis containing publications or prepared for publication are appended below for Chapters 3, 4, 5, 6, 7, and 8.

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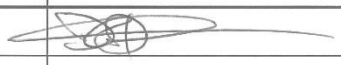
GRADUATE RESEARCH SCHOOL

# STATEMENT OF CONTRIBUTION
# DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

| | |
|---|---|
| Name of candidate: | Stacey Johanna Hendriks |
| Name/title of Primary Supervisor: | Prof. Danny Donaghy |
| Name of Research Output and full reference: | |
| Graduate Student Literature Review: Considerations and understanding the appropriate use of wearable accelerometers to monitor lying behaviors of dairy cows. | |
| In which Chapter is the Manuscript /Published work: | Chapter 3 |
| Please indicate: | |
| • The percentage of the manuscript/Published Work that was contributed by the candidate: | 85% |
| and | |
| • Describe the contribution that the candidate has made to the Manuscript/Published Work: | |
| Development of the scope of the review, collation of literature, manuscript write-up, and development of review narrative was completed by S. J. Hendriks. | |
| For manuscripts intended for publication please indicate target journal: | |
| Submitted to Journal of Dairy Science. November 2019. | |
| Candidate's Signature: |  |
| Date: | 10/02/2020 |
| Primary Supervisor's Signature: |  |
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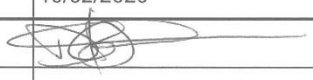
| | |
|---|---|
| Name of candidate: | Stacey Johanna Hendriks |
| Name/title of Primary Supervisor: | Prof. Danny Donaghy |
| Name of Research Output and full reference: | |
| Technical Note: A comparison of editing criteria applied to behavior data recorded by two different IceRobotics three-dimensional accelerometer devices. | |
| In which Chapter is the Manuscript /Published work: | Chapter 4 |
| Please indicate: | |
| • The percentage of the manuscript/Published Work that was contributed by the candidate: | 80% |
| and | |
| • Describe the contribution that the candidate has made to the Manuscript/Published Work: | |
| Exploratory analysis and study design was completed by S. J. Hendriks with technical assistance. Development of the scope of the review, manuscript write-up and results interpretation was completed by S. J. Hendriks | |
| For manuscripts intended for publication please indicate target journal: | |
| Submitted to Proceedings of the New Zealand Society of Animal Production | |
| Candidate's Signature: |  |
| Date: | 10/02/2020 |
| Primary Supervisor's Signature: |  |
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|---|---|
| Name of candidate: | Stacey Johanna Hendriks |
| Name/title of Primary Supervisor: | Prof. Danny Donaghy |
| Name of Research Output and full reference: | |
| Effect of weather on activity and lying behaviour in clinically healthy grazing dairy cows during the transition period. Anim. Prod. Sci. 60 :148–153. https://doi.org/10.1071/AN18569. | |
| In which Chapter is the Manuscript /Published work: | Chapter 5 |
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| • The percentage of the manuscript/Published Work that was contributed by the candidate: | 85% |
| and | |
| • Describe the contribution that the candidate has made to the Manuscript/Published Work: | |
| Editing, summarizing, interpretation and analysis of data, interpretation of results and manuscript write-up was completed by S. J. Hendriks. | |
| For manuscripts intended for publication please indicate target journal: | |
| Journal of Dairy Science (Published) | |
| Candidate's Signature: |  |
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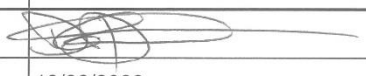
| Primary Supervisor's Signature: |  |
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| Name of candidate: | Stacey Johanna Hendriks |
| Name/title of Primary Supervisor: | Prof. Danny Donaghy |
| Name of Research Output and full reference: | |
| Lying behavior and activity during the transition period of clinically healthy grazing dairy cows. J. Dairy Sci. 102: 7371–7384. https://doi.org/10.3168/jds.2018-16045. | |
| In which Chapter is the Manuscript /Published work: | Chapter 6 |
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| • The percentage of the manuscript/Published Work that was contributed by the candidate: | 80% |
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| Editing, summarizing, interpretation and analysis of data, interpretation of results and manuscript write-up was completed by S. J. Hendriks. | |
| For manuscripts intended for publication please indicate target journal: | |
| Journal of Dairy Science (Published) | |
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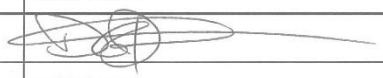
|---|---|
| Name of candidate: | Stacey Johanna Hendriks |
| Name/title of Primary Supervisor: | Prof. Danny Donaghy |
| Name of Research Output and full reference: | |
| Associations between lying behavior and activity and hypocalcemia in grazing dairy cows during the transition period. | |
| In which Chapter is the Manuscript /Published work: | Chapter 7 |
| Please indicate: | |
| • The percentage of the manuscript/Published Work that was contributed by the candidate: | 85% |
| and | |
| • Describe the contribution that the candidate has made to the Manuscript/Published Work: | |
| Editing, summarizing, interpretation and analysis of data, interpretation of results and manuscript write-up was completed by S. J. Hendriks. | |
| For manuscripts intended for publication please indicate target journal: | |
| Submitted to Journal of Dairy Science. December 2019. | |
| Candidate's Signature: |  |
| Date: | 10/02/2020 |
| Primary Supervisor's Signature: |  |
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| Name of candidate: | Stacey Johanna Hendriks |
| Name/title of Primary Supervisor: | Prof. Danny Donaghy |
| Name of Research Output and full reference: | |
| Changes in lying behavior and activity during the transition period could be indicative of risk of hyperketonemia in grazing dairy cows | |
| In which Chapter is the Manuscript /Published work: | Chapter 8 |
| Please indicate: | |
| • The percentage of the manuscript/Published Work that was contributed by the candidate: | 85% |
| and | |
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| Editing, summarizing, interpretation and analysis of data, interpretation of results and manuscript write-up was completed by S. J. Hendriks. | |
| For manuscripts intended for publication please indicate target journal: | |
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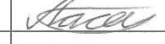
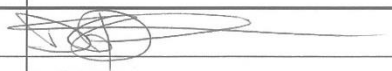
| Candidate's Signature: |  |
| Date: | 10/02/2020 |
| Primary Supervisor's Signature: |  |
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