

DISSERTATION

LEVERAGING PRECISION TECHNOLOGIES FOR IMPROVED HEALTH,
WELFARE, AND PERFORMANCE OF DAIRY COWS

Submitted by

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ABSTRACT

LEVERAGING PRECISION TECHNOLOGIES FOR IMPROVED HEALTH, WELFARE, AND PERFORMANCE OF DAIRY COWS

In recent years, the incorporation of precision technologies, in particular the automated health monitoring systems, into dairy herd management has opened new opportunities for understanding how health, productivity, and reproductive performance interact in cows throughout lactation. This dissertation, of an introduction and four observational studies, leverages data derived from automated body condition scoring (ABCS) systems and automatic milking systems (AMS) to investigate critical aspects of cow health, welfare, and performance. Conducted across different production systems and geographical settings, these studies explore how behavioral and physiological indicators can help detect and even anticipate important health and productive outcomes in Holstein cows.

Chapter 1, the introduction, explores highlighted aspects of the effects of the rise in global demand for dairy, which demands farms to increase milk production, often at the expense of animal health and welfare. The period around calving, known as the transition period, is especially delicate because cows undergo intense physical and metabolic adjustments that can put them at risk for developing several diseases. These disorders, such as metabolic imbalances and infectious diseases, frequently occur together and can have lasting effects on milk production, fertility, and overall welfare. In large operations, early signs of health problems often go unnoticed. To address this issue, many farms are turning to precision technologies such as automated health monitoring systems, designed to identify cows at risk of disease due to changes in normal patterns of behavior

or physiology. Studies in Chapter 2 and Chapter 3 utilize tools like ABCS systems to monitor changes in fat reserves that reflect energy status, while studies in Chapter 4 and Chapter 5 use AMS to record changes in milking behavior that can indicate underlying health issues. When used together, these systems allow farmers to detect problems earlier and make more informed decisions, ultimately supporting both productivity and animal welfare.

The first study, in Chapter 2, focused on characterizing the dynamics of BCS during early lactation, with particular attention to the nadir BCS (nBCS) as the lowest BCS value observed within the first 100 days in milk (DIM). Exploring the factors associated with the timing and depth of nBCS in early lactation, and its effect on reproductive outcomes. Analyzing more than 12,000 lactations in a commercial dairy herd in Colorado using an ABCS system, this study identified key variables associated with both the timing and magnitude of nBCS, such as initial BCS at dry-off, average milk yield in early lactation, and occurrence of periparturient diseases. Also, it was observed that cows with lower nBCS and those who lost more condition between calving and the nadir had lower chances of conceiving at first artificial insemination. Interestingly, cows that did become pregnant at first service reached their nBCS earlier, which may suggest that a faster physiological recovery improves reproductive success. While cows that later experienced pregnancy loss often had lower nBCS, this pattern was not consistent across all analyses. These findings highlight the usefulness of tracking continuous BCS values using ABCS system as part of routine monitoring to support reproductive efficiency and transition management.

The second study in Chapter 3 shifted the focus to a pasture-based system in southern Chile, where year-round calvings and seasonal forage variation introduce additional complexity. Analyzing over 2,000 lactations, the study examined how BCS patterns, also using the ABCS system, varied with calving season and how these patterns were related to health status and milk

production. Seasonal effects were evident, especially in primiparous cows, who showed the lowest BCS at calving in early spring and the highest in late fall. Conversely, multiparous cows experienced their greatest losses in condition and lowest nadir scores during late spring and early summer, periods likely aligned with increased nutritional demands and potentially reduced pasture quality. The time taken for cows to reach their lowest BCS also varied by season and parity. Higher peak milk yields were associated with deeper drops in BCS, particularly in multiparous cows, highlighting a trade-off between milk output and energy reserves. The study emphasizes the importance of adapting feeding and breeding strategies to seasonal dynamics and lactation stage, especially in grazing systems.

In Chapter 4, the third study focused on health, examining how clinical mastitis affects cow behavior and milking performance in herds managed with AMS. By analyzing nearly 3,000 lactations and hundreds of thousands of individual milking events, the study revealed that cows diagnosed with mastitis during the early, mid, or late stages of early lactation (first 100 DIM) displayed clear deviations in behavior before diagnosis. Cows with mastitis had higher chances of incomplete milkings and more frequent teat placement issues. They also tended to have longer milking intervals, slower milk flow rates, and reduced milk yield compared to healthy cows. These patterns were especially pronounced around the day of diagnosis, suggesting that AMS data can help flag animals at risk even before clinical signs become obvious. The ability to detect these subtle changes early on opens the door to more timely interventions, potentially reducing the severity and impact of mastitis on both welfare and productivity.

In the fourth and final study in Chapter 5, the scope of health monitoring was expanded to include a range of postpartum disorders, such as retained fetal membranes (RFM), clinical hypocalcemia, subclinical ketosis, metritis, and displaced abomasum (DA), diagnosed during the

postpartum transition period (first three weeks after calving). Drawing on data from more than 2,500 lactations, the study examined how these conditions affected behavior and performance indicators measured by AMS. Each disorder had its own distinctive profile; for instance, cows with DA were much more likely to have incomplete milkings, while those with RFM showed a higher probability of having teat not found. Across nearly all health issues, affected cows had longer milking intervals and produced less milk. Cows with metritis had longer and less efficient milkings, while those with subclinical ketosis showed more subtle changes. These differences underscore how AMS can be used not just for identifying disease, but also for characterizing its impact on individual cows. The ability to detect these changes in behavior and performance early on could be critical for improving animal care and management efficiency.

Together, these four studies illustrate the powerful role that automated data collection systems can play in improving the way we manage dairy herds. Whether in housed or pasture-based systems, real-time information on body condition, milk production, and behavior offers a window into the physiological status of each animal, helping farmers detect problems sooner, make more informed decisions, and ultimately improve both performance and welfare. These technologies provide a valuable complement that enhances our understanding of what cows experience during key stages of lactation and recovery. By integrating this kind of data into everyday management, producers can work toward more proactive, responsive, and sustainable dairy systems.

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CHAPTER 1 – INTRODUCTION

TRANSITION PERIOD AND PRECISION TECHNOLOGIES

In recent years, the dairy industry has expanded to meet the dairy consumption needs of the growing population (IDF, 2025). This creates challenges for the dairy sector when trying to increase production levels while maintaining animal welfare and environmental sustainability. Global cow milk production has reached nearly 680 million tons, supported mainly by advances in genetic selection and nutritional improvements aimed at increasing milk yield. However, the rising demand for milk yield predisposes cows to develop health problems and increases culling rates (Fleischer et al., 2001). These indicators not only compromise farm profitability but also raise serious concerns about animal welfare, an issue that continues gaining significant importance for the dairy industry (von Keyserlingk et al., 2009). Additionally, economic limitations and labor shortages further compound these pressures.

Among the many challenges faced by modern dairy systems, one of the most critical stages in a cow's productive cycle is the transition period, which covers the weeks before and after calving (Grummer, 1995; Drackley, 1999). This period is characterized by intense physiological and metabolic changes that enable the cow to move from a pregnant, non-lactating state to a non-pregnant, lactating one. These long-term adaptations, known as homeorhetic processes, involve coordinated changes across multiple systems to support lactation, including rapid fetal growth, the initiation of colostrum and milk production, mammary gland development, and the mobilization of body reserves to meet energy demands (Bauman and Bruce Currie, 1980). However, during this time, a combination of physiological, nutritional, and management challenges places dairy cows at high risk of developing metabolic and infectious diseases (Lean et al., 2013; Sordillo and

Raphael, 2013; Van Saun, 2023). These issues often result in lower milk production, impaired reproductive performance, higher culling rates, and reduced animal welfare (Goff and Horst, 1997; Drackley et al., 2005; Roche et al., 2013a).

As a result of these stressors, it is estimated that more than half of cows will experience one or more health clinical or subclinical events around calving (LeBlanc et al., 2006; LeBlanc, 2010). Most diseases that occur during the transition period are etiologically interconnected and related to inflammatory responses, nutrition management, and productivity stress. Metabolic diseases, defined as those diseases where controls to maintain specific nutrient homeostasis have been lost (Van Saun, 2023), are especially prevalent. These included mineral imbalances (related to calcium, magnesium, phosphorus) and energetic disorders (e.g., negative energy balance, hypoglycemia, hyperketonemia, hepatic lipidosis). They are also highly linked to infectious diseases such as mastitis and metritis (LeBlanc, 2010; Caixeta and Omontese, 2021).

Given the critical risks associated with this stage, effective management and timely interventions are crucial for preventing disease development during this phase. Additionally, careful monitoring of cows during the transition period is essential for detecting early signs of disease.

Due to the intensification of the production systems and the lower human-animal interaction over recent decades, the dairy industry has implemented several technologies to improve herd management and overall efficiency (Schillings et al., 2021; Simitzis et al., 2022). To support these management strategies, both the dairy industry and the scientific community have adopted a variety of precision technologies aimed at improving herd management and early detection of health issues, particularly during early lactation.

Among these technologies, monitoring daily animal-level metrics plays a critical role in evaluating health, fertility, and productivity in dairy cows. According to Kleen and Guatteo (2023), precision farm technologies can be categorized by their applications in animal husbandry, including feeding, reproduction, health, and herd management. However, these categories are not exclusive; for example, activity and location sensors such as pedometers and accelerometers could be used for feeding, health, and reproductive purposes. The precision farm technologies associated with early disease diagnosis are also known as automated health monitoring systems (**AHMS**). For example, the AHMS can identify cows at risk of disease around calving by detecting changes in metrics such as dry matter intake, water consumption, rumination time, body condition score (**BCS**), locomotion score, lying behavior, milk yield and performance parameters, milking behavior, and physiological indicators like rumen pH and body temperature, often several days before a clinical diagnosis is possible (Caixeta and Omontese, 2021; Perez et al., 2023). This is particularly significant in large herds, where reduced human contact makes visual detection of physical or behavioral changes nearly impossible (Barkema et al., 2015). The AHMS are designed to detect deviations from normal behaviors or physiological patterns in individual animals and to generate alerts to warn the farm personnel about possible health issues (Barkema et al., 2015; Caixeta and Omontese, 2021). This allows for earlier intervention and improved animal welfare, as well as enhanced reproductive and productive outcomes.

This document will explore two precision farm technologies: the first related to BCS dynamics, and the second related to milking behavior and performance.

Given its close relationship with energy status and postpartum disorders, BCS remains one of the most valuable metrics for informing energy metabolism balance during the transition period (Roche et al., 2013b). The BCS enables the estimation of subcutaneous fat reserves on a numeric

scale due to the direct effect of fat mobilization during the transition period (Edmonson et al., 1989). These fat reserves are closely associated with metabolic imbalances resulting from the increased nutrient demands of the mammary gland at the start of lactation, along with a slower increase in dry matter intake (Ferguson et al., 1994; Roche et al., 2013a). Thus, BCS provides essential information for decision-making in nutritional and health management, helping to prevent metabolic imbalances and optimize productive and reproductive performance (Roche et al., 2013a). Therefore, periodic assessment of BCS during the transition period is a crucial tool for monitoring the energy status of dairy cows (Roche et al., 2009).

To monitor daily values of BCS, recent technological advances have led to the development of automated systems known as automated body condition scoring (**ABCS**) systems, which are designed to help reduce subjectivity and minimize the effort involved in assessing BCS (Mullins et al., 2019). In general, the ABCS systems rely mainly on image analysis through cameras placed in strategic locations, such as milking parlors or exit lanes. These systems utilize computer vision and machine learning algorithms to estimate BCS by analyzing specific anatomical landmarks, providing an objective, consistent, and high-frequency assessment of body condition that does not require direct human intervention (Mullins et al., 2019; Silva et al., 2021; Truman et al., 2022). Additionally, they offer the valuable opportunity to monitor daily BCS values, enabling early detection of subtle changes in body condition and facilitating timely interventions that can greatly enhance postpartum health and productivity during lactation (Silva et al., 2021). Therefore, Chapters 2 and 3 of this document assess the dynamics of BCS in Holstein cows during lactation using ABCS systems, focusing on the BCS nadir and its relationship with health, production, and reproduction. Chapter 2, conducted in a conventional system in the United States, aims to identify factors associated with the timing and magnitude of the BCS nadir and their relationship with

fertility. Chapter 3, conducted in a pastoral system in southern Chile, aims to characterize seasonal variations in automated BCS and analyze their relationship with peak milk production across different calving seasons.

On the other hand, the use of behavioral changes as a tool to identify diseases is based on the premise that animals, when experiencing physiological changes or states of discomfort, modify their behavior in observable ways (Huzzey et al., 2005; Tsai et al., 2021). However, cattle, as prey animals, tend to display a stoic behavior during illness, often masking early signs of disease to avoid appearing vulnerable (Weary et al., 2009). This natural tendency makes it challenging for farmers to detect subtle behavioral changes through visual observation alone, especially in large herds where individual monitoring is limited (Barkema et al., 2015). Therefore, behavioral monitoring, aided by precision technologies, represents a powerful tool for earlier diagnosis (Barkema et al., 2015; Dittrich et al., 2019). Milking behavior is particularly sensitive to factors such as pain, inflammation, or stress, making it an indirect but valuable indicator of the animal's health (King and DeVries, 2018). In fact, deviations in milking patterns, such as reduced milking frequency, altered milking duration, increased kicking frequency, or changes in milk flow, can serve as early indicators of underlying health issues (Bach et al., 2007; King et al., 2017, 2018). However, milking behavior has received less attention in the literature compared to other behaviors, such as eating, ruminating, lying, resting, and social interactions, mainly because the precision technologies for evaluation are limited. In this context, the automatic milking system (AMS) has emerged as a rapid and effective tool for continuously and objectively monitoring milking behavior, enabling the timely detection of abnormalities and contributing to improved animal health management (Barkema et al., 2015; King and DeVries, 2018).

The use of AMS has increased in recent decades, primarily due to its advantages, including reduced labor, minimal human-animal interactions, and consistency in the milking procedure (Svennersten-Sjaunja and Pettersson, 2008). In addition to these operational benefits, improved fertility and overall health and welfare have also been observed in cows managed under AMS conditions (Jacobs and Siegford, 2012; Tse et al., 2018). In AMS, cows have greater autonomy over their daily routines and behaviors, including feeding, lying, and milking activities, as they can voluntarily access the milking system and establish their own milking schedules (Jacobs and Siegford, 2012). This voluntary interaction not only supports natural behavioral expression but also provides an opportunity to explore individual milking behavior patterns. Also, the individualization of each cow is a unique advantage of AMS for evaluating different parameters (Jacobs and Siegford, 2012; Barkema et al., 2015). Thus, the copious data from the AMS can be used to assess the cow's behavior, especially inside the robot during the milking process, allowing for individual measures per each AMS visit. In this context, both Chapters 4 and 5 in this document investigate how data collected by AMS can detect early changes in the behavior and performance of dairy cows, including parameters such as milking interval, milking duration, milk flow, and events of incomplete milking and milking unit kick-off, that are associated with transition diseases. Chapter 4 focuses on evaluating the effects of clinical mastitis during the first third of lactation, while Chapter 5 explores various postpartum diseases, including clinical hypocalcemia, retained fetal membranes, subclinical ketosis, metritis, and displaced abomasum, all of which are diagnosed within the first 21 days of lactation.

Finally, addressing body condition score and milking behavior in dairy cows through the use of automated precision farm technologies allows for a more complete view of the challenges that cows face during the transition period. This integration not only improves the understanding

of the processes affecting animal health and welfare but also creates new opportunities for more timely and efficient interventions. The findings presented in these studies aim to provide concrete tools to support decision-making in modern dairy systems, whether in confined or grazing systems, promoting sustainable production focused on animal welfare.

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CHAPTER 2 – FACTORS ASSOCIATED WITH THE TIME AND MAGNITUDE OF THE NADIR BODY CONDITION SCORE IN EARLY LACTATION AND ITS SUBSEQUENT EFFECTS ON FERTILITY OF HOLSTEIN COWS

Abstract

The objective of this study was to identify variables associated with the time and magnitude of nadir BCS (**nBCS**) during early lactation and to characterize the effects of these two measurements on the subsequent pregnancy at first artificial insemination (**P/AI1**) and pregnancy loss of Holstein cows. A retrospective observational study was completed using data collected from 12,042 lactations in 7,626 Holstein cows calving between April 2019 and January 2022 in a commercial dairy operation located in Colorado, USA. Scores generated by an automated BCS camera system at dry-off (**BCSdry**), calving (**BCSc**), nadir [**nBCS**; defined as the lowest daily BCS from calving to 100 days in milk (DIM)], and 21 d post nadir were selected for the analyses and subsequently categorized into quartiles (Q1 = lower nBCS). Changes in BCS were calculated from dry-off to calving (multiparous cows); from calving to nadir; and from nadir to 21 d post nadir and assigned into quartile categories considering Q1 as the 25% of cows with greater loss (pre nadir) or smaller gain (post nadir) of BCS. Data were examined using logistic regression, ANOVA, and time to event analyses. Initial univariable models were followed by multivariable models that considered parity category, calving season, BCSc, and average milk yield up to 30 DIM (**M30**) as covariables. Sick cows and high producing cows had increased DIM at nadir, while lower BCSdry, greater M30, and occurrence of disease were associated with lower nBCS. Cows that conceived at AI1 had their nBCS earlier (45.1 ± 0.32 d) than cows that remained open after first AI (46.4 ± 0.35 d). On the contrary, DIM at nBCS did not differ by pregnancy loss status. The

logistic regression analyses reported smaller odds of P/AI1 in cows in the lower nBCS categories. Likewise, cows with larger loss in BCS between calving and nadir had smaller odds of P/AI1. Although cows that lost their pregnancy had lower nBCS, this association was not consistent across the analyses. The time to and magnitude of nBCS, together with the Δ BCS from calving to nadir could be useful measures to evaluate the transition and early lactation status of dairy cows.

Introduction

The transition from gestation to lactation requires adjustments in nutrient consumption that are challenging for the postpartum dairy cow (Horst et al., 2021). As milk energy output increases more rapidly than energy intake, most cows experience a negative energy balance that extends for 30 to 100 d (Drackley, 1999; Coffey et al., 2002). In response, fat and labile protein are mobilized from body energy reserves, which results in reductions in body condition score (**BCS**) during early lactation (Bauman and Currie, 1980; Lean et al., 2013).

Interestingly, research presents variable levels in BCS changes postpartum, including cows that maintain or gain body condition during early lactation (Britt, 1992; Middleton et al., 2019; Pinedo et al., 2022a). Proposed factors associated with this variability include genetic selection for milk production (Mao et al., 2004; Lucy et al., 2009), management (McNamara, 1991), parity (Coffey et al., 2004), season of calving (Pryce et al., 2001), and health status (Chebel et al., 2018; Melendez et al., 2020; Pinedo et al., 2022a), among others. However, as most studies performed BC scoring at predetermined time points in the lactation, there are limitations on their ability to precisely describe the dynamics of BCS change, especially the time and magnitude of the nadir BCS (**nBCS**) post calving.

As automated camera systems developed in recent years provide daily BCS (Truman et al., 2022), potential factors affecting the nadir BCS, as well as its effects on subsequent performance, can be explored in greater detail. Since the nadir BCS represents the lowest level in BC, identifying this point would provide a single measurement that could be used as a reference for decision making at the farm. Nonetheless, the relevance of this value as compared to the change in BCS relative to calving and the importance of the time when the lowest BCS is reached have not been explored. In consequence, it becomes relevant to investigate factors affecting the time and magnitude of the nadir, as well as defining the value of this measurement as a predictor of future cow's performance.

Although the interrelationship between changes in BCS and occurrence of metabolic imbalances and early lactation diseases has been reported (Roche et al., 2009; Chebel et al., 2018; Stevenson et al., 2020), the lack of high frequency BC scoring has limited the potential for addressing event timing when exploring these associations. On the one hand, cows with inadequate BCS at dry-off and calving are more likely to be affected by disease (Roche et al., 2009; Barletta et al., 2017; Chebel et al., 2018). Conversely, diseases that are associated with reduced dry matter intake (**DMI**) would result in increased loss of body reserves (Bach et al., 2007; Miguel-Pacheco et al., 2014) and inefficient feed utilization due to increased glucose use during infection (Lang and Dobrescu, 1991; Kvidera et al., 2017).

The physiological effects of energy status and energy balance on the resumption of postpartum ovarian cyclicity and the likelihood of a successful pregnancy are widely recognized (Roche et al., 2009; Carvalho et al., 2014; Chebel et al. 2018; Pinedo et al., 2022a). Considering the potential interrelations between the cow's ability to properly transition to early lactation and

the magnitude of the BCS loss, exploring the time and magnitude of nBCS as predictors of subsequent performance is plausible.

Hence, the hypothesis of this study was that the time and magnitude of the change in BCS following parturition would be affected by variables such as health status and milk yield. Moreover, time and magnitude of nadir BCS would impact subsequent fertility. Therefore, the objective of this study was to identify variables associated with the time and magnitude of nBCS during early lactation and to characterize the effects of these two measurements on the subsequent pregnancy at first artificial insemination (**P/AI1**) and pregnancy loss of Holstein cows.

Materials and methods

Study population

This retrospective observational study is part of a research effort investigating the dynamics of automated BCS during the dry period and early lactation and their subsequent effects in reproductive performance (Pinedo et al., 2022a, b). The analyses included data collected from 12,042 lactations in 7,626 Holstein cows calving between April 2019 and January 2022 in a commercial dairy operation located in Windsor, Colorado, USA. In addition to the aforementioned time period for the calving's, the inclusion criteria required availability of daily BCS and at least one AI for the current lactation.

Cows were housed in a cross ventilated barn, milked 3× in a 90 units rotary parlor, and subject to first AI at about 80 days in milk (**DIM**; primiparous) and 60 DIM (multiparous), following a double OvSynch protocol. Pregnancy diagnosis was performed via transrectal ultrasonography on day 32 ± 3 after artificial insemination (**AI**) and reconfirmed at day 80 ± 3 of gestation. Cows determined non pregnant were administered prostaglandin F2 α if a corpus luteum

was visible and were submitted for AI based on estrus detection using the DeLaval activity meter system (DelPro Farm Manager software; DeLaval International AB, Tumba, Sweden).

Data were collected from dry-off (multiparous) or calving (primiparous) until dry-off or culling. Cow demographic, reproductive, and health data were extracted from on-farm software (DairyComp 305; Valley Ag Software, Tulare, CA). Daily milk yield and BCS were extracted from DelPro Farm Manager software.

Information originated from DairyComp 305 and DelPro Management software was combined in a single file with records organized by cow and lactation number. Each record started with the cow's unique identification number, followed by the calving date and ending with the date of dry-off, live culling, or death. Date of and BCS at the previous dry-off were also included in multiparous cows.

The dataset included cows' individual identification (**ID**), date of calving, lactation number, calving-related and disease events, breeding dates, pregnancy diagnosis outcomes, daily milk yield for the first 30 DIM (**M30**), and daily BCS.

Assessment and selection of body condition score

Scores were generated by BCS video cameras (DeLaval International AB, Tumba, Sweden) previously validated by Mullins et al. (2019) that were mounted on the sorting-gate at each exit ($n = 2$) of the milking parlor. As the cow passed under the mounted camera, a continuous video (30 FPS, 32,000 captured reference points) was taken and a 3D image from the video was automatically created and saved by the BCS camera software (Mullins et al., 2019). In a secondary step, the saved 3D images were processed through an algorithm and analyzed to locate the key physical characteristics (pins, tail head ligaments, sacral ligaments, short ribs, and hooks) of the

cow to calculate the automated BCS, viewable in DelPro Farm Manager. The proprietary algorithm used the BCS scoring scale proposed by earlier studies, modified to report BCS in 0.1 increments in a scale from 1 to 5 in a ¼ unit increment (Ferguson et al., 1994).

All automated BCS data were recorded in and downloaded from DelPro Farm Manager and BCS at dry-off (**BCS_{dry}**), calving (**BCS_c**), nadir DIM (nBCS; defined as the lowest daily BCS from calving to 100 DIM), and 21 d post nadir (**nBCS+21d**) were selected for the analyses. As BCS from the camera system were not reported as a continuous variable, but in 0.1 intervals, scores were categorized using the quartile distribution at each time point in analysis. Individual cow values for nBCS were categorized as Q1 ($\leq$ lower quartile), Q2, Q3, and Q4 ($\geq$ upper quartile), separately for primiparous and multiparous cows (modified from Pinedo et al., 2022a).

Changes in BCS (**Δ BCS**) were calculated by periods of interest subtracting the BCS at the earliest time from the BCS at the latest time as follows: Δ dry-off to calving = $\text{BCS}_c - \text{BCS}_{dry}$; Δ calving to nadir = $\text{nBCS} - \text{BCS}_c$; and Δ nadir to 21 d post nadir = $\text{nBCS}+21d - \text{nBCS}$. Furthermore, based on the magnitude of the BCS change between time points, cows were assigned into quartile categories considering Q1 as the 25% of cows with greater loss (pre nadir) or smaller gain (post nadir) of BCS.

Time to nadir was defined as the DIM when the cow reached nBCS. Values were categorized using the quartile distribution (Q1 = lower DIM), separately for primiparous and multiparous cows.

Covariables, categorization, and study outcomes

Calving-related events and diseases were obtained from farm records stored in on-farm software. Only health events diagnosed before or at the day nBCS were considered in the analyses. When a cow had repeated events for the same disease, only the first event was considered.

Reproductive health events of interest included retained fetal membranes (membranes not expelled after 24 h postcalving, Kelton et al., 1998), metritis (7 ± 4 DIM; watery, reddish/brownish fetid vaginal discharge, independent of fever; McDougall et al., 2007), and pyometra (25 ± 3 DIM; ultrasound examination evidencing accumulation of purulent material within the uterine lumen in the presence of a persistent corpus luteum and a closed cervix, Sheldon et al., 2006). Non reproductive disorders included clinical hypocalcemia (down cow or cow unsteady prior to calving to 1 or 2 dafter calving with no other abnormal physical exam findings and responsive to calcium administration), subclinical ketosis (6 ± 5 DIM; blood BHB > 1.3 mmol/L), left displaced abomasum (off feed, scant pasty manure, ping in left flank, usually within 30 DIM), lameness (assessed weekly; score > 2 ; Bicalho et al., 2007), clinical mastitis (abnormal milk or udder inflammation); digestive problem (off feed, altered feces), injury (visible body trauma including wounds, ulcerations, and swelling), and pneumonia (nasal discharge, respiratory distress, altered lung sounds; Pinedo et al., 2022b).

Health-related events occurring before BCSn were categorized into reproductive (**REP**; dystocia, retained fetal membranes, metritis, and pyometra), metabolic (**MTB**; clinical hypocalcemia, subclinical ketosis, left displaced abomasum) or other disorders (**OTH**; lameness, clinical mastitis, digestive problem, injury, and respiratory disease). Lactations were classified as affected by REP, MTB, OTH, multiple categories (**MLT**), or unaffected (**HLT**). In addition, a

second classification based on the number of health events before nadir was completed as healthy, one event, or multiple events.

Parity was created as a binary variable including primiparous (lactation number = 1) and multiparous (lactation number ≥ 2) cows. Calving's were grouped by season (spring, summer, fall, or winter). Finally, a milk yield category was added as a covariable in the models using the quartile distribution of the average daily milk yield in the first 30 DIM (M30) obtained from DelPro Farm Manager. Cows were assigned into quartiles as Q1 (primiparous: < 22.5 kg; multiparous: < 35.8 kg), Q2 (primiparous: 22.5 to 26.1 kg; multiparous: 35.8 to 40.6 kg), Q3 (primiparous: 26.2 to 29.7 kg; multiparous: 40.7 to 45.1 kg), and Q4 (primiparous: > 29.7 kg; multiparous: > 45.1 kg).

Reproductive outcomes included the probability of pregnancy per AI1 (**P/AI1**), time from calving to pregnancy, and pregnancy loss (d 80 ± 3 of gestation). Cows diagnosed pregnant at first examination and subsequently diagnosed not pregnant at the following examination were considered to have undergone pregnancy loss.

Statistical analyses

The approach was to enroll the largest number of cows fitting our inclusion criteria to obtain adequate levels of variation in the variables under analysis. Due to our interest on multiple explanatory variables and outcomes, a single overall sample size determination was not performed. Separate sample size calculations for the analyses of time and magnitude of nBCS, as well as for reproductive outcomes were performed using the data analysis application SAS Power and Sample Size (release 9.4; SAS Institute Inc., Cary, NC). The calculations considered power = 80% and confidence = 95%. For testing associations between health status and magnitude and time of nBCS, differences of 0.05 points in nBCS and 3 d between healthy and sick (multiple events) cows were

considered. For reproductive outcomes, a difference of 0.05 points in nBCS between pregnant and non-pregnant cows was considered. The reference values (mean and standard deviation [**SD**]) for nBCS and time to nBCS were 2.90 (0.24) and 40 (25) d, respectively (health status analyses). The reference value for nBCS in the reproductive analysis was 2.80 (0.24) (BCS by pregnancy at AI1 outcome). The resulting numbers of cows per group required to show significant differences for nBCS and time to nBCS by health status were 394, and 1,092, respectively. A total of 369 cows per group were required for the reproductive analysis.

Descriptive statistics for BCS at specific time points by parity category were calculated using the PROC UNIVARIATE in SAS 9.4. Descriptive time-to-event analyses for calving to pregnancy by category of nBCS and by category of BCS change from calving to nadir were performed for primiparous and multiparous cows using PROC LIFETEST.

Initial univariable models using only nBCS or Δ BCS as explanatory variables were followed by multivariable models that considered parity category, calving season, BCS_c, and M30 as covariables. Estimated marginal means (least square means, **LSM**) for nBCS and Δ BCS were calculated and compared using ANOVA (PROC GENMOD).

Odds ratios (**OR**) and predicted probabilities for pregnancy at AI1 were estimated for nBCS category, Δ BCS between calving and nadir category, Δ BCS between nadir and 21 d post nadir category, and for DIM at nadir category using PROC GLIMMIX. A backward stepwise elimination procedure was used considering four categories of nBCS [Q1 = lower BCS; Q4 = greater BCS (reference)] and Δ BCS [Q1 as the 25% of cows with greater loss (pre nadir) or smaller gain (post nadir) of BCS; Q4 = reference], the covariables (**COV**), and their interaction in the initial model. The logistic equation to investigate the effects of nBCS/ Δ BCS can be expressed as presented by de Mutsert et al. (2009):

$$\ln [p/(1-p)] = \beta_0 + \beta_1(\text{BCS}/\Delta\text{BCS}) + \beta_2(\text{COV}) + \beta_3(\text{BCS}/\Delta\text{BCS} \times \text{COV})$$

where $\ln$ is the natural logarithm, p is the proportion of cows pregnant at AI/presenting pregnancy loss and $[p/(1-p)]$ are the odds of these outcomes, β_0 is the model intercept for each study outcome, β_1, β_2 , and β_3 are the regression parameters for BCS, ΔBCS , the proposed covariables (COV), and the interaction term $\text{BCS}/\Delta\text{BCS} \times \text{COV}$.

From the final logistic regression models, predicted probabilities and 95% confidence intervals (CI) were calculated using the $p=\text{pred}$ and the $l=\text{lower}$ and $u=\text{upper}$ options in the output statement of the procedure. Predicted probabilities from study outcomes were modeled using PROC GENMOD with time to nBCS, nBCS and ΔBCS from calving to nadir categories as predictors.

For all outcome variables, significant predictors were selected at $p < 0.05$; interaction terms and controlling variables remained in the models at $p \leq 0.10$.

Results

The study included data from 12,042 lactations (primiparous = 4,361; multiparous = 7,681) from cows that had at least one AI after calving. All the lactations had BCS records at calving and at nadir available. A total of 4,661 and 10,706 lactations had BCS at dry-off and 21 d after nadir, respectively. Overall, the distribution of calvings across seasons was spring 23.2%, summer 30.9%, fall 29.2%, and winter 16.7%.

Descriptive statistics for BCS at specific time points and time periods are presented in Table 2.1. Multiparous cows had lower mean nBCS (2.86 vs. 3.03) and greater mean reductions in BCS from calving and nadir (-0.46 vs. -0.34) compared with primiparous cows. Gain in BCS from nadir to 21 d post nadir were similar in primiparous (0.12) and multiparous cows (0.14). Overall,

7.32% of the cows had their nBCS within 3 d postpartum, indicating that this group of cows evidencing an early nadir gained BC during early lactation.

Primiparous cows reached nBCS earlier than multiparous cows. Mean $\pm$ SD DIM at nadir for primiparous and multiparous cows were 43.1 ± 26.8 DIM (Q1 = 23 DIM, Q2 = 39 DIM, Q3 = 62 DIM, and Q4 = 100 DIM) and 47.8 ± 25.1 DIM (Q1 = 30 DIM, Q2 = 46 DIM, Q3 = 66 DIM, and Q4 = 108 DIM; $p < 0.0001$), respectively.

Health status

Time to nBCS and nBCS least square means by health status group and by specific diseases are presented in Table 2.2. Mean time to nBCS was associated with health status. Cows classified as healthy reached nBCS earlier (40.9 ± 0.33 DIM) than cows that develop one (49.5 ± 0.41 DIM) or multiple health events before nadir (53.8 ± 0.49 DIM; $p < 0.0001$). Similarly, DIM at nadir differed by system affected, with healthy cows presenting the shortest time (40.9 ± 0.33 DIM) and OTH and MUL the longest (54.2 ± 0.58 DIM and 53.4 ± 0.58 DIM, respectively; Table 2.2).

Time to nBCS was greater for cows in the low nBCS quartile (Q1 = 59.0 d), as compared with cows in Q2 (50.1 d), Q3 (41.4 d) and Q4 (31.0 d; $p < 0.0001$). Likewise, nBCS decreased from cows with earlier nBCS (Q1 = 3.04), to cows in Q2 (2.95), Q3 (2.89), and Q4 (2.86; $p < 0.0001$). Time to nBCS was also different by M30 category, with low producing cows consistently reaching nBCS earlier (Q1 = 41.8 DIM) than cows with greater milk yield (Q2 = 45.0 DIM, Q3 = 48.1 DIM, and Q4 = 51.6 DIM; $p < 0.0001$).

Healthy cows had the highest nBCS, while cows that developed multiple health events had the lowest nBCS. When cows were grouped by specific disease, affected cows had the lowest nBCS (except for injury; Table 2.2).

The differences in nBCS by BCS at dry-off category, calving season, and M30 are presented in Table 2.3. Cows with lower BCS at dry-off had the lowest nBCS (2.78), while cows that had higher BCS at dry-off had the highest nBCS (2.90).

Milk yield

Milk yield (M30) was associated with nBCS. Cows in the lowest M30 quartile had the greatest nBCS values (2.97), while cows in the highest M30 quartile had the lowest nBCS (2.90). This trend for lower nBCS when milk production increased remained when cows were grouped by health status (Table 2.3).

Fertility outcomes

Least square means for time to nBCS and for nBCS and Δ BCS by P/AI1 and pregnancy loss categories are presented in Table 2.4. Cows that conceived at AI1 had their nBCS earlier (45.1 ± 0.32 d) than cows that remained open after first AI (46.4 ± 0.35 d; $p < 0.001$). On the contrary, DIM at nBCS did not differ by pregnancy loss status ($p = 0.22$). Nadir BCS was greater in cows that conceived at first AI (2.86) compared with cows that did not conceive at first AI (2.82). Similarly, cows non-pregnant after first AI had greater loss in BCS between calving and nadir (-0.48) than cows pregnant after first AI (-0.44).

The logistic regression analyses identified significant associations between nBCS category and P/AI1, with smaller odds of pregnancy in cows with low nBCS. Similarly, greater loss in BCS from calving to nadir was associated with lower P/AI1 (Table 2.5). On the contrary, no association was identified between Δ BCS from nadir to 21 d post nadir and P/AI1. The association between pregnancy loss and nBCS or Δ BCS was partial and only evident for the Q1 vs. Q4 categories.

Figures 2.1 and 2.2 show the predicted probabilities of P/AI1 by category of time to nBCS, nBCS, and Δ BCS between calving and nadir. No differences in probabilities were detected among time to nBCS categories in primiparous cows, while for multiparous cows the only difference was identified between Q1 and Q4 categories. When nBCS was analyzed in primiparous cows, the main differences were established between low nBCS (Q1) vs. the other three categories of nBCS. A trend to greater P/AI1 was also observed as the Δ BCS from calving to nadir was less pronounced. For multiparous cows, a more sequential trend for greater P/AI1 from cows in Q1 to cows in Q4, in both nBCS and Δ BCS between calving and nadir, was evident.

Figures 2.3 and 2.4 present the Kaplan–Meier survival curves for the interval from calving to pregnancy in primiparous and multiparous cows by category of nadir BCS. Consistently, cows with greater nBCS and cows with smaller changes in BCS between calving and nadir conceived sooner than cows with lower nBCS or larger loss in BCS.

Mean $\pm$ SD DIM to AI1 for primiparous and multiparous cows were 90.7 ± 19.5 d (Q1 = 80 d, Q2 = 80 d, and Q3 = 96 d) and 69.9 ± 17.6 d (Q1 = 60 d, Q2 = 60 d, and Q3 = 73 d; $p < 0.0001$), respectively. Pregnancy at AI1 was 53.4% (primiparous = 63.2%; multiparous = 47.8%; $p < 0.0001$) and DIM at conception was 113.3 ± 0.4 d (primiparous = 118.3 d; multiparous = 110.3 d; $p < 0.0001$). The overall incidence of early pregnancy loss was 9.1%, with primiparous cows evidencing lower pregnancy loss than multiparous cows (5.4% vs. 11.4%; $p < 0.0001$).

Discussion

The recent development of BCS automated systems and their implementation in commercial farms has allowed for daily assessment of large populations of dairy cows. The availability of high frequency data provides detailed information on the dynamics of BCS, which

permits a more precise evaluation of potential factors affecting BCS and facilitates the analysis of the effect of BCS on performance variables. Nonetheless, the large amounts of data produced by the systems pose a challenge for timely and effective interpretation and application at the farm level. The availability of BCS in this large population of dairy cows allowed for the detection of small differences among groups of interest. Nonetheless, the modest level of variation among the categories in study should be considered when addressing the biological relevance of these differences.

Inadequate transition to early lactation and occurrence of diseases are associated with reduced DMI and result in increased loss of body condition (Bach et al., 2007; Miguel-Pacheco et al., 2014). Moreover, redirection of resources due the activation of the immune system in response to infection also plays a role in loss of weight and body condition, especially in high producing cows (Lang and Dobrescu, 1991; Kvidera et al., 2017). In this study, we hypothesized that the time and magnitude of the change in BCS following parturition would be affected by variables such as health status and milk yield.

Additionally, we anticipated that time and magnitude of nadir BCS would impact subsequent fertility. As reviewed by Roche et al. (2009), most reports studying the physiological effects of energy status and energy balance on fertility suggest a negative association between BCS loss during early lactation and resumption of ovarian cyclicity and subsequent pregnancy. Previous research has explored the ability of multiple time points and periods for body condition scoring as potential predictors of future performance with varying results (Lopez-Gatius et al., 2003; Carvalho et al., 2014, 2019; Chebel et al., 2018; Pinedo et al., 2022a).

The post calving nadir BCS, together with the magnitude of the change in BCS from calving to this point, represent the most extreme BCS change during early lactation, which makes

them plausible candidates for the assessment of cow's energy balance and subsequent performance. Moreover, this point signals the time when cows were able to revert the descending trend for BC.

The BCS originated by the camera system were not reported as a continuous variable, but at 0.1 intervals. Although this represents a limitation for the statistical analysis, in practical terms a difference smaller than 0.1 points in body condition would be difficult to assess.

As expected, most cows in the current study lost BCS during early lactation. However, about 7% of the cows had their lowest BCS at calving and gained BCS afterwards. The proportion of cows maintaining or gaining body condition in our study population is smaller than that reported in previous studies presenting values over 20% (Carvalho et al., 2014; Barletta et al., 2017; Middleton et al., 2019). In addition to differences among management and cow populations, possible explanations for this discrepancy include the variable times for assessment and the fact that in this study we identified the nadir BCS, which could have been missed by other studies. The requirement of first AI for inclusion is another factor that differentiates this study population from those in previous reports. The idea of adding this requisite was to facilitate comparisons in the proposed reproductive parameters and to give time to the cows to reach their nBCS. Nonetheless, the first AI requisite for inclusion left out of the analysis the group of cows that were not able to reach the time for submission to AI (80 DIM in primiparous and 60 DIM in multiparous), following the double OvSynch protocol. It is plausible to envision that this would be the group evidencing the most severe loss in BCS following parturition. In consequence, our findings may not represent the cows that were in the worst condition during their transition period.

Cows usually return to positive energy balance between 40 and 80 DIM (Sutter and Beever, 2000; Coffey et al., 2002). Coincidentally, in this study, primiparous and multiparous cows reached

their nBCS at 43.1 DIM and 47.8 DIM, respectively. This finding agrees with data presented by Truman et al. (2022), where nadir BCS was earlier in primiparous cows (38 DIM) compared with multiparous cows (54 DIM). Nonetheless, other studies have reported nadir BCS later in lactation, with values between 60 and 80 DIM and even up to 120 DIM (Gallo et al., 1996; Pryce et al., 2001; Mao et al., 2004).

Interestingly, in both Truman et al. (2022) and the current study, multiparous cows had greater BCS loss from calving to nadir, compared with primiparous cows. Correspondingly, other studies indicate that second lactation cows may lose significantly less body condition within 7 wks post-calving compared to older cows, and multiparous cows may have greater losses in body condition from dry-off to nadir compared with primiparous cows (Mao et al., 2004; Sakaguchi, 2009; Berry et al., 2011). Moreover, primiparous cows tended to lose less body condition compared to multiparous cows during the first months after parturition (Ruegg and Milton, 1995). Besides the differences associated with parity, the time of nBCS seems to reflect the ability of the cow to start recovering from the challenges in the transition period. In fact, cows with lower nBCS took longer to reach their nBCS (Q1 = 59 d vs. Q4 = 31 d). Moreover, cows with disease also required more time to reach nBCS (40.9 d vs. 53.8 d for healthy and multiple diseases). Interestingly, some diseases had significant impacts on the delay to reach nBCS. Compared with unaffected cows, animals diagnosed with pyometra, mastitis, and lameness took 11, 11, and 10 extra days to reach nadir and revert their BCS loss trend (Table 2.2). On the other hand, high producing cows, which had lower values for nBCS, consistently reached this point later during their lactation (Q1 = 41.7 d; Q4 = 51.6 d), suggesting a delay in their return to positive energy balance.

Aligned with the findings associated with the time to nBCS, both the magnitude of nBCS, as well as the change from calving to nBCS were significantly affected by disease. Notably, the

association between BCS and disease can take place in both directions. In a recent study by Melendez et al., (2020), loss of BCS during the late dry period increased the odds of several postpartum diseases compared to cows that maintained or gained BCS during the entire dry period. Similarly, cows that lost BCS during the dry period had increased culling or death during the first 90 DIM.

Although it is intuitive to consider that disease will result in greater loss of body condition during early lactation, the lack of high frequency BC scoring in previous studies makes it difficult to establish cause-effect associations between these two variables. The availability of precise disease records together with daily BCS was a strength in this study that facilitated the exploration of temporal associations between disease occurrence and nBCS. As expected, cows affected by multiple disease events before nadir had significantly smaller BCS at that point and this was also the case for most of specific diseases in analysis. In fact, healthy cows had the highest nBCS (2.97), while cows that developed multiple health events (2.85) or that were affected in multiple systems (2.86) had the lowest nBCS.

Nonetheless, it should be considered that fat is also deposited in the abdominal cavity around vital organs. These deposits and their mobilization are not evident to the BCS system used in this study and could play a role during the transition period. Interestingly, the distribution of fat deposits and their mobilization dynamics appear to be affected by a genetic component (Melendez et al., 2019) and cows favoring deposits abdominal fat seem to be at greater risk of releasing excesses of NEFA, developing more diseases, such as fatty liver, left displaced abomasum, or ketosis (Hostens et al., 2013).

Milk yield (M30) was also associated with nBCS, with cows in the highest M30 quartile evidencing the lowest nBCS. Previous research has described an association between change in

BCS between calving and nadir and milk yield. In Berry et al. (2007) and Roche et al. (2007a, 2007b), greater post calving BCS loss increased the height of the lactation profile and the rate of milk yield increase to peak. To clarify the confounding role of disease in these associations, we analyzed this relationship in cows that were healthy and in cows with at least one disease event. Interestingly, this trend for lower nBCS when milk production increased remained when cows were grouped by health status (Table 2.3).

Pregnancy at AI1 was associated with nBCS and Δ BCS from calving to nadir. Nadir BCS was greater (2.86) in cows that conceived at first AI compared to cows that did not conceive at first AI (2.82). Similarly, cows non-pregnant after first AI had larger changes in BCS between calving and nadir (-0.48) than cows pregnant after first AI (-0.44). Notably, post nadir changes in BCS (21d) were not associated with P/AI1, indicating that the rate of BCS recovery may not be a significant factor affecting fertility (Table 2.4).

In agreement with later nadir for cows with disease, cows that remained open after first AI had their nBCS later (46.4 d) than cows that conceived at AI1 (45.1 d). These findings agree with previous reports where the magnitude in the change in BCS (Δ BCS) following dry-off and during the transition period was a relevant factor impacting subsequent pregnancy rate (Carvalho et al., 2014). In the study by Barletta et al. (2017), lower numbers of cyclic cows at 50 DIM and decreased P/AI were reported when loss of BCS occurred during the transition period. Moreover, Carvalho et al. (2014) established that cows with the greatest BCS loss during early postpartum displayed increased impairments of embryo development during the first week after AI.

In the current study, nBCS was lower in cows that lost their pregnancy. Similarly, BCS loss from calving to nadir was more pronounced in these cows. Nonetheless, BCS variables were not consistently associated with pregnancy loss and the logistic regression analyses were only

indicative of greater pregnancy loss in cows in the lowest quartile for nBCS and for animals with greater BCS loss after nBCS.

In contrast with this inconsistency in our results, previous research has identified the effect of BCS in maintenance of pregnancy. Ribeiro et al. (2016) reported similar results for cows with low BCS ($BCS < 3.0$) at the moment of AI, compared to those with moderate BCS ($BCS \geq 3$). Lopez-Gatius et al. (2002) reported that the risk of pregnancy loss increased 2.4-fold for each unit decline in BCS from calving to 30 dpostpartum, while Middleton et al. (2019) indicated that BCS loss during the first 30 DIM increased the risk of pregnancy loss from 35 to 60 d after first AI (0.0 vs. 6.7%). Similarly, extensive reductions in BCS between parturition and AI following synchronized ovulation increased the risk of pregnancy loss, compared with cows that had less than one unit loss of BCS or no loss in BCS (Santos et al., 2009). Nonetheless, some research have failed to fully demonstrate consistent impacts of both low BCS or loss of BCS on pregnancy loss (Barletta et al., 2017; Manríquez et al., 2021; Pinedo et al., 2022b).

This study included a large number of lactation records. However, as only one herd was considered in the analyses, the external validity of the reported findings is limited. Moreover, this is a well-managed operation housing high producing Holstein cows, where extreme BCS were not common, which also represents a limitation for this research.

Although this study identified variables associated with the time and magnitude of nadir BCS during early lactation, the size of the differences was small, which should be considered when translating these findings into management strategies at the farm level.

Conclusions

Lower BCS at dry-off, greater milk yields up to 30 DIM, and occurrence of disease were associated with lower nBCS, while sick and high producing cows had increased DIM at nadir. An association between nBCS and P/AI1 was established, with shorter DIM at nadir, greater nBCS, and smaller Δ BCS between calving and nadir in cows that become pregnant at AI1. The associations between BCS category and P/AI1 indicated smaller odds of P/AI1 in cows in the low nBCS category. Likewise, cows with large loss in BCS between calving and nadir had smaller odds of P/AI1. Although cows that lost their pregnancy had lower nBCS, this association was not consistent across the analyses. The magnitude of and time to nBCS, together with the Δ BCS from calving to nadir, could be useful measures to evaluate the transition and early lactation status of dairy cows.

Tables and figures

Table 2.1 Descriptive statistics for BCS at multiple time points and time periods by category.

Time point (BCS)	Q1	Q2	Q3	Mean	SD
Primiparous					
Calving	3.20	3.30	3.45	3.31	0.22
Nadir	2.90	3.07	3.20	3.03	0.20
21d after nadir	3.00	3.20	3.30	3.15	0.19
Multiparous					
Dry-off	3.30	3.40	3.50	3.39	0.49
Calving	3.28	3.40	3.50	3.37	0.15
Nadir	2.70	2.90	3.00	2.86	0.25
21d after nadir	2.90	3.00	3.20	3.00	0.24
Overall					
Calving	3.20	3.35	3.47	3.34	0.20
Nadir	2.80	2.90	3.10	2.92	0.25
21d after nadir	2.90	3.10	3.20	3.06	0.31
Time period (Δ BCS)					
Primiparous					
Calving to nadir	-0.444	-0.303	-0.200	-0.340	0.18
Nadir to 21d after nadir	0.000	0.100	0.200	0.116	0.11
Multiparous					
Dry-off to calving	-0.200	-0.100	0.000	-0.097	0.49
Calving to nadir	-0.600	-0.433	-0.300	-0.455	0.22
Nadir to 21d after nadir	0.088	0.100	0.200	0.143	0.15
Overall					
Calving to nadir	-0.533	-0.400	-0.267	-0.413	0.21
Nadir to 21d after nadir	0.032	0.100	0.200	0.133	0.31

A total of 4,661, 12,042, and 10,706 lactations had BCS at dry-off, nadir, and 21 days after nadir, respectively.

Table 2.2 Least square means (SE) for BCS at nadir by health status groups and specific health disorders.

Health status	nBCS		Time to nBCS (DIM)	
	LSM ¹	SE	LSM	SE
Number of disease events (n)				
Healthy (6,541)	2.97a	0.002	40.9a	0.33
One event (3,218)	2.91b	0.003	49.5b	0.41
Multiple events (2,283)	2.85c	0.004	53.8c	0.49
System affected (n)				
Healthy (6,541)	2.97a	0.002	40.9a	0.33
Reproductive disorders (1,882)	2.92b	0.004	47.8b	0.54
Metabolic disorders (397)	2.89c	0.009	48.6b	0.99
Other disorders (1,623)	2.90c	0.005	54.2c	0.58
Multiple disorders (1,599)	2.86e	0.005	53.4c	0.58

Specific diseases	nBCS			Time to nBCS (DIM)		
	No	Yes	P-value	No	Yes	p-value
Dystocia	2.93 (0.002)	2.91 (0.006)	0.01	45.6	45.9	0.75
Retained fetal membranes	2.94 (0.002)	2.88 (0.008)	<0.0001	45.6	48.6	<0.0001
Metritis	2.94 (0.002)	2.89 (0.004)	<0.0001	44.9	50.0	<0.0001
Pyometra	2.94 (0.005)	2.87 (0.002)	<0.0001	44.5	55.5	<0.0001
Milk fever	2.93 (0.002)	2.87 (0.020)	0.006	45.7	51.1	0.055
Subclinical ketosis	2.93 (0.002)	2.88 (0.005)	<0.0001	45.2	50.3	<0.0001
Left displaced abomasum	2.93 (0.002)	2.81 (0.020)	<0.0001	45.6	53.4	<0.0001
Lameness	2.94 (0.002)	2.92 (0.003)	<0.0001	44.8	55.0	<0.0001
Mastitis	2.93 (0.002)	2.92 (0.003)	0.003	44.9	55.9	<0.0001
Respiratory disorders	2.94 (0.002)	2.91 (0.003)	<0.0001	45.6	54.6	<0.0001
Digestive disorders	2.94 (0.002)	2.89 (0.004)	<0.0001	45.1	52.3	<0.0001
Injury	2.93 (0.002)	2.93 (0.020)	0.82	45.7	44.7	0.77

The full model included parity category, calving season, BCS_c, and average milk yield up to 30 DIM as covariables. Interactions tested were not significant and removed from the models.

¹Different letters within column indicate significant differences ($p < 0.05$)

Table 2.3 Least square means (SE) for BCS at nadir by BCS category at dry-off (multiparous), calving season, and milk yield average at 30 DIM.

Category	LSM ¹	SE
BCS at dry-off (n = 4,661)		
Q1 (lowest 25% BCS)	2.78a	0.005
Q2	2.87b	0.005
Q3	2.88bc	0.006
Q4	2.90c	0.006
Calving season (n)		
Fall (3,514)	2.92a	0.003
Winter (2,009)	2.93a	0.004
Summer (3,731)	2.91b	0.003
Spring (2,788)	2.85c	0.003
Milk yield (up to 30 DIM; n = 12,042)		
Overall population		
Q1 (primiparous <22.5 kg; multiparous <35.8 kg)	2.97a	0.003
Q2 (22.5-26.1 kg; 35.8-40.6 kg)	2.95b	0.003
Q3 (26.2-29.7kg; 40.7-45.1 kg)	2.93c	0.003
Q4 (>29.7 kg; >45.1 kg)	2.90d	0.003
Healthy cows (n = 6,541)		
Q1 (primiparous <22.5 kg; multiparous <35.8 kg)	3.04a	0.006
Q2 (22.5-26.1 kg; 35.8-40.6 kg)	2.98b	0.006
Q3 (26.2-29.7kg; 40.7-45.1 kg)	2.95c	0.006
Q4 (>29.7 kg; >45.1 kg)	2.91d	0.006
Cows with at least one disease event (n = 5,501)		
Q1 (primiparous <22.5 kg; multiparous <35.8 kg)	2.94a	0.004
Q2 (22.5-26.1 kg; 35.8-40.6 kg)	2.91b	0.005
Q3 (26.2-29.7kg; >40.7-45.1 kg)	2.89c	0.005
Q4 (>29.7 kg; >45.1 kg)	2.86d	0.006

The full model included parity category, calving season, BCS_c, and average milk yield up to 30 DIM as covariables. Interactions tested were not significant and removed from the models.

¹Different letters within column indicate significant differences ($p < 0.05$)

Table 2.4 Least square means (SE) for BCS at nadir and for change in BCS from calving to nadir, and nadir to 21d after nadir by pregnancy at first AI and pregnancy loss status.

		Time point/period (BCS)		
	Time to nBCS (DIM)	nBCS	ΔBCS calving to nadir	ΔBCS nadir to 21d after nadir
Pregnancy at 1st AI (n)				
Yes (6,431)	45.1 (0.32)	2.86 (0.004)	-0.44 (0.004)	0.14 (0.003)
No (5,611)	46.4 (0.35)	2.82 (0.004)	-0.48 (0.004)	0.14 (0.003)
<i>p</i> -value	<0.0001	<0.0001	<0.001	0.5
Pregnancy loss (n)				
Yes (918)	47.0 (0.79)	2.83 (0.009)	-0.47 (0.009)	0.14 (0.007)
No (9,172)	46.0 (0.29)	2.85 (0.003)	-0.45 (0.003)	0.14 (0.007)
<i>p</i> -value	0.22	0.048	0.049	0.87

The full model included parity category, calving season, BCSc, and average milk yield up to 30 DIM as covariables.

Table 2.5 Adjusted odds ratios (OR) and 95% CI for pregnancy at first AI and pregnancy loss by category of BCS at nadir, and for category of change in BCS¹ from calving to nadir, and nadir to 21d after nadir.

Time point/period	Nadir		Δ Calving to nadir		Δ Nadir to 21d post nadir	
	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value
Pregnancy at AI1						
BCS/ΔBCS Q1	0.63 (0.55-0.70)	<0.0001	0.63 (0.56-1.70)	<0.0001	0.98 (0.87-1.11)	0.86
BCS/ΔBCS Q2	0.80 (0.72-0.89)	<0.0001	0.83 (0.74-0.92)	0.0004	1.06 (0.94-1.19)	0.36
BCS/ΔBCS Q3	0.91 (0.81-1.01)	0.09	0.86 (0.78-0.96)	0.006	0.97 (0.86-1.09)	0.6
BCS/ΔBCS Q4	Ref.	-	Ref.	-	Ref.	-
Pregnancy loss						
BCS/ΔBCS Q1	1.33 (1.09-1.63)	0.047	1.28 (1.05-1.55)	0.01	0.97 (0.78-1.21)	0.81
BCS/ΔBCS Q2	1.22 (0.99-1.49)	0.053	1.17 (0.96-1.42)	0.11	0.84 (0.68-1.04)	0.11
BCS/ΔBCS Q3	1.07 (0.85-1.34)	0.54	1.05 (0.85-1.28)	0.66	0.77 (0.61-0.96)	0.02
BCS/ΔBCS Q4	Ref.	-	Ref.	-	Ref.	-

The full model included parity category, calving season, BCS_c, and average milk yield up to 30 DIM as covariables.

¹Values for BCS at each time point were categorized using the quartile distribution (Q1 = lower BCS), separately for primiparous and multiparous cows.

Changes in BCS were assigned into quartile categories considering Q1 as the 25% of cows with greater loss (pre nadir) or smaller gain (post nadir) of BCS.

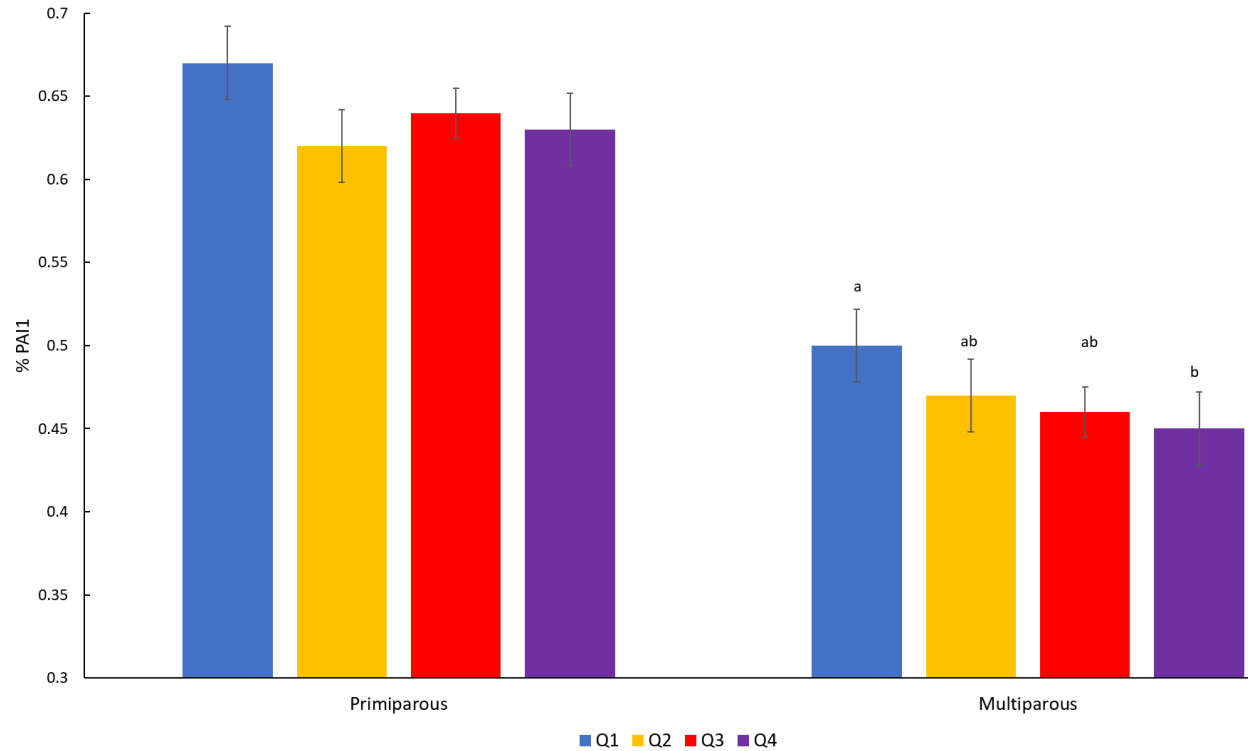


Figure 2.1 Predicted probabilities (LSM and SE bars) for pregnancy at first AI (P/AI1) by time to nBCS category in primiparous and multiparous cows.

The full model included parity category, calving season, BCSc, and average milk yield up to 30 DIM as covariables. Time to nadir was defined as the DIM when the cow reached nBCS. Values were categorized using the quartile distribution (Q1 = lower DIM), separately for primiparous and multiparous cows. Different letters indicate statistical differences at $p < 0.05$.

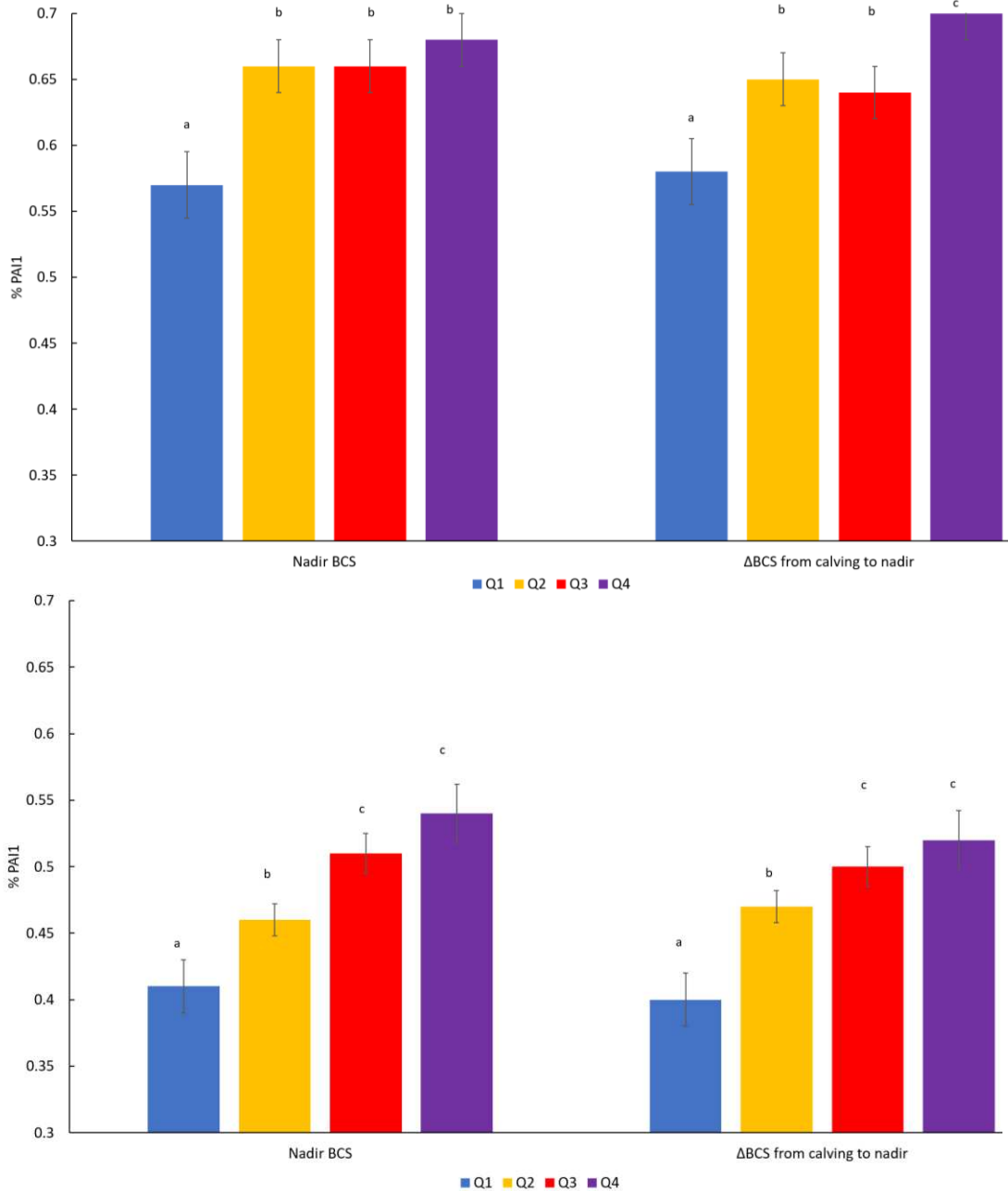


Figure 2.2 Predicted probabilities (LSM and SE bars) for pregnancy at first AI (P/AI1) by nBCS category and Δ BCS from calving to nadir category in primiparous (top panel; n = 4,361) and multiparous cows (bottom panel; n = 7,681).

The full model included parity category, calving season, BCS_c, and average milk yield up to 30 DIM as covariables. Values for BCS at each time point were categorized using the quartile distribution [Q1 = lower BCS (blue); Q2 = orange; Q3 = red; Q4 = greatest quantile (purple)], separately for primiparous and multiparous cows.

Based on the magnitude of the BCS change between time points, cows were assigned into quartile categories considering Q1 (blue) as the 25% of cows with greater loss of BCS, Q2 (orange), Q3 (red), and Q4 [25% of cows with the smallest loss of BCS (purple)].

Different letters indicate statistical differences at $p < 0.05$.

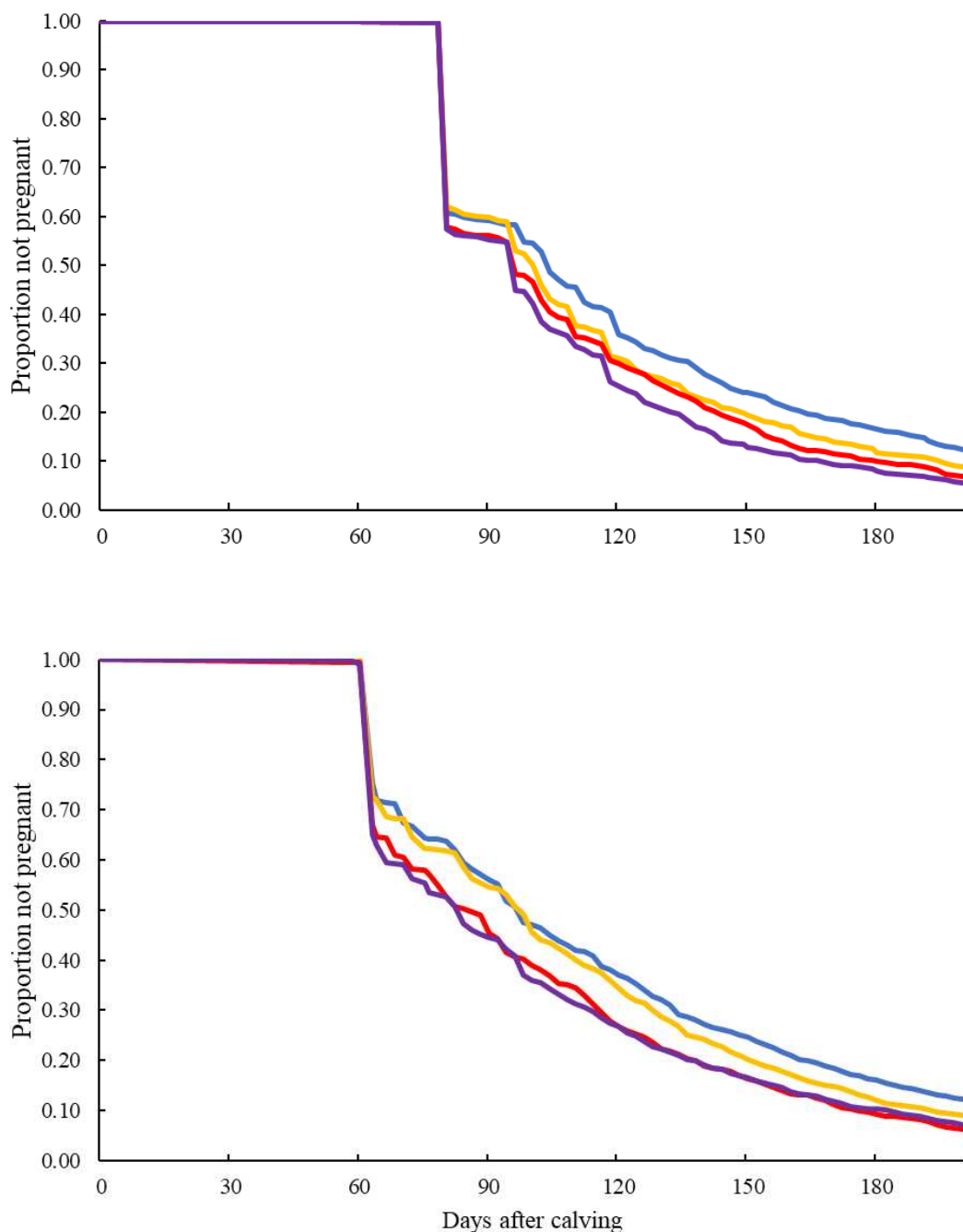


Figure 2.3 Kaplan-Meier survival curves for the interval from calving to pregnancy in primiparous (top panel; n = 4,361) and multiparous (bottom panel; n = 7,681) cows by category of nadir BCS.

Values for nBCS were categorized using the quartile distribution [Q1 = lowest nBCS (blue); Q2 = orange; Q3 = red; Q4 = greatest n BCS (purple)].

Mean days to pregnancy in primiparous cows were 126.3 d, 119.8 d, 115.8 d, and 110.8 d for Q1, Q2, Q3, and Q4, respectively. Mean days to pregnancy in multiparous cows were 118.3 d, 110.9 d, 104.8 d, and 102.1 d for Q1, Q2, Q3, and Q4, respectively.

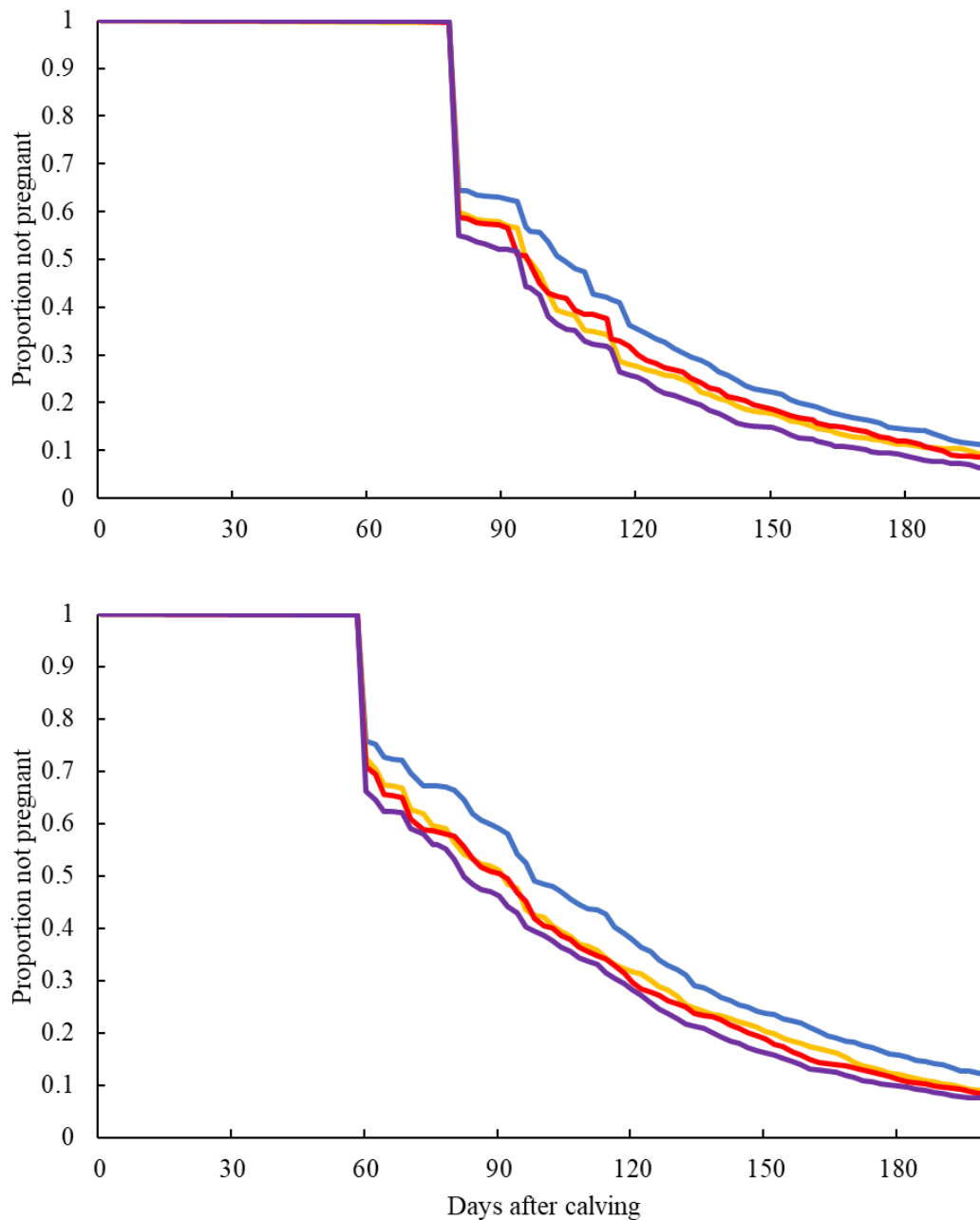


Figure 2.4 Kaplan-Meier survival curves for the interval from calving to pregnancy in primiparous (top panel; n = 4,361) and multiparous (bottom panel; n = 7,681) cows by category of BCS change between calving and nadir [Q1 = greatest loss of BCS (blue); Q2 = orange; Q3 = red; Q4 = smallest loss of BCS (purple)].

Mean days to pregnancy in primiparous cows were 125.7 d, 117.3 d, 118.8 d, and 112.0 d for Q1, Q2, Q3, and Q4, respectively. Mean days to pregnancy in multiparous cows were 119.3 d, 109.9 d, 107.6 d, and 104.2 d for Q1, Q2, Q3, and Q4, respectively.

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CHAPTER 3 — DYNAMICS OF AUTOMATED BODY CONDITION SCORES IN GRAZING HOLSTEIN COWS IN A YEAR-ROUND CALVING SYSTEM

Abstract

This observational study aimed to characterize the seasonal dynamics of automated BCS throughout the lactation of Holstein cows in a pasture-based system with year-round calvings. Examining the association between nadir BCS (**nBCS**; defined as the lowest daily BCS after calving) and peak milk yield within each calving period (calendar seasons equally divided in early and late) was a secondary objective of this research. Retrospective data included 2,164 lactations in 539 primiparous (**PP**) and 1,625 multiparous (**MP**) Holstein cows that calved from July 2021 to June 2023 in a commercial dairy farm located in Southern Chile. Individual BCS were generated in every milking by an automated BCS camera system. Body condition score at calving (**BCSc**), nBCS, and the change in BCS from calving to nadir (**ΔBCS**) were considered for the analysis. Peak milk yield (greatest daily milk yield) values were retrieved from on-farm software and lactations were categorized considering quartiles (Q1 = 25% lowest peak milk yield; Q4 = 25% greatest peak milk yield). Data were examined in PP and MP cows separately using multivariable linear models that considered calving period and peak milk yield as the main explanatory variables of interest, whereas BCSc and health status were included as covariables when appropriate. The differences in BCSc among calving periods were more evident in primiparous cows that had the lowest BCSc in early spring (3.13 ± 0.03 ; $\pm$ SE) and the greatest BCSc in late fall (3.51 ± 0.03). In MP cows, the lowest and greatest BCSc were recognized in late fall (3.07 ± 0.04) and early summer (3.23 ± 0.03), respectively. Only MP cows had differences in nBCS and ΔBCS across

calving periods: The lowest nBCS were identified in late spring (2.71 ± 0.02), early summer (2.71 ± 0.02), and late summer (2.71 ± 0.02). The greatest losses in BCS also occurred in early summer (-0.41 ± 0.02) and in late spring (-0.41 ± 0.02). Time from calving to nBCS varied among calving periods and depended on parity category. Primiparous cows reached nBCS earlier in late summer (65 ± 8 days in milk; **DIM**) and late spring (68 ± 7 DIM), whereas MP had the shortest times to nBCS in late and early fall (67 ± 5 DIM; 68 ± 5 DIM). Greater values at milk peak were associated with lower nBCS only in multiparous cows during the late spring period. We concluded that the dynamics of BCS were associated with calving period in both parity groups. These data could help guide decisions on nutrition and breeding strategies in farms facing comparable conditions.

Introduction

The use of pasture as the main source of nutrients is a common practice in many regions of the world and consumers express a positive perception toward grazing systems associated with sustainable farming practices (USDA, 2022). Universal to most management systems, postpartum dairy cows are challenged by metabolic imbalances resulting from an increased demand for nutrients to initiate lactation, accompanied by slower increments in dry matter intake (**DMI**). The resulting energetic deficit triggers metabolic adjustments favoring the mobilization of fat and labile protein from body energy reserves (Sordillo and Raphael, 2013). As pronounced and extended periods of negative energy balance have multiple detrimental effects, including reduced likelihood of resumption of postpartum ovarian cyclicity (Roche et al., 2009), lowered pregnancy rate (Carvalho et al., 2019), and increased pregnancy loss (Pinedo et al., 2022a), the evaluation of the energy reserves by body condition scoring is a common monitoring practice implemented in dairy farms (Roche et al., 2009).

As pasture-based systems are very dependent on the availability of pasture resources throughout the year, continuous monitoring of the energy balance of cows is crucial. Nonetheless, the ability to precisely investigate the dynamics of BCS is limited when body condition scoring is performed at predetermined time points in the lactation. Recently developed automatic BCS systems provide consistent and more detailed information on the variations of BCS across the entire lactation (Mullins et al., 2019; Pinedo et al., 2022). Moreover, daily body condition assessment allows for the identification of specific landmarks, such as the magnitude of and the time to nadir BCS (**nBCS**; defined as the lowest daily BCS after calving), that previous research has highlighted as associated with cow performance and health (Hernandez-Gotelli et al., 2023; Pinedo et al., 2023). In specific, the aforementioned studies reported that greater milk yield was associated with lower nBCS, whereas cows affected by disease had greater days in milk (DIM) at nBCS in addition to lower nBCS.

System-level and nutrition-related features make the energetic dynamics of grazing transition cows different from those of cows maintained in confinement systems (Roche, 2023). Research suggests that postpartum cows in pasture-based systems lose BCS at a faster rate than cows receiving total mixed ration (**TMR**; Roche et al., 2007). Previous studies have described that, unlike cows in confined systems, pasture cows that calved in spring had a second decline in BCS in mid lactation due to low pasture availability during late summer and fall (Pryce and Harris, 2006; Roche et al., 2007).

As reviewed by Roche (2023), due to the variability of grass growth rate during the year, the season of calving is relevant in the BCS changes occurring throughout the lactation. Notably, because most pasture-based farms group their calvings to take advantage of forage growth curves, the dynamics in BCS for calving periods other than spring have not been widely investigated.

In Chile, 86% of the dairy farms are located in the Southern region, with 75% of the Chilean dairies maintaining cows grazing throughout the year (ODEPA, 2019; Chilean Dairy Consortium, 2024). Remarkably, due to milk price incentives during the winter, a significant proportion of the operations located in Southern Chile have calvings during the whole year. In consequence, the availability of automated BCS systems in commercial farms in this area provides opportunity for exploring the BCS dynamics of grazing dairy cows with year-round calvings.

We hypothesized that calving periods would affect the dynamics of BCS in both primiparous (**PP**) and multiparous (**MP**) cows maintained in a pasture-based system with year-round calvings. Therefore, the objective of this study was to characterize the seasonal dynamics of automated BCS throughout the lactation of Holstein cows in a pasture-based system with year-round calvings. Examining the association between nBCS and peak milk yield within each calving period was a secondary objective of this research.

Materials and methods

This study used retrospective data from a commercial dairy and the research procedures were approved by the Colorado State University Institutional Animal Care and Use Committee (IACUC) Waiver Subcommittee (protocol #5933). This observational study analyzed data collected from 2,164 lactations (539 PP and 1,625 MP) of Holstein cows calving from July 2021 to June 2023 in a grazing dairy located in Puerto Octay, Region de Los Lagos, Chile (latitude: -41.0 ; longitude: -72.6 ; altitude: 160 m above sea level). Average minimum and maximum temperatures by season were 5.6°C to 15.9°C (fall), 2.9°C to 10.8°C (winter), 5.3°C to 17.4°C (spring), and 8.7°C to 23.9°C (summer). Monthly rainfalls were 91.7, 152.4, 53.8, and 43.1 mm for fall, winter, spring, and summer, respectively (Chilean Weather Center, 2024).

The size of the herd varied from 1,000 to 1,150 milking cows during the study period. Lactating cows grazed perennial ryegrass (*Lolium perenne*) and were managed with a daily rotational grazing method using pasture paddocks of varying sizes. Due to the fluctuation in grass growth rates throughout the year, the percentage of grass consumed varied by season with 8 to 10 kg DM/cow in summer, 8 kg DM/cow in winter, 2 to 3 kg DM/cow in fall, and 10 to 12 kg DM/cow in spring. The diet was supplemented with corn (30%) and grass (70%) silage offered in feeding barns after milking and concentrate given in the milking parlor, depending on the level of milk production of individual cows. These supplements were offered throughout the whole year, but in variable amounts, depending on grass availability and quality. Cows remained all day at the pasture, except in winter when they were maintained in barns during the nighttime (12 h) to receive supplementary feed. The average milk production per lactation was 7,300 kg/cow and the cows were milked in a 50-unit rotary milking parlor twice per day at approximately 0600 and 1600 h.

Data were collected from calving until dry-off or culling, considering cow as the experimental unit. Cow demographics and health events were extracted from on-farm software (CliWin Software; COOPRINSEM, Osorno, Chile). Milk production and BCS were obtained from DelPro Farm Manager software (DeLaval International AB, Tumba, Sweden). The dataset for the analyses included cow identification number, calving date, lactation number, calving-related and disease events, culling date, and peak milk yield. Information from lactations that started outside the study period was not included. Health events were diagnosed by farm personnel and recorded in the management software when a treatment requiring a milk withdrawal period was administered. Main disorders included retained placenta, metritis, mastitis, pneumonia, lameness, diarrhea, colic, and acidosis. Lactations were categorized by health status as healthy (no-events), with one event, or with multiple events, considering only health disorders occurring before nBCS.

For the analysis, cows were categorized into PP and MP. Due to the variability of the grass growth rate and management within seasons (Demanet et al., 2015), each calendar season was divided into early (first 45 d) and late (last 45–46 d), resulting in a total of 8 calving periods.

Body condition scores were generated by an automated BCS camera system (DeLaval International AB) validated previously by Mullins et al. (2019) located at the exit of the milking parlor. The BCS calculated by the algorithm was based on the 1 to 5 scale proposed by Ferguson et al. (1994) but was reported in 0.1-point increments. Body condition scores from July 2021 to September 2023 were downloaded from DelPro Farm Manager software. Body condition score at calving (**BCSc**) and nBCS were selected for the analyses, and changes in BCS (**ΔBCS**) were calculated as nBCS – BCSc. Time to nBCS was defined as the DIM when the cow reached nBCS. Peak milk was categorized into quartiles as Q1 (< 22.7 kg/d), Q2 (22.7 – 25.2 kg/d), Q3 (25.3 – 27.9 kg/d), and Q4 (> 27.9 kg/d) in PP and as Q1 (< 28.6 kg/d), Q2 (28.6 – 31.9 kg/d), Q3 (32.0 – 35.4 kg/d), and Q4 (> 35.4 kg/d) in MP.

Statistical analyses were performed separately for PP and MP cows in R, version 4.2.2 (R Core Team, 2022), using the *lmer4*, *lmerTest*, and *emmeans* packages. Descriptive statistics for BCSc, nBCS, ΔBCS, and DIM at nBCS were calculated using ANOVA. Multivariable linear models were fit using BCSc, nBCS, ΔBCS, and DIM at nBCS as the response variables, with calving period as the main predictor. Other fixed effects included in the models were BCSc, peak milk yield, and health status. Potential interactions were tested and removed from the models when $p > 0.10$. Days in milk (**DIM**) at the milk yield peak was included as covariable when the association between milk yield peak category and nBCS by calving period was tested. Least squares means were calculated for daily BCS by category of calving period, using repeated measures analysis for linear mixed models, considering cow's individual identification (**ID**) as a

random effect and a compound symmetric/exchangeable correlation with random intercept. The resulting estimated marginal means (least square means, **LSM**) values were presented as BCS curves from calving to 305 DIM (Figure 3.1). Assumptions of constant variance and normal residuals were assessed using residual diagnostic plots. Results for multivariable linear models are reported in LSM and standard errors (**SE**). Statistical significance was established at a $p < 0.05$ level using a likelihood ratio test.

Results

A total of 2,164 lactations (PP = 539; MP = 1,625) and 461,779 daily BCS were included in the analysis. The distribution of calvings by period (early and late) was 7.76% and 11.2% (fall), 9.75% and 21.7% (winter), 16.7% and 11.2% (spring), and 13.1% and 8.64% (summer).

Daily BCS curves by calving period are shown in Figure 3.1. Among PP, cows that calved in early spring had the lowest BCS during the first 150 DIM. In MP, the lowest BCS during the first 150 DIM was identified in cows that calved during early and late spring and early and late winter.

Overall, mean $\pm$ SE BCS_c was greater in PP than MP (3.34 ± 0.01 vs. 3.12 ± 0.01 ; $p < 0.0001$). Similarly, nBCS was greater in PP (2.91 ± 0.01 vs. 2.77 ± 0.01 ; $p < 0.0001$). However, the reduction in BCS until nadir was greater in PP than in MP (-0.43 ± 0.011 vs. -0.36 ± 0.006 ; $p < 0.0001$). In contrast, MP cows reached nBCS earlier than PP cows (76 ± 1 DIM vs. 81 ± 2 DIM; $p = 0.006$).

Least squares means for BCS_c, nBCS, Δ BCS, and time to nBCS by calving period are reported in Table 3.1. In PP cows, the lowest BCS_c was in early spring, whereas MP cows had the lowest BCS_c in late fall. For MP cows, both the lowest nBCS and the greatest Δ BCS between

calving and nadir occurred during the summer and during late spring. Primiparous cows reached nBCS earlier in late summer and late spring, whereas MP reached nBCS in late and early fall.

No differences were identified in BCS_c, nBCS, and Δ BCS by health status in PP or MP cows. Time to nBCS varied by health status only in MP, with healthy cows evidencing the earliest nBCS (Table 3.1).

Milk yield peak means (SE) in PP were 22.8 (1.2) kg for early fall, 26.4 (0.4) kg for late fall, 28.2 (0.8) kg for early winter, 25.6 (0.3) kg for late winter, 25.7 (0.5) kg for early spring, 25.0 (0.7) kg for late spring, 23.5 (0.4) kg for early summer, and 22.3 (0.7) kg for late summer ($p < 0.0001$). In MP cows, milk yield peak means were 30.7 (0.4) kg for early fall, 32.8 (0.5) kg for late fall, 32.7 (0.4) kg for early winter, 34.0 (0.3) kg for late winter, 32.3 (0.3) kg for early spring, 32.1 (0.4) kg for late spring, 30.2 (0.4) kg for early summer, and 28.2 (0.5) kg for late summer ($p < 0.0001$). As presented in Figure 3.2, greater values at milk peak were associated with lower nBCS only in MP cows during the late spring period.

Discussion

Studies exploring the BCS dynamics of grazing dairy cows with year-round calvings are scarce. The dairy analyzed in the current study represents a considerable proportion of dairies in Southern Chile and allows for the analysis of BCS dynamics of grazing dairy cows with year-round calvings (Chilean Dairy Consortium, 2024).

Due to the effect of precipitation and temperature in the growing cycle of the pasture throughout the year, the amounts of DM available for consumption vary considerably by season. Hence, dairy farms in Southern Chile strategically concentrate the calvings during late winter to early spring and late summer to early fall to meet the highest grass growth rate and the cows'

energy demands. Nonetheless, unlike grazing-based dairy systems in New Zealand and Ireland, which concentrate the calving season only in spring (Berry et al., 2006; Roche et al., 2007), due to milk price incentives during the winter in Southern Chile, calvings occur throughout the year.

In agreement with previous research on pasture systems (Berry et al., 2006; Roche et al., 2007), in the current study, PP cows calved at a greater BCS and had a more moderated nBCS compared with MP cows. However, PP cows lost more BCS during their early lactation. Roche et al. (2009) suggested that during the first-lactation cows add to their BCS losses the energy required to grow, causing them to have extended BCS losses throughout their early lactation. Proudfoot and Huzzey (2022) speculated that PP cows have their feed consumption reduced in early lactation because they experienced more displacements from the feeder by the MP due to their lower social status, which would reduce their intake of supplements in the feeding barn. The consequences are appreciated in second-parity animals, which evidence the lowest precalving BCS (Roche, 2023). In PP cows in this specific farm, competition could be a key factor in their postpartum loss of BCS, as these cows were supplemented together with older animals.

Primiparous cows also reached nBCS later in the lactation compared with MP cows in this and other grazing studies (Berry et al., 2006; Roche et al., 2007). Notably, in confined systems, the opposite has been reported (Truman et al., 2022; Hernandez-Gotelli et al., 2023). In the current study, cows reached nBCS later in their lactation. Due to the differences in feeding and diet management between confined and grazing systems, cows receiving TMR lose BCS at a slower rate than cows on pasture (Roche et al., 2007) and grazing cows require more feed and time to revert the downward trend on their postpartum BCS (Roche, 2023).

In PP cows, calving period was associated with BCS_c but not with nBCS or Δ BCS. However, when BCS_c was removed from the nBCS and Δ BCS models the effect of calving period

was significant (not shown here), indicating that the effect of BCSc on the BCS losses after calving would be relevant. Time to nBCS was associated with calving period, with later nBCS in PP cows calved in late (96 DIM) and early (91 DIM) fall. In contrast, in MP cows, BCSc, nBCS, Δ BCS, and time to nBCS were associated with calving period (Table 3.1). Multiparous cows that calved during late spring and summer had the lowest nBCS and higher losses in BCS between calving to nadir. This could be explained by the availability of grass and cows' feeding behavior changes during summer. Cows in this geographic area decrease their grazing activity by 17% in summer because they are exposed to heat stress (Arias et al., 2021), and this low DMI during early lactation negatively affects the BCS, increasing BCS losses (Roche et al., 2009). In addition, during summer the grass growth rate is reduced and the vegetative state of the plant changes, decreasing its nutritional value by increasing NDF and reducing ME (Anrique et al., 2008). Thus, early summer seems to be the most challenging period for MP cows, with the highest BCSc because they ended the previous lactation during spring when the grass had the greatest growth rate and better nutritional value, which decreased significantly during summer.

Although this study did not seek to statistically compare the dynamics of daily BCS by calving period, Figure 3.1 shows different curve patterns by calving period for PP and MP cows. Weather and season effects on the nutrient composition of the grass, environmental conditions, and cows' grazing/feeding behavior affecting DMI (Arias et al., 2021; Roche, 2023) are factors to consider (Berry et al., 2006).

Specific to grazing systems, Roche et al. (2007) described a W-shaped BCS profile on cows that calved during spring, with a second BCS reduction in mid lactation around 122 and 171 DIM. In an earlier study, Berry et al. (2006) reported a plateau profile for BCS until 200 DIM for cows calving during spring. Although these patterns were not evident in our study, PP cows calving in

winter and late fall had extended periods (even reaching 280 DIM) of low BCS after their nBCS. This was most evident in PP cows calving in late fall that were unable to return to greater BCS. Interestingly, extended periods of low BCS were evident in MP cows calving in spring, fall, and winter. Notably, BCS curves for PP calving in spring and MP calving in summer are similar to those reported in confined systems, with quicker returns to greater BCS after nBCS (Pinedo et al., 2022).

Although the study cows were maintained in a grazing regimen during the whole year, as highlighted by Roche et al. (2007), the BCS patterns can be also affected by the different diet supplementation levels. In our study, MP cows calving in spring and summer did not recover BCS until they entered a half-day confinement to receive supplementation during fall and winter. Similarly, this halfday confinement could explain the moderated losses in BCS after calving in MP cows that calved during fall and winter, who had a slower and less pronounced BCS recovery.

In the current study, PP and MP cows that calved in early winter and late winter had the greatest milk peak yield. Notably, contrary to previous reports analyzing cows in confinement (Pinedo et al., 2023), only in MP cows that calved during late spring greater nBCS was associated with lower milk yield (Figure 3.2).

A limitation of this study is that only health events treated with a product that required a milk withdrawal period were recorded, which likely resulted in an underestimation of the impact of disease on the variables in analysis.

Conclusions

In conclusion, the dynamics of BCS were associated with calving period in both parity groups. Nonetheless, the role of supplementary feeding in the study farm is a factor that should be

considered when analyzing the current findings. The information originated from this study could help guide decisions on nutrition and breeding strategies in farms facing comparable conditions.

Tables and figures

Table 3.1 Least square means (SE) for BCS at calving, BCS at nadir, change in BCS (Δ BCS), and time to BCS nadir (nadir DIM) by calving period (dividing calendar seasons in early and late periods) and health status in primiparous and multiparous cows¹.

	BCS at calving	BCS nadir	Δ BCS	nadir DIM
Primiparous				
Calving period				
Early fall	3.42 (0.13) ^{abc}	2.94 (0.01)	-0.34 (0.01)	91 (21) ^{ab}
Late fall	3.51 (0.03) ^c	2.90 (0.03)	-0.44 (0.03)	96 (6) ^b
Early spring	3.13 (0.03) ^a	2.86 (0.03)	-0.48 (0.03)	74 (6) ^a
Late spring	3.18 (0.04) ^{ab}	2.91 (0.04)	-0.43 (0.04)	68 (7) ^a
Early summer	3.44 (0.03) ^c	2.89 (0.03)	-0.45 (0.03)	81 (6) ^a
Late summer	3.47 (0.05) ^c	2.93 (0.04)	-0.42 (0.04)	65 (8) ^{ab}
Early winter	3.47 (0.05) ^c	2.97 (0.04)	-0.37 (0.04)	87 (8) ^{ab}
Late winter	3.30 (0.03) ^b	2.88 (0.02)	-0.46 (0.02)	84 (5) ^{ab}
Health status				
Healthy	3.36 (0.02)	2.93 (0.02)	-0.42 (0.02)	76 (3)
1 event	3.37 (0.06)	2.92 (0.02)	-0.42 (0.02)	81 (5)
≥ 2 events	3.36 (0.03)	2.89 (0.05)	-0.45 (0.05)	84 (11)
Multiparous				
Calving period				
Early fall	3.14 (0.03) ^a	2.82 (0.02) ^{bc}	-0.29 (0.02) ^{bc}	68 (5) ^a
Late fall	3.09 (0.04) ^a	2.85 (0.03) ^c	-0.27 (0.03) ^c	67 (5) ^a
Early spring	3.13 (0.02) ^a	2.72 (0.02) ^a	-0.39 (0.02) ^a	90 (4) ^c
Late spring	3.18 (0.03) ^{ab}	2.71 (0.02) ^a	-0.41 (0.02) ^a	92 (4) ^c
Early summer	3.27 (0.03) ^b	2.71 (0.02) ^a	-0.41 (0.02) ^a	87 (4) ^{bc}
Late summer	3.16 (0.03) ^a	2.71 (0.02) ^a	-0.40 (0.02) ^a	74 (5) ^{ab}
Early winter	3.16 (0.03) ^a	2.82 (0.02) ^c	-0.30 (0.02) ^c	78 (4) ^{ab}
Late winter	3.10 (0.02) ^a	2.76 (0.02) ^{ab}	-0.36 (0.02) ^{ab}	93 (3) ^c
Health status				
Healthy	3.12 (0.01)	2.77 (0.01)	-0.35 (0.01)	71 (1) ^a
1 event	3.14 (0.03)	2.76 (0.02)	-0.35 (0.02)	76 (4) ^a
≥ 2 events	3.19 (0.05)	2.76 (0.04)	-0.35 (0.04)	97 (8) ^b

¹ The multivariable linear regression models, for both parity categories (primiparous and multiparous), included calving period, health status, BCS at calving, and peak milk yield as covariables. Interactions tested were not significant and removed from the models.

^{a-c} Different superscripts within columns indicate significant differences ($p < 0.05$).

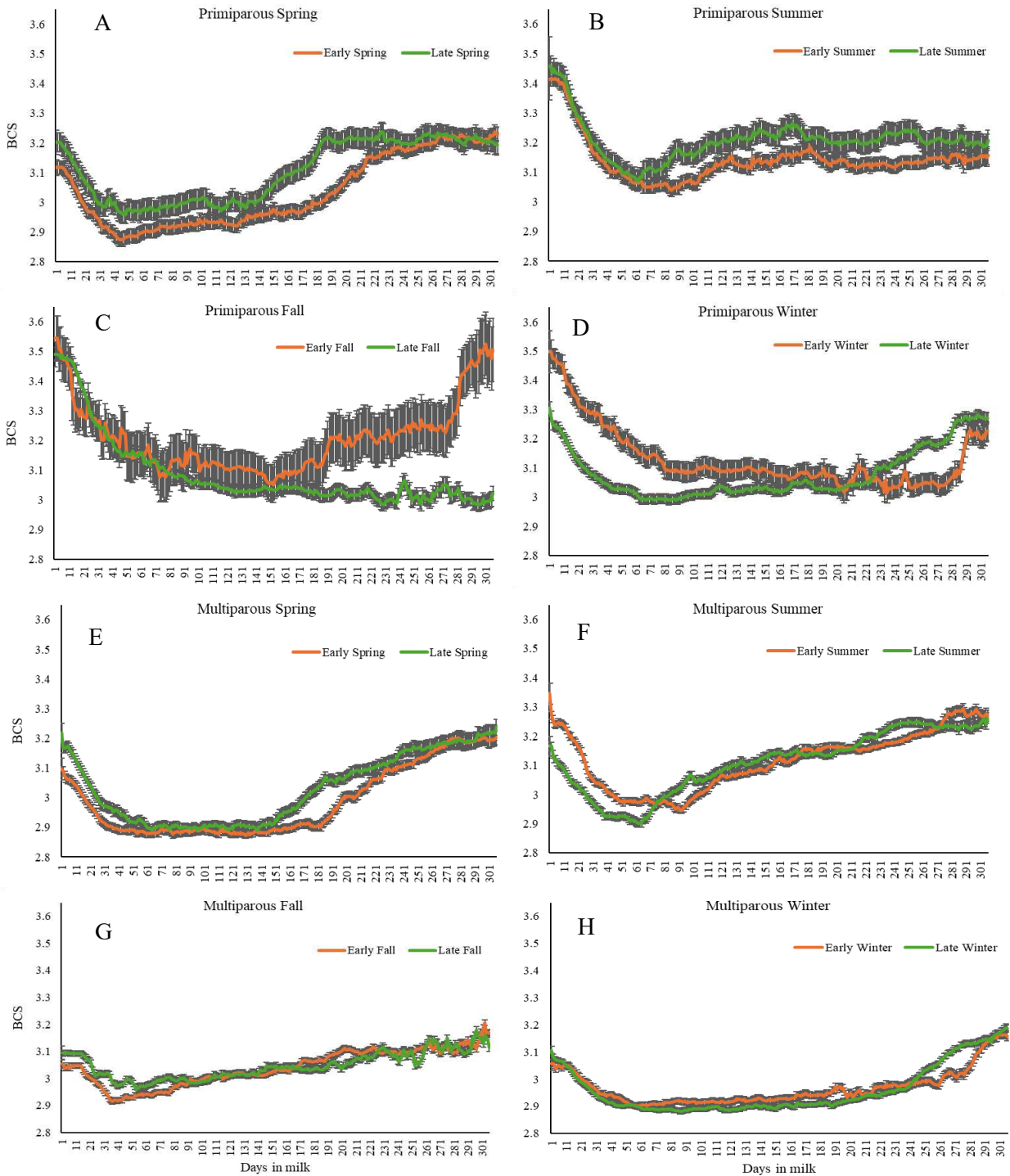


Figure 3.1 Dynamics of average ($\pm$ SE) daily automated BCS from calving to 305 DIM by calving period (calendar seasons equally divided in early and late) for primiparous (A: spring, B: summer, C: fall, and D: winter) and multiparous (E: spring, F: summer, G: fall, and H: winter) cows.

The linear mixed models for repeated measures consider cow's ID as a random effect, for both parity categories.

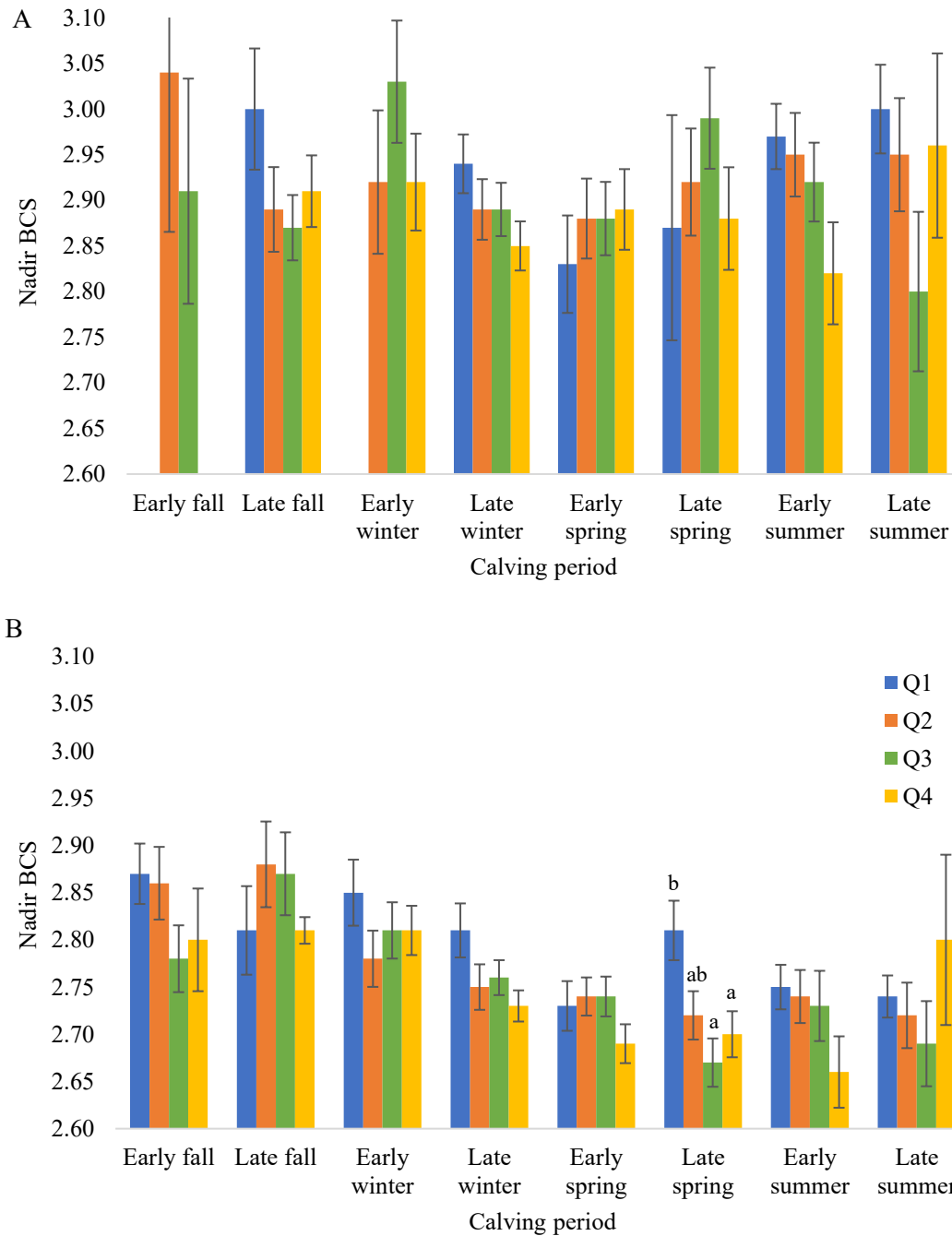


Figure 3.2 Least square means ($\pm$ SE) for BCS at nadir by peak milk yield category (Q1 = lowest milk yield quartile) and calving period in primiparous (A) and multiparous (B) cows.

The full model included BCS_c and DIM at milk yield peak as covariables.

Peak milk yield values were categorized using the quartile distribution [Q1 = lowest milk yield (blue), Q2 = orange; Q3 = green; Q4 = greatest milk yield (yellow)].

Note that some calving periods do not have all the peak milk yield quantiles resented.

Different superscripts within periods indicate $p < 0.05$.

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CHAPTER 4 – ASSOCIATION OF CLINICAL MASTITIS WITH MILKING BEHAVIOR AND PERFORMANCE OF COWS IN AN AUTOMATIC MILKING SYSTEM

Abstract

The continuous growth in the use of automatic milking systems (**AMS**) that provide detailed individual cow data creates the opportunity for continuous monitoring of deviations in milking behavior and performance that could be associated with diseases, such as mastitis. This study aimed to investigate the dynamics of milking behavior and performance in Holstein cows around clinical mastitis diagnosis occurring during the first third of lactation, and to compare these patterns with those observed in healthy cows under the same conditions. Data considered 2,983 lactations from January 2023 to April 2024 and included 367,686 milking events from cows milked using 62 AMS. Milking behaviors included incomplete milking, milking unit kick-off, teat not found, and milking interval (h). Performance variables were milk yield (kg), milking duration (min), milking duration by milk yield ratio (min/kg), milk flow duration (min), mean milk flow rate (kg/min), and peak milk flow rate (kg/min). Mastitis cases were categorized by days in milk (**DIM**) at diagnosis into early (0 to 30 DIM; **MAS1**), mid (31 to 60 DIM; **MAS31**), and late (61 to 100 DIM; **MAS61**) periods. The early period analyzed milking data from 1 to 30 DIM, while for the MAS31 and MAS61, milking data included -14 to 0 days relative to the mastitis diagnosis. A healthy group was created as a reference for comparisons in each period. Multivariable mixed linear regression models and multivariable mixed logistic regression models were fit using milking variables as the outcomes of interest. Compared with healthy controls, MAS1 cows had greater probabilities of incomplete milking and teat not found, and extended milking intervals. Affected cows had smaller milk yield, longer milking durations, greater milking duration by milk yield ratio,

longer milk flow durations, and reduced mean and peak milk flow rates than the healthy group through the early period. Similarly, MAS31 and MAS61 had a significant increase in the probabilities of incomplete milking and teat not found, and extended milking intervals, milking duration, milking duration by milk yield ratio, and milk flow duration around the time of diagnosis. These cows also showed a sharp decline in milk yield, mean milk flow, and peak milk flow around the diagnosis day. Milking behavior and performance were altered in cows that experienced mastitis during the first third of lactation. These variables may serve as early indicators for detecting mastitis in cows managed under AMS.

Introduction

Mastitis remains one of the most relevant diseases affecting dairy cows (Ruegg, 2017), resulting in significant economic losses and reduced cow welfare (Petersson-Wolfe et al., 2018). The painful and debilitating effects of mastitis have been recognized (Leslie and Petersson-Wolfe, 2012; Fitzpatrick et al., 2013; Petersson-Wolfe et al., 2018), often leading to behavioral changes collectively referred to as sickness behavior (Fogsgaard et al., 2012; Dittrich et al., 2019). These changes usually involve increased lethargy, anorexia, and fatigue or depression (Tizard, 2008).

Although prey animals, including cattle, may exhibit stoicism and mask overt signs of discomfort, previous studies reported that activity, resting, feeding, and rumination behavioral patterns changed in cows affected by mastitis before, during and after the mastitis diagnosis, demonstrating greater restlessness, variations in lying time (Sepúlveda-Varas et al., 2014; Fogsgaard et al., 2015), reduced physical activity (Liboreiro et al., 2015; Stangaferro et al., 2016), decreased feeding (Huzzey et al., 2007; Fogsgaard et al., 2015; Sepúlveda-Varas et al., 2016) and

water (Huzzey et al., 2007) intake, and shorter rumination times (Fogsgaard et al., 2012; Liboreiro et al., 2015; Stangaferro et al., 2016; King et al., 2018).

Although less explored, changes in milking behavior have also been associated with the occurrence of mastitis. In conventional parlors, cows with clinical mastitis expressed more restlessness during milking and often kicked off the milking units (Fogsgaard et al., 2015), while in herds using automatic milking systems (AMS), affected cows had reduced frequency of AMS visits (King et al., 2017, 2018).

As cows in AMS have more autonomy over their daily activities, variables such as milking interval, milking duration, incomplete milking, or milking unit kick-off provide opportunities for cow health assessment. Although some of these parameters have been explored in the development of mastitis prediction models (Khatun et al., 2018; Tian et al., 2024) and are also utilized by AMS to generate health alerts, the dynamics of these variables regarding how they evolve over time in cows with mastitis compared to healthy animals, or when meaningful changes in these indicators first appear, remain insufficiently characterized in the literature. In addition, unlike conventional systems that detect clinical mastitis during milking, AMS primarily relies on alerts issued by the milking equipment, which provides additional information to create timely and even more accurate alerts.

We hypothesize that milking behavior and performance are altered by the occurrence of clinical mastitis in cows maintained in an AMS. In consequence, the objective of this study was to investigate the dynamics of milking behavior and performance in Holstein cows around clinical mastitis diagnosis occurring during the first third of lactation, and to compare these patterns with those observed in healthy cows housed under the same automatic milking system conditions.

Materials and methods

Study population

This retrospective observational study was conducted in a commercial dairy farm located in northeast Colorado, housing 3,700 lactating cows in free-stall barns. All research procedures were approved by the Colorado State University IACUC Waiver Subcommittee (protocol #4907). Cows were milked using 62 automatic milking units (VMS, DeLaval International AB, Tumba, Sweden) in a guided-flow system (milking first) and the final dataset included records from 2,983 Holstein cows that calved between January 2023 and April 2024.

Cows calved in a maternity pen, where they stayed for 24 to 48 hours before being transferred to the fresh pen equipped with free stalls. Cows remained in the fresh pen until 18–20 days in milk (**DIM**), after which they were moved to the lactation pen. However, during this period, primiparous cows were milked in a conventional milking parlor, while multiparous cows were milked using an AMS before being transferred to the lactation pen. Once in the lactation pen, primiparous and multiparous cows were housed together and milked using AMS until the end of the lactation.

The average stocking density during the study was 62 ± 5 cows per AMS unit. The vaccination protocol for all cows included Envirocor J5 (Zoetis Animal Health, Parsippany, NJ, USA), Bovishield Gold (Pfizer Animal Health, New York City, NY, USA), and Ultrabac-8 (Pfizer Animal Health, New York City, NY, USA) vaccines. Cows were provided *ad libitum* access to clean water and fed a total mixed ration twice daily, formulated according to the National Research Council's recommendations (NRC, 2001). The diet for lactating cows included corn silage (25.5 to 32.1 %), hay (2.7 to 3.4%), haylage (3.2 to 4.0%), base mix (20.5 to 25.8%), supplement (5.3 to 6.6%), flaked corn (19.4 to 24.4%) and brews (2.1 to 2.7%). Additionally, the AMS delivers

concentrate that ranges from 0.2 to 5.7 kg DM/day/cow, depending on each cow's productivity level and lactation stage. Farm personnel utilized the DeLaval Activity Meter System and DelPro Farm Management system (DeLaval International AB, Tumba, Sweden) to monitor total activity for reproductive and health management purposes.

Study design and milking variables

The period of analysis extended from calving (multiparous) or from 31 DIM (primiparous) to 100 DIM. Cow health information was collected from the on-farm software (DairyComp 305; Valley Ag Software, Tulare, CA, USA), while milking behavior and performance data were extracted from DelPro Farm Manager software for each AMS visit. Both databases were combined into a single file, organized by each cow's unique identification (**ID**) and lactation number. The final dataset included the cows' ID, calving date, lactation number, calving-related and health disorder events, as well as performance and milking behavior data for each AMS visit.

Milking behaviors included the occurrence of incomplete milking, milking unit kick-off, teat not found, and milking interval (h). Performance variables were milk yield (kg), milking duration (min), milking duration by milk yield ratio (min/kg), milk flow duration (min), mean milk flow (kg/min), and peak milk flow (kg/min; Table 1).

Milking interval was affected by milking permits that were adjusted by DIM. From calving to 66 DIM, cows were allowed to enter the AMS after 4 h since their last milking. Cows could visit the AMS every 4.5h, between 67 and 72 DIM, and after 72 DIM, the time between milkings was individually adjusted based on the cow's production level, ranging from 5 to 8 h. If a cow's milking interval exceeded her permitted time by 4 or more hours, farm personnel would intervene and ensure the cow was milked.

Mastitis diagnosis

Farm personnel performed disease diagnoses following farm health protocols. Alerts from the AMS based on the DeLaval Mastitis Detection index (**MDi**), and/or reductions in general activity (measured by activity collars) were checked daily by farm personnel. Potential cows were sorted to be clinically examined. Mastitis was determined if any sign of inflammation of the udder (e.g., heat, swelling, or discoloration of the skin) or visually abnormal milk secretion (e.g., clots, flakes, or watery) from one or more quarters (Kelton et al., 1998) was present.

After identifying cows with clinical mastitis, farm personnel aseptically collected a milk sample from the affected quarter for subsequent bacteriological culture. On-farm cultures were performed using tri-plates from the AccuMast On-Farm Mastitis Culture Test Kit (FERA Diagnostics and Biologicals, Ithaca, NY, USA). Based on culture results, cows were treated according to the farm's established protocol. Cows with mastitis were moved to the hospital pen if a milk withdrawal period following treatment was required. Once moved to the hospital pen, cows were milked separately in a conventional milking parlor until full recovery or when the drug withdrawal period ended. As a result, milking behavior and performance during their time in the hospital following diagnosis were not available and not included in the analysis.

Data editing and variable categorization

Data management, editing, and statistical analyses were conducted using R, version 4.2.2 (R Core Team, 2022). Milking events with a milk yield of 0.0 kg or a duration longer than 20 minutes were excluded from the final analysis. Lactations with missing milking data for the evaluation periods (1 to 30 DIM, or -14 to 0 days relative to diagnosis) were also excluded. Hence, a total of 367,686 milking events in 2,983 lactations started between January 2023 and April 2024

were included in the analysis. The early, mid, and late periods included 97, 74, and 93 lactations for mastitic cows.

Mastitis cases were categorized by DIM at diagnosis into three periods: early (0 to 30 DIM; **MAS1**), mid (31 to 60 DIM; **MAS31**), and late (61 to 100 DIM; **MAS61**). If a cow was diagnosed with mastitis multiple times during the first 100 DIM, only the first case was considered for the analysis. Similarly, cows that were diagnosed with mastitis in one period were excluded from the analysis in the subsequent periods. Finally, cows diagnosed with other diseases, such as retained fetal membranes, clinical hypocalcemia, metritis, subclinical ketosis, displaced abomasum, pneumonia, lameness, and digestive disorders were removed from the final data set. Cows that remained healthy between calving to 100 DIM were assigned to the healthy control group.

The analysis of milking data in the early period covered 1 to 30 DIM, while for mid and late periods, it included -14 to 0 days relative to mastitis diagnosis. Since primiparous cows began to use the AMS milking after 18–21 DIM, the analyses for the early period only included multiparous cows (healthy and sick), while mid and late period analyses included both primiparous and multiparous cows. The calving day was excluded from the analyses because of the high variability of the data. Cow data following diagnosis of mastitis cases were unavailable, as diagnosed cows were moved to receive treatment at the hospital pen, where cows were milked in a conventional milking parlor with no milking information available.

For comparisons in the mid and late periods, the healthy group used the mean DIM of diagnosis for mastitis in each period as a mock day of diagnosis. The average DIM for mastitis diagnosed in the mid period (MAS31) was 45 DIM, while the average DIM for the diagnosis of mastitis in the late period (MAS61) was 81 DIM.

Due to behavioral and performance daily variation at the beginning of the lactation, in the early period, DIM was used instead of days to diagnose, to ensure more consistent comparisons. Using days relative to diagnosis as the reference for comparisons would have aligned mastitic cows (mean diagnosis at 13.8 DIM for MAS1) with healthy cows during their first 14 postpartum days, a period already influenced by significant physiological changes associated with the calving and early lactation.

Statistical analysis

The statistical analyses were conducted using the *nlme* (Pinheiro et al., 2024), *lme4* (Bates et al., 2015), and *emmeans* (Lenth, 2022) packages of R. Multivariable mixed linear and mixed logistic regression models for repeated measures were used, with milking behavior and performance variables as the main outcomes, and health status as the main explanatory variable of interest. To address heteroscedasticity and unequal group sizes (healthy vs. mastitis), continuous milking-related variables were transformed using either logarithmic or square root functions, depending on which transformation had the best effect on model fit and residual homogeneity. Specifically, logarithmic transformations were applied to milking interval, milking duration, and milk flow duration, while square root transformations were used for milk yield, milking duration by milk yield ratio, mean milk flow, and peak milk flow. Linear regression models also included a variance structure (`weights = varIdent`) to explicitly model group-specific residual variances. Robust standard errors were used to improve the reliability of statistical inferences in the presence of heteroscedasticity. For logistic regression models, given the low occurrence of incomplete milkings, milking unit kick-offs, and teat not found events during the early period, the DIM were

grouped by 3 days into ten categories for analysis of these variables: 1–3, 4–6, 7–9, 10–12, 13–15, 16–18, 19–21, 22–24, 25–27, and 28–30 DIM.

In all models, health status (mastitis vs. healthy), DIM or days relative to diagnosis, and their interactions were considered as the primary predictors. Lactation number (continuous; 2 to 5 for early period analyses; 1 to 5 for mid and late periods analyses) was included as a fixed effect in all linear models. Milk yield per milking (continuous) was included in the linear models as a fixed effect when appropriate. Cow's ID was treated as a random effect in all linear and logistic models. Milking events with missing data for a specific behavior or performance variable were excluded only when the missing variable was the variable of interest. Milking behavior and performance were compared by health status using Tukey-adjusted pairwise comparisons for each DIM or day relative to diagnosis. To facilitate interpretation of the linear regression results, estimated marginal means (least squares means, **LSM**) were back-transformed to the original scale using the corresponding inverse transformation, while standard errors (**SE**) were approximated using the delta method. For the logistic regression models, results are presented as LSM probabilities along with their associated SE. Assumptions of constant variance and normality of residuals were evaluated using diagnostic plots. Milking behavior and production variables were also assessed for normality and outliers. Statistical significance was set at $p < 0.05$ using a likelihood ratio test, with differences of $0.05 \leq p < 0.10$ considered as tendencies.

Results

The adjusted probabilities of incomplete milking, milking unit kick-off, and teat not found by health category are presented in Figure 1 (early period) and Figure 2 (mid and late periods).

During the first 30 DIM, the probability of incomplete milking in healthy cows ranged from 0.006 ± 0.001 (LSM $\pm$ SE) to 0.016 ± 0.001 , while the MAS1 group consistently exhibited greater probability of incomplete milkings compared to the control group, with values ranging from 0.023 ± 0.01 to 0.087 ± 0.01 (Figure 1A). During the mid and late periods the probability of incomplete milking in healthy cows varied from 0.007 ± 0.001 (LSM $\pm$ SE) to 0.011 ± 0.001 . The patterns observed in MAS31 and MAS61 indicated greater or increased probabilities of incomplete milkings only sporadically between -14 and -4 days relative to diagnosis, with an average of 0.025 ± 0.01 (LSM $\pm$ SD) and 0.014 ± 0.001 , respectively. From day -3 onward, these probabilities significantly increased, peaking at 0.82 ± 0.05 (MAS31) and 0.75 ± 0.06 (MAS61) on the day of diagnosis (Figure 2A and Figure 2B).

During the early period, the probability of milking unit kick-off for the healthy group was greatest at 0–3 DIM [0.05 ± 0.003 (LSM $\pm$ SE)], before decreasing to 0.02 ± 0.001 at 28–30 DIM. For the MAS1 group, the average probability of milking unit kick-off was 0.041 ± 0.015 (LSM $\pm$ SD), with greater values than the healthy group only during the 22–30 DIM period (Figure 1B). During the mid and late periods, the average probabilities of milking unit kick-off for healthy cows were 0.016 ± 0.009 (LSM $\pm$ SD) and 0.014 ± 0.002 , respectively. While this probability was significantly greater for MAS31 at -1 (0.040 ± 0.02 ; LSM $\pm$ SE) and 0 (0.041 ± 0.02) days relative to mastitis diagnosis (Figure 2C and Figure 2D).

In the healthy group, the greatest probability of teat not found observed during the early period was at 1–3 DIM, reaching 0.008 ± 0.0009 (LSM $\pm$ SE) and then declining to 0.004 ± 0.0005 at 28–30 DIM. Over the early period, the MAS1 group consistently showed a greater probability of teat not found than the control group, ranging between 0.009 ± 0.004 (LSM $\pm$ SE) and 0.038 ± 0.013 (Figure 1C). As lactation progressed into the mid and late periods, the average teat not found

probabilities for the healthy group further decreased to an average of 0.0007 ± 0.0001 (LSM $\pm$ SD) and 0.0006 ± 0.0001 , respectively. The MAS31 group showed a greater teat not found probability during almost the 14 days before the diagnosis (days -14 to -3 average of 0.004 ± 0.003 ; LSM $\pm$ SD), while the MAS61 group only showed a greater probability on some specific days. A noticeable increase in the teat not found probability was observed in both the MAS31 and MAS61 groups beginning on day -2 [0.009 ± 0.006 (LSM $\pm$ SE) and 0.005 ± 0.003 , respectively], reaching a peak at day 0 (0.054 ± 0.033 and 0.016 ± 0.009 , respectively; Figure 2E and Figure 2F).

Figure 3 illustrates the dynamics of milking interval, milk yield per milking, milking duration, and milking duration by milk yield ratio for the early period, while Figure 4 presents these variables for the mid and late periods.

Healthy cows in the early period had the longest milking interval at 1 DIM with 11.1 ± 0.05 h (LSM $\pm$ SE). Values gradually decreased to 6.44 ± 0.02 h by 30 DIM. During the same period, MAS1 cows consistently exhibited longer milking intervals compared to healthy cows (Figure 3A). In the mid and late periods, the average milking interval for healthy cows was 7.9 ± 0.08 (LSM $\pm$ SD) h and 7.4 ± 0.01 h, respectively. In contrast, MAS31 exhibited a steadily longer milking interval throughout the entire mid period (-14 to 0 days relative to diagnosis), whereas MAS61 showed no significant differences in milking interval between day -14 and day -4 relative to diagnosis during the late period. However, both MAS31 and MAS61 cows demonstrated a sharp increase in the milking interval starting on day -3, reaching peaks of 16.2 ± 0.4 (LSM $\pm$ SE) h and 13.9 ± 0.3 h, respectively, on the day of diagnosis (Figure 4A and Figure 4B).

As expected, during the early period, the healthy group experienced a constant increase in milk yield, rising from 5.56 ± 0.05 kg/milking (LSM $\pm$ SE) on the first day after calving to 18.3 ± 0.08 kg/milking at 30 DIM. The MAS1 group consistently produced less milk than the healthy

group throughout days 4 to 30 after calving (Figure 3B). During the mid and late periods, milk yield was relatively similar between healthy cows and those with MAS31 and MAS61 from day -14 to day -3 relative to diagnosis. However, a decline began on day -6 for MAS31 and on day -2 for MAS61, reaching its lowest point on the day of diagnosis (day 0), with 8.17 ± 0.35 (LSM $\pm$ SE) kg/milking for MAS31 and 8.34 ± 0.33 kg/milking for MAS61 (Figure 4C and Figure 4D).

The average milking duration for healthy cows was 6.6 ± 0.1 min (LSM $\pm$ SD) during the early period; in comparison, cows in the MAS1 group exhibited longer milking durations throughout the entire early period (Figure 3C). Healthy cows had an average milking duration of 7.4 ± 0.01 (LSM $\pm$ SD) min in the mid period, and 7.2 ± 0.03 min in the late period. Milking duration was consistently longer in MAS31 cows compared to controls from day -14 to 0 relative to diagnosis, while MAS61 cows exhibited a reduced milking duration between days -14 and -3. However, in both the MAS31 and MAS61 groups, a noticeable increase in milking duration began around day -3, reaching a peak on the day of diagnosis, with durations of 9.45 ± 0.16 (LSM $\pm$ SE) and 8.68 ± 0.15 min, respectively (Figure 4E and Figure 4F).

During the early period, both healthy and MAS1 cows showed a consistent decline in the milking duration by milk yield ratio during the first week after calving. However, throughout the first 30 DIM, this ratio remained higher in the MAS1 group compared to healthy cows (Figure 3D). Interestingly, no differences in the milking duration by milk yield ratio were observed between mastitis-affected and healthy cows from day -14 to day -4 in the MAS31 group, and from day -14 to day -3 in the MAS61 group. However, from those points onward, the ratio began to rise, reaching 1.22 ± 0.04 (LSM $\pm$ SE) in MAS31 and 1.18 ± 0.04 in MAS61 on the day of diagnosis (Figure 4G and Figure 4H).

Figure 5 (early period) and Figure 6 (mid and late periods) present the dynamics of milk flow duration, mean milk flow, and peak milk flow across different study periods.

Healthy cows in the early period showed an increase in milk flow duration from 4.5 ± 0.03 min (LSM $\pm$ SE) on day 1 postpartum to 5.0 ± 0.03 min by 30 DIM. Throughout this period, cows in the MAS1 group consistently exhibited longer milk flow durations (Figure 5A). In the mid and late periods, the average milk flow duration in healthy cows was 5.5 ± 0.01 (LSM $\pm$ SD) and 5.3 ± 0.03 min, respectively. During the same periods, MAS31 cows exhibited a longer milk flow duration compared to healthy cows on some days during the mid period, whereas MAS61 cows showed a shorter milk flow duration between days -14 and -3 relative to diagnosis in the late period. Conversely, cows in the MAS31 and MAS61 groups experienced a sharp increase in milk flow duration on the day of diagnosis, reaching 7.2 ± 0.16 (LSM $\pm$ SE) and 6.8 ± 0.15 min, respectively (Figure 6A and Figure 6B).

During the early period, mean milk flow increased in healthy cows during the first week, rising from 1.12 ± 0.01 kg/min (LSM $\pm$ SE) on day 1 DIM to 1.34 ± 0.01 kg/min by day 7, followed by a slight decline to 1.26 ± 0.01 kg/min by day 17, after which it remained stable through 30 DIM. A similar trend was observed for peak milk flow in healthy cows, which increased from 1.71 ± 0.01 kg/min on day 1 to 1.83 ± 0.01 kg/min on day 7, and then slightly declined to 1.75 ± 0.01 kg/min by day 17, remaining steady thereafter. Throughout this time, MAS1 cows consistently showed smaller mean and peak milk flow values compared to healthy cows, with an average of 1.20 ± 0.02 (LSM $\pm$ SD) kg/min and 1.68 ± 0.02 kg/min, respectively (Figure 5B and Figure 5C). In the mid and late periods, healthy cows maintained average mean milk flow rates of 1.20 ± 0.001 and 1.21 ± 0.006 kg/min, respectively, while their peak milk flow remained relatively stable at 1.68 ± 0.004 and 1.68 ± 0.007 kg/min. MAS31 cows exhibited smaller mean and peak milk flow

rates compared to control cows throughout the entire mid period, whereas MAS61 cows showed greater mean and peak milk flow rates than healthy cows between days -14 and -3 during the late period. In contrast, MAS31 and MAS61 cows experienced a sharp drop in both mean and peak milk flow on the day of diagnosis, reaching 0.97 ± 0.02 (LSM $\pm$ SE; Figure 6C and Figure 6D) kg/min and 1.51 ± 0.02 kg/min (Figure 6E and Figure 6F), respectively.

Discussion

This study examined milking behaviors and performance indicators in Holstein cows around the time of clinical mastitis diagnosis during the first third of lactation, comparing affected vs. healthy cows housed under the same AMS conditions. Overall, mastitic cows had significantly altered milking patterns. These differences were particularly evident close to the time of mastitis diagnosis, suggesting that this disorder negatively influences both milking behavior and performance in early lactation.

During the early period, as significant behavioral and performance variations are expected daily, the initial 30 days in milk were used instead of days relative to diagnosis to ensure a more consistent comparison between healthy and mastitic cows. In consequence, aligning cows based on days to diagnosis, as done in the mid and late periods, would have matched the MAS1 cows group (with a mean diagnosis at 13.8 DIM) to the first 14 postpartum days of healthy cows, a timeframe heavily influenced by the physiological adaptations of early lactation, such as metabolism changes and hormonal shifts.

One of the most noticeable changes at the beginning of lactation is the rapid increase in milk production levels, which occurs within the first few days after calving (Hostens et al., 2012). Given that milk yield is strongly correlated with multiple aspects of milking behavior and

performance (Rodriguez et al., 2023), fluctuations in production during this period are likely to be accompanied by substantial day-to-day variation in these parameters among healthy cows. As reported, hair cortisol concentrations in healthy cows increase at calving, remaining high for the first three weeks after calving, and return to normal values after 30 days (Burnett et al., 2015; Hayashi et al., 2021). High cortisol is a stress indicator that affects behavior (Bristow and Holmes, 2007) and milk ejection (Bruckmaier, 2005). In addition, during this time (18–21 DIM), cows were regrouped and moved from the fresh pen to the lactation pen, which can also affect their behavior and cortisol levels (Schirmann et al., 2011; Mazer et al., 2020). These factors could independently influence the milking behavior and performance in healthy cows, making comparisons with mastitis-affected cows inappropriate. Furthermore, they would also affect cows that develop mastitis, making it inappropriate to group them based on days relative to diagnosis when the first month postpartum is so variable, which could potentially confound the effects attributed to mastitis itself. Therefore, using DIM as the reference point allowed a more accurate assessment of early lactation dynamics in milking behavior and performance, regardless of the DIM when the mastitis diagnosis occurred during the early period.

In this study, the greatest probabilities of incomplete milkings and teats not found in cows with mastitis may be attributed to reduced milk yield and udder discomfort associated with this disease. Other studies have identified incomplete milking as a risk factor for the development of mastitis, promoting milk retention within the mammary gland, facilitating bacterial invasion (Hovinen and Pyörälä, 2011). However, in this study, the term "incomplete milking" refers to a reduction in the harvested milk by the AMS, which could be primarily attributed to decreased milk production during the sickness phase in cows affected by mastitis.

Nevertheless, in this study, milking unit kick-offs did not show a significant association with mastitis as incomplete milkings did. Therefore, the idea of linking incomplete milkings to reduced milk production appears to be more plausible. In this context, our findings during the mid and late periods are consistent with those reported by Khatun et al. (2018), who found that incomplete milkings were associated with mastitis and represented a relevant variable for achieving significant mastitis detection capability.

In the case of the teat not found and milking unit kick-off variables, it has been reported that issues with teat cup attachment and increased kicking occur more frequently in cows with a more nervous or anxious temperament under stressful conditions (Hedlund and Løvlie, 2015; Carlström et al., 2016). Indeed, cows with mastitis have been shown to exhibit restless behaviors (Fogsgaard et al., 2015), which are commonly used as indicators of stress during milking (Rushen et al., 2001). In this idea, in conventional milking parlors, Okkema et al. (2023) reported that cows affected by udder edema after calving exhibited stepping and kicking behaviors at different stages of the milking process, while Medrano-Galarza et al. (2012) reported greater frequencies of stepping, leg lifting, and kicking during milking in cows with mastitis, particularly in the days following diagnosis. Therefore, the increased probabilities of teat not found, particularly during the mid and late periods in this study, may suggest that signs of discomfort and udder sensitivity begin prior to the clinical diagnosis of mastitis. Similarly, this study showed that the probability of milking unit kick-off events also diverged from the healthy group in the days leading up to diagnosis, although this was observed only as a trend during the late period.

These weaker associations may be due to the fact that the AMS registers a kick-off event whenever the liner detaches from the teat, an event that does not necessarily result from a real kick by the cow. Even though Ginger et al. (2023) noted that kicking was one of the most specific

behaviors for detecting udder inflammation, its sensitivity was low, suggesting that not all cows with mastitis displayed this behavior. In addition, previous research has reported that cow movement inside the milking box or inappropriate udder conformation and teat placement may contribute to premature liner detachment (Carlström et al., 2016). Hence, the low ability in identifying the actual cause when a liner detachment occurs, as recorded by the AMS, may be hindering the detection of more significant differences between healthy cows and those with mastitis for this variable in this study. This is further supported by another study that also used DeLaval AMS and found no associations between mastitis and milking unit kick-off events (Khatun et al., 2018). Associated with this, differences in milking unit kick-off probability between healthy and mastitic cows with mastitis during the early postpartum days may be biased, as these initial days of lactation coincide with the cows' adaptation period to the AMS. The greatest probabilities of milking unit kick-off in healthy cows were observed in the first days postpartum, which is when these variables associated with milking discomfort were most likely to occur (Munoz-Boettcher et al., 2025).

Regarding the milking interval variable, previous studies have reported an association between clinical mastitis and milking interval, indicating that increases in the milking interval would be a risk factor for developing clinical mastitis (Hammer et al., 2012; Penry et al., 2017). However, this study focuses on evaluating how milking interval patterns differ in cows with mastitis compared to those that did not become sick. Cows in the MAS1 and MAS31 groups exhibited consistently longer milking intervals throughout the entire evaluation period. Rasmussen et al. (2007) reported that cows with clinical mastitis increased their milking intervals by approximately 2 hours per day during the month preceding treatment, a trend that closely reflects the MAS31 group, where cows with mastitis showed about 1 hour/day longer milking interval,

beginning at least 14 days prior to diagnosis. It has been suggested that prolonged milking intervals may contribute to the progression from subclinical to clinical mastitis in the affected quarters (Hovinen and Pyörälä, 2011). Conversely, this pattern in MAS31 was not observed in the MAS61 group; however, both the MAS31 and MAS61 groups demonstrated noticeable increases in milking intervals around the time of diagnosis. This could also be attributed to the cows being sorted for diagnosis, spending time in the shoot or other areas for clinical examination and treatment. Interestingly, the rising trend started around day -2 relative to diagnosis, indicating the onset of behavioral change before diagnosis as a result of the development of the disease, including udder inflammation and pain. Consequently, the potential discomfort associated with the milking process in cows affected by mastitis could result in fewer voluntary milking visits (King et al., 2018), thereby contributing to an overall increase in the milking interval.

Additionally, it has been suggested that an increase in the time between milkings might indicate that the cows' daily rhythms are affected by the clinical phase of the disease (Hovinen and Pyörälä, 2011). Thus, the sickness behavior could be a plausible reason for the extended milking intervals observed in cows affected by mastitis in this study. During clinical mastitis, cows develop an inflammatory process in the mammary gland (Sordillo, 2018), which triggers the release of cytokines that interact with the central nervous system and result in behavioral alterations, including reduced locomotion, decreased appetite, increased fatigue, and signs of depression (Tizard, 2008). In agreement with our findings, several studies have documented negative alterations in behavioral patterns related to activity, resting, feeding, and rumination in cows diagnosed with mastitis before, during, and after the clinical signs become apparent. These behavioral shifts include decreased consumption of feed and water (Huzzey et al., 2007; Fogsgaard et al., 2015; Sepúlveda-Varas et al., 2016), along with shorter durations times spent eating and

ruminating (Huzzey et al., 2007; Fogsgaard et al., 2012; Liboreiro et al., 2015; Stangaferro et al., 2016; King et al., 2018). Affected cows have also shown increases in lying time (Sepúlveda-Varas et al., 2014) and marked declines in physical activity (Liboreiro et al., 2015; Stangaferro et al., 2016). This state of lethargy may diminish the cows' willingness to walk voluntarily to the milking station, while reduced feed motivation may impact their drive to access food, both at the AMS and in the feeding area. This is especially relevant under guided-flow traffic systems like the one used in this study, where such behavioral changes can result in prolonged intervals between milkings.

The effects of clinical mastitis on milk yield have been widely reported (Lescourret and Coulon, 1994; Rajala-Schultz et al., 1999; Gröhn et al., 2004; Wilson et al., 2004; Sguizzato et al., 2024), which aligns with the results of this study. Our results evidence that cows with mastitis during the early period had lower production per milking than healthy cows, with the difference increasing as the days in lactation progressed. It could be assumed that this difference would be maintained throughout lactation beyond what was reported in this study. This finding is supported by other studies that report negative effects on production in early lactation and long-term effects throughout lactation (Rajala-Schultz et al., 1999), where the production of cows with mastitis never fully returned to the levels of healthy cows (Wilson et al., 2004), as a result of the damage in the milk-secretory epithelium of the mammary gland that increases the rate of cell apoptosis because of the infection (Capuco et al., 2003).

Likewise, the mid and late periods showed rapid and sharp declines in milk yield on the day of diagnosis, similar to what has been previously reported (Wilson et al., 2004). However, in our study, this decline began 6 to 2 days before diagnosis, whereas other studies report that these declines began 2 to 4 weeks prior to diagnosis (Rajala-Schultz et al., 1999; Sguizzato et al., 2024) or could begin 1 to 2 weeks before diagnosis, depending on the pathogen involved (Gröhn et al.,

2004). This finding is consistent with that reported by Fogsgaard et al. (2012), who observed a significant reduction in milk production 24 hours after intramammary inoculation of *E. coli*. Considering this study explores the behavior of cows with mastitis in general, future research could further examine the dynamics of milking behavior and performance in relation to the specific mastitis-causing pathogen, as cows may respond differently depending on the infectious agent, as suggested by Gröhn et al. (2004).

The milking duration variable (also known as box time) reflects the AMS usage efficiency, so it can be considered the best indicator of milkability for cows in AMS (Carlström et al., 2014). Nonetheless, the total duration of the milking process is influenced by various factors, including teat preparation, milk harvest, and post-milking procedures such as liner removal and dipping application (Rodriguez et al., 2023). In this study, cows with MAS1 and MAS31 showed longer milking durations, as well as peaks around diagnosis in MAS31 and MAS61. On the contrary, cows with MAS61 had shorter milking durations between days -14 and -3 relative to diagnosis. However, this difference disappears in the milking duration by milk yield ratio, as does the difference between MAS31 and the group of healthy cows between -14 and -4 during the mid period. Because this variable is standardized by milk yield, it provides a better interpretation of the factors affecting milk duration. Consequently, it can be assumed that cows with MAS1 throughout the study period and MAS31 and MAS61 close to diagnosis have longer milking durations, regardless of the milk produced during that milking. Therefore, it seems plausible to assume that these longer milking durations are linked to changes in behavior during the handling time or variations in milk flow that prolong the time spent in the AMS unit.

Milking duration depend on factors associated with the individual cow, since this process can be further prolonged in cows with a nervous temperament (Carlström et al., 2016) or those

experiencing illness-related discomfort, as is the case with mastitis, which is considered a painful disease for cows (Underwood, 2002; Leslie and Petersson-Wolfe, 2012).

Likewise, any factor that interferes with milk ejection may alter milk flow duration and milk flow rate, ultimately affecting overall milking duration. Since milk flow parameters, such as milk flow duration and mean and peak milk flow rates, are indicative of milking efficiency and are closely associated with milk ejection (Bruckmaier and Blum, 1996; Sandrucci et al., 2007), changes in these variables could help explain variations in milking duration. In the three periods evaluated in this study, the dynamics of milking duration and milk flow duration were very similar, supporting this association.

In this study, particularly for MAS31 and MAS61, the mean milk flow and peak milk flow rates exhibited similar patterns, both following an inverse relationship with milk flow duration. This is due to the strong correlation between the mean milk flow and peak milk flow (Weiss et al., 2004; Penry et al., 2018) and the inversely proportional correlation of these variables with milk flow duration (Weiss et al., 2004). Similar to this study, which observed significant drops in mean and peak flow rates in cows with mastitis around the diagnosis day, Khatun et al. (2018) demonstrated a reduction in the mean flow rate as one of the predictor variables of mastitis in AMS. Consequently, delayed milk ejection was correlated with a decreased mean milk flow rate and an extended milk flow duration (Singh et al., 2024).

Milk ejection is induced by the release of oxytocin through teat stimulation. However, elevated levels of glucocorticoids, particularly cortisol, can interfere with this process by inhibiting or delaying oxytocin secretion (Bruckmaier, 2005). Such cortisol elevations are typically linked to stress-inducing conditions, including exposure to unfamiliar environments (Bruckmaier and Blum, 1998; Bruckmaier, 2005) and physiological stressors, such as pain and discomfort caused by

clinical diseases, such as mastitis (Underwood, 2002; Leslie and Petersson-Wolfe, 2012). Supporting this, cows suffering from clinical mastitis have been shown to exhibit higher plasma and milk concentrations of cortisol compared to healthy cows (Shuster and Harmon, 1992; Waldron et al., 2006; Ginger et al., 2023; Ma et al., 2023). Moreover, milk ejection could be affected by reduced milk yield or a lower relative udder filling degree in cows with mastitis (Bruckmaier and Hilger, 2001; Wieland et al., 2022), which could explain the longer milk flow duration, and smaller mean and peak milk flow of cows developing MAS1 and around the diagnostic day for MAS31 and MAS61.

Interestingly, the mean and peak milk flow rates were greater than those of healthy cows only in MAST61 between -14 and -3 days before diagnosis. Although some previous studies have suggested that high milk flow rates may increase the risk of mastitis (Grindal et al., 1991; Hogeveen et al., 2001), others have found no such association (Penry et al., 2017). In the present study, no relationship between milk flow rate and mastitis risk was identified.

Conclusions

The results of this study showed that cows with mastitis during the first third of lactation had a greater probability of incomplete milking and teat not found, along with longer milking intervals, milking duration, milking duration by milk yield ratio, and milk flow duration. Furthermore, a decrease in milk yield and a reduction in the mean and peak milk flow rate were observed. These findings suggest that mastitis is significantly associated with both milking behavior and performance, especially close to the time of diagnosis. These changes may have implications for production efficiency and animal welfare and could assist in the early detection of mastitis in cows managed in automatic milking systems.

Tables and figures

Table 4.1 Definitions of the variables considered for the assessment of milking behavior and performance in Holstein cows in an automatic milking system (adapted from Munoz-Boettcher et al., 2025).

Variable	Definition
<i>Binary variables</i>	
Incomplete milking	A quarter producing $\leq 50\%$ of the expected milk (only if a quarter is expected to produce > 1 kg of milk)
Milking unit kick-off	Abrupt interruption in the milk flow during the milking process of one or more quarters
Teat not found	One or more teats were not found and not milked by the automatic milking system liner
<i>Continuous variables</i>	
Milking interval (h)	Total time elapsed from the previous to the current milking.
Milk yield (kg/milking)	Total milk produced from all quarters in an automatic milking system visit
Milking duration (min)	Time from cow identification by the milking unit sensor until the cow moved away from the sensor leaving the milking unit
Milking duration by milk yield ratio (min/kg)	Milk duration divided by milk yield at the same automatic milking system visit
Milk flow duration (min)	Time from the first milk ejected until the last milk collected
Mean milk flow rate (kg/min)	Average velocity from which the milk was extracted from each quarter
Peak milk flow rate (kg/min)	Maximum velocity at which milk was extracted per milking quarter

MAS1

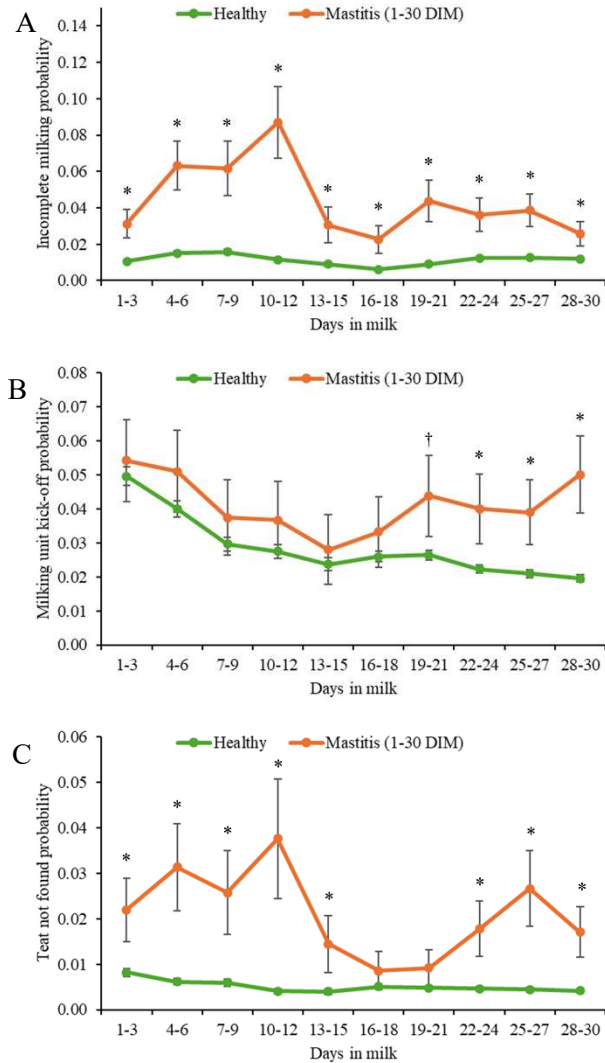


Figure 4.1 Adjusted probabilities (LSM and SE) for incomplete milking (A), milking unit kick-off (B), and teat not found (C) in healthy cows (green) and cows diagnosed with mastitis (orange; n = 97) during the early period (1–30 DIM, MAS1).

Logistical mixed-effects models for repeated measures were used, with cow ID as a random effect. The early period only included multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

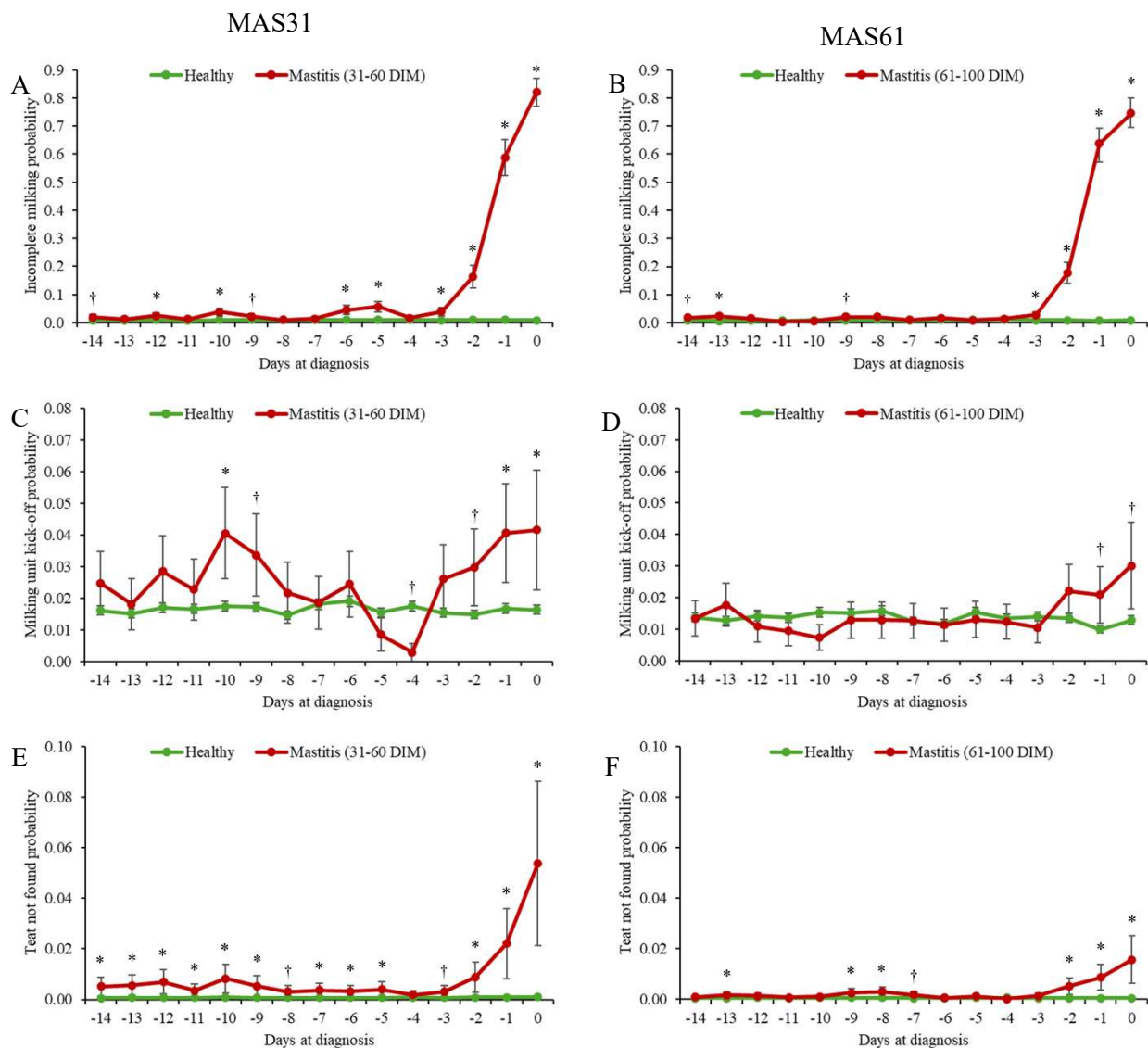


Figure 4.2 Adjusted probabilities (LSM and SE) for incomplete milking (top row: A and B), milking unit kick-off (middle row: C and D), and teat not found (bottom row: E and F), in healthy cows (green) and cows diagnosed with mastitis (red) during -14 to 0 days relative to the mastitis diagnosis. Panels on the left (A, C, and E) represent mastitis diagnosed in the mid period (31–60 DIM, MAS31; n = 74), and panels on the right (B, D, and F) represent the late period (61–100 DIM, MAS61; n = 93). For healthy cows, a "mock" diagnosis day was assigned based on the average diagnosis DIM (mid: 45 DIM; late: 81 DIM).

Logistical mixed-effects models for repeated measures were used, with cow ID as a random effect. Both mid and late periods included primiparous and multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

MAS1

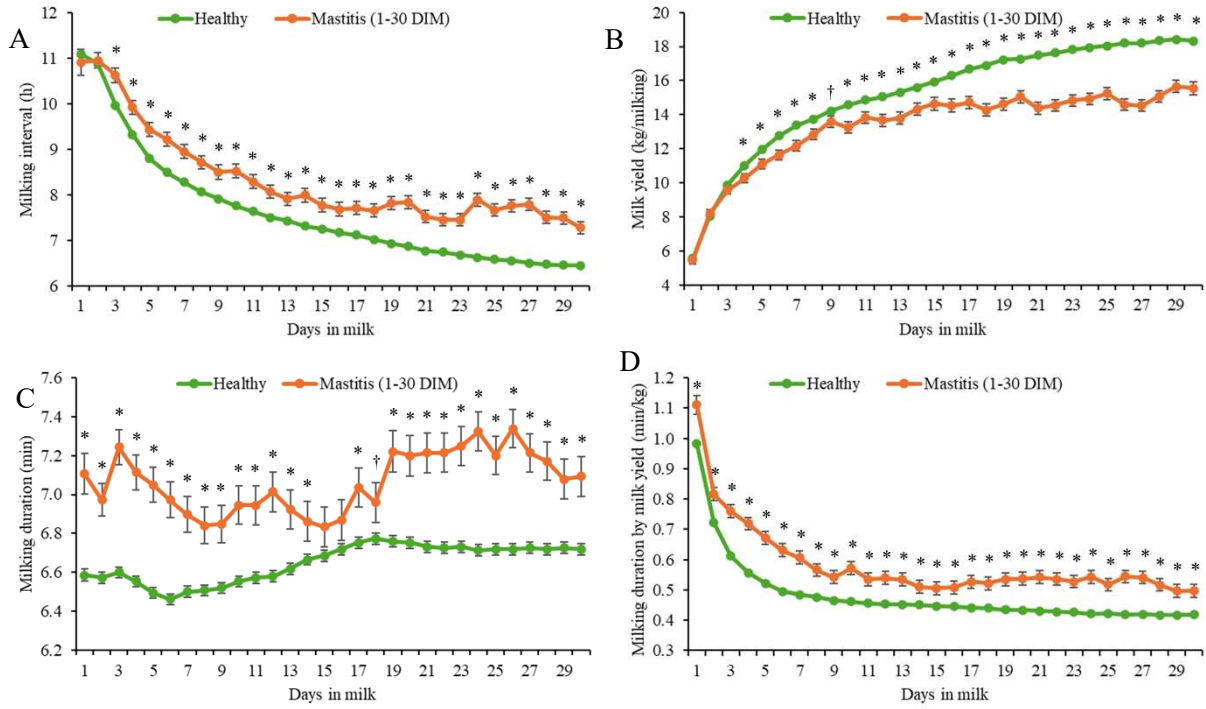


Figure 4.3 Least square means (SE) for milking interval (hours; A), milk yield (kg/milking; B), milking duration (minutes; C), and milking duration by milk yield ratio (min/kg; D), in healthy cows (green) and cow diagnosed with mastitis (orange; n = 97) during the early period (1–30 DIM, MAS1).

Linear mixed-effects models for repeated measures were used, with cow ID as a random effect. Parity was included as a fixed effect in all models, and milk yield was included as a fixed effect in the model for milking interval and milking duration. The early period only included multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

MAS31

MAS61

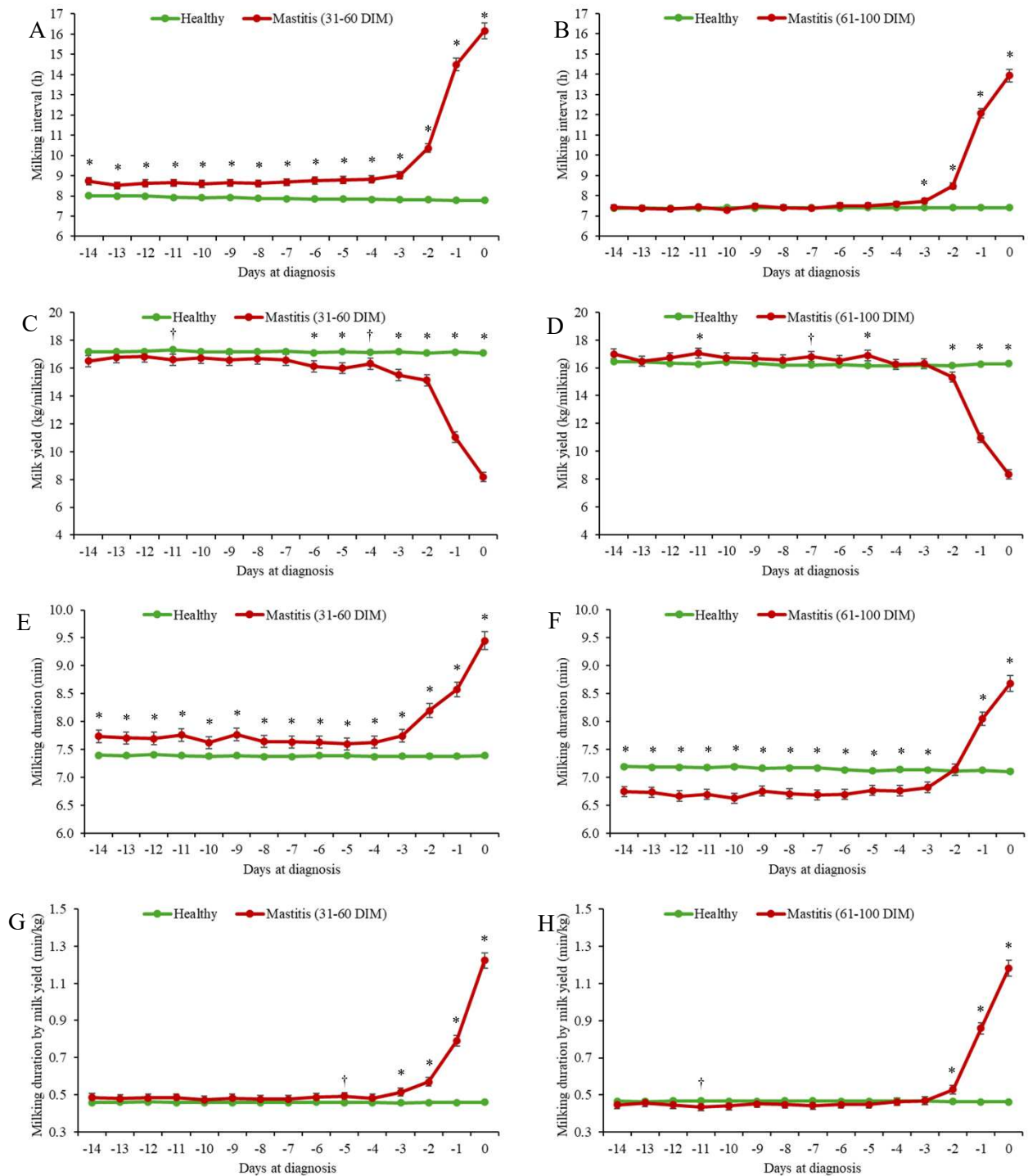


Figure 4.4 Least square means (SE) for milking interval (hours; top row: A and B), milk yield (kg/milking; second row: C and D), milking duration (minutes; third row: E and F), and milking duration by milk yield ratio (min/kg; bottom row: G and H), in healthy cows (green) and cows diagnosed with mastitis (red) during -14 to 0 days relative to the mastitis diagnosis. Panels on the left (A, C, E, and G) represent mastitis diagnosed in the mid period (31–60 DIM, MAS31;

n = 74), and panels on the right (B, D, F, and H) represent the late period (61–100 DIM, MAS61; n = 93). For healthy cows, a "mock" diagnosis day was assigned based on the average diagnosis DIM (mid: 45 DIM; late: 81 DIM).

Linear mixed-effects models for repeated measures were used, with cow ID as a random effect. Parity was included as a fixed effect in all models, and milk yield was included as a fixed effect in the model for milking interval and milking duration. Both mid and late periods included primiparous and multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

MAS1

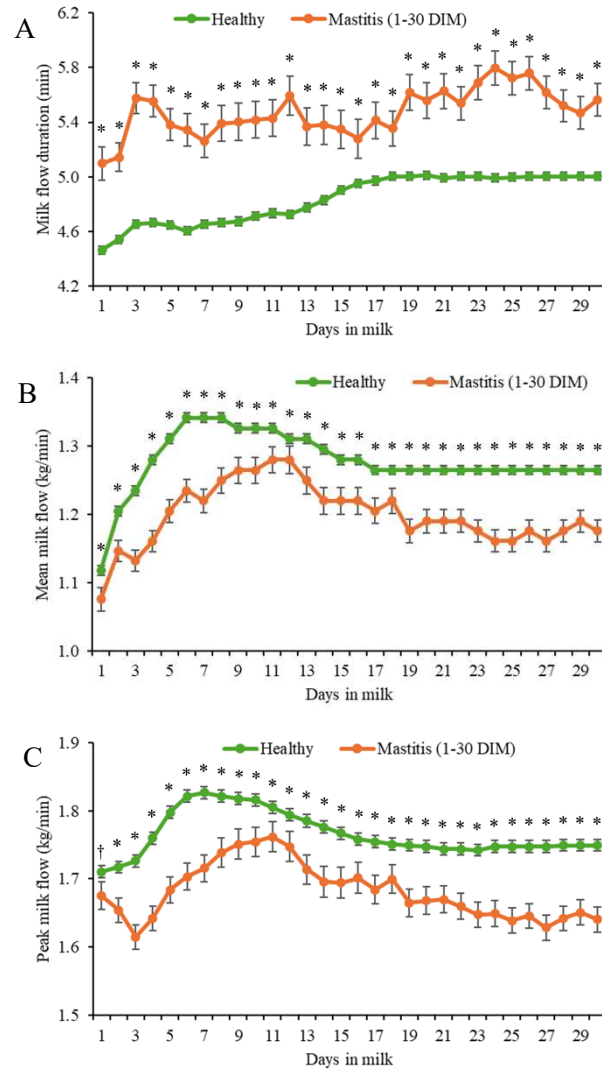


Figure 4.5 Least square means (SE) for milk flow duration (minutes; A), mean milk flow (kg/min; B), and peak milk flow (kg/min; C), in healthy cows (green) and cow diagnosed with mastitis (orange; n = 97) during the early period (1–30 DIM, MAS1).

Linear mixed-effects models for repeated measures were used, with cow ID as a random effect. Parity and milk yield were included as fixed effects. The early period only included multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

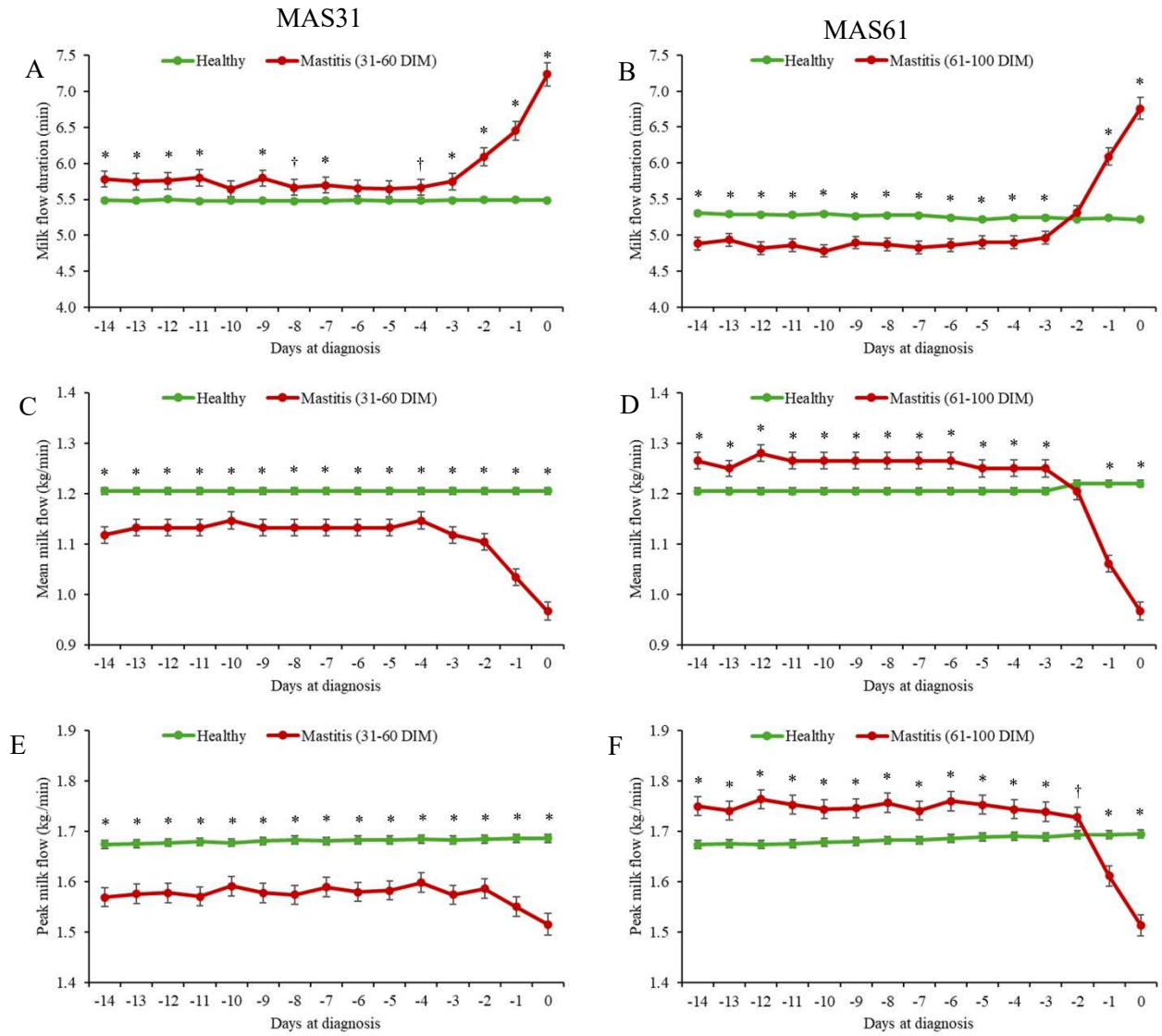


Figure 4.6 Least square means ($\pm$ SE) of milk flow duration (minutes; top row: A and B), mean milk flow (kg/min; middle row: C and D), and peak milk flow (kg/min; bottom row: E and F), in healthy cows (green) and cows diagnosed with mastitis (red) during -14 to 0 days relative to the mastitis diagnosis. Panels on the left (A, C, and E) represent mastitis diagnosed in the mid period (31–60 DIM, MAS31; $n = 74$), and panels on the right (B, D, and F) represent the late period (61–100 DIM, MAS61; $n = 93$). For healthy cows, a "mock" diagnosis day was assigned based on the average diagnosis DIM (mid: 45 DIM; late: 81 DIM).

Linear mixed-effects models for repeated measures were used, with cow ID as a random effect. Parity and milk yield were included as fixed effects. Both mid and late periods included primiparous and multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

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CHAPTER 5 – HEALTH CONDITIONS AND THE DYNAMICS OF MILKING BEHAVIOR AND PERFORMANCE DURING THE EARLY LACTATION OF COWS UNDER AN AUTOMATIC MILKING SYSTEM

Abstract

Automatic milking systems (**AMS**) collect extensive cow-level data, which can be utilized to identify behavioral and performance changes indicative of developing diseases. The objective of this study was to investigate the relationship between various milking behaviors and performance metrics and health disorders diagnosed within the first 21 days in milk (**DIM**) in Holstein cows kept in an automatic milking system on a commercial dairy operation. Data included information from 2,511 lactations started between January 2023 and April 2024, in multiparous cows milked in 62 AMS. Milking behaviors included incomplete milking, milking unit kick-off, teat not found, and milking interval (h). Performance variables were milk yield (kg), milking duration (min), milking duration by milk yield ratio (min/kg), milk flow duration (min), and mean milk flow rate (kg/min). Health disorders diagnosed during the first three weeks postpartum included retained fetal membranes (**RFM**), clinical hypocalcemia (**HCa**), metritis, mild subclinical ketosis (**M-SCK**), severe subclinical ketosis (**S-SCK**), and displaced abomasum (**DA**). Milking data covered 1 to 21 DIM, and healthy cows were identified to serve as a reference group for comparisons. For the categorical milking variables, DIM were categorized into three periods (week 1, 2, and 3 after calving). Multivariable mixed linear models and multivariable mixed logistic models were fit using milking variables as the outcomes of interest. In comparison with the healthy group, cows with DA in weeks 1 and 2 after calving showed the greatest probability of incomplete milking. The probability of milking unit kick-off was significantly smaller in HCa at week 3, in S-

SCK at weeks 1 and 3, and in mastitic cows at week 1 after calving. Furthermore, the probability of teat not found was greater in cows with RFM throughout the entire period, while in cows with HCa, it was elevated only in weeks 1 and 2 after calving. No differences were found in the probability of teat cleaning failure for any disease. Compared with the control group, cows with any health disorder had longer milking intervals. Milk yield was smaller in cows with RFM, HCa, metritis, and DA. Cows that developed metritis had longer milking durations, whereas those with M-SCK had shorter milking durations. The ratio of milking duration by milk yield was greater in cows with RFM, metritis, and DA. Additionally, cows with metritis and DA had longer milk flow durations, whereas cows affected by RFM and metritis showed smaller mean milk flow. Overall, our findings support the use of AMS data as a tool for identifying changes in cow behavior and performance linked to early health disorders, offering a feasible approach for improved monitoring and cow welfare in dairy herds.

Introduction

Despite the continuous advances in disease prevention in dairy herds, almost half of high-producing cows present at least one clinical disease during their lactation, with the greatest incidence during the postpartum transition period (LeBlanc et al., 2006; Carvalho et al., 2019). The transition period is marked by dramatic hormonal, metabolic, and immune changes (Drackley, 1999; Van Saun, 2023) making cows particularly vulnerable to a range health disorders, including metabolic (e.g., ketosis, hypocalcemia), infectious (e.g., metritis, mastitis), and digestive issues (e.g., displaced abomasum; Sordillo and Raphael, 2013; Van Saun, 2023). In addition to the detrimental effects on animal welfare, diseases during early lactation lead to reduced cow performance and heightened risk of death and culling (De Vries et al., 2010), accompanied by

impaired fertility (Ribeiro et al., 2016; Pinedo et al., 2020) and loss of body condition (Roche et al., 2009; Hernández-Gotelli et al., 2023). Given the close link between health and behavior, early lactation disorders highlight the need to better understand behavioral alterations as potential indicators of distress and impaired welfare (von Keyserlingk et al., 2009; Weary et al., 2009).

Most health disorders cause behavioral changes in dairy cows, largely driven by the underlying inflammatory and immune responses associated with illness. Some significant behavioral deviations include clinical signs such as lethargy, anorexia, and fatigue or depression (Tizard, 2008), which are used in routine health monitoring programs at dairy farms (Espadamala et al., 2016). However, cattle often mask early indicators of disease due to their prey-animal instincts, particularly during the initial stages of illness (Weary et al., 2009). This behavioral masking makes early detection particularly challenging in large commercial herds. In response, precision livestock technologies have emerged as promising tools for accurately identifying deviations from normal behavioral patterns and supporting timely health interventions, generating alerts to warn farm staff of potential disease events (Caixeta and Omontese, 2021).

Commonly reported behavioral changes in sick cows include changes in rumination, feeding, activity, and standing and lying behavior. These behavioral alterations have been reported in cows affected by several health conditions, including mastitis, metritis, lameness, subclinical ketosis, and hypocalcemia (González et al., 2008; Schirmann et al., 2016; Dittrich et al., 2019), highlighting the strong link between physiological stress and deviations from normal behavioral rhythms. Notably, these patterns appear to be generally consistent across management systems, including both conventional and automatic milking systems (AMS) herds (King and DeVries, 2018).

In recent years, AMS has progressively become more popular in the dairy industry. One of the advantages of these systems is the greater autonomy for cows over their daily routines and behaviors, as the cow-human interactions are reduced and the AMS allows cows to set their own milking schedule (Jacobs and Siegford, 2012). In consequence, voluntary milking in AMS herds has opened the exploration of some specific milking behavioral variables that could be of value in the identification of health issues in cows. An additional advantage of automated monitoring is that it does not require human presence, which is a relevant point, given that animals may suppress visible signs of illness or vulnerability when people are nearby (Weary et al., 2009). Examples of these alterations include cows that develop lameness, subclinical ketosis, displaced abomasum, and metritis that had reduced frequencies of AMS visits (Bach et al., 2007; Borderas et al., 2008; Miguel-Pacheco et al., 2014; King et al., 2017b; a, 2018), suggesting that changes in milking behavior could serve as early warning signs of disease.

Interestingly, most studies exploring changes in milking behavior in transition diseases in cows using AMS have relied on moderate sample sizes, which may limit the generalizability of their findings. Expanding the number of animals in the analysis could provide a more robust and comprehensive understanding of the variation associated with the onset of specific health conditions. In addition, although AMS continuously collects a wide variety of milking-related variables, such as milking interval, milking unit kick-off, and occurrence of incomplete milking, many of these variables associated with milking behavior have not been extensively investigated in the literature.

We hypothesized that milking behavior and milking performance are influenced by the presence of early lactation health disorders, including retained fetal membranes (**RFM**), clinical hypocalcemia (**HCa**), metritis, subclinical ketosis, and displaced abomasum (**DA**), and that these

changes can be detected in cows milked within an AMS. Specifically, we anticipated alterations in a range of AMS-recorded variables, including the occurrence of incomplete milkings, milking unit kick-off, teat not found, and teat cleaning failure, milking interval, milk yield, milking duration, milking duration by milk yield ratio, milk flow duration, and mean milk flow rate. Therefore, the objective of this study was to investigate the relationship between various milking behaviors and performance metrics and health disorders diagnosed within the first 21 days in milk (DIM) in Holstein cows kept in an AMS on a commercial dairy operation.

Materials and methods

Study population

This retrospective observational study was conducted on a commercial dairy operation in northeast Colorado that houses 3,700 lactating Holstein cows in free-stall barns. All the research procedures were approved by the Colorado State University IACUC Waiver Subcommittee (protocol #4907). Cows were milked using 62 AMS (Voluntary Milking Systems, DeLaval, Sweden) in a guided-flow system (milking first). The final data set includes information from 137,284 milking events in 2,511 lactations from multiparous cows, started between January 2023 and April 2024.

All cows calved in a maternity pen and were moved to the fresh pen after 24 to 48 hours. Primiparous and multiparous cows were managed similarly but kept apart in the fresh pen during the first 21 DIM. During this period, multiparous cows were housed in free-stall pens and milked using AMS, while primiparous fresh cows were milked in a conventional milking parlor before being moved to their lactation pen. Once in the lactation pen, primiparous and multiparous cows were housed together and milked using AMS until the end of the lactation. The average stocking

density during the study was 62 ± 5 cows per AMS unit. Cows were provided with ad libitum access to clean water and were fed a total mixed ration twice daily, formulated in accordance with the National Research Council's recommendations (NRC, 2001). The diet consisted of corn silage (25.5 to 32.1% DM), hay (2.7 to 3.4%), haylage (3.2 to 4.0%), base mix (20.5 to 25.8%), supplement (5.3 to 6.6%), flaked corn (19.4 to 24.4%) and brews (2.1 to 2.7%). Furthermore, the AMS provides concentrate varying from 0.2 to 5.7 kg DM/day per cow, depending on the productivity level and lactation stage of each cow. The vaccination protocol for all cows included Enviracor J5 (Zoetis Animal Health, Parsippany, NJ, USA), Bovishield Gold (Pfizer Animal Health, New York City, NY, USA), and Ultrabac-8 (Pfizer Animal Health, New York City, NY, USA) vaccines. The farm personnel used the DeLaval activity meter system and DelPro Farm Management systems (DeLaval Activity Meter System; DeLaval International AB, Tumba, Sweden) to assess total activity for reproductive and health management purposes.

Study design and milking variables

Data collected included information from calving to 100 DIM. Cow demographic and health data were extracted from on-farm software (DairyComp 305; Valley Ag Software, Tulare, CA, USA). Milking behavior and performance were extracted from DelPro Farm Manager software for each AMS visit. Information was combined in a single file with records organized by the cow's unique identification (**ID**) and lactation number. The combined data set included cows' ID, calving date, lactation number, calving-related and health disorder events, and milking behavior and performance per AMS visit.

Milking behaviors included occurrence of incomplete milking, milking unit kicking-off, teat not found, and milking interval (h). Performance variables were milk yield (kg), milking

duration (min), milking duration by milk yield ratio (min/kg), milk flow duration (min), mean milk flow (kg/min; Table 5.1).

From calving to 66 days in milk (**DIM**), cows were allowed to enter the AMS again after 4 h since the last milking. Later in the lactation, the permission time for the next milking was adjusted individually for each cow depending on her productive level. When the milking interval of a cow was 4 h longer than her permitted time, the farm staff went to push these cows to be milked.

Health variables

Disease events and diagnosis dates were extracted from DairyComp 305 and included RFM, HCa, metritis, subclinical ketosis, and DA. If a cow was diagnosed with the same health disorder multiple times during the first 21 DIM, only the first one was considered for the analysis.

Farm staff performed all the disease diagnoses following the farm health protocol. Cows were considered with RFM when there was a failure to expel the fetal membranes before 24 h postpartum (Kelton et al., 1998). Clinical hypocalcemia was defined as any cow that was recumbent within 72 h after calving, exhibiting anorexia, nervous clinical signs, staggering, varying degrees of unresponsiveness, and that had a good response to intravenously administered calcium (Kelton et al., 1998). Subclinical ketosis was monitored daily in the fresh pen by testing ketone bodies in urine using urine reagent strips (Ketostix strip, Bayer, Leverkusen, Germany) for semi-quantitative detection of acetoacetic acid (**AcAc**) concentrations. The scale was categorized into 6 color blocks that represent different AcAc concentrations in urine. The categories are negative, trace or grade 1 (490 μmol of AcAc/L, shown as 5 mg/dL on the scale), grade 2 (1470 μmol /L or 15mg/dL), grade 3 (3920 μmol /L or 40 mg/dL), grade 4 (7840 μmol /L or 80 mg/dL),

and grade 5 (15,700 $\mu\text{mol/L}$ or 160 mg/dL ; Carrier et al., 2004; Tufarelli et al., 2024). Cows graded 2 to 4 were categorized with mild subclinical ketosis (**M-SCK**), and cows with at least one result in grade 5 were categorized with severe subclinical ketosis (**S-SCK**). Cows with M-SCK received oral propyleneglycol, while cows with S-SCK were treated with oral propyleneglycol and intravenous dextrose and dexamethasone. Metritis diagnosis was assessed based on the appearance and smell of the vaginal discharge, and body temperature; if the vaginal discharge was foul-smelling, purulent, or watery red-brown, and with or without fever ($> 39.5^{\circ}\text{C}$), then the cows were considered metritic (Sheldon et al., 2006). Cows were diagnosed with DA when they showed a reduction or selective appetite, a sudden drop in milk yield, pasty feces, and the auscultation of a “ping” sound on the abdomen percussion (Kelton et al., 1998; Van Winden et al., 2003). All the cows with DA were moved to the hospital pen to receive surgical treatment. Cows that did not develop any diseases (including other diseases such as mastitis and lameness) between calving and 100 DIM were assigned to the healthy group.

Cows were moved to the hospital pen when antibiotic or anti-inflammatory treatment with a withdrawal period was required or when a major procedure, such as surgery, was indicated. Once in the hospital pen, cows were milked in a conventional milking parlor, separate from the rest of the herd, until complete recovery or the end of the drug withdrawal period. Therefore, the analysis did not include milking behavior and performance during their stay in the hospital pen.

Statistical analysis

Data management, data editing, and statistical analyses were conducted using R, version 4.2.2 (R Core Team, 2022). During the data editing process, milking events resulting in 0.0 kg of milk yield or with a duration of more than 20 minutes were excluded from the final analysis.

Milking data covered 1 to 21 DIM. The calving day was omitted from the analyses due to the high variability and inconsistency of the data. Since primiparous cows began using the AMS after 18 – 21 DIM, the analyses only included multiparous cows (both healthy and sick). Lactations involving health disorders not included in this study (such as mastitis, lameness, and other digestive events), as well as those lacking milking data for the evaluation period (1 to 21 DIM), were excluded from the analysis. A total of 158,691 milking events across 2,914 lactations in multiparous cows, which began between January 2023 and April 2024, were included in the analysis. Of these, 1,906 lactations corresponded to healthy cows, 770 to cows diagnosed with one of the diseases of interest in this study, and 238 to cows diagnosed with two or more diseases. Cows diagnosed with health disorders that differed from the disease of interest were excluded from the total number of lactations analyzed for each health disorder, while the healthy group remained consistent across all comparisons (Table 5.2).

The statistical analysis was completed using the *nlme* (Pinheiro et al., 2024), *lme4* (Bates et al., 2015), and *emmeans* (Lenth, 2022) packages. Multivariable mixed linear regression models and multivariable mixed logistic regression models for repeated measures were fit using milking behavior and milking performance variables as the response. To address heteroscedasticity and stabilize variance, as well as to manage the imbalance in sample sizes between groups (healthy and sick), continuous variables related to milking behavior and performance were transformed using logarithmic or square root functions. These transformations were chosen based on which one most effectively improved model fit and ensured residual homogeneity. Specifically, logarithmic transformations were applied to milking interval, milking duration, and milk flow duration, whereas square root transformations were used for milk yield, milk duration by milk yield ratio, and mean milk flow. Additionally, linear regression models included a variance structure (weights

= *varIdent*) to explicitly model the residual variances specific to each group. Robust standard errors were employed to enhance the reliability of statistical inferences under conditions of heteroscedasticity. For logistic regression models, given the low occurrence of incomplete milkings, milking unit kick-offs, teat not found, and teat cleaning failures, the DIM were grouped into three categories for analysis of these variables: 1–7 DIM, 8–14 DIM, and 15–21 DIM as week 1, 2, and 3 after calving.

In all models, the health status, DIM or DIM category, and their interactions were examined as main predictors. Lactation number (continuous; 2 to 5) was included in all models as a fixed effect. Milk yield per milking was included in the multivariable mixed linear models as a fixed effect when appropriate. For all the models, the cow's ID was considered as a random effect. Milking events with missing records for a specific milking behavior or performance variable were excluded from the analysis only when the missing variable was the variable of interest.

Milking behavior and milking performance by health status were compared using Tukey-adjusted pairwise comparisons for each DIM or each DIM category. To facilitate interpretation of the results of the linear regression models, the estimated marginal means (least square means, LSM) obtained from the model were back-transformed to the original scale using the appropriate function, and standard errors (SE) were approximated using the delta method. Results from the logistic regression model are reported as LSM probabilities and SE. Assumptions of constant variance and normal residuals were assessed using residual diagnostic plots. Milking behavior and performance variables were evaluated for normality and outliers. Statistical significance was established at a $p < 0.05$ level using a likelihood ratio test, while differences of $0.05 \leq p < 0.10$ were considered tendencies.

Results

The probabilities of incomplete milking by health category are shown in Figure 5.1. Healthy cows showed a reduction in the incomplete milking probability over time, from 0.013 ± 0.001 (LSM $\pm$ SE) at the 1–7 DIM period to 0.008 ± 0.0006 at the 15–21 DIM period. The incomplete milking probability was numerically greater during the evaluation period in most diseases compared to the healthy group. However, no statistical differences were observed, except that cows diagnosed with DA had the greatest probabilities at 8–14 DIM (0.057 ± 0.020) and 15–21 DIM (0.029 ± 0.016), both significantly greater than those of healthy cows ($p < 0.05$). Additionally, cows with HCa at 1–7 DIM (0.026 ± 0.011) and with metritis at both 1–7 DIM (0.017 ± 0.003) and 15–21 DIM (0.012 ± 0.003) showed a tendency for increased incomplete milking probability compared to healthy cows.

Figure 5.2 illustrates the probability of milking unit kick-off by health category. For the healthy group, the probability of milking unit kick-off was greatest at the 1–7 DIM period, measured at 0.042 ± 0.002 (LSM $\pm$ SE), and subsequently decreased in the 8–14 DIM and 15–21 DIM periods, with values of 0.026 ± 0.002 and 0.025 ± 0.001 , respectively. Compared to healthy cows, the probability of milking unit kick-off was significantly smaller in cows with HCa at 15–21 DIM (0.007 ± 0.004 ; $p = 0.024$), in S-SCK at 1–7 DIM (0.033 ± 0.004 ; $p = 0.021$) and 15–21 DIM (0.019 ± 0.002 ; $p = 0.009$), and in mastitis at 1–7 DIM (0.030 ± 0.004 ; $p = 0.016$). Additionally, the probability tended to be smaller in S-SCK (0.021 ± 0.003) and greater in M-SCK (0.033 ± 0.004) during the 8–14 DIM period.

The probabilities of teat not found are presented according to health category in Figure 5.3. Healthy cows exhibited the greatest probability during the 1–7 DIM period (0.006 ± 0.001 , LSM $\pm$ SE), which decreased to 0.004 ± 0.001 during both the 8–14 and 15–21 DIM periods.

Throughout the entire period, cows with RFM consistently greater compared to controls throughout the entire period (0.020 ± 0.009 at 1–7 DIM, 0.025 ± 0.023 at 8–14 DIM, and 0.019 ± 0.009 at 15–21 DIM; $p < 0.05$ for all periods). In cows with HCa, the probability was elevated during the 8–14 DIM (0.022 ± 0.014 , $p = 0.041$) and 15–21 DIM (0.012 ± 0.009 , $p = 0.095$) periods, with the latter showing a trend toward significance. Although cows with M-SCK and DA had numerically greater probabilities than controls, these differences were not statistically significant ($p > 0.10$). Conversely, cows with S-SCK showed smaller probabilities, with a trend at 1–7 DIM (0.005 ± 0.001 , $p = 0.057$) and a significant decrease at 15–21 DIM (0.002 ± 0.001 , $p = 0.032$).

The occurrence of teat cleaning failure was the smallest among the binary variables assessed (Figure 5.4). In healthy cows, the probability of teat cleaning failure started at 0.001 ± 0.0002 (LSM $\pm$ SE) during the 1–7 DIM period, decreased to 0.0004 ± 0.0001 at 8–14 DIM, and then increased slightly to 0.0007 ± 0.0001 at 15–21 DIM. Due to the low incidence, no events of teat cleaning failure were recorded for RFM and HCa during 8–14 DIM, nor for DA during both the 8–14 and 15–21 DIM periods. No statistically significant differences were detected between health categories throughout the study period.

Milking intervals for healthy and sick cows are shown in Figure 5.5. Healthy cows exhibited the longest milking interval at 1 DIM, with a duration of 10.6 ± 0.05 h (LSM $\pm$ SE). This interval gradually decreased by the end of the study period, resulting in a mean of 6.2 ± 0.02 h at 21 DIM. Longer milking intervals between AMS visits were observed throughout the first 21 DIM in all health disorders analyzed, except for S-SCK and DA, which showed increases primarily on days 1 and 2 after calving. The most substantial average differences in milking interval over the 21-day period were found in cows with RFM, metritis, and DA, with increases of 1.94, 1.75, and

2.15 hours, respectively, compared to healthy cows. The smallest differences were observed in M-SCK (0.09 h) and S-SCK (0.38 h), while cows with HCa showed an intermediate increase of 0.81 h.

The dynamics of milk yield per AMS visit for cows that remained healthy or were affected by the diseases in the analysis are presented in Figure 5.6. Milk yield (LSM $\pm$ SE) for healthy cows increased from 5.6 ± 0.05 kg/milking at day 1 after calving to 17.5 ± 0.08 kg/milking at 21 DIM. Overall, affected cows had smaller milk yields throughout the first 21 DIM, with the most significant reductions observed in cows diagnosed with RFM, HCa, metritis, and DA. Compared to healthy cows, the average milk yield per AMS visit over the 21-day period was reduced by 2.64 kg in RFM, 1.36 kg in HCa, 2.35 kg in metritis, and 3.09 kg in DA. In contrast, cows with M-SCK and S-SCK showed smaller reductions, averaging 0.24 kg and 0.51 kg less per visit, respectively. Notably, during the first 4 days postpartum, cows with M-SCK, S-SCK, and DA produced more milk per visit than healthy controls, although this early advantage diminished as lactation progressed.

Figure 5.7 shows the milking duration by health category. For healthy cows, the milking duration began at 6.48 ± 0.03 minutes (LSM $\pm$ SE) on day 1 of lactation. This duration decreased to 6.27 ± 0.03 minutes by approximately day 6, before showing a slight increase thereafter. In comparison with healthy cows, M-SCK cows had shorter milking durations, while the opposite was observed for cows with metritis, which had longer milking durations throughout the 21 DIM. Cows with RFM, HCa, and DA exhibited greater fluctuations in milking duration across time. Interestingly, no differences in milking duration were evident in cows with S-SCK.

As presented in Figure 5.8, the healthy group experienced a strong reduction in milking duration by milk yield ratio during the first week after calving. The ratio decreased from $0.98 \pm$

0.005 (LSM $\pm$ SE) at 1 DIM to 0.48 ± 0.003 at 7 DIM. This trend stabilized later, with a consistent reduction to 0.43 ± 0.003 by 21 DIM. Compared to healthy cows, greater milking duration by milk yield ratio was evident for cases of RFM, HCa (initial week), metritis (whole 21-d period), and DA (starting at 5 DIM). Cows with M-SCK and S-SCK showed smaller milking duration by milk yield ratio during the first 5 and 3 days after calving, respectively.

Figure 5.9 illustrates the milk flow duration for each health category. In the healthy group, milk flow duration increased from 4.33 ± 0.03 min at 1 DIM to 4.69 ± 0.03 min at 18 DIM, before slightly decreasing to 4.66 ± 0.03 min by the end of the study period (21 DIM). Compared with healthy controls, cows that developed metritis and DA had longer milk flow duration on most days after calving, while cows with S-SCK had shorter milk flow duration. The duration of milk flow varied in cows that developed RFM, HCa, and M-SCK, showing statistical differences in just a couple of days.

Mean milk flow values according to health category are shown in Figure 5.10. For healthy cows, mean milk flow started at 1.10 ± 0.007 kg/min (LSM $\pm$ SE) at 1 DIM, increasing during the first week up to 1.32 ± 0.007 kg/min (6 and 7 DIM) and then decreasing until 21 DIM (1.24 ± 0.007 kg/min). Cows that developed RFM and metritis exhibited smaller mean milk flow, while cows that developed S-SCK had greater mean milk flow from calving to 21 DIM. Additionally, greater mean milk flow was observed in cows with HCa from 7 to 21 DIM, cows with M-SCK during the first 5 DIM, and in cows with DA only during the third week after calving.

Discussion

This study analyzed the milking behavior and performance of cows housed in an AMS that developed common diseases during their early lactation. Given the increased autonomy cows have

in AMS environments to regulate their own milking routines, we anticipated that cows experiencing health disorders would exhibit distinct behavioral and production patterns compared to their healthy counterparts. The results confirmed this expectation, revealing clear associations between specific diseases and changes in milking behavior and performance.

In this study, the probabilities of incomplete milking, milking unit kick-off, teat not found, and teat cleaning failure during the first 21 DIM after calving were generally similar between sick cows and those that remained healthy. Additionally, Munoz-Boettcher et al. (2025) reported that teat clean failure and teat not found had a greater probability of occurring within the first three weeks after calving in multiparous cows, regardless of their health status. These findings suggest that these behaviors are more closely connected to the adaptation to robot use during the postpartum period, which is the same timeframe assessed in this study. Nonetheless, when evaluating milking unit kick-off, healthy cows had smaller probabilities of kick-off than cows with HCa, S-SCK, or metritis. In addition, the lack of differences in the probability of incomplete milking and milking unit kick-off could be attributed to the fact that these behaviors are more closely associated with pain and discomfort caused by udder inflammation and edema (Khatun et al., 2018; Okkema et al., 2023), rather than the diseases analyzed in this study. Furthermore, the AMS classifies any incident that leads to the detachment of the teat cup as a “kick-off” event, even though it doesn’t necessarily indicate that the cow actually kicked, which could explain the weak associations with disease occurrence.

Displaced abomasum was the only disorder associated with a greater incidence of incomplete milkings in this study. Incomplete milking, which refers to milk production falling below the expected 50%, may be linked to the abrupt decline in milk production typically seen in cows with DA (Van Winden et al., 2003; Edwards and Tozer, 2004). This could explain the

increased probability of incomplete milkings observed during weeks 2 and 3 postpartum, as this period coincides with the average DIM at the time of DA diagnosis, which was 16 ± 6.5 DIM.

Interestingly, only cows with RFM and HCa exhibited a greater probabilities of teat not found. While the literature reports teat liner attachment issues in cows with a nervous or anxious temperament, especially in stressful conditions (Hedlund and Løvlie, 2015; Carlström et al., 2016), there is no evidence to suggest that cows with RFM are more prone to nervousness. However, stress and anxiety during calving, which a retained placenta can trigger, may lead to changes in a cow's behavior, potentially making her more nervous (Mordak and Stewart, 2015). On the other hand, cows with HCa have been reported to exhibit increased activity in the first two days postpartum compared to healthy cows or those with subclinical hypocalcemia (Hendriks et al., 2020), which might suggest more nervous behavior during milking that prevents the AMS liners from properly attaching to the teat during the 1–3 DIM period.

Overall, the behaviors in analysis occurrence in low frequencies, which may explain the absence of significant differences between groups, alongside the high variability observed in the sick cows group. This was especially important for teat cleaning failure, where RMF, HCa, and DA did not record events in some of the evaluation periods. Furthermore, a limitation of this study was the inability to have records of milking behavior when cows were in the hospital pen, where cows were milked in a conventional parlor. Some changes in behavior could be underestimated due to these unavailable data.

The milking interval LSM for healthy cows showed a constant reduction from 11 h to 6 h between 1 to 21 DIM. This time reduction in the milking interval could be influenced by the increment in milk yield in healthy cows (Hogeveen et al., 2001; Penry et al., 2018). As previously reported (Edwards and Tozer, 2004), the milk production curve for healthy cows began low and

rose rapidly during the first week after calving, followed by a gradual increase thereafter. Similar to healthy cows, a reduced milk yield in sick cows due to diseases could lengthen the milking interval, making it longer. The combination of longer milking intervals and smaller milk yield per visit in sick cows indicates a decline in both behavior and productivity. These signals can serve as health markers in the early postpartum days, allowing for earlier and more accurate identification of at-risk cows. Notably, these patterns persist through the 21 DIM, and it is possible that the differences may continue later in the lactation.

Unlike the other milking variables, cows affected by any health disorders evaluated had a longer milking interval, although in different magnitudes. These findings are in agreement with previous reports that sick cows during early lactation exhibit a reduced frequency of AMS visits (Steensels et al., 2016; King et al., 2018), which could be interpreted as longer times between milkings. The extended intervals between milkings indicate a possible decrease in their motivation or capacity to voluntarily visit the AMS. This may be associated with pain, fever, lethargy, or metabolic changes that impair activity and exploration, which are the manifestations of sickness behavior during the sickness stage. The cytokines released during inflammation and the immune response affect the central nervous system, causing changes in behavior by reducing activity and appetite and increasing fatigue and depression (Tizard, 2008). The effect of transitional diseases on feeding and lying behavior has been widely studied, reducing feeding and rumination times and increasing resting times before, during, and after the disease diagnosis (Dittrich et al., 2019). The lethargy could also affect the motivation to walk to be milked, and the inappetence could affect the motivation to receive food from the milking unit and in the feed area, particularly in this study where cows were in guided flow traffic, lengthening the times of milking interval. Indeed, effects on activity and laying behavior, such as reduction in physical activity and increase in lying

duration, have been widely reported in RMF (Cattaneo et al., 2020), HCa (Sepúlveda-Varas et al., 2014; Barraclough et al., 2020), subclinical ketosis (Liboreiro et al., 2015; Steensels et al., 2016; Piñeiro et al., 2019), metritis (Sepúlveda-Varas et al., 2014; Liboreiro et al., 2015; Stangaferro et al., 2016; Neave et al., 2018), and DA (King et al., 2017a). Besides, pain and discomfort during the milking process could reduce the milking frequency (King et al., 2018) and, in consequence, increase the milking interval. Interestingly, DA and RFM, the diseases that were reported as associated with potential pain (Underwood, 2002; Stojkov et al., 2015), are the ones that exhibited longer intervals between milkings compared to healthy cows. Surprisingly, cows with M-SCK and S-SCK exhibited longer milking intervals, despite not showing clinical signs of illness. This suggests that even subclinical diseases can influence cow milking behavior. Overall, these findings imply that tracking milking intervals might serve as an early and practical indicator of postpartum diseases.

The negative impact of diseases on milk yield has been widely reported (Fourichon et al., 1999; Carvalho et al., 2019). Similar to this study, reduced milk yield has been reported for cases of RFM (Dubuc et al., 2011), HCa (Bareille et al., 2003; Venjakob et al., 2018), metritis (Dubuc et al., 2011; Wittrock et al., 2011), and DA (Van Winden et al., 2003; Asgari et al., 2024). Previous studies propose multiple mechanisms by which early lactation diseases affect milk production. Bertoni et al. (2008) reported that inflammation during the first month after calving has a direct negative impact on milk production. Similarly, Carvalho et al. (2019) suggested that infections or injuries occurring outside the mammary gland could lead to physiological responses that affect the activity, cell proliferation rate, and apoptosis of the epithelial cells in the mammary gland. In addition, it is suggested that during the infectious response, resources would be redirected toward the immune response, to the detriment of the mammary gland. Both immune defense and milk

synthesis demand significant energy, primarily derived from glucose, which can create competition between these processes when glucose availability is restricted (Habel and Sundrum, 2023), especially when the appetite and intake are reduced as a consequence of the sickness stage (Tizard, 2008). Likewise, decreases in dry matter intake (DMI) due to the reductions on in feeding duration, feeding intake, and rumination duration have been widely reported in cows diagnosed with RMF (Huzzey et al., 2007; Luchterhand et al., 2016), HCa (Jawor et al., 2012), subclinical ketosis (Goldhawk et al., 2009; Steensels et al., 2016; King et al., 2018), metritis (Huzzey et al., 2007; Stangaferro et al., 2016; Steensels et al., 2016; Neave et al., 2018), and DA (Van Winden et al., 2003; Pérez-Báez et al., 2021).

Milk yield reductions of up to 2–3 kg per visit in cows affected by conditions such as RFM, HCa, metritis, and DA were consistent with previous findings, which reported daily losses ranging from 2.19 to 3.7 kg during early lactation (Dubuc et al., 2011; Venjakob et al., 2018). These losses are significant not only from an economic perspective but also as objective indicators of compromised animal welfare. This reinforces the value of AMS records as effective tools for proactive health monitoring and management (King and DeVries, 2018). Especially because AMS systems offer continuous and objective data on both cow behavior and milk production, enabling constant monitoring without the need for direct human observation (Ozella et al., 2023). This is particularly valuable in large herds, where individual animal monitoring is limited, as they can detect subtle changes that may otherwise go unnoticed during routine visual assessments.

In this study, greater milk yield was observed during the first 4 days postpartum in cows with M-SCK and S-SCK. Greater milk yields could elevate the likelihood of cows experiencing subclinical ketosis because an increase in milk yield may be associated with elevated fat mobilization and hyperketonemia (Duffield, 2000). Interestingly, the average lactation days for M-

SCK and S-SCK diagnoses were 9 and 8 DIM, respectively, and the milk yield began to decline from 8 DIM for both diseases, compared to healthy cows; this suggests that cows start producing less milk after being diagnosed. A similar pattern, where cows with DA had greater milk yield only during the first 2 DIM, was observed here as reported by Edwards and Tozer (2004).

Milking duration dynamics over the study period were different among diseases, with metritis showing the largest milking durations, whereas in RFM, HCa, and DA, the milking duration varied over time. The duration of the milking process is affected by several components, including teat preparation, milk ejection, and the time required for detaching the liners and performing post-milking dipping (Rodriguez et al., 2023). Cows with a nervous temperament (Carlström et al., 2016) or who experience discomfort due to illness further exacerbate delays during the milking process. Metritis may result in pain and discomfort, resulting in behavioral and physiological responses (Stojkov et al., 2015), accompanied by longer milking times. Similarly, any factors that influence milk ejection could affect the milk flow duration and related flow variables, ultimately impacting the overall milking time.

To improve the accuracy of milking duration interpretation in relation to health conditions, we included the milking duration by milk yield ratio variable. As greater milk yield is associated with longer machine-on times (Neijenhuis et al., 2001; Rodriguez et al., 2023), this ratio provides a more precise measure than the milking duration alone because it is standardized by milk yield. This was particularly relevant for RFM, HCa, and DA, which showed more variability in the milking duration variable. However, the differences by health category were more evident in the milking duration by milk yield ratio variable. This suggests that the behavior of sick cows inside the AMS unit may partially contribute to the increased total milking time (milk flow duration and/or handling time). Probably because, similar to metritis, DA and RFM can be related to visceral

pain (Underwood, 2002) and generate behavioral responses during milking. On the contrary, for M-SCK the differences in milking duration with the healthy group disappeared for milking duration by milk yield ratio. This would attribute the differences in milking time on these diseases to variations in milk yield and not to changes in behavior.

The milk flow measurements, such as milk flow duration and mean milk flow, are considered indicative of milking efficiency because they are associated with milk ejection (Bruckmaier and Blum, 1996; Sandrucci et al., 2007). Delayed milk ejection was linked to a smaller mean milk flow rate and a longer milk flow duration (Singh et al., 2024). It is known that the release of oxytocin by teat stimulation induced milk ejection, and high concentrations of glucocorticoids, such as cortisol, disturbed the milk ejection during milking by delaying or reducing oxytocin release (Bruckmaier, 2005). The high cortisol concentration during milking is commonly associated with stress events, such as unfamiliar surroundings (Bruckmaier and Blum, 1998; Bruckmaier, 2005). However, pain and discomfort caused by clinical diseases, such as metritis and DA, could act as stressors (Underwood, 2002; Stojkov et al., 2015). Indeed, cows that develop clinical diseases (i.e., RFM, HCa, DA, and metritis) had greater hair cortisol concentrations than clinically healthy cows (Comin et al., 2013; Burnett et al., 2015). In addition, Wieland et al. (2022) proposed that cows with reduced milk yield or a smaller relative udder filling degree, which occurs in sick cows, could affect milk ejection. This could explain the longer milk flow duration and smaller mean milk flow of cows developing RFM, metritis, and DA. Surprisingly, contrary to these hypotheses, in this study, cows that developed S-SCK had shorter milk flow duration, while cows with HCa, M-SCK, and S-SCK had greater mean milk flow.

Conclusions

This study demonstrates that several milking behavior and performance variables recorded by AMS differ between healthy cows and those affected by specific health disorders during early lactation. In particular, cows diagnosed with DA had the greatest probabilities of incomplete milkings. An increased occurrence of teat not found events was observed in cows with RFM and HCa, while a reduced probability of milking unit kick-off was associated with HCa, S-SCK, and metritis. Although no disease was associated with teat cleaning failure, all health disorders were linked to longer milking intervals. Reduced milk yield, with consistent patterns observed in cows diagnosed with RFM, HCa, metritis, and DA. Notably, milking duration and milk flow variables displayed disease-specific patterns. Metritic cows had longer milking durations, while those with M-SCK had shorter ones. The milking duration by milk yield ratio was elevated in cows with RFM, metritis, and DA, suggesting smaller milking efficiency. Additionally, milk flow duration was prolonged in cows with metritis and DA, and mean milk flow was reduced in animals affected by RFM and metritis. Overall, these findings support the use of AMS data as a valuable tool for identifying changes in cow behavior and performance linked to early health disorders. Incorporating these indicators into herd health monitoring programs could enhance early disease detection and support improved welfare management in high-producing dairy herds.

Tables and figures

Table 5.1 Definitions of the variables considered for the assessment of milking behavior and performance in Holstein cows in an automatic milking system (adapted from Munoz-Boettcher et al., 2025).

Variable	Definition
<i>Binary variables</i>	
Incomplete milking	A quarter producing $\leq 50\%$ of the expected milk (only if a quarter is expected to produce > 1 kg of milk)
Milking unit kick-off	Abrupt interruption in the milk flow during the milking process of one or more quarters
Teat not found	One or more teats were not found and not milked by the automatic milking system liner
Teat cleaning failure	The automatic milking system teat cleaning cup did not clean one or more teats before the milking
<i>Continuous variables</i>	
Milking interval (h)	Time from the previous milking
Milk yield (kg/milking)	Total milk produced from all quarters in an automatic milking system visit
Milking duration (min)	Time from cow identification by the milking unit sensor until the cow moved away from the sensor leaving the milking unit
Milking duration by milk yield ratio (min/kg)	Milk duration divided by milk yield at the same automatic milking system visit
Milk flow duration (min)	Time from the first milk ejected until the last milk collected
Mean milk flow rate (kg/min)	Average velocity from which the milk was extracted from each quarter

Table 5.2 Health disorders (n and DIM) diagnosed in the first three weeks postpartum included in the analysis for milking behavior and performance in multiparous Holstein cows using an automatic milking system.

Health disorder	Lactations analyzed (n) ¹			DIM at diagnosis
	Total	Healthy	Sick	Mean (SD)
Retained fetal membranes	1983	1906	77	2.0 (1.4)
Clinical hypocalcemia	1946	1906	40	1.0 (0.8)
Mild subclinical ketosis ²	2338	1906	432	9.3 (4.9)
Severe subclinical ketosis ³	2261	1906	355	7.8 (4.7)
Metritis	2161	1906	255	6.9 (3.1)
Displaced abomasum	1955	1906	49	16.0 (6.5)

¹ Period of evaluation between 1 to 21 DIM.

² Cows graded 2 to 4 on the urine reagent strips for semi-quantitative detection of acetoacetic acid concentrations in urine.

³ Cows with at least one result in grade 5 on the urine reagent strips for semi-quantitative detection of acetoacetic acid concentrations in urine.

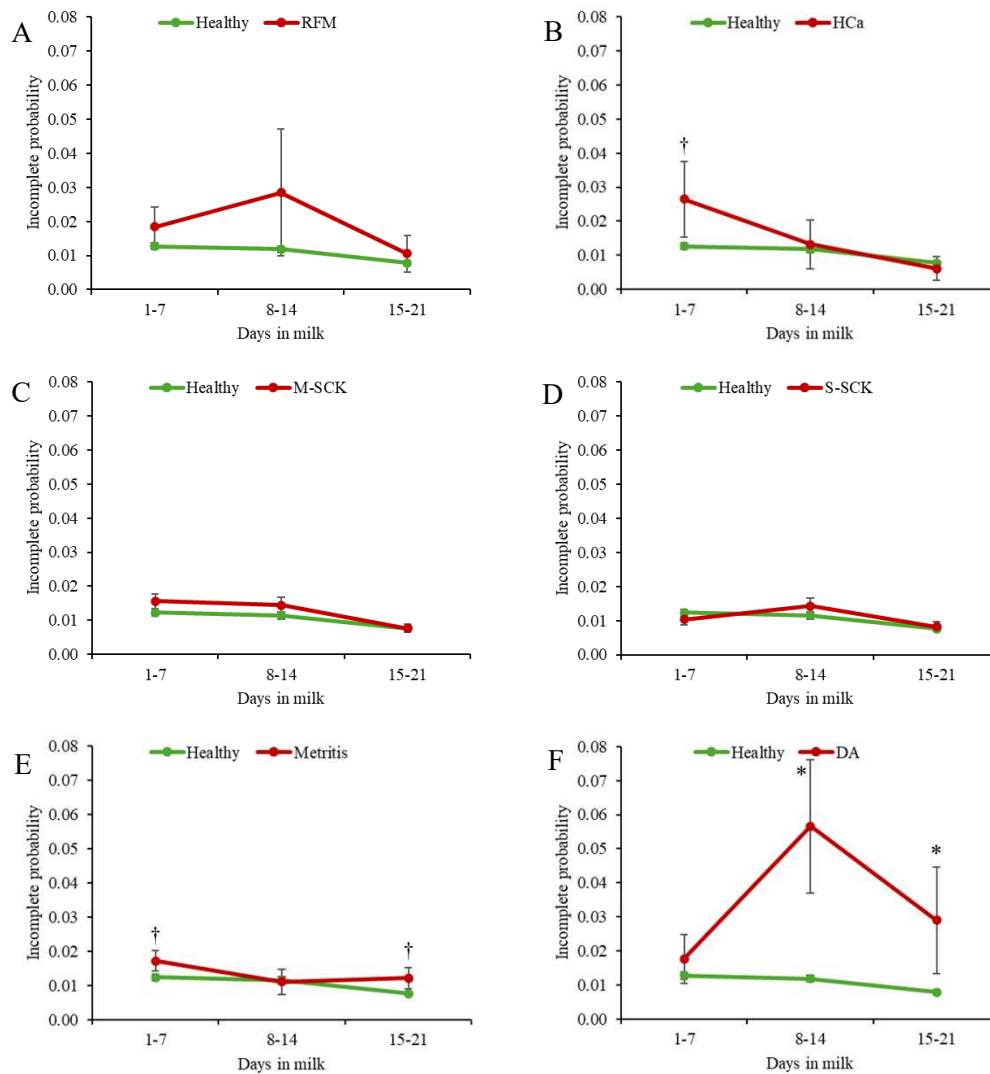


Figure 5.1 Adjusted probabilities (LSM $\pm$ SE) incomplete milking at 1–7 DIM, 8–14 DIM, and 15–21 DIM periods by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HCa), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The regression mixed models for repeated measures consider the cow's ID as a random effect for multiparous cows. Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

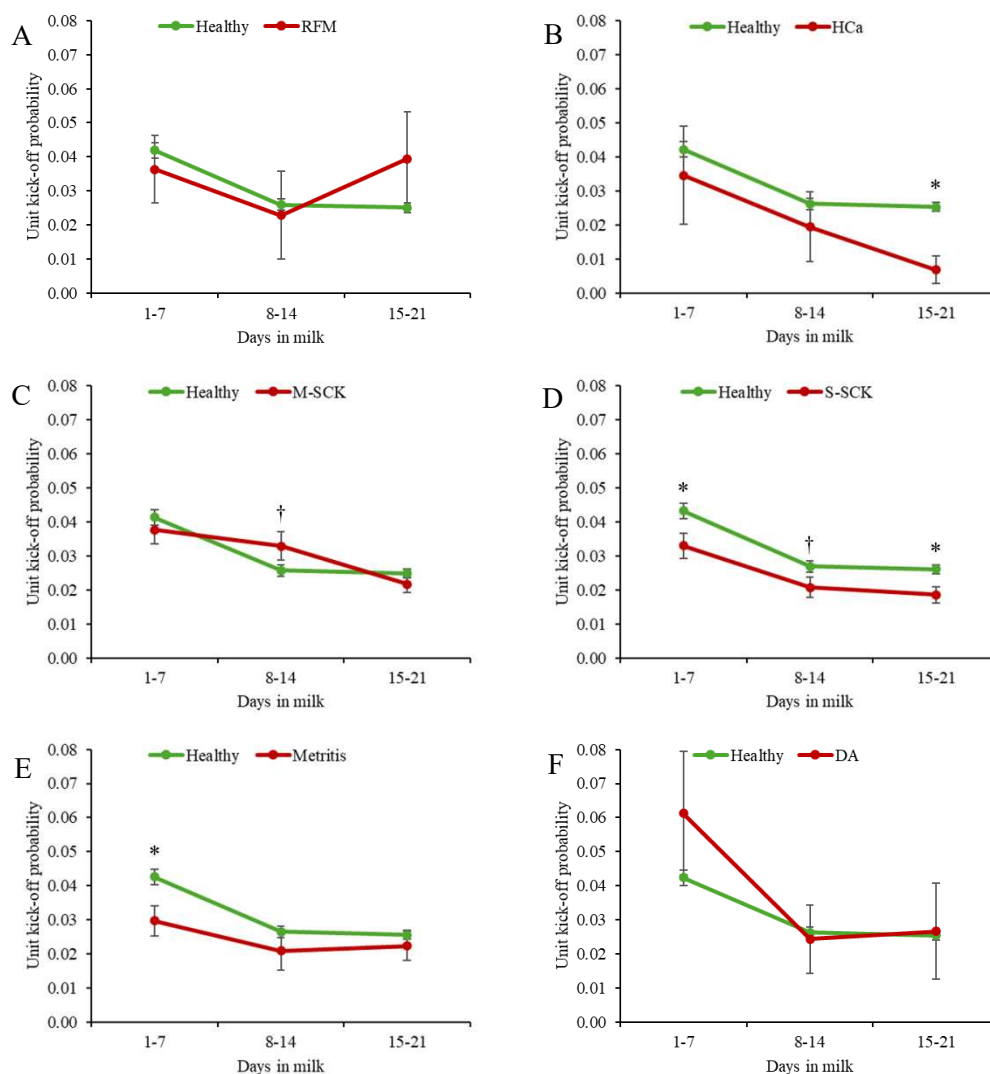


Figure 5.2 Adjusted probabilities (LSM ± SE) milking unit kick-off at 1–7 DIM, 8–14 DIM, and 15–21 DIM periods by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HCa), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The regression mixed models for repeated measures consider the cow's ID as a random. Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

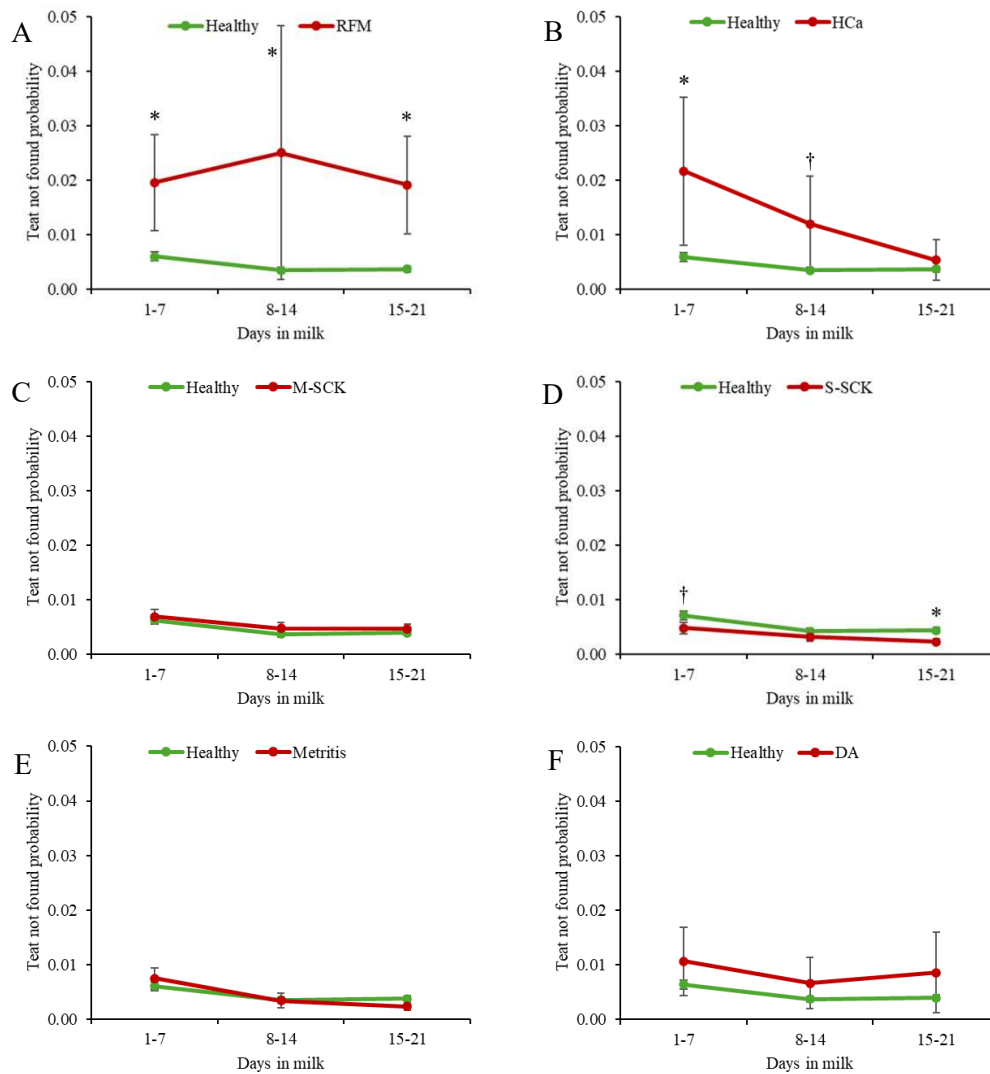


Figure 5.3 Adjusted probabilities (LSM ± SE) teat not found at 1–7 DIM, 8–14 DIM, and 15–21 DIM periods by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HCa), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The regression mixed models for repeated measures consider the cow's ID as a random effect for multiparous cows. Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

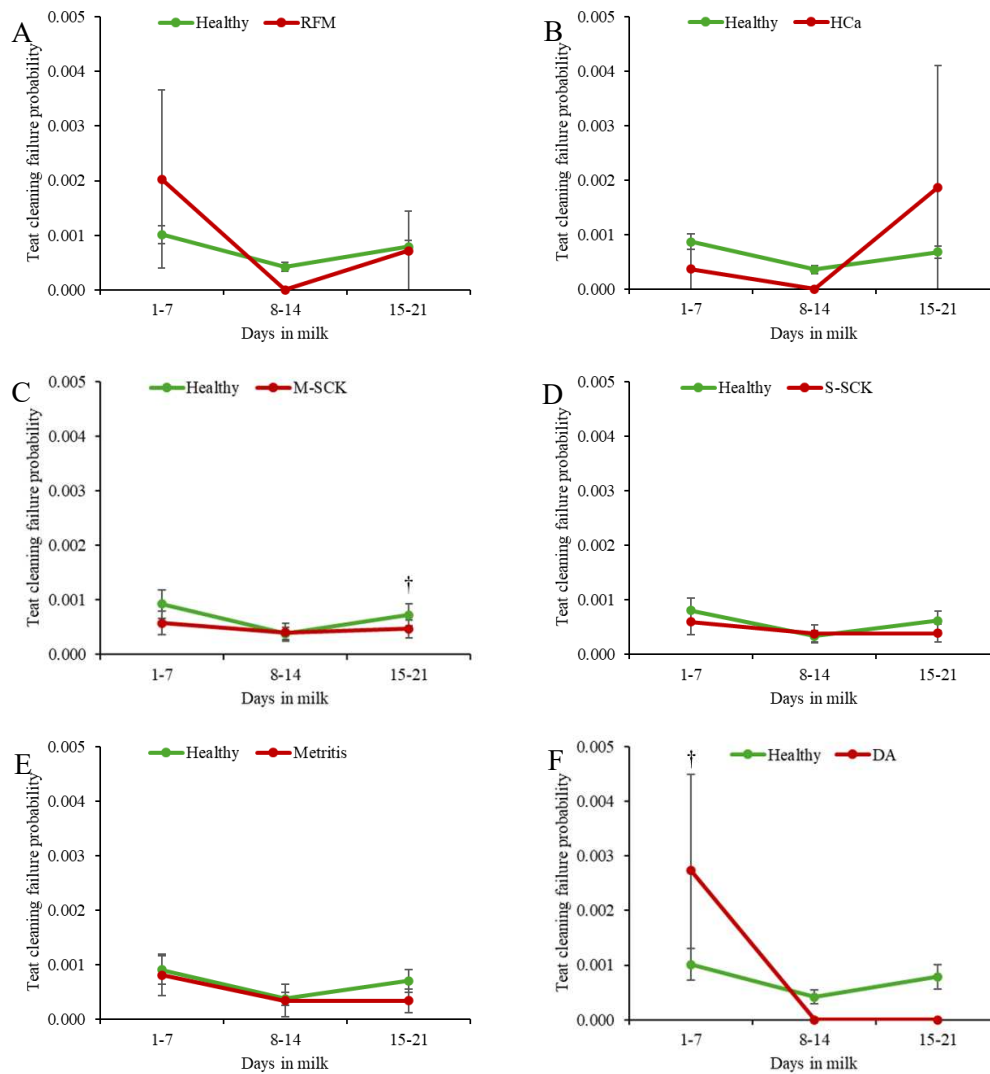


Figure 5.4 Adjusted probabilities (LSM ± SE) teat cleaning failure 1–7 DIM, 8–14 DIM, and 15–21 DIM periods by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HCa), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The regression mixed models for repeated measures consider the cow's ID as a random effect for multiparous cows. Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

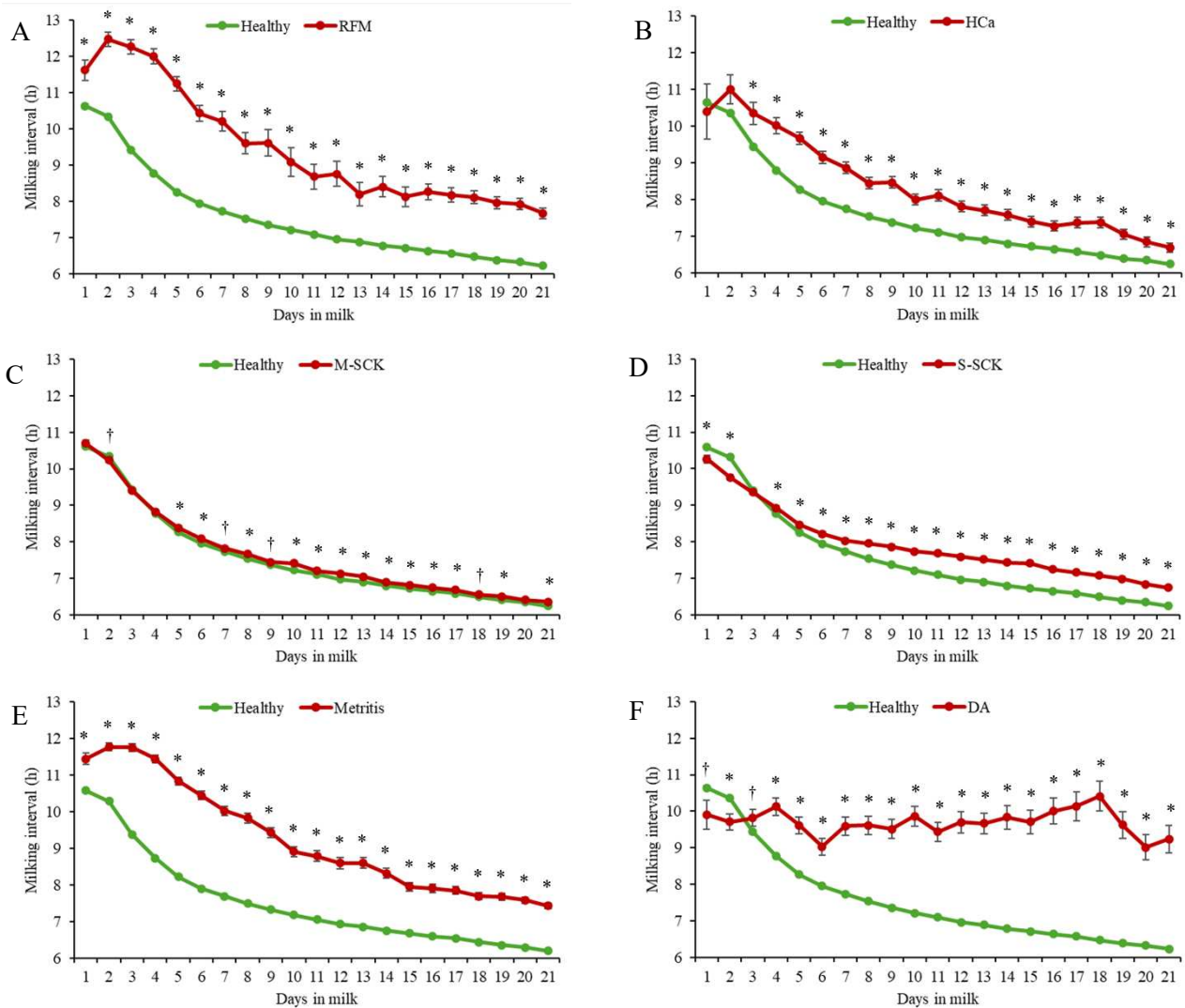


Figure 5.5 Least square means ($\pm$ SE) for milking interval (hours) between 1 to 21 DIM by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HCa), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The linear mixed models for repeated measures consider the cow's ID as a random effect and parity and milk yield as fixed effects for multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

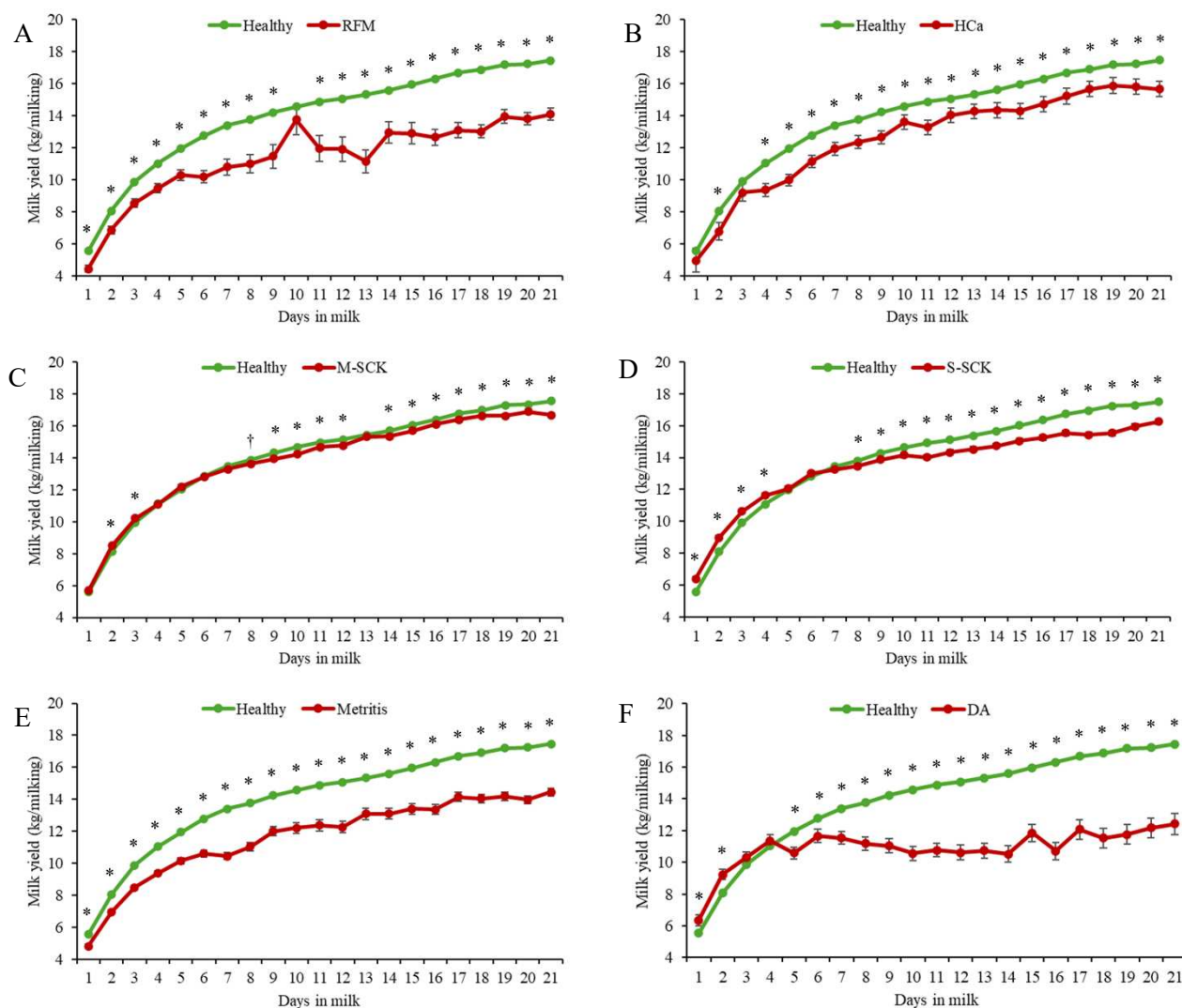


Figure 5.6 Least square means ($\pm$ SE) for milk yield (kg/milking) between 1 to 21 DIM by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HCa), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The linear mixed models for repeated measures consider the cow's ID as a random effect and parity as a fixed effect for multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

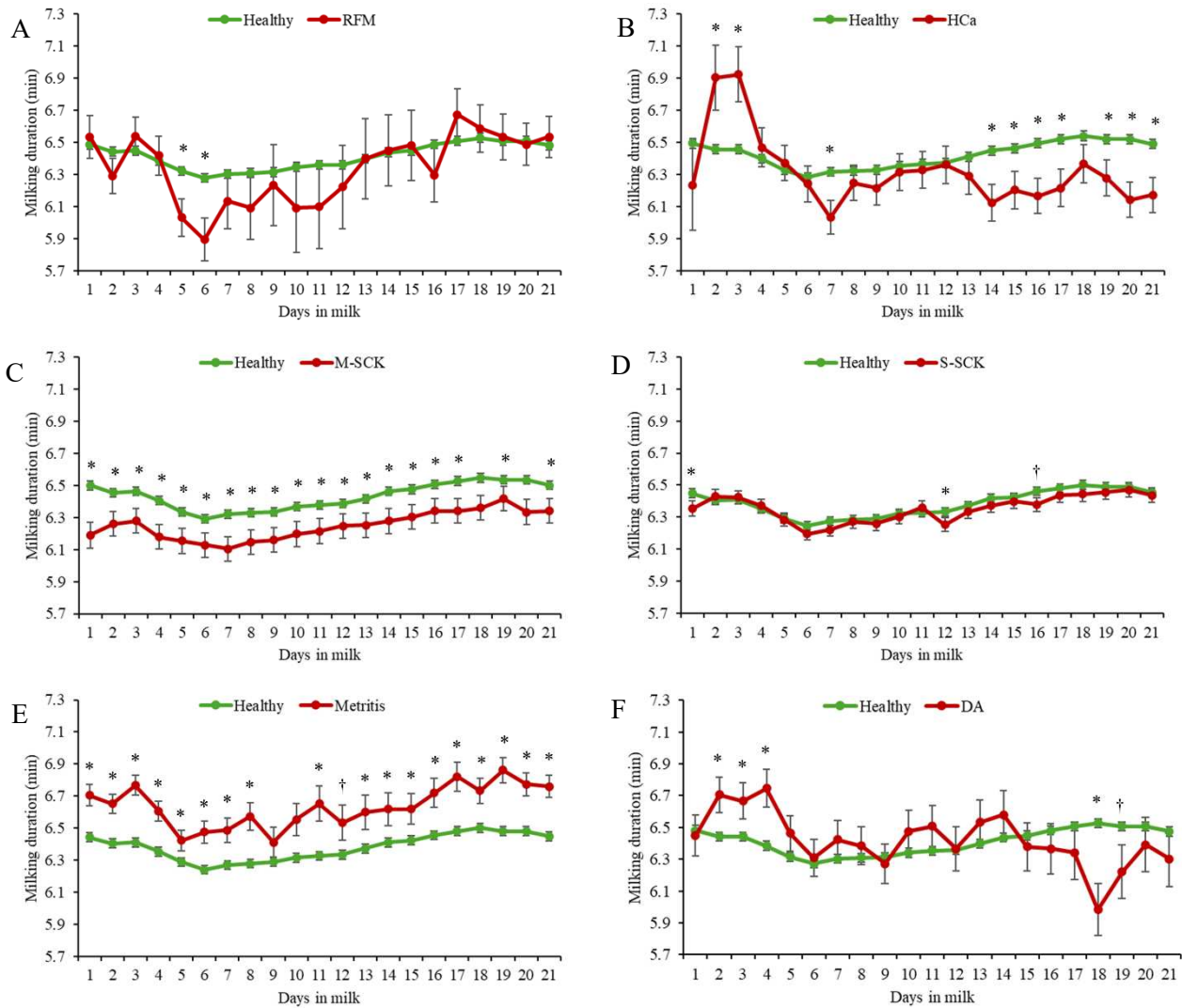


Figure 5.7 Least square means ($\pm$ SE) for milking duration (minutes) between 1 to 21 DIM by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HcA), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The linear mixed models for repeated measures consider the cow's ID as a random effect and parity and milk yield as fixed effects for multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

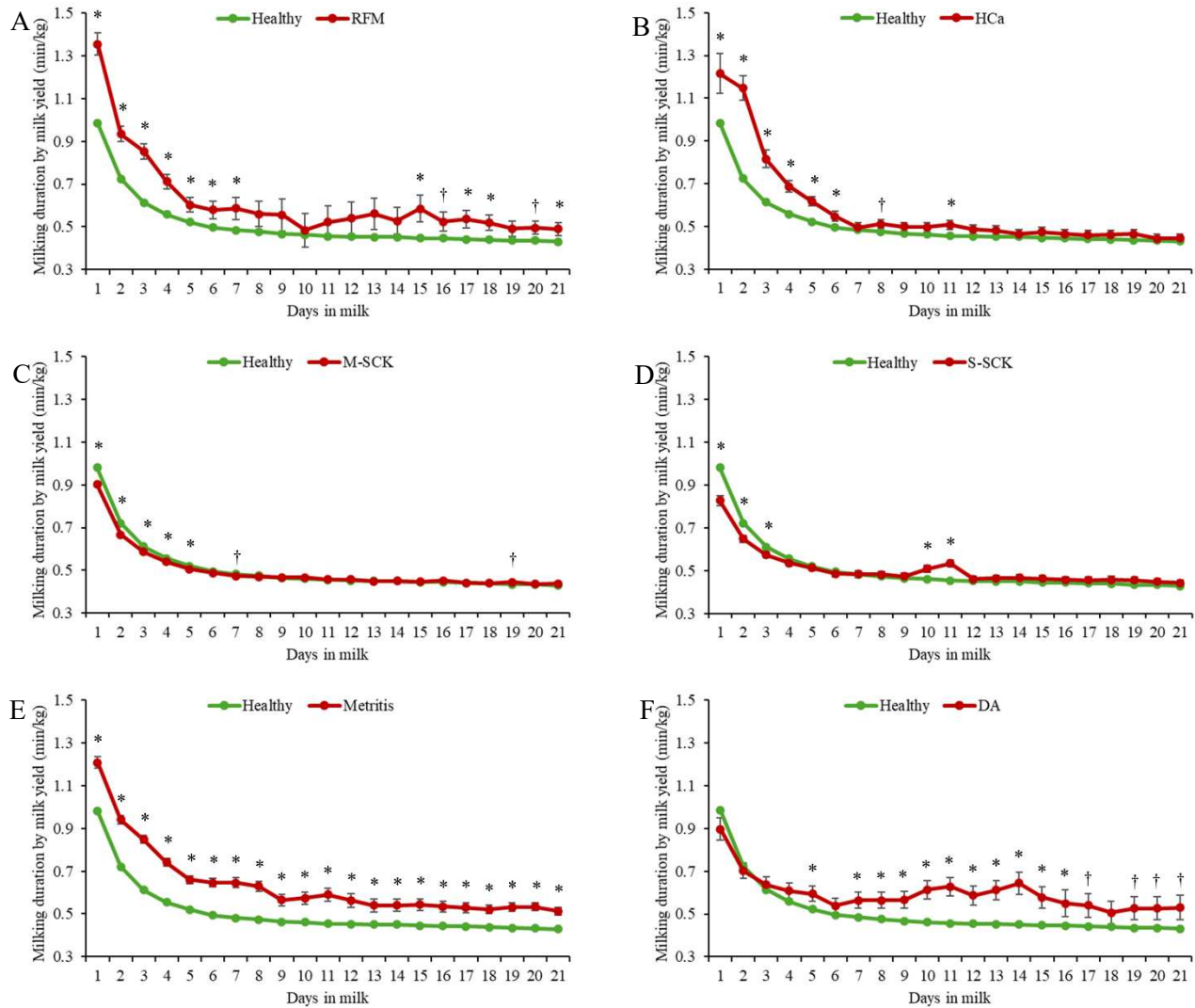


Figure 5.8 Least square means ($\pm$ SE) for milking duration by milk yield ratio (min/kg) between 1 to 21 DIM by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HcA), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The linear mixed models for repeated measures consider the cow's ID as a random effect and parity as a fixed effect for multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

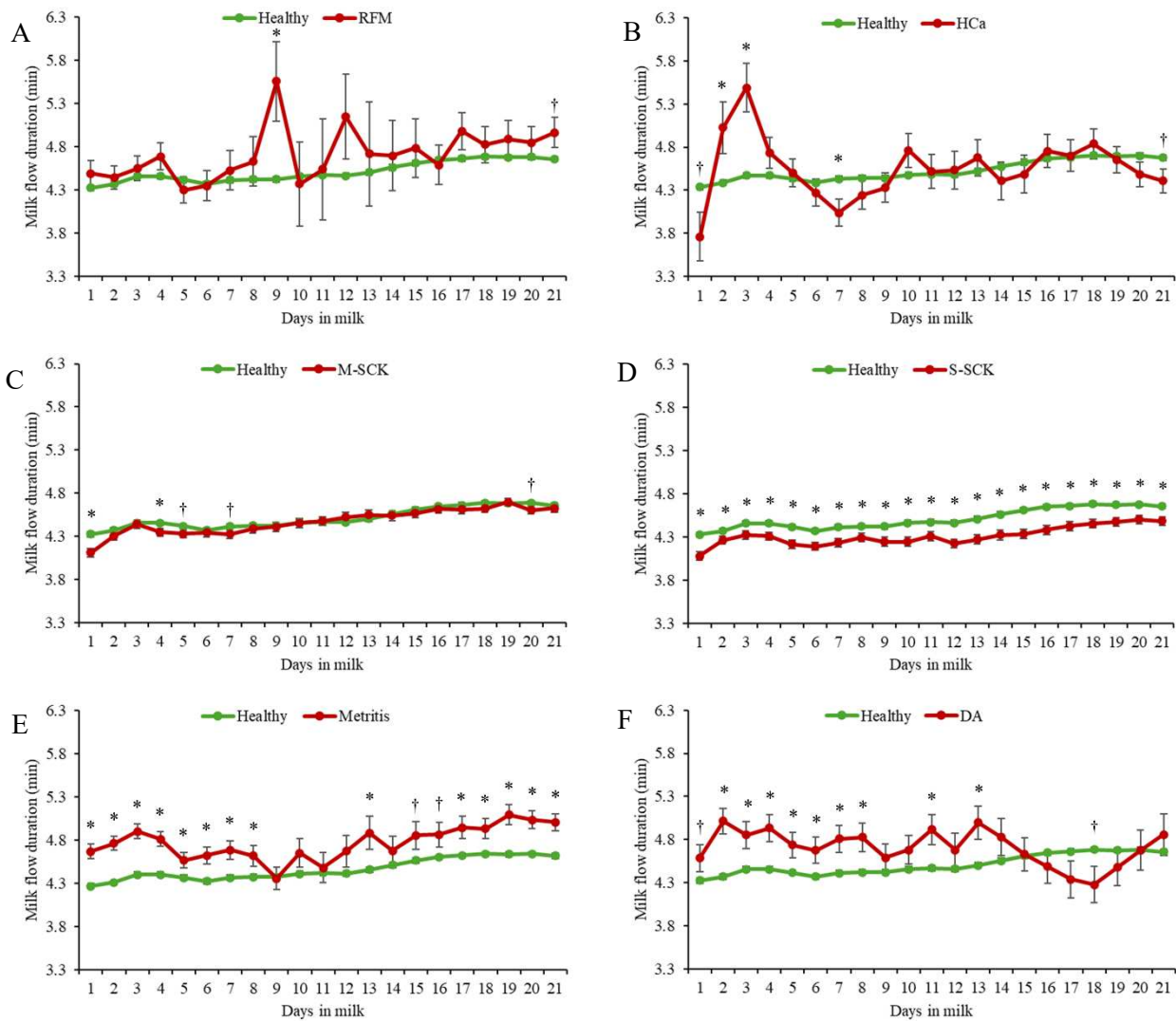


Figure 5.9 Least square means ($\pm$ SE) for milk flow duration (minutes) between 1 to 21 DIM by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HCa), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The linear mixed models for repeated measures consider the cow's ID as a random effect and parity and milk yield as fixed effects for multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

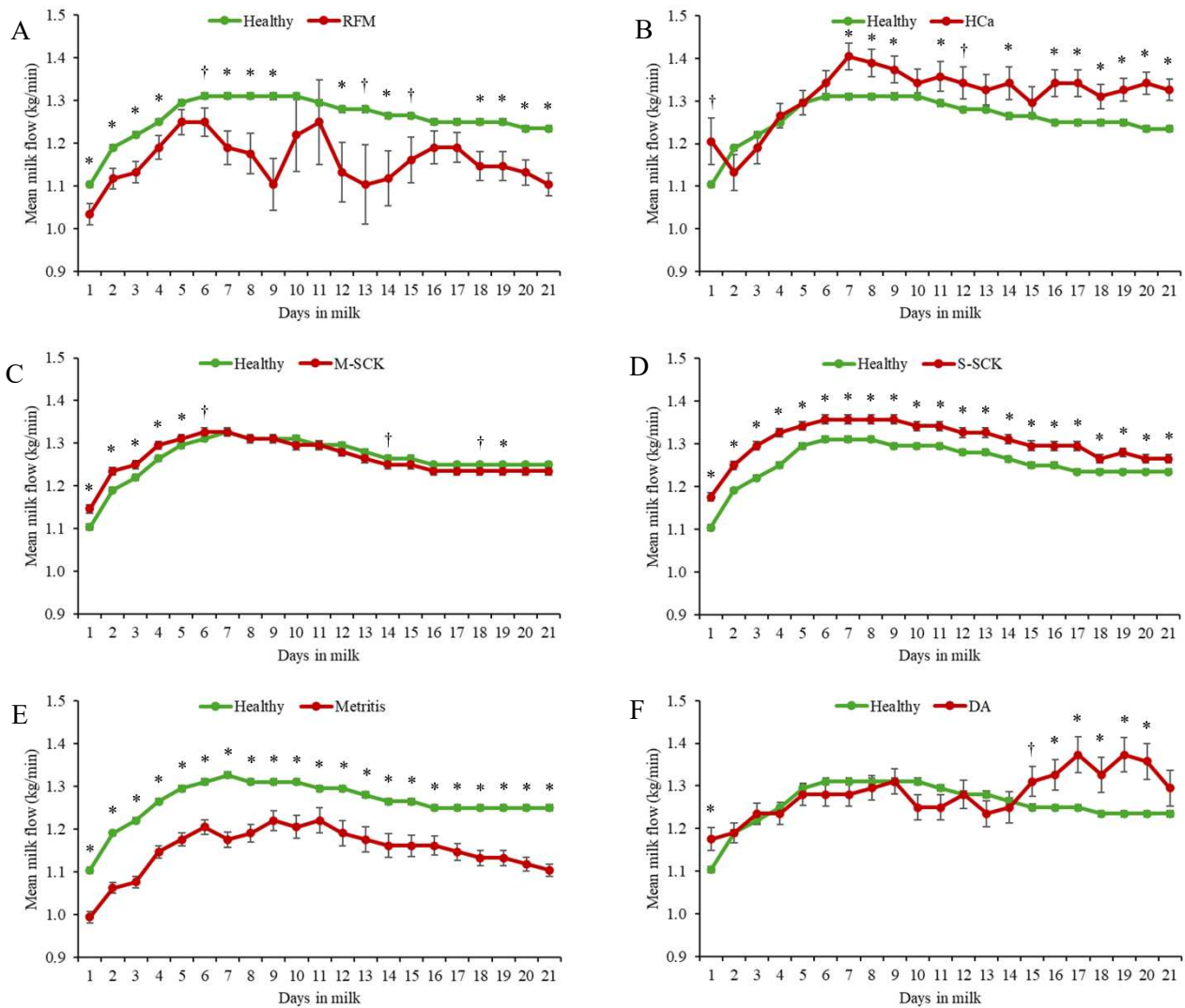


Figure 5.10 Least square means ($\pm$ SE) for mean milk flow (kg/min) between 1 to 21 DIM by health status category (green = healthy; red = sick) for diseases diagnosed in the first month postpartum [A: retained fetal membranes (RFM), B: clinical hypocalcemia (HcA), C: mild subclinical ketosis (M-SCK), D: severe subclinical ketosis (S-SCK), E: metritis, and F: displaced abomasum (DA)].

The linear mixed models for repeated measures consider the cow's ID as a random effect and parity and milk yield as fixed effects for multiparous cows.

Asterisk (*) indicates $p < 0.05$, dagger (†) indicates $0.05 \leq p < 0.10$.

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LIST OF ABBREVIATIONS

ABCS: automated body condition scoring

AcAc: acetoacetic acid

AHMS: automated health monitoring systems

AI: artificial insemination

AMS: automatic milking system

ANOVA: analysis of variance

BCS: body condition score

BCSc: body condition score at calving

BCSdry: body condition score at dry-off

Δ BCS: BCS change

BHB: beta-hydroxybutyrate

CI: confidence interval

COV: covariables

DA: displaced abomasum

DIM: days in milk

DM: dry matter

DMI: dry matter intake

HCa: clinical hypocalcemia

HLT: healthy category

IACUC: institutional animal care and use committee

ID: identification number

LSM: least square means (estimated marginal means)

M-SCK: mid subclinical ketosis

M30: average daily milk yield in the first 30 din milk

MAS1: mastitis diagnosed between 1 to 30 days after calving

MAS31: mastitis diagnosed between 31 to 60 days after calving

MAS61: mastitis diagnosed between 61 to 100 days after calving

MDi: mastitis detection index

ME: metabolizable energy

MLT: multiple disease categories

MP: multiparous

MTB: metabolic disease

NEFA: non-esterified fatty acid

nBCS: nadir BCS

nBCS+21d: body condition score 21 d post nadir

NDF: neutral detergent fiber

OR: odds ratio

OTH: other disorders (non-reproductive and non-metabolic)

P/AI1: pregnancy at first artificial insemination

PP: primiparous

Q: quartile

REP: reproductive disease

RFM: retained fetal membranes

S-SCK: severe subclinical ketosis

SD: standard deviation

SE: standard error

TMR: total mixed ration