THE EFFECT OF PREPARTUM SYNTHETIC ZEOLITE A SUPPLEMENTATION ON THE EATING, LYING, AND ACTIVITY BEHAVIORS OF MULTIPAROUS GRAZING DAIRY COWS

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ABSTRACT

Synthetic zeolite A is a precalving feed supplement that improves parturient blood calcium concentrations, thereby reducing hypocalcemia risk. Zeolite is associated with altered feeding behavior in housed cows and may affect lying and activity behaviors due to their established relationships with hypocalcemia. Furthermore, these responses may be affected by parity. This study determined the effect of feeding zeolite prepartum on eating, lying, and activity behaviors in multiparous grazing dairy cows during the transition period. Forty-three cows were randomly allocated to either a Zeolite treatment group (n = 21; individually fed 500 g/d zeolite in 2 kg DM/d maize silage for 18.2 ± 3.6 d prepartum) or a Control group (n = 22; fed maize silage only for 20.6 ± 4.1 d prepartum). Behavior data obtained from accelerometers were analyzed using repeated-measures ANOVA to determine the effects of treatment (Zeolite vs. Control), parity (Parity 2−3 vs. 4+), day, and their interactions, during 3 periods: PRE (-21 to -3 d), PERI (-2 to 2 d) and POST (3 to 28 d) relative to the day of calving (d 0). Parity 4+ cows spent a similar amount of time eating PRE (6.9 h/d) irrespective of treatment, whereas Parity 2–3 Zeolite cows varied in their eating time PRE (7.6 h/d), which was generally lower than that of Parity 2–3 Control cows (7.9 h/d). Zeolite cows also ate for 24 min/d less than Control cows PERI and lay down for 30 min/d longer POST. Regardless of treatment, Parity 2–3 cows ate for 0.6 – 5 min/h longer at night (1700 – 0559 h) and were more active than Parity 4+ cows, especially PRE. They also had shorter lying times PRE (30 min less/d; 1.6 – 4.7 min/h less at night) and PERI (48 min less/d), indicating younger cows ate more at night while their older herdmates were resting. These results suggest a subtle anorexic effect of zeolite in younger grazing dairy cows during precalving supplementation, whereas longer lying times may indicate improved cow comfort and welfare postcalving. Results also reflect possible competitive interactions and differences in time budgets between younger and older cows during the transition period.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber
AIC	Akaike’s Information Criterion
ANOVA	Analysis of Variance
BCS	Body Condition Score
BHB/BOH	β-hydroxybutyrate
BrW	Breeding Worth
BW	Body Weight
Cday	Day Relative to Calving Day
CP	Crude Protein
DCAD	Dietary Cation-Anion Difference
DIM	Days in Milk
DM	Dry Matter
DMI	Dry Matter Intake
ECM	Energy-Corrected Milk
HF	Holstein Friesian
LB	Lying Bout
ME	Metabolizable Energy
mol	Molar
NAD^+/NADH	Nicotinamide Adenine Dinucleotide
NDF	Neutral Detergent Fiber
NEFA	Non-esterified Fatty Acids

PMR	Partial Mixed Ration
PTH	Parathyroid Hormone
SD	Standard Deviation
SED	Standard Error of the Difference
SSS	Soluble Sugars and Starch
Tday	Day Relative to Treatment Start Date
TMR	Total Mixed Ration

UNITS AND TERMS

˚C	Degrees Celsius
Ca	Calcium
Cl^-	Chloride
d	Day(s)
g	Grams
g	Gravity
h	Hour(s)
ha	Hectare
J	Joules
k (prefix)	Kilo
K	Potassium
L (unit)	Liters
m (prefix)	Milli
M (unit)	Mega
m	Meters
Mg	Magnesium
min (unit)	Minute(s)
N or n	Number of
Na	Sodium
no.	Number

P	Probability
P	Phosphorus
pH	potential of hydrogen
PO_4^{3-}	Phosphate
s (unit)	Seconds
SO_4^{2-}	Sulfate
t (unit)	Tonne(s)
x	Crossed with, Times
yr	Year(s)

CHAPTER 1. GENERAL INTRODUCTION

The dairy industry is a major contributor to New Zealand's economy, with the sector producing 21.1 billion liters of milk during the 2019/20 farming season (LIC, and DairyNZ, 2020), employing over 50,000 people, and generating $19.7 billion in exports (DairyNZ, 2020). The approximately 4.92 million dairy cows in New Zealand (LIC, and DairyNZ, 2020) are a cornerstone of the dairy industry, with the industry aiming to improve dairy cow production efficiently and sustainably. New Zealand has a unique pasture-based dairy system, whereby cows graze pastures *in situ* and are managed to seasonally calve during the late winter/early spring. Animal health is an important aspect of dairy farming due to its direct impacts on milk production (Fourichon et al., 1999) and reproductive performance (Fourichon et al., 2000), the farmer's own time and money, the animal's quality of life and longevity, the consumer and public's perception of the dairy industry (Cardoso et al., 2016), and the marketability of dairy products. These factors drive the improvement of farm practices and the introduction of regulations by which to improve animal health and welfare.

Much focus is, therefore, placed on the prevention and treatment of diseases in dairy cattle, especially during the transition period (the three weeks before and after calving) due to the high incidence of disease at this time (LeBlanc et al., 2006). This period is marked by intense metabolic, physiological, and management-related changes as the cow transitions from late gestation, through calving, and to early lactation (Grummer, 1995; Drackley, 1999). At parturition, there is a drastic increase in demand for energy and calcium (Ca) to support the onset of milk production; however, the cow is not immediately able to support this demand through feed intake alone and experiences drastic changes to her energy and mineral metabolism. This metabolic and physiological stress is also associated with impaired immune function (Kehrli et al., 1989; Kehrli and Goff, 1989; Heiser et al., 2015; Crookenden et al., 2016). Failure to adapt to these changes can result in metabolic disorders and infectious diseases (Drackley, 1999).

Parturient hypocalcemia is one such metabolic disorder experienced by most dairy cows in the first few days following parturition. It occurs when cows fail to adapt to the sudden increased physiological demand for Ca to support milk production, and blood Ca concentrations decline below the normal physiological range of between 2.1 – 2.5 mmol/L (Horst et al., 1994; Goff, 2008). The severity, timing of onset, and duration of hypocalcemia can differ between individuals. Milk fever, or clinical hypocalcemia with paresis, is a severe form of the disorder where the lack of muscle contractility associated with hypocalcemia leaves the cow unable to rise to her feet and may result in fatality without treatment interventions. While these cases are easily identifiable because of their clinical signs, clinical hypocalcemia without paresis (blood Ca < 1.4 mmol/L; Lindsay and Pethick, 1983) and subclinical hypocalcemia (generally defined as Ca ≤ 2.0 – 2.2 mmol/L in one or more samples within 1 to 4 days postpartum; Roberts and McDougall, 2019; McArt and Neves, 2020; Seely et al., 2021) are more difficult for the farmer to detect.

Although only a small percentage (between 2 and 5%) of grazing dairy cows experience clinical hypocalcemia (Roche, 2003; Roberts and McDougall, 2019), a greater percentage experience subclinical hypocalcemia (range: 30 to 40% of cows with blood Ca < 2.0 mmol/L; 52% with blood Ca ≤ 2.14 mmol/L; Roche, 2003; Roberts and McDougall, 2019). A similar prevalence of clinical and subclinical hypocalcemia has been reported in higher-yielding cows managed in housed systems (Wilkens et al., 2020). Furthermore, hypocalcemia is a “gateway disorder”, with numerous studies in housed cows associating the severity, timing of onset, and duration of hypocalcemia with other health disorders around the time of calving (e.g., Goff, 2008; Rodríguez et al., 2017; McArt and Neves, 2020) as well as with early removal from the herd (McArt and Neves, 2020). These outcomes can lead to added costs to the farmer, as well as negatively affecting the production, health, and welfare of the dairy cow. Therefore, due to the severity and prevalence of hypocalcemia, strategies to prevent this disorder have been extensively explored.

Several strategies involve changing the mineral composition of the cow’s prepartum diet to improve blood Ca concentrations postcalving (e.g., Goff, 2006; Goff, 2008; Santos et al., 2019). Although this is effective in housed systems where cows are fed total-mixed rations (TMR), they are often impractical to use in grazing dairy systems due to the difficulty of adjusting the mineral balance of a predominantly pasture-based diet. Recently, prepartum supplementation with synthetic zeolite A (sodium aluminosilicate) has been investigated as an alternative approach. Zeolite binds dietary Ca, as well as other ions (e.g., magnesium (Mg) and phosphorus (P)), in the digestive tract, minimizing the absorption of these minerals into the blood and creating a state of negative Ca balance (Thilsing-Hansen et al., 2002; Grabherr et al., 2009). Supplementing between 500 to 1000 g/d of zeolite for 2 to 4 weeks prepartum can effectively reduce the risk of hypocalcemia in both housed (Thilsing-Hansen and Jørgensen, 2001; Grabherr et al., 2009; Kerwin et al., 2019) and grazing (Roche et al., 2018; Crookenden et al., 2020) dairy cows.

Previous studies regarding the use of zeolite in dairy cows have focused on its effects on blood mineral concentrations and, to a lesser extent, on milk yield and composition. Several researchers studying housed cows have also noted that zeolite-fed cows have lower dry matter intakes (DMI) during the precalving supplementation period (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009; Kerwin et al., 2019), possibly because of zeolite-induced hypophosphatemia, poor palatability of zeolite, altered digestion rate, or a combination thereof. Further, Kerwin et al. (2019) determined zeolite-fed cows had reduced daily rumination time prepartum. These reported effects on DMI and rumination indicate that the feeding behavior of zeolite-supplemented cows may be altered in grazing systems. In addition, hypocalcemia (without paresis) has been associated with longer lying times and reduced activity postcalving in both grazing dairy cows (Hendriks et al., 2020a) and housed cows (Jawor et al., 2012; Barraclough et al., 2020b; Tsai et al., 2021). Therefore, it is possible that supplementing cows with zeolite prepartum may be linked with differences in lying behavior and activity, which are increasingly used as possible indicators of animal health and functional welfare (Munksgaard and Simonsen, 1996; Maselyne et al., 2017; Hendriks et al., 2019a). To my knowledge, the effects of zeolite on the behaviors expressed by grazing dairy cows have not been investigated. Given that parity is independently associated with both hypocalcemia risk and behavior, whereby older cows are at increased risk of developing hypocalcemia (Roche and Berry, 2006; Roberts and McDougall, 2019), typically have longer lying times (Sepúlveda-Varas et al., 2014; Barraclough et al., 2020a), take fewer lying bouts (Calderon and Cook, 2011; Barraclough et al., 2020a) and steps (Hendriks et al., 2019a; Barraclough et al., 2020a), and have altered feeding behaviors (Proudfoot et al., 2009b; Neve et al., 2017) relative to younger cows, this factor must also be considered when investigating cow behaviors.

The objectives of this thesis are to: 1) review the literature on dairy cow behavior during the transition period, with particular emphasis on grazing systems, and 2) investigate the effect of precalving synthetic zeolite A supplementation and parity on the feeding, lying, and activity behaviors of multiparous grazing dairy cows.

CHAPTER 2. REVIEW OF LITERATURE

2.1. Introduction

A large and growing body of literature has recognized cow behavior as an important tool to identify and monitor disease in transition dairy cows (Jawor et al., 2012; Sepúlveda-Varas et al., 2014; Hendriks et al., 2020a; Tsai et al., 2021). Behavior is also regarded as an indicator of animal welfare (e.g., Webster et al., 2008; Vasseur et al., 2012), with its importance highlighted in the incorporation of a gold standard in recent legislature for the welfare of dairy cows in New Zealand (New Zealand Ministry for Primary Industries, 2019). It is important, then, to understand what comprises "typical" behavior in dairy cows when relating behavioral parameters to both cow health and welfare.

Behavior is influenced by a multitude of variables. While experimental design may account for relevant sources of variation in dairy cow behavior among different studies, variation may still exist due to uncontrollable factors (e.g., weather and photoperiod), social factors (e.g., competition and remixing), or due to differences in the subjects selected (e.g., breed, milk yield, body condition score [BCS], parity, and disease). It is well known that cow-level and environmental factors can influence cow phenotypic variance and these factors are typically taken into consideration when production-related outcomes (e.g., milk yield and BCS) are the biological measures of interest. Substantial within- and between-cow variation in behavior exists and, therefore, it is important to consider and understand possible sources of variation when behavior measures are the outcomes of interest (Ito et al., 2009).

This literature review will describe typical changes in feeding, lying, and activity behaviors during the transition period and how system- and cow-level factors can influence these behaviors. In particular, behaviors in cows managed in grazing systems will be compared with those reported in housed systems. I will also briefly characterize the most common transition cow metabolic disorder, hypocalcemia, and how this condition and associated mitigation strategies may influence animal behaviors.

2.2. Typical behavior in dairy cattle

The importance of feeding and lying behaviors. It is widely accepted that lying is an important behavior in dairy cows. Dairy cows are highly motivated to lie down, placing priority on lying time before eating (Metz, 1985; Munksgaard et al., 2005) and socialization (Munksgaard et al., 2005); they also attempt to recover lying time after periods of lying deprivation (Metz, 1985; Munksgaard and Simonsen, 1996; Cooper et al., 2007). Furthermore, behavioral and physiological observations indicate cows become stressed and frustrated when deprived of lying time (Munksgaard and Simonsen, 1996; Fisher et al., 2002; Cooper et al., 2007). Lying time has recently been included in the New Zealand Dairy Cattle Code of Welfare (New Zealand Ministry for Primary Industries, 2019). This code specifies that ‘dairy cattle must be able to lie and rest comfortably for sufficient periods to meet their behavioral needs’ and suggests the “gold standard” for lying time in dairy cattle given comfortable lying surfaces and kept out of adverse weather (i.e., housed systems) is between 10 and 12 h/d (New Zealand Ministry for Primary Industries, 2019).

Feeding is also an important behavior due to its immediate connection with survival as well as with parameters of milk production and body condition. The effort required to prehend and ingest feed is greater in grazing than in housed systems, which may affect the time available for lying and socialization. Most of a cow’s day is allocated into time spent lying, feeding, and ruminating, with time also spent idle, walking, grooming, and socializing. The time an individual spends engaged in each of these behaviors each day is defined as the cow’s time budget, with feeding and lying behaviors comprising a significant proportion of a dairy cow’s time budget.

Many studies have assessed typical feeding, lying and activity behaviors and the factors affecting these behaviors in housed dairy cows; however, studies in grazing dairy cows are sparse. While studies in housed cows are important to improve our understanding of behavior in dairy cows, housing systems have fundamental differences from grazing systems, and it is, therefore, important that we quantify typical eating, lying, and activity behaviors for grazing dairy cows and consider system-level differences.

Daily feeding, lying, and activity behaviors in dairy cows. Behaviors such as feeding time, the number and duration of meals (bouts of feeding/grazing), the rate of feed intake, bite mass, and bite rate all contribute to the overall dry matter intake (DMI) and energy. Feed intake is described by Gibb (1998) as the product of the number of minutes spent feeding and the rate of intake per minute of feeding. Therefore, DMI is positively correlated with both feeding time and the rate of feed intake; however, feeding time and the rate of intake are inversely correlated. Breaking these components down further, the total time spent feeding is a product of the number and duration of meals, and the rate of feed intake is the product of bite rate and bite mass. Cows, like most ruminants, exhibit a distinct pattern of feeding behavior. Cows have multiple meals per day separated by inter-meal intervals: periods of ruminating or idling lasting > 5 min (Rook and Huckle, 1997; Gibb, 1998). An individual meal consists of consecutive bouts of eating (biting, masticating, and swallowing) punctuated by short periods (< 5 min) of jaw inactivity (i.e., "intra-meal intervals") when the cow is either searching for food or idling (Gibb, 1998).

A summary of studies with grazing or feeding times in dairy cows reported is presented in Table 2.1. Grazing dairy cows have between 3 and 5 meals per day (Gibb et al., 1998) with total grazing time ranging between 5.2 and 11.8 h/d (Table 2.1), similar to the ranges previously reported in other reviews in grazing dairy and beef cattle (range: 6.0 and 13.0 h/d; Krysl and Hess, 1993; Kilgour, 2012). Grazing times are generally slightly shorter in cows that are supplemented with concentrate (ranging from 6.5 to 8.8 h/d; Table 2.1), and, similarly, feeding times are shorter in housed cows fed freshly-harvested grass (6.6 h/d; Table 2.1). In comparison, the feeding times of housed cows that are fed either total-mixed rations (TMR) or partial-mixed rations (PMR) are much lower than those of grazing cows, ranging between 1.0 and 5.2 h per day (Table 2.1). In cows fed TMR, the number of meals ranged between 7 to 12 per day (Dado and Allen, 1994; Huzzey et al., 2005; Crossley et al., 2017; Neave et al., 2017), much greater than the number reported for grazing dairy cows. Taken together, these studies suggest that grazing dairy cows feed more intensely and for longer than housed dairy cows, which is possibly related to the greater amount of work undertaken by grazing cows in searching for, prehending, and chewing food.

The activity of grazing dairy cows is also much greater than housed cows, as they need to walk long distances within and between paddocks in search of food, as well as to and from the farm dairy during lactation. These extra activities (Neave et al., 2021), coupled with the longer feeding times, may place restrictions on the time budget of the grazing dairy cow, leading to relatively short lying times both pre- (~10.3 h/d; Table 2.2) and postcalving (between 7.5 to 8.6 h/d; Table 2.2). Although the lying times of housed cows vary considerably both pre- (range: 7.0 to 15.7 h/d) and postcalving (range: 6.0 to 14.0 h/d; Table 2.2), the majority of daily lying times reported are longer than those reported for grazing dairy cows. Whether reduced time available to lie compromises the welfare of grazing cows, however, has not yet been thoroughly investigated.

Diurnal patterns of feeding, lying, and activity. It has been well noted that ruminants have a distinct pattern of behavior. Both housed (DeVries et al., 2003; Proudfoot et al., 2010; Schirmann et al., 2012) and grazing cows (Hafez, 1962; Rook et al., 1994; Linnane et al., 2001; Sheahan et al., 2011) feed most intensely during the daytime, and inversely are less active and lie more at night (Proudfoot et al., 2010; Schirmann et al., 2012). The within-day patterns of grazing and ruminating are presented in Figure 2.1 (Sheahan et al., 2011) and within-day patterns for lying time are presented in Figure 2.2 (Hendriks et al., 2019a). A review by Krysl and Hess (1993) determined that grazing between 0600 h to 1700 h makes up 60 to 100% of the total daily grazing time. Several grazing events occur throughout the day, the biggest of which is just after sunrise and just before sunset. These peak grazing events correspond with periods of reduced lying times (Schirmann et al., 2012; O'Driscoll et al., 2019), such that only about 20% of the total time spent lying is achieved during the daytime (based on results from Hendriks et al. (2019a)).

The morning grazing event is relatively shorter than that at dusk, possibly due to the interaction between particle size, pasture nutrient composition, rumen fill, and the feeling of satiation (Linnane et al., 2001). The dawn grazing event coincides with the allocation of fresh pasture (Gregorini et al., 2006a), or returning to the paddock after the morning milking (Rook et al., 1994; Gibb et al., 1998). This is also associated with short lying times and an increase in the number of steps due to grazing or walking before and after milking (Hendriks et al., 2019a). Prior to the morning allocation of pasture, plasma ghrelin concentrations are at their greatest (Sheahan et al., 2013). This combined with low rumen fill and other factors likely drive the cow to initiate feeding-related behavior (Sheahan et al. (2013)) and to quickly ingest large amounts of feed as it becomes available. Furthermore, pasture nutrient composition changes throughout the day, accumulating photosynthates and losing water through evapotranspiration (Orr et al., 1997). This means that the pasture consumed during the morning grazing event will generally have a higher water content, which would also influence rumen fill and the feeling of satiety. Smaller grazing bouts occur during the day (Gibb et al., 1998), along with a small peak in lying time during the middle of the day (Figure 2.2; Schirmann et al., 2012; Hendriks et al., 2019a; O'Driscoll et al., 2019) when the cow is likely ruminating (Figure 2.1; Sheahan et al., 2011; Schirmann et al., 2012).

The longest and most intense grazing bout occurs at dusk (Rook et al., 1994; Gibb et al., 1998; Linnane et al., 2001) when cows fulfill a large proportion of their dietary requirements before nighttime. This is also a period of shorter lying times (Figure 2.2; Hendriks et al., 2019a). Several studies have suggested that the intensity of the dusk grazing event may be related to diurnal changes in the external environment, such as changes in light intensity (Linnane et al., 2001) or changes in pasture nutrient composition (Linnane et al., 2001; Gregorini et al., 2006a; Gregorini et al., 2009). Sunset usually acts as the signal to stop grazing (Sheahan et al., 2011) and while short bouts of grazing can occur at night (Gibb et al., 1998; Gregorini et al., 2006b), Krysl and Hess (1993) suggest that these grazing events contribute minimally to feed intake.

Nighttime grazing may be unfavorable or disadvantageous to the cow: the ability to distinguish between pasture swards may be reduced, and, from an evolutionary point of view, the risk of predation is greater (Rook et al., 1994; Gregorini et al., 2006a). Furthermore, as pasture is progressively grazed, the surface height of the sward is reduced and the preferred parts of the pasture are likely eaten, meaning the food available to graze at nighttime is likely of lower quantity and quality. Similar behaviors have been reported (DeVries et al., 2003) in housed cows, who spent little time at the feed bunk during the late evening and early morning. The reason for these similarities between systems is unclear but, as with grazing cows, could include an evolutionary adaptation to avoid predation, poor visibility to sort feed, or lower feed quality or quantity at this time. Instead of grazing at nighttime, cows may prefer to ruminate and rest, as demonstrated by the increase in lying times (Figure 2.2; Schirmann et al., 2012; Hendriks et al., 2019a; O'Driscoll et al., 2019), and a greater percentage of cows ruminating (Figure 2.1; Sheahan et al., 2011; Sheahan et al., 2013) or idling (Sheahan et al., 2013) after sunset.

While dairy cows typically express a distinct diurnal pattern of eating, lying, and activity behaviors, it is not inflexible, and cows may adapt these behaviors to better suit changes in the external environment. An example is the adaptation of feeding behavior to changes in animal husbandry (Gregorini et al., 2006b). Periods of short-term food deprivation occur in both housed and grazing systems, either when cows have eaten their allocated feed or are physically removed from the feeding environment (e.g., during milking where in-shed feeding isn't available). Cows are highly motivated to feed following short-term feed deprivation, with greater durations of deprivation resulting in a greater drive to eat (Schütz et al., 2006). In housed cows, both feeding time (DeVries et al., 2003; Schirmann et al., 2012) and DMI (Huzzey et al., 2007; Schirmann et al., 2012) peak just after the feed is delivered, with smaller increases in feeding time occurring just after the feed is pushed up (Schirmann et al., 2012). Similarly, the grazing time of beef heifers is the longest after the allocation of fresh pasture, regardless of it being allocated in the morning or afternoon (Gregorini et al., 2006a). Peaks in feeding time can also be observed after the milking event in both grazing (Rook et al., 1994; Gibb et al., 1998) and housed (Dado and Allen, 1994; DeVries et al., 2003) dairy cows. Similarly, the percentage of cows grazing is at its greatest after the morning and afternoon milking events (Sheahan et al., 2011; Sheahan et al., 2013). Fresh feed allocation and returning to the feeding environment after milking can occur simultaneously in most dairy systems. However, the stimulatory effects of these two events on feeding behavior individually, the extent to which cows will deviate from their natural rhythms of feeding behavior under changed management, and the flow-on effects of changing animal husbandry on lying and social behaviors, are unclear.

Table 2.1. Summary of studies reporting feeding time in dairy cows. The time spent feeding/grazing in dairy cows of different feeding and housing systems, and different stages of pregnancy or lactation (PRE = prepartum; POST = postpartum; DIM = days in milk), as well as the method used to record feeding behavior.

System/Study	Study Description		Method of recording behavior	Feeding/grazing time (h/d)
	N cows and status	Stage		
Housed fed total mixed ration				
Dado and Allen, 1994	2 6 primiparous 6 multiparous	Lactating (approx. 63 to 84 DIM)	Water filled tube connected to a pressure transducer	Primiparous 4.7 Multiparous 5.2
Huzzey et al., 2005	15 5 primiparous 10 multiparous	10 d PRE to 10 d POST	Electronic transponders at feedbunk (GrowSafe System)	PRE (-10 to -2 d): 1.4 POST (2 to 10 d): 1.0
Huzzey et al., 2007	62 12 with severe metritis[1] 27 with mild metritis[1] 23 healthy	-13 d PRE to 21 d POST relative to calving (d 0)	Electronic transponder (Insentec electronic feeding system)	PRE (wk -2 and -1): Healthy 3.2 – 3.6 Mild metritis 2.8 – 3.4 Severe metritis 2.4 – 3.1 POST (wk 1 to 3): Healthy 2.6 – 3.1 Mild metritis 2.2 – 2.9 Severe metritis 1.5 – 2.7
Hill et al., 2009	136 44 primiparous 92 multiparous	146 DIM at enrollment, observed for 28 d	Video recordings	4.8 – 5.0

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Table 2.1 (continued). Summary of studies reporting feeding time in dairy cows.

System/Study	Study Description		Method of recording behavior	Feeding/grazing time (h/d)
	N cows and status	Stage		
Housed fed total mixed ration				
Proudfoot et al., 2009b	36 16 primiparous 20 multiparous	9 d PRE to 18 d POST	Insentec system	PRE (wk -1): Primiparous 3.3 – 3.6 Multiparous 2.6 – 3.0 POST (wk 1 and 2): Primiparous 2.3 – 3.1 Multiparous 2.1 – 2.9
Proudfoot et al., 2010	26 (multiparous) 13 with claw horn lesions 13 without lesions	14 d PRE to 21 d POST	Video recordings	PRE (wk -2): Without lesions 3.8 With lesions 3.6 POST (wk 1 – 2): Without lesions 2.6 – 3.5 With lesions 2.9 – 3.5
Schirmann et al., 2011	48 (multiparous)	Pregnant non-lactating (enrolled 40 d PRE)	Insentec system	4.0 – 4.5
Schirmann et al., 2013	11 (multiparous)	96 h PRE to 22 h POST	Insentec system	2.1 – 3.4
Lobeck-Luchterhand et al., 2015	756 (primiparous and multiparous)	28 d PRE to the day of calving	Video recordings	Primiparous: 4.1 – 4.3 Multiparous 4.9 – 5.0

Table continued over the page.

Table 2.1 (continued). Summary of studies reporting feeding time in dairy cows.

System/Study	Study Description		Method of recording behavior	Feeding/grazing time (h/d)
	N cows and status	Stage		
Housed fed total mixed ration				
Crossley et al., 2017	18 5 primiparous 13 multiparous	Lactating (77 DIM at enrollment)	Insentec system	3.1 – 3.4
Neave et al., 2017	100 38 primiparous 62 multiparous	d 14 PRE to 21 POST	Insentec system	PRE (wk -2 and -1): Primiparous 3.6 – 3.9 Multiparous 3.6 – 3.9 POST (wk 1 to 3): Primiparous 2.4 – 3.2 Multiparous 3.0 – 3.8
Housed fed partial mixed ration				
Munksgaard et al., 2020	255 99 primiparous 156 multiparous 123 Jersey 132 Holstein	Between 1 and 240 DIM	RIC system (activity and LT AfiTagII)	Primiparous: Holstein 2.9 Jersey 2.6 Multiparous: Holstein 3.4 Jersey 2.8
Grazing pasture with or without added concentrates				
Linane et al., 2001	12 (2- and 3-year-old heifers) No concentrates	Non-pregnant (between July and December)	Kienzle vibracorders	9.5 – 11.8

Table continued over the page.

Table 2.1 (continued). Summary of studies reporting feeding time in dairy cows.

System/Study	Study Description		Method of recording behavior	Feeding/grazing time (h/d)
	N cows and status	Stage		
Grazing pasture with or without added concentrates				
Hancock, 1954b	20 (primiparous)	90 – 273 DIM	Visual observation	8.4
Hancock, 1954a	20 (> 2-years-old)	During lactation	Visual observation	No area restriction 7.0 – 8.8 Restricted grazing area[1] 5.3 – 5.6 Restricted time at pasture 5.2 – 6.5 Unrestricted time at pasture 5.7 – 8.2 Pasture maturity[2] 4 wks 6.3 – 6.9 6 wks 6.0 – 6.7 8 wks 7.7 – 8.4
Hancock and McMeekan, 1954	14 10 4-year-olds 4 2-year-olds	73 – 89 DIM at experiment start	Visual observation	Rotational grazing 8.1 Continuous grazing 9.1

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[1] Grazing area restricted by allocating a smaller area per break and fewer breaks per day.

[2] Pastures of different maturity achieved by not grazing pastures for 4, 6 and 8 weeks before the start of the experiment.

Table continued over the page.

Table 2.1 (continued). Summary of studies reporting feeding time in dairy cows.

System/Study	Study Description		Method of recording behavior	Feeding/grazing time (h/d)
	N cows and status	Stage		
Grazing pasture with or without added concentrates				
Phillips and Rind, 2001	32 16 primiparous 16 multiparous + 2 kg DM / cow / d concentrates	Lactating (primiparous average 191 DIM, multiparous average 205 DIM)	Observer	Primiparous 8.4 Multiparous 8.1 Primiparous and multiparous 7.8
Phillips and Rind, 2001	32 16 primiparous 16 multiparous + 2 kg DM / cow / d concentrates	Lactating (primiparous average 191 DIM, multiparous average 205 DIM)	Observer	Primiparous 8.4 Multiparous 8.1 Primiparous and multiparous 7.8
Sheahan et al., 2011	113 (primiparous and multiparous) + 0, 3 or 6 kg DM/cow/d concentrates	Peak (DIM = 80), mid (DIM = 170) and late (DIM = 250) lactation	Observer (?)	Peak No concentrates 7.9 Concentrates 6.5 – 7.2 Mid No concentrates 7.9 Concentrates 6.7 – 7.3 Late No concentrates 8.5 Concentrates 7.1 – 8.0
Dohme-Meier et al., 2014	14 (multiparous) + 5.1 kg DM/cow/d concentrates	Between 38-52 DIM, 94-108 DIM, and 171-185 DIM	Jaw movement recorders (IGER Behaviour Recorder)	Grazing: 8.8 Zero-grazing: 6.6

[1] Severe metritis classified as at least one vaginal discharge score of 4 plus one recording of fever (≥39.5°C) within 21 DIM. Mild metritis was classified as a vaginal discharge score of 2 or 3 within 21 DIM (Huzzey et al., 2007).

Table 2.2. Summary of studies reporting lying time or the number of lying bouts during the transition period in dairy cows. The time spent lying (h/d), number of lying bouts (bouts/d) and duration of lying bouts (min/bout) in dairy cows of different feeding and housing systems, and different stages of pregnancy or lactation (PRE = prepartum; POST = postpartum; DIM = days in milk), as well as the method used to record lying behavior.

Authors	Study Description		Recording device	Lying time	Number of lying bouts (bouts/d)	Duration of lying bouts (min/bout)
	N Cows	Stage				
Housed fed total mixed ration						
Huzzey et al., 2005	15 5 primiparous 10 multiparous	10 d PRE to 10 d POST	Gemini Dataloggers	(Standing time) PRE: 12.3 POST: 13.4	(Standing bouts) PRE: 11.7 POST: 13.1	Not reported
Proudfoot et al., 2009 b	36 16 primiparous 20 multiparous	9 d PRE to 18 d POST	Gemini Dataloggers	PRE (wk -1): Primiparous 10.0 Multiparous 10.0 – 11.7 POST (wk 1 and 2): Primiparous 10.4 – 12.9 Multiparous 10.8 – 13.8	Not reported	Not reported
Proudfoot et al., 2010	26 (multiparous)	14 d PRE to 21 d POST	Gemini Dataloggers	(Standing time) PRE (wk -2): 11.9 – 13.9 POST (wk 1 to 2): 13.3 – 14.2	(Standing bouts) PRE: 9 – 10 POST: 9	Not reported
Calderon and Cook, 2011	57 15 nonlame 42 lame[2]	16 d PRE to 16 d POST	IceTag accelerometers	PRE (-16 to -3): Nonlame 10.0 - 13.0 Lame 11.5 – 14.5 POST (3 to 16): Nonlame 9.0 to 12.0 Lame 9.0 to 13.0	PRE: Nonlame 11 – 16 Lame 8.5 – 16 POST: Nonlame 9 – 14 Lame 8.5 – 14	PRE: Nonlame 40 – 65 Lame 46 – 90 POST: Nonlame 45 – 61 Lame 48 – 68

[2] Lameness classified as either hemorrhage in the sole or white line, claw lesion, or infectious lesion (Calderon and Cook, 2011).

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Table 2.2 (continued). Summary of studies reporting lying time or the number of lying bouts during the transition period in dairy cows.

Authors	Study Description		Recording device	Lying time	Number of lying bouts (bouts/d)	Duration of lying bouts (min/bout)
	N Cows	Stage				
Housed fed total mixed ration						
Steensels et al., 2012	246 120 Parity 2 126 Parity ≥ 3	1 to 28 d POST	Pedometers (Pedometer Plus)	Parity 2: 8.2 – 8.9 Parity ≥ 3: 8.7 – 9.7	Parity 2: 10.8 – 11.2 Parity ≥3: 11.0 – 11.4	Not reported
Brzozowska et al., 2014	193 93 primiparous 59 Parity 2 41 Parity ≥ 3	Lactating (between 1 and 305 DIM)	IceQube accelerometers	Primiparous: 10.7 Parity 2: 10.7 Parity ≥ 3: 10.5	Primiparous: 13.0 Parity 2: 11.6 Parity ≥ 3: 11.2	Primiparous: 51 Parity 2: 56 Parity ≥ 3: 57
Lobeck-Luchterhand et al., 2015	756 (primiparous and multiparous)	Between 35 to 0 d PRE	Video recordings	Primiparous 12.9 Multiparous 13.2	Primiparous 16.5 Multiparous 13.7	Primiparous 54 Multiparous 78
Neave et al., 2017	100 38 primiparous 62 multiparous	d 14 PRE to 21 POST	HOBO Pendant	PRE (wk -2 and -1): Primiparous 10.4 – 11.6 Multiparous 11.6 – 11.9 POST (wk 1 to 3): Primiparous 9.1 – 10.5 Multiparous 9.8 – 10.8	PRE: Primiparous 12.4 – 13.7 Multiparous 10.6 – 10.9 POST: Primiparous 13.6 – 16.8 Multiparous 9.0 – 10.0	PRE: Primiparous 50 – 58 Multiparous 72 – 74 POST: Primiparous 42 to 45 Multiparous 72 to 76

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Table 2.2 (continued). Summary of studies reporting lying time or the number of lying bouts during the transition period in dairy cows.

Authors	Study Description		Recording device	Lying time	Number of lying bouts (bouts/d)	Duration of lying bouts (min/bout)
	N Cows	Stage				
Housed fed total mixed ration						
Piñiero et al., 2019	1052 401 primiparous 651 multiparous	14 d PRE to 14 d POST	IceQube accelerometers	PRE (-14 to -3 d) Primiparous 10.4 – 11.7 Multiparous 12.5 – 13.0 POST (3 to 14 d) Primiparous 8.8 – 9.8 Multiparous 10.8 – 12.1	Not reported	Not reported
Barraclough et al., 2020b	72 21 primiparous 51 multiparous	14 d PRE to 21 d POST	IceQube accelerometers	PRE (-14 to -1 d): Primiparous 10.0 – 14.0 Multiparous 10.0 – 15.7 POST (1 to 21 d): Primiparous 6.0 – 10.0 Multiparous 8.6 – 10.7	PRE: Primiparous 11.5 – 16.5 Multiparous 9.5 – 12.0 POST: Primiparous 8.5 – 15.0 Multiparous 9.0 – 11.0	Not reported
Housed fed total mixed ration with access to pasture						
Borchers et al., 2017	53 cows 20 primiparous 33 multiparous + access to pasture	14 d to 1 PRE	IceQube accelerometer	Primiparous 7.0 – 11.3 Multiparous 10.2 – 12.9	9.1 to 13.6	Not reported

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Table 2.2 (continued). Summary of studies reporting lying time or the number of lying bouts during the transition period in dairy cows.

Authors	Study Description		Recording device	Lying time	Number of lying bouts (bouts/d)	Duration of lying bouts (min/bout)
	N Cows	Stage				
Housed fed partial mixed ration						
Munksgaard et al., 2020	255 99 primiparous 156 multiparous 123 Jersey 132 Holstein	Between 1 and 240 DIM	AfiTagII activity pedometers	Primiparous: Holstein 11.8 Jersey 10.8 Multiparous: Holstein 11.9 Jersey 10.6	Not reported	Not reported
Grazing pasture						
Sepulveda-Varas et al., 2014	274 47 primiparous 227 multiparous	3 to 21 d POST	Hobo Pendant	Primiparous 7.5 Multiparous 8.5	Primiparous 9.7 Multiparous 8.4	Primiparous 51 Multiparous 63
Hendriks et al., 2019	310 (multiparous)	21 d PRE to 21 d POST	IceTag and IceQube accelerometers	PRE 10.3 h/d POST 8.6 h/d	PRE 8.2 POST 7.7	PRE 77 POST 69
Grazing pasture and supplemented with total mixed ration						
Rice et al., 2017	16	6 d PRE	IceTag accelerometers	10.3	10.0	97

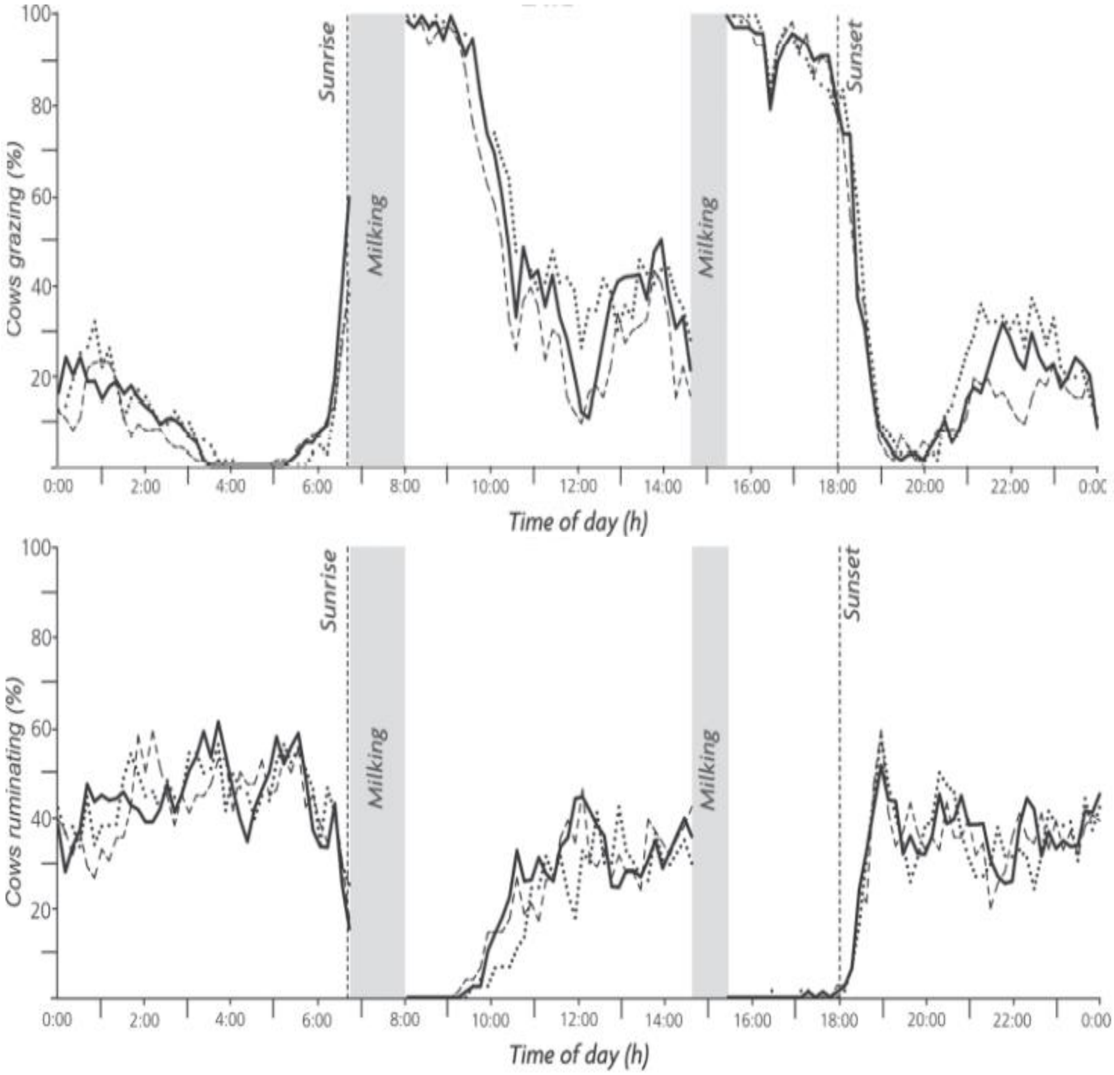

Figure 2.1. Within-day profile of grazing and rumination behavior in grazing dairy cows. The percentage of cows grazing (top) and ruminating (bottom) across the day during late lactation. Dotted lines represent the amount of concentrate offered (0 kg DM/d = dotted line, 3 kg DM/d = solid line, and 6 kg DM/d = dashed line). From Sheahan et al. (2011).

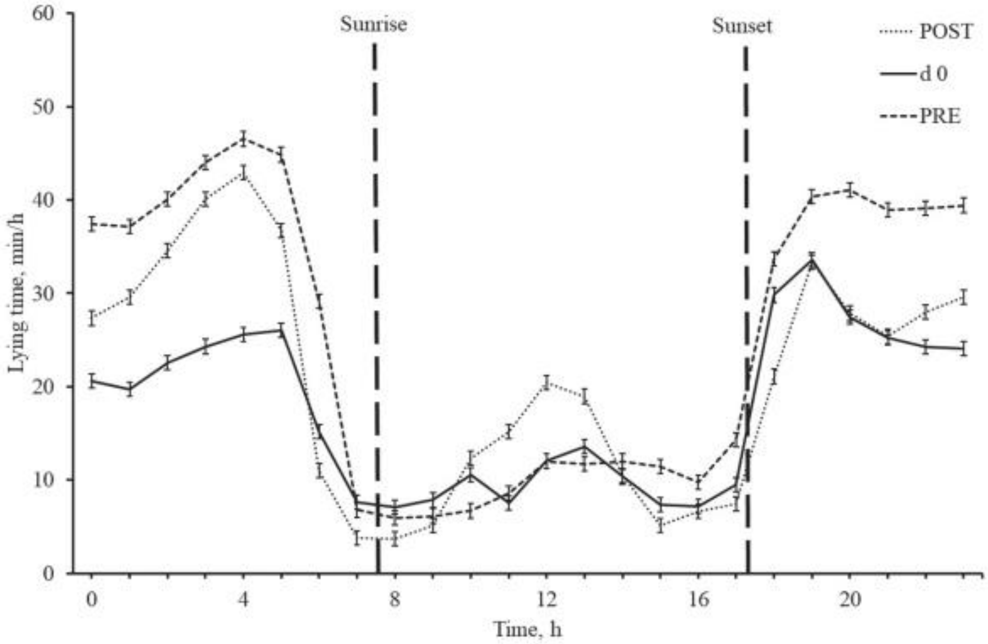

Figure 2.2. The within-day profile of lying time in grazing dairy cows. Hourly lying times of grazing dairy cows during the 3 to 21 d before calving (PRE; d -21 to -3), the day of calving (d 0), and the 3 to 21 d postcalving (POST). Figure from Hendriks et al. (2019a).

2.2.1. Before calving

Feeding behavior. To my knowledge, no studies have been undertaken in grazing dairy cows to assess changes in grazing behavior during the transition period. This is likely due to the limited availability of technology available to record grazing behavior and the otherwise labor-intensive and expensive methods to collect grazing behavior data (Dela Rue et al., 2020). Several studies indicate that housed cows fed TMR eat, on average, between 11.2 and 17.0 kg of dry matter (DM)/d in the precalving period (e.g., Huzzey et al., 2007; Proudfoot et al., 2010; Neave et al., 2017). However, the levels of DMI in these cows decrease in the 2 to 3 weeks before calving (Bertics et al., 1992; Hayirli et al., 2002; Huzzey et al., 2007; Neave et al., 2017). In comparison, estimations of prepartum DMI in grazing cows (based on measures of pre- and post-grazing pasture mass) are lower than that of housed cows fed TMR, ranging from 6.6 to 10.7 kg DM per d in grazing dairy cows depending on pasture allowance and body condition score (Roche et al., 2015). Similarly, the prepartum DMI of housed cows fed fresh-cut pasture was reportedly between 8.8 – 10.1 kg DM/d (Roche, 2006). The lower DMI reported in these studies may be attributed to the smaller size of the cows and the relative difficulty in prehension and ingestion of feed compared to studies in TMR-fed housed cows.

Feeding time in housed cows fed TMR is highly variable in the 2 weeks before calving, ranging between 1.4 and 3.9 h/d (Table 2.1). A decrease in feeding time has also been observed in several studies, beginning 2 to 3 weeks before calving (Huzzey et al., 2005; Huzzey et al., 2007; Neave et al., 2017; Piersma, 2017), and becoming more pronounced in the final few days of gestation (Huzzey et al., 2005; Huzzey et al., 2007; Lobeck-Luchterhand et al., 2015; Neave et al., 2017; Piersma, 2017). The decrease in feeding time likely drives the decline in DMI, as the rate of feed intake during the prepartum period remains relatively constant (between 76 and 77 g of DM/min; Neave et al., 2017). The number of meals also remains constant (between 7.2 and 9.8 meals/d; Huzzey et al., 2005; Neave et al., 2017), indicating that the lower feeding times are due to shorter meal durations. Lower DMI and feeding times along with shorter meal durations may be associated with an increase in discomfort and restlessness as calving approaches; however, the growing fetus and reduction in abdominal space (National Research Council, 2001) and postpartum illnesses (e.g., metritis; Huzzey et al., 2007) have also been hypothesized as reasons for the prepartum reduction in DMI. In contrast, Roche (2006) did not observe a decline in DMI during the 14 to 2 d precalving in housed cows fed fresh-cut pasture and suggested that declines in prepartum DMI observed in other studies may also be at least somewhat attributed to the addition of concentrates in the precalving diet.

Lying and activity behavior. Precalving lying times in housed dairy cows range from 7.0 to 15.7 h/d (Table 2.2) but decrease as calving approaches (Calderon and Cook, 2011; Lobeck-Luchterhand et al., 2015). Shorter daily lying times (approximately 10.3 h/d) have been observed in grazing dairy cows and in cows kept on pasture and supplemented with TMR (Table 2.2) which reflects the different time budgets of cows in housed compared with grazing systems. Although the feeding times of precalving dairy cows at pasture are currently unknown, grazing dairy cows generally spend longer amounts of time eating during lactation compared with housed cows fed TMR (Table 2.1). More time spent actively eating (e.g., accessing feed and prehension) in grazing dairy cows may place demands on their time budgets that housed cows fed TMR do not experience to the same degree. Motivation to perform other behaviors, such as socialization and grooming, may also place restrictions on the time budgets of dairy cows during the prepartum period. To my knowledge, this has not been fully investigated in grazing dairy cows during the dry period.

A lying bout is defined as the number of transitions from standing to lying and is analogous to a standing bout. The number of precalving lying bouts ranges from 8.6 to 16.5 bouts/d in housed cows (Table 2.2), and is similar in cows kept on pasture and supplemented with TMR (10.0 bouts/d; Rice et al., 2017). However, the number of lying bouts precalving is reportedly lower in grazing dairy cows (8.2 bouts/d; Hendriks et al., 2019a). The duration of lying bouts, defined as the period between two consecutive standing events, is similar between studies in housed and grazing cows, ranging from 40 to 90 min/bout (Table 2.2). Hence, it appears that the shorter daily lying times precalving in grazing cows can be mostly explained by fewer lying bouts, rather than shorter durations of lying per bout, compared with housed cows.

Few studies have investigated step counts in conventionally housed cows, where access to paddocks or exercise areas is limited. Although some authors report trends in activity (Huzzey et al., 2005; Stangaferro et al., 2016), quantitative information on activity measures is limited. Barraclough et al. (2020b) reported that the precalving step count of housed cows was not only lower (843 steps/d at d –14) than previous studies, but also tended to decrease as calving approached. Of the few studies with activity data for cows with access to pasture, precalving step count was similar in housed cows with access to pasture (Borchers et al., 2017), cows kept on pasture and fed TMR (Rice et al., 2017) and grazing cows (Hendriks et al., 2019a), ranging from 2122 to 3369 steps/d. Borchers et al. (2017) also determined that the number of steps taken was consistent across the last 14 d of gestation. The lack of reported step counts throughout literature may be due in part to the activity monitor being used in the study. For example, neck-worn collars (e.g., HR Tags, SCR Dairy Netanya, Israel) and some leg-mounted monitors (e.g., HOBO, Onset Computer Corp., Pocasset, MA), collect activity data based on the acceleration recorded across the 3 axes of the accelerometer and, therefore, activity is presented in arbitrary units, whereas some leg worn monitors such as IceQubes and IceTags record step counts in 15 min and 1 h intervals, respectively (Hendriks et al., 2020b).

2.2.2. Immediately before, during, and after calving

Feeding behavior. The most dramatic decline in feeding time occurs on the day of calving. Feeding times in housed cows on the day of calving are between 32 and 50% shorter than during the 2 to 4 d before calving, reaching a nadir of between ~1.8 and ~2.7 h/d (Proudfoot et al., 2009a; Schirmann et al., 2013; Lobeck-Luchterhand et al., 2015). Similarly, Neave et al. (2017) reported that feeding time was between 28 and 37% shorter in housed cows during the immediate periparturient period (d -1 to 1) than during the week before calving (d -7 to -1). Dry matter intake also continues to decrease in both housed cows fed TMR (Proudfoot et al., 2009a; Schirmann et al., 2013) and fresh-cut pasture (Roche, 2006) and is between 24 and 43% lower in housed cows in the 24 h before calving compared with the last 2 to 4 d of gestation (Proudfoot et al., 2009a; Schirmann et al., 2013). The most dramatic declines in feeding time (Miedema et al., 2011; Jensen, 2012; Schirmann et al., 2013) and DMI (Schirmann et al., 2013) are detected in the 6 to 8 h before calving, which is possibly related to an increase in restlessness, discomfort, and pain immediately before calving. To my knowledge, however, there are no published studies on the changes in feeding behaviors that occur immediately surrounding the calving event in dairy cows grazing pasture.

The cow prioritizes cleaning and nursing the calf in the first 2 hours after calving, which occurs at the expense of other behaviors such as feeding and lying (Jensen, 2012; Piersma, 2017). These calf-directed behaviors decrease in the subsequent several hours and cows resume feeding (Jensen, 2012; Schirmann et al., 2013). The daily feeding times remained low for the 2 days after calving in the studies by Schirmann et al. (2013) and Proudfoot et al. (2009a); however, the feeding time of cows in both studies could have been influenced by changes in diet composition between the pre- and postcalving periods. Changes in behavior surrounding the calving event for lying and activity behaviors are similar in housed and grazing dairy cows (see the section below) and, therefore, it is plausible that similar reductions in feeding time due to reduced grazing activity would occur in grazing dairy cows.

Lying and activity behavior. There are marked changes in lying and activity behaviors around the time of parturition. Numerous studies in housed cows (Calderon and Cook, 2011; Miedema et al., 2011; Jensen, 2012; Piñeiro et al., 2019), grazing cows (Hendriks et al., 2019a) and cows kept on pasture and supplemented with TMR (Rice et al., 2017) indicate lying time decreases by 1.0 to 3.0 h/d in the 24 h before calving. In most of these studies, authors also noted an increase in the number of lying bouts on the day of calving; however, the duration of lying bouts decreased (Calderon and Cook, 2011; Neave et al., 2017; Rice et al., 2017; Hendriks et al., 2019a). Furthermore, the activity of fully-housed cows (Jensen, 2012; Barraclough et al., 2020a), housed cows with access to pasture (Borchers et al., 2017), and grazing cows (Hendriks et al., 2019a) typically increases on the day of calving, particularly in the 2 h immediately before parturition (Owens et al., 1985). In contrast, Rice et al. (2017) did not detect any significant differences in step count on the day of calving relative to the 6 d prior; however, this study was conducted with small groups of 2 to 18 cows kept on pasture and fed TMR. These conditions may not accurately represent those experienced by dairy cows in typical grazing systems throughout Australia, New Zealand, and Ireland that are kept in small to large herds (ranging: 50 to 1000+ cows; LIC, and DairyNZ, 2020; Dairy Australia, 2021). Hence, changes in lying and activity behaviors around the time of parturition appear consistent across housed and grazing systems in most studies, and likely indicate agitation and discomfort associated with the calving process.

2.2.3. During lactation

Feeding behavior. In TMR-fed housed cows, dry matter intake slowly increases from approximately 8 – 15 kg DM during the week of calving to between 20 – 25 kg DM during the 2 to 3 weeks postcalving (e.g., Kertz et al., 1991; Bertics et al., 1992; Neave et al., 2017; Munksgaard et al., 2020). This increase in DMI is required for the cow to meet the greater energy requirements associated with the onset of milk production. Intakes continue to increase into lactation, reaching peak DMI between 3 and 4 months postcalving, about 1 to 2 months after milk yield peaks (Kertz et al., 1991; Munksgaard et al., 2020). However, several studies indicate that the pattern of feeding behavior during the first few weeks postcalving is more complex. Surprisingly, feeding times in the 3 weeks postcalving (range: ~1.0 to 3.8 h/d) are not greater than those measured during the 10 to 14 d precalving (Huzzey et al., 2005; Huzzey et al., 2007; Neave et al., 2017). This indicates that a greater feeding rate immediately after calving is needed to support a greater DMI (Huzzey et al., 2005; Neave et al., 2017). It is also possible that the feeding rate in these studies was influenced by changes in diet composition at the time of calving (Huzzey et al., 2005), with the postcalving diets having greater amounts of energy than the precalving diets. Furthermore, although feeding times are initially lower in the immediate postcalving period compared with precalving, they gradually increase during early lactation (Huzzey et al., 2005; Huzzey et al., 2007; Jensen, 2011; Neave et al., 2017; Munksgaard et al., 2020), which contributes to the increase in DMI. Nevertheless, Munksgaard et al., 2020) reported that the feeding time of multiparous cows plateau by about 30 days of lactation, indicating that the rate of feed intake must again increase between 4 and 16 weeks of lactation for cows to continue achieving increasing DMI.

Lying and activity behavior. Daily lying time is lower during early lactation compared with late gestation in both housed and grazing cows, but the extent of this decrease between pre- and postcalving periods varies between studies. Considerable variation exists in the postcalving lying time reported in housed cows, which ranges between 6.0 and 14.0 h/d (Table 2.2). Many different factors could influence lying time in these studies including, for example, parity (Steensels et al., 2012; Piñeiro et al., 2019; Barraclough et al., 2020b), calving season (Steensels et al., 2012), and competition (Proudfoot et al., 2009b). These factors will be discussed in the next sections of this review. Other lying behavior parameters also vary between studies conducted in early lactating housed cows. For example, housed cows have been reported to engage in between 8.5 and 16.8 lying bouts per day with bouts ranging between 42 and 76 mins in duration (Table 2.2) and take between 802 to 1,720 steps/d (Brzozowska et al., 2014; Barraclough et al., 2020b).

Lying times in grazing dairy cows during early lactation are generally much lower than housed cows, ranging from 7.5 to 8.6 h/d, which is split over 7.7 to 9.7 lying bouts/d of between 51 and 69 mins in duration (Table 2.2). Daily lying times during early lactation also appear more consistent in grazing cows as Hendriks et al. (2019a) combined data from 4 experiments across different dry cow management strategies, farms, years, and regions and discerned little variation across these pasture-based systems. Grazing dairy cows are also more active than housed dairy cows during the postcalving period, achieving, on average, 4,424 steps/d during the first three weeks of lactation (Hendriks et al., 2019a). The shorter lying times and greater activity in grazing dairy cows are likely due to longer feeding times (Table 2.1), the need to walk to and from the milking shed (Hendriks et al., 2019a), and increased time away from pasture (Neave et al., 2021).

It is worth noting that lying time and activity are dynamic and change across the course of lactation. Hendriks et al. (2019) reported that daily lying time steadily declined between 5- and 34-days postcalving in grazing cows, and Maselyne et al. (2017) demonstrated that the lying time of housed cows decreases to a nadir of 10.5 h/d at 33 days of lactation, then steadily increases to a maximum lying time of 12.4 h/d at 265 days of lactation. In agreement, several studies in housed cows indicate similar lying profiles during early to late lactation (Nielsen et al., 2000; Munksgaard et al., 2005; Bewley et al., 2010; Vasseur et al., 2012; Brzozowska et al., 2014; Chaplin and Munksgaard, 2016; Westin et al., 2016; Munksgaard et al., 2020); however, others have also reported that this relationship may be influenced by parity (Neave et al., 2017; Munksgaard et al., 2020). Both Munksgaard et al. (2020) and Neave et al. (2017) reported that primiparous cows increase their lying time in the first 30 days in milk (DIM), starting lactation with a lower daily lying time compared with multiparous cows. Parity-driven differences in the trajectory of lying time during early lactation are discussed further in section 2.4.4. Cows may achieve longer lying times in later lactation through taking more lying bouts (Brzozowska et al., 2014) or longer average lying bout durations (Nielsen et al., 2000; Munksgaard et al., 2005; Vasseur et al., 2012; Chaplin and Munksgaard, 2016; Westin et al., 2016). A corresponding decrease in activity is observed across lactation (Brzozowska et al., 2014; Munksgaard et al., 2020).

The shorter lying times at the start of lactation could be attributed to an increase in the time spent feeding (Vasseur et al., 2012); however, Munksgaard et al. (2005) did not detect any association between eating time and DIM, even though cows had greater DMI during late lactation. Social mixing may influence these behaviors during early lactation when individual cows that have calved are moved from the dry or springer cow group into the milking herd. The social stressors that come with social mixing can have marked effects on their behavior including increased activity, reduced lying times, and increased agonistic behavior. Other possible explanations are that cows experience greater udder discomfort when lying during early lactation (Vasseur et al., 2012; Chaplin and Munksgaard, 2016; Maselyne et al., 2017), or that cows in early lactation spend more time engaging in other behaviors such as socializing (Munksgaard et al., 2005).

2.3. System-level factors that influence transition dairy cow behavior

Dairy farming in New Zealand is predominantly pasture-based and is characterized by seasonally concentrated calving in late winter/early spring, with cows grazing pasture outdoors year-round as their primary feed source (Roche et al., 2017). Grazing systems are also common in Australia and Ireland, with growing interest in managing cows on grazed pasture in other countries in Europe and North and South America, due to its perceived environmental and animal welfare benefits (Roche et al., 2017). In these latter countries, confinement housing systems are historically predominant, with cows kept indoors and fed PMR or TMR. Understandably, there are features of grazing systems that differ significantly compared with housed systems and many of these factors are known to influence aspects of dairy cow behavior, health, and welfare.

A major characteristic unique to grazing systems is the exposure to changing environmental conditions that cannot be controlled. Being outdoors, grazing dairy cows have limited shelter and are, therefore, largely exposed to a range of weather conditions typical of temperate climates e.g., wind, rain, and solar radiation (Webster et al., 2015), in contrast to cows kept in housed systems. They may be more protected from extremes in temperature and weather events due to the ability to design cow housing that minimizes the effects of cold or heat stress (Jones and Kammel, 2017), although the latter is often problematic in hot climates and not all housed systems have adequate heat abatement (Becker and Stone, 2020). Grazing dairy cows can also express behaviors that are influenced by changes in photoperiod due to outside exposure; however, housed cows may experience changes in behavior due to disruptions in photoperiod caused by artificial lighting (e.g., Phillips and Schofield, 1989). Furthermore, diurnal and seasonal fluctuations in pasture nutrient composition and variation in pasture quantity and quality can influence the grazing behavior of grazing cows. In contrast, in housed systems, other factors influence eating behavior such as feed delivery and diet composition, both of which can be more easily controlled by farm management. As space is restricted in housing systems, the competition for space to eat and lie down may be greater and the freedom of cows to express their entire range of behaviors may be more limited compared with cows at pasture. The effects of weather exposure, photoperiod, and social interactions between cows can drive variability in cow behavior between and within herds. Studies indicate that these system-level factors can influence behavior and differ across systems, and therefore, should be considered when studying cow behavior.

2.3.1. Photoperiod

It is generally accepted that melatonin mediates the response to photoperiod in dairy cattle (Dahl et al., 2000). The exposure to light, which is perceived by the retina, initiates a cascade of events resulting in the inhibition of melatonin synthesis in the pineal gland. Therefore, exposure to darkness results in melatonin secretion. The relative lengths of ‘daylight’ and ‘darkness’ (i.e., the length of elevated melatonin secretion) allow the animal to perceive short days (darkness > daylight) or long days (daylight > darkness) (Dahl et al., 2000).

Behavior in grazing dairy cows is affected by photoperiod. As discussed previously, cows prefer to graze during the daytime (Krysl and Hess, 1993; Linnane et al., 2001; Sheahan et al., 2011) and the change in the intensity and quality of light at sunset act as a cue for cows to stop their afternoon grazing bout (Linnane et al., 2001; Sheahan et al., 2011). Therefore, the time available to graze during the daylight decreases as the daylength gets shorter. The literature indicates two ways in which grazing dairy cows can respond to this. First, is an increase in grazing intensity during daylight hours, achieved through taking fewer but longer meals (Gregorini et al., 2006b), and second is to compensate by increasing the time spent feeding at night (Linnane et al., 2001; Sheahan et al., 2011). This effect of photoperiod in grazing dairy cows may be reinforced by seasonal fluctuations in, for example, pasture nutrient composition (Linnane et al., 2001). It has also been suggested that temperature, as well as cow-level factors (e.g., breed and milk production), can influence the behavioral response to photoperiod (Phillips and Schofield, 1989).

In housed cows, photoperiod may be influenced due to the use of artificial light in cow housing. However, the productive and behavioral responses to photoperiod are still very complex, illustrated in the study by Phillips and Schofield (1989). Dairy cows exposed to an artificial increase in daylength had both increased milk yield, and DMI, as well as an increase in the frequency of meals. However, the length of light exposure did not affect the overall feeding time in this study, indicating that these meals must also be shorter in duration. Interestingly, the less intense feeding during long days here is consistent with the previous observation that grazing intensity increases during short days in cows at pasture (Gregorini et al., 2006b). This leaves the door open to the possibility that, under the right conditions, housed and grazing dairy cows may similarly respond to photoperiod. Phillips and Schofield (1989) also reported that housed dairy cows exposed to artificial long days spent longer lying and had lower activity than those exposed to artificial short days, which they reasoned could influence energy expenditure, resulting in more energy available for milk production. The experiment by Phillips and Schofield (1989), however, indicated no effect of photoperiod on milk yield, DMI, or behavior, regardless of the intensity of artificial light. Inconsistencies between these two experiments could relate to differences in experimental design (i.e., continuous versus crossover), the length of treatment, and the time of year the experiment was conducted.

To my knowledge, the effects of photoperiod on cow behavior during the transition period have not been examined in grazing systems. It is possible that the grazing and lying behaviors of transition dairy cows at pasture are influenced by daylength and these differences may be more pronounced between individuals that calve at the very start and very end of the calving season, or between spring- and autumn-calving herds. Seasonal factors other than photoperiod, such as changes in weather (discussed further in section 2.3.2) and pasture nutrient composition (Linnane et al., 2001) during the calving season, may also influence these behaviors, and add a further layer of complexity to the understanding of how photoperiod influences dairy cow behavior.

2.3.2. Inclement weather

In New Zealand grazing systems, the transition period coincides with late winter/early spring and, therefore, increased exposure to inclement weather. Rain, wind, and decreasing ambient temperature are associated with a decrease in skin temperature (Schütz et al., 2010a). Changes in cow behavior, alongside changes in physiology, occur in response to deteriorating weather conditions (Webster et al., 2008). Cows attempt to minimize heat loss to the environment during periods of inclement weather, both through seeking shelter (Redbo et al., 2001; Legrand et al., 2009; Charlton et al., 2011) and changing their lying behavior. Housed cows minimize heat loss by lying, resulting in longer lying times during cold weather (Steensels et al., 2012; Brzozowska et al., 2014). Conversely, grazing dairy cows decrease their lying time in response to wet weather (Tucker et al., 2007; Webster et al., 2008; Schütz et al., 2010b; Hendriks et al., 2019b), a response that is further exacerbated by wind and low air temperatures (Schütz et al., 2010a; Hendriks et al., 2019b). This response can also be observed in grazing cows exposed to cold weather only (Malechek and Smith, 1976). Furthermore, cows prefer to lie down on clean dry surfaces, rather than on muddy or wet ones (Chen et al., 2017; Schütz et al., 2019), indicating that the condition of the lying surface may be driving differences between studies in housed and grazing cows. In pasture-based systems, the ground outside becomes wet, muddy, and cold during inclement weather, and a cow may conserve more body heat, and be more comfortable, standing than she would lying under these conditions.

Previous studies in housed and grazing cows indicate inconsistent effects of weather on activity. In housed cows, activity was greatest during winter than in summer (Brzozowska et al., 2014), an observation inconsistent with the reported increase in lying time during periods of cold weather. However, the effect of weather on the activity of grazing dairy cows reportedly changes across the transition period. Hendriks et al. (2019b) reported that cold and wet periods were associated with increased activity precalving, while wet weather was associated with decreased activity during the postcalving period. The reason for this difference across the transition period is currently unknown but could be related to differences in paddock surface conditions, feed requirements, motivation to graze and differences in baseline activity levels in the pregnant versus lactating dairy cow (Hendriks et al., 2019b).

Cows also modify their feeding behavior in response to inclement weather to conserve heat and energy. While grazing is associated with energy intake, it is not without an energy cost. This energy cost is proportional to the time spent feeding (Malechek and Smith, 1976), and cows may instead choose to save energy during periods of inclement weather by reducing their grazing time. This is supported by the observations that cows will reduce their grazing time in response to wet and windy (Redbo et al., 2001; Webster et al., 2008), or cold (Malechek and Smith, 1976) conditions. Although Schütz et al. (2010b) did not detect an effect of simulated wet conditions on the eating time of housed cows, they did observe reduced feeding rates and DMI. The response of cows to inclement weather also interacts with their BCS, likely because cows with greater fat stores are more insulated from the cold. Tucker et al. (2007) reported that thinner cows have lower body temperatures and tend to reduce their feeding times in response to colder weather more than cows with a greater BCS.

Overall, the published literature indicates that changes in feeding, lying and activity behaviors occur in response to inclement weather. Given the increased exposure of transition dairy cows to cold, wet, or windy conditions in seasonal, pasture-based systems, it is important to account for variations in weather when interpreting behavioral parameters (Hendriks et all., 2019b).

2.3.3. Influences of social interactions

Cattle are social animals that display both positive (e.g., affiliative behaviors such as allogrooming) and negative (e.g., aggression and avoidance) social behaviors. Cows also form complex social hierarchies, with dominance being related to many factors including increasing parity (González et al., 2003; Val-Laillet et al., 2008a; Neave et al., 2017), body weight (BW; Phillips and Rind, 2002; Neave et al., 2017), milk production (Val-Laillet et al., 2008a), and experience (González et al., 2003). Although time spent socializing is relatively more flexible than the times needed to eat or rest (Munksgaard et al., 2005), it is still an important behavior. This is demonstrated in socially isolated dairy cows who show behavioral signs of frustration and stress (Munksgaard and Simonsen, 1996). Social interactions can be influenced by farm management including, but not limited to, competition and access to food and lying space, regrouping of cows, and herd size. Further, these social interactions are important components of the time budgets of dairy cows and, therefore, may influence other behaviors, such as feeding and lying. While research into the social behavior of grazing dairy cows is limited, studies in housed cows are more extensive. Due to similar management practices surrounding the transition period, where dairy cows experience social mixing and possible changes to competitive interactions for feed when moved from the dry cow group into the milking herd, it is plausible that some of the behavioral differences reported in housed cows may be relevant to grazing systems. The associations between competition and access to food and lying space and regrouping of cows and social, feeding, and lying behaviors are discussed below.

Competition for resources. Access to feed presents one of the main sources of negative interactions between cows (Val-Laillet et al., 2008b). As stocking rate increases or access to feed decreases (e.g., fewer feed bins available), the competition for food in housed cows also increases, and cows alter their feeding behavior. This increased competition has been demonstrated where cows are displaced from the feeding bins more frequently (Olofsson, 1999; Proudfoot et al., 2009b; Krawczel et al., 2012; Lobeck-Luchterhand et al., 2015), feeding times decrease (Olofsson, 1999; Huzzey et al., 2006; Crossley et al., 2017), and feeding rates increase (Olofsson, 1999), particularly in more subordinate cows (Proudfoot et al., 2009b; Crossley et al., 2017) possibly as an attempt to reduce negative interactions at the feed bunk. The effect of competition on feeding behaviors may be further complicated by the motivation of a cow to eat; as satiation increases, more dominant cows are likely to be replaced from the feeding area by less dominant cows (Val-Laillet et al., 2008a) and, therefore, changes in behavior may be inconsistent depending on the duration and severity of the competition to access feed along with other factors affecting the motivation to eat such as BCS and energy requirements. This may explain, in part, inconsistencies between studies, where other studies indicate no effect of increased competition (e.g., stocking density) on feeding time (Hill et al., 2009; Krawczel et al., 2012).

Lying times in housed cows also decrease as competition increases (Huzzey et al., 2006; Hill et al., 2009; Krawczel et al., 2012; Lobeck-Luchterhand et al., 2015; Crossley et al., 2017), as cows spend more time standing idle (Olofsson, 1999). Proudfoot et al. (2009b) also reported this, but only in multiparous cows in the week before and after calving. It was suggested that the decrease in lying time may reflect cows spending more time waiting for access to the feed bunk (Proudfoot et al., 2009b; Crossley et al., 2017), or accessing lying bunks and engaging in more social interactions. It appears that the effects of restricting access to lying bunks may be exacerbated in subordinate primiparous cows, where these animals are more likely to be displaced from their cubicles compared with more dominant animals (González et al., 2003).

Therefore, when competition increases due to restrictions to feed and lying space, subordinate animals may be more adversely affected and alter their lying and feeding behaviors more substantially than dominant animals.

Evidence from studies in housed cows (Fregonesi and Leaver, 2002) and grazing cows on stand-off pads (Schütz et al., 2015) indicates that a decrease in space allowance (i.e., an increase in competition) results in reduced lying times and increased agonistic interactions. Therefore, the differences in feeding and lying behaviors observed due to increased competition in housed dairy cows may not be seen to the same extent in grazing dairy cows as they have more space to feed and lie. However, there could still be an element of competition for specific grazing sites and areas to lie, especially during the precalving period where the area allowance is often smaller than postcalving to match the lower energy requirements.

The effects of social interactions, hierarchy, and competition may affect how efficient an individual cow is at grazing and digesting its food. For example, dominant cows reportedly increase their rate of feed intake and decrease their time spent grazing when the amount of pasture available to consume is limited, instead allocating more time to ruminate and possibly defend their position in the hierarchy (Phillips and Rind, 2002). Similarly, Ungerfeld et al. (2014) reported that more dominant cows spend less time grazing and walking, but more time ruminating than subordinate cows. Gregorini et al. (2013) also noted that primiparous dairy cows, who are possibly more subordinate, not only spend less time ruminating but ruminate, and likely graze, at different and possibly less-favorable times of the day to multiparous cows. More dominant cows are also seemingly more relaxed while ruminating, as they spend more time ruminating whilst lying and regurgitate their boluses less frequently than subordinate cows (Phillips and Rind, 2002). To my knowledge, the associations between feeding, lying, and social behaviors influenced by competitive environments have not been extensively studied in grazing dairy cows and further work in this area is needed.

Re-grouping. A significant amount of re-grouping occurs during the transition period. Multiple groups of cows (e.g., dry, springer, colostral, and main milking mobs) are formed during the transition period to facilitate management. These mobs may also be split based on other attributes such as age (Hubbard et al., 2021). Further, sick cows may be kept separate from the main mobs. After regrouping, social relationships need to be re-formed, involving an adjustment period where cows learn about new individuals and possibly re-evaluate affiliative or competitive relationships with known herd mates. This is a potentially stressful process and likely requires reallocation of time towards social behavior. The amount of time spent engaging in aggressive or agonistic behavior in re-grouped cows is known to increase (von Keyserlingk et al., 2008; Schirmann et al., 2011), while participation in affiliative behavior (i.e., allogrooming) reportedly decreases (von Keyserlingk et al., 2008). It is also possible that some cows may refrain from participating in certain behaviors to avoid conflict (e.g., Rioja-Lang et al., 2009). This results in cows spending less time eating and lying down after re-grouping (Phillips and Rind, 2001; von Keyserlingk et al., 2008), and possibly increasing activity (Hubbard et al., 2021). Similarly, Schirmann et al. (2011) demonstrated that regrouping affects feeding behavior, particularly in the cows introduced both to a new herd and environment; these cows ate more slowly, had lower intakes, and spent less time ruminating than cows that stayed in their home pen. This indicates that additional stressors, such as a novel environment, may further affect how dairy cows cope with changing social situations.

2.4. Cow-level factors that influence transition dairy cow behavior

Several cow-level factors reportedly influence dairy cow behavior, such as breed, milk yield, BCS, and parity. Furthermore, the effects of disease or health status on behavior are an important, and well-researched, area. These factors can drive differences both between herds and between individuals within a herd (Ito et al., 2009). Hence, the effects of these factors on cow behavior must be explored.

2.4.1. Breed

Few studies have investigated the possible effects of breed on feeding, lying, and activity behaviors in dairy cows. Munksgaard et al. (2020) reported that housed lactating Holstein cows had longer daily eating times and greater intakes, compared with Jersey cows. They attributed this difference to the greater BW and milk production of the Holstein cows. Similar observations during mid and late lactation are reported by Henriksen et al. (2019); however, while Holstein cows had greater DMI during the first 14 d postpartum, they did not differ from Jersey cows in their feeding time, and instead had a greater rate of feed intake during this period. A study investigating the effects of feeding system on the grazing behavior of North American versus New Zealand Holstein-Friesian dairy cows indicated that while cows of North American ancestry were heavier, produced more milk, and, consequently, had greater DMI than those with New Zealand ancestry, they spent less time grazing, especially between sunset and midnight (Sheahan et al., 2011). The authors explained that, rather than increase their feeding time to achieve greater intakes, the North American Holstein-Friesian cows increased their feeding rate through having greater bite mass (Sheahan et al., 2011). Similar results in North American versus New Zealand Holstein-Friesian cows have were reported by other authors (Thorne et al., 2003;

McCarthy et al., 2007). Collectively, these studies indicate that while breed has consistent effects on milk production, BW and DMI, the relationship between breed and the expression of feeding-related behaviors is not consistent, and in these studies might potentially be influenced by other factors such as differences in management systems or the effects of competition.

Holstein cows are also reportedly less active than Jersey cows during lactation, spending longer lying and taking fewer steps (Henriksen et al., 2019; Munksgaard et al., 2020). The differences in step count were attributed to the Holstein cows having a greater body size and, therefore greater stride length than the Jersey cows (Munksgaard et al., 2020). Other studies, however, indicate no effect of breed on dairy cow lying behavior (Stone et al., 2017; Hendriks et al., 2019a) or activity (Hendriks et al., 2019a), although this may reflect less-divergent breed groups in the study by Hendriks et al. (2019a) and adjusting for milk production and BW, resulting in the masking of breed effects in the study by Stone et al. (2017). The inconsistencies of breed effects between studies indicate that this factor requires consideration along with other system- and cow-level variables when investigating various behaviors.

2.4.2. Milk yield

Unsurprisingly, numerous studies have identified that high-yielding dairy cows have greater feed intakes than those with lower milk yields (e.g. Dado and Allen, 1994; Fregonesi and Leaver, 2002; Norring et al., 2012; Munksgaard et al., 2020). While greater feed intakes could be a result of increased energy demand to support greater milk production, the opposite could also be true, i.e., greater feed intake itself drives greater milk production. Cows with high milk yields may need more time to ingest and process food, resulting in longer daily feeding (Løvendahl and Munksgaard, 2016; Munksgaard et al., 2020) and rumination times (Dado and Allen, 1994; Fregonesi and Leaver, 2002; Stone et al., 2017).

Many studies have shown that housed cows with higher milk yields had shorter daily lying times than lower-yielding cows (e.g., Bewley et al., 2010; Løvendahl and Munksgaard, 2016; Stone et al., 2017), with more lying bouts (Løvendahl and Munksgaard, 2016) of shorter duration (Deming et al., 2013; Løvendahl and Munksgaard, 2016; Rajala-Schultz et al., 2018). These shorter lying times may be the result of a trade-off for longer eating times to support milk production. Norring et al. (2012) also reported shorter lying times in cows with greater milk yield; however, milk yield did not affect the total time spent eating or ruminating. Instead, they suggested that high-yielding cows spend less time lying down due to greater discomfort because of more milk in their udders. To my knowledge, the associations between milk yield and lying behavior in grazing dairy cows have not been investigated.

2.4.3. Body condition score

There is very little research on the relationship between BCS and feeding time in dairy cattle. Adipose tissue is likely involved in regulating DMI (Hervey, 1959; Bray, 1991; Archer et al., 2002) so that the animal maintains a particular level of body condition. This premise is supported by Hayirli et al. (2002), who reported prepartum DMI (expressed as a % of BW) was negatively correlated with BCS. Similarly, Matthews et al. (2012) observed grazing dairy cows of a higher BCS had a lower feed intake during mid- and late-lactation. Furthermore, these cows spent less time grazing and ruminating than cows with a lower BCS (Matthews et al., 2012). Westin et al. (2016) reported high BCS cows (BCS > 3.5; 5-point scale) lay down an hour more than thin cows (BCS < 2.25). They suggested that this effect was due to thin cows experiencing greater discomfort when lying on hard surfaces, being lower in the social hierarchy and more likely to be displaced from lying stalls, or possibly producing more milk and spending more time eating (Westin et al., 2016). A positive association between BCS and lying time was also reported in grazing dairy cows, but only during late lactation (Matthews et al., 2012). In a study by Calderon and Cook (2011), thin cows (BCS < 3.0; 5-point scale) had fewer lying bouts of longer duration than moderate (3.0 to 3.75) or fat (> 4.0) cows; however, there was no effect of BCS on overall daily lying time. Other studies found no effect of BCS on lying behavior or activity in housed (Bewley et al., 2010) or grazing dairy cows (Hendriks et al., 2019a).

2.4.4. Parity

Several studies have investigated the associations between parity and lying behavior and these studies are summarized below. To my knowledge, however, no studies have been published on the associations between parity and feeding behavior in grazing dairy cows, but a study on grazing time in beef cows has been conducted (Dunn et al., 1988). Feeding behavior has been more extensively studied in housed cows due to the ability to easily measure DMI and feeding behavior when cows receive a TMR. Therefore, studies investigating feeding behavior in housed cows are reported below.

Associations between parity and feeding behaviors. The effects of parity on feeding behaviors vary between the pre- and postcalving periods. During the precalving period, multiparous housed cows have a greater DMI than primiparous cows (Proudfoot et al., 2009b; Neave et al., 2017), as well as spend less time feeding spread across fewer meals per day

(Proudfoot et al., 2009b; Neave et al., 2017). To achieve this greater DMI while spending fewer minutes feeding, multiparous cows must have a greater rate of feed intake. Similarly, Dunn et al. (1988) report older beef cows (between 5- and 7-years old) have shorter grazing times and lower activity than younger (3-year-old) cows and suggested this could be a result of the older cows being more experienced grazers.

This pattern contrasts with what is observed during early lactation. Multiparous cows continue to have a greater DMI compared with primiparous cows (Proudfoot et al., 2009b; Neave et al., 2017; Henriksen et al., 2019; Munksgaard et al., 2020); however, this is associated with longer feeding times (Neave et al., 2017; Henriksen et al., 2019; Munksgaard et al., 2020) rather than increased rates of feed intake. Dry matter intake is influenced by the number and duration of meals (i.e., feeding time), and the bite rate and bite size (i.e., rate of feed intake) (Gibb, 1998). A cow may adjust one or more components of its feeding behavior to ensure they meet its daily energy requirements. Dry matter intake can, therefore, be affected by the motivation to feed, especially in a competitive environment, which is driven by greater BW and milk yield, and thus greater energy requirements (Neave et al., 2017). However, evidence suggests that feeding behavior can also be affected by increased social pressure in the feeding area that may be experienced by both multiparous and primiparous animals (Proudfoot et al., 2009b) and may change in response to demands on the time budgets of cows such as adequate time to lie and ruminate (Munksgaard et al., 2020) as animals transition from gestation to early lactation. It is difficult to disentangle exactly what is driving differences in feeding behaviors between multiparous and primiparous cows pre- and postcalving and further research on parity and social, feeding, and lying interactions are needed, particularly in grazing dairy cows.

Associations between parity and lying and activity behaviors. Housed primiparous cows steadily decrease their daily lying times in the 4 to 10 d before calving, resulting in shorter lying times than multiparous cows (Borchers et al., 2017; Piñeiro et al., 2019; Barraclough et al., 2020a). Primiparous cows also have more lying bouts (Calderon and Cook, 2011; Neave et al., 2017; Barraclough et al., 2020a) of shorter duration (Neave et al., 2017) during the precalving period. To investigate whether BW or milk yield in the subsequent lactation influenced lying bout number and duration, Neave et al. (2017) compared a model controlling for these 2 factors with a simple model and reported that parity differences were still apparent after controlling for these factors. Instead, Neave et al. (2017) proposed that, as primiparous cows are experiencing the lead-up to calving for the first time, a lack of experience may lead to greater restlessness. This hypothesis is supported by Wehrend et al. (2006), who reported that precalving primiparous cows exhibited a greater degree of restlessness compared with multiparous cows, characterized by a greater number of transitions between different activities. Other authors also report greater step counts in younger housed cows during the 4 d before calving (Barraclough et al., 2020a) and in younger grazing dairy cows in the 2 d before calving (Hendriks et al., 2019a). Nevertheless, longer lying times and reduced activity observed in multiparous cows may be a result of alternative factors such as longer rumination times (Stone et al., 2017) and increased incidences of metabolic disease (Steensels et al., 2012), which are further discussed below.

During lactation, increasing parity in both housed and grazing cows is associated with longer lying times (Steensels et al., 2012; Sepúlveda-Varas et al., 2014; Westin et al., 2016; Stone et al., 2017; Henriksen et al., 2019; Piñeiro et al., 2019), and longer, less frequent lying bouts (Vasseur et al., 2012; Brzozowska et al., 2014; Sepúlveda-Varas et al., 2014; Neave et al., 2017). Furthermore, the trajectory of lying time during early lactation differs for primi- and multiparous cows. While primiparous cows have shorter daily lying times at the start of lactation, they increase their daily lying times as lactation progresses (Vasseur et al., 2012; Sepúlveda-Varas et al., 2014; Munksgaard et al., 2020), such that there are no parity differences in lying time in housed cows after 6 weeks (Munksgaard et al., 2020) or in grazing cows after 3 weeks (Sepúlveda-Varas et al., 2014). Housed multiparous cows are also reportedly less active than primiparous cows during lactation (Henriksen et al., 2019; Munksgaard et al., 2020). Similarly, in grazing dairy cows, parity 2 to 3 cows are more active than parity 8+ cows, but only during the first 5 d of lactation (Hendriks et al., 2019a), with no further differences within 35 d postcalving.

The longer lying times both pre- and postcalving in multiparous cows have been attributed to longer rumination times (Stone et al., 2017), and are possibly related to greater DMI, BW, and milk yield (Steensels et al., 2012). Furthermore, the longer lying times and fewer lying bouts in multiparous cows may be a result of increased incidence of subclinical metabolic issues (Steensels et al., 2012), increased difficulty to transition from standing to lying (Vasseur et al., 2012), or less disrupted resting behaviors (Vasseur et al., 2012). First-parity cows may be more subordinate and more likely to struggle with changes in management associated with early lactation (e.g., regrouping after calving), which could affect their lying behavior, particularly in a competitive environment (Brzozowska et al., 2014; Sepúlveda-Varas et al., 2014; Neave et al., 2017).

It is worth noting that the effect of parity on pre- and postcalving lying behaviors and activity is not consistent between studies in housed cows, with some studies reporting no effect of parity on precalving lying time (Jensen, 2012; Neave et al., 2017), the number of lying bouts

(Jensen, 2012), or step count (Jensen, 2012; Borchers et al., 2017). Similarly, parity reportedly did not affect the daily lying time, the frequency of lying bouts, lying bout duration, or step count of grazing dairy cows during the three weeks to three days before calving (Hendriks et al., 2019a). However, the study by Hendriks et al. (2019a) did not include primiparous cows, and differences in prepartum lying and activity behavior may not be apparent within multiparous cows only.

During the postcalving period, Brzozowska et al. (2014) reported that lactating housed multiparous cows had shorter daily lying times than primiparous cows, which they reasoned could be due to the multiparous cows possibly having longer postcalving feeding times. Other studies in housed and grazing dairy cows did not indicate any effects of parity on daily lying time (Jensen, 2012; Vasseur et al., 2012; Neave et al., 2017; Hendriks et al., 2019a), the number of lying bouts (Jensen, 2012; Hendriks et al., 2019a), lying bout duration (Hendriks et al., 2019a), and step count (Jensen, 2012) postcalving. Inconsistent differences reported in the literature may be due, in part, to differences in experimental designs, competition within the environment (particularly in housed cows), and the range of ages investigated. Irrespective of these inconsistencies, future studies should consider parity when investigating behavior.

2.4.5. Disease

Dairy cows can experience a range of interrelated metabolic disorders and infectious diseases around calving. A cow that is unable to adapt to the physiological and metabolic demands of the calving event and the onset of lactation may be more at risk of experiencing health issues. It is well known that sick animals alter their behavior to improve the likelihood of recovery, with sickness behaviors commonly used to clinically diagnose disease including paralysis, lethargy, reduced appetite, and restlessness (Dantzer and Kelley, 2007). These behaviors are also influenced by the severity of the disease; hence, animals experiencing subclinical disease may go undetected (Sepúlveda-Varas et al., 2014). Wearable behavior-monitoring technologies have the advantage of detecting subtle changes in behavior via continuous measurements. Therefore, several studies have investigated the associations between behavior and a range of diseases in dairy cows to test the ability of these technologies to detect subtle behavior differences in dairy cows of different health statuses (Weary et al., 2009). Associations between metabolic diseases (e.g., hypocalcemia and hyperketonemia; González et al., 2008; Jawor et al., 2012; Liboreiro et al., 2015; Hendriks et al., 2020a) and infectious diseases (e.g., metritis and endometritis; Huzzey et al., 2007; Liboreiro et al., 2015) and a range of behaviors including feeding, lying, social, and drinking behaviors have been investigated. While studies have been undertaken in both housed and grazing dairy cows, studies in grazing dairy cows are scarce and our understanding of transition cow behavior is continually evolving. For this thesis, I will focus on the associations between periparturient hypocalcemia and feeding, lying, and activity behaviors. The reader is referred to Tucker et al. (2021) and Beauchemin (2018) for recent reviews of the associations between lying and eating behaviors and diseases such as ketosis, mastitis, and lameness.

Hypocalcemia is the most common of the many possible disorders a dairy cow can experience during the transition period (Goff, 2008). It is characterized by low circulating Ca concentrations but is identified on farm largely based on visual observations. In severe cases, a clinically-hypocalcemic cow becomes recumbent and is unable to get to her feet due to weakened muscle contractility, a condition termed ‘parturient paresis’ or ‘milk fever’ (Goff, 2008). However, in milder forms of hypocalcemia, changes in behaviors and symptoms may only be subtle (Jawor et al., 2012), resulting in many subclinically hypocalcemic animals and those with less severe cases of clinical hypocalcemia (i.e. without paresis) going undetected. Wearable technologies could allow greater detection of hypocalcemia as the condition is associated with restlessness, hypersensitivity, ataxia, tachycardia, subnormal body temperature, reduced DMI, poor rumen and intestine motility, and increased susceptibility to other metabolic and infectious diseases (Goff, 2008), which can alter cow behaviors.

Associations between hypocalcemia and lying and activity behaviors. To my knowledge, only one study in grazing dairy cows (Hendriks et al., 2020a), but five studies in housed dairy cows (Jawor et al., 2012; Liboreiro et al., 2015; Barraclough et al., 2020b; Tsai et al., 2021), have investigated the associations between periparturient hypocalcemia and lying behavior. Descriptions of these studies are presented in Table 3. While some studies in housed cows indicate no associations between hypocalcemia and lying behavior (Piñeiro et al., 2019) or activity (Liboreiro et al., 2015), others (Jawor et al., 2012; Barraclough et al., 2020b; Tsai et al., 2021) indicate similar associations to those reported in grazing dairy cows (Hendriks et al., 2020a).

Table 2.3. Summary of studies of the associations between blood Ca status and transition dairy cow behavior. The calcium (Ca) status classification for clinical (CLIN) and subclinical (SUB) hypocalcemia and normocalcemia (NORM), the timing of blood samples in the immediate postpartum period, and the effects on behavior (lying time, number of lying bouts (LB), LB duration, dry matter intake (DMI) or rumination time) during the transition period, of dairy cows fed pasture or total mixed rations (TMR).

Authors	Study Description	Ca Status Classification	Timing of Blood Sampling	Effects on Behavior
Jawor et al. (2012)	30 cows, housed fed TMR	Hypocalcemic = serum Ca $\leq$ 1.8 mmol/L, clinical signs not observed NORM = serum Ca $>$ 1.8 mmol/L	Within 24 h postcalving	Hypocalcemic: Longer standing times on the day before calving (+ 2.6 h/d) and shorter standing times on the day after calving (+ 2.7 h/d).
Liboreiro et al. (2015)	296 cows, housed fed TMR	SUB = serum Ca $<$ 8.55 mg/dL ($<$ 2.13 mmol/L); univariate analysis using continuous data	Within 72 h postcalving (single sample)	SUB: Lower rumination times on the day of calving (~0.3 h/d) and d 3 postcalving (~0.4 h/d). No association with activity.
Piñeiro et al. (2019)	1,052 cows, housed fed TMR	Hypocalcemic = serum Ca $\leq$ 8.0 mg/dL ($<$ 2.0 mmol/L) NORM = serum Ca $\geq$ 8.0 mg/dL ($\geq$ 2.0 mmol/L)	Within 48 h postcalving	Hypocalcemic: No association with lying time.
Hendriks et al. (2020)	72 cows, grazing pasture	CLIN = plasma Ca $\leq$ 1.4 mmol/L, clinical signs not observed SUB = plasma Ca $>$ 1.4 and $<$ 2.0 mmol/L NORM = plasma Ca $\geq$ 2.0 mmol/L	Within 48 h postcalving	CLIN: longer lying on the day before (+ 1.4 h) and day of (+ 2.6 h) calving. More LB on the day of calving (+ 2.9 bouts). SUB: more LB on the day of calving (+ 1.9 bouts).

Table continued over the page.

Table 2.3 (continued). Summary of studies of the associations between blood Ca status and transition dairy cow behavior.

Authors	Study Description	Ca Status Classification	Timing of Blood Sampling	Effect on Behavior
Barraclough et al. (2020b)	72 cows, housed fed TMR	CLIN = clinical signs and treatment SUB = serum Ca < 2.0 mmol/L, clinical signs not observed NORM = serum Ca ≥ 2.0 mmol/L	Within 24 h postcalving	CLIN: more LB in the 14 d precalving (+2.5 bouts/d) in multiparous cows. Less active and longer lying times (between 1.5 and 2.1 h/d) d 1 to 21 postcalving compared with SUB and NORM cows SUB: more LB in the 14 d precalving (+ 2.5 bouts/d) in multiparous cows
Seely et al. (2021)	78 cows, housed fed TMR	NORM = serum Ca > 1.95 mmol/L on d 1 and > 2.2 mmol/L on d 4 postcalving Transient SUB = serum Ca ≤ 1.95 mmol/L on d 1 and > 2.2 mmol/L on d 4 postcalving Delayed SUB = serum Ca > 1.95 mmol/L on d 1 and ≤ 2.2 mol/L on d 4 postcalving Persistent SUB = serum Ca ≤ 1.95 mmol/L on d 1 and ≤ 2.2 mmol/L on d 4 postcalving.	Daily for the first 6 d postcalving	Persistent/delayed SUB: lower DMI than NORM and transient SUB cows during the 21 d postpartum. NORM and transient SUB cows did not differ in postpartum DMI.
Tsai et al. (2021)	90 cows, housed fed TMR	Hypocalcemic = serum Ca < 8.6 mg/dL (< 2.14 mmol/L)	Samples on days 3, 7, 14 and 21 postcalving	CLIN/SUBCLIN: less time ruminating (- 0.7 h/d) and longer lying times (+ 1.2 h/d) during the 21 d postpartum

A recent study undertaken in grazing dairy cows indicated that clinically hypocalcemic cows (without paresis) spent longer lying on the day before (+1.4 h) and on the day of calving (+2.6 h), were less active and had more lying bouts compared with normocalcemic cows (Hendriks et al., 2020a). Similarly, Jawor et al. (2012) reported that housed cows experiencing hypocalcemia (without paresis) spent ~2.7 h less time standing on the day after calving compared with normocalcemic cows, but spent more time standing on the day before calving, which could be due to discomfort and restlessness. Differences between hypocalcemic and normocalcemic groups did not extend beyond 2 DIM in the study by Hendriks et al. (2020a); however, other studies have reported longer (between 0.6 and 2.1 h/d) lying times in clinically and subclinically hypocalcemic cows during the first 21 DIM (Barraclough et al., 2020b; Tsai et al., 2021).

Associations between hypocalcemia and feeding behaviors. Dry matter intake was reportedly lower in nonpregnant dry cows when subclinical hypocalcemia was induced (Martinez et al., 2014). Similarly, Seely et al. (2021) reported lower postcalving blood Ca concentrations are associated with lower postcalving intakes. These authors also recognized that subclinical hypocalcemia is dynamic across the first few days postpartum (Table 3). Cows below the blood Ca threshold (serum Ca ≤ 1.95 mmol/L) on the day after calving had lower DMI than those above the blood Ca threshold. Cows who recovered their blood Ca by the blood sample at 4 d postcalving (i.e. transient hypocalcemia) also recovered their DMI by ~2 d postcalving to amounts similar to normocalcemic cows. However, those who stayed subclinically hypocalcemic (i.e. persistent hypocalcemia; serum Ca ≤ 2.2 mmol/L at 4 DIM) or developed delayed onset of subclinical hypocalcemia (serum Ca > 1.95 mmol/L at 1 DIM and ≤ 2.2 mmol/L at 4 DIM), had reduced DMI across the 2 to 21 d postcalving. These results indicate that the relationships between Ca status and DMI are influenced by the timing and duration of periparturient hypocalcemia.

Associations between blood Ca status and DMI may be related to changes in feeding behavior. Jawor et al. (2012) reported subclinically hypocalcemic (serum Ca ≤ 1.8 mmol/L) cows had slightly fewer visits to the feed bins during weeks 1 and 3 of lactation; however, this did not affect their DMI, nor was the rate of feeding affected by Ca status. While this study didn't report the duration of time spent at the feeder, these results suggest that the feeding time in the subclinically hypocalcemic may be longer than normocalcemic cows to maintain their DMI with fewer visits. In contrast, the study by Tsai et al. (2021) did not observe any difference in eating time or time spent at the feed bunk between hypocalcemic (serum Ca < 2.14 mmol/L) and normocalcemic cows during early lactation (d 1 to 21). The study by Tsai et al. (2021) classified subclinically hypocalcemic cows using a greater blood Ca threshold and later timing of the first blood sample (3 d postpartum) than the study by Jawor et al. (2012) (Table 3). This means there is less of a difference in blood Ca concentrations between the subclinically hypocalcemic and normocalcemic groups in the study by Tsai et al. (2021), and indicates that differences in feeding behavior may only be distinguished between more extreme differences in blood Ca concentration.

Other studies have found associations between rumination times and Ca status. For example, Liboreiro et al. (2015) found subclinically hypocalcemic cows spent less time ruminating on the day of calving and day 3 postcalving (Table 3). Although differences in rumination times did not persist after day 3 of lactation in the study by Liboreiro et al. (2015), the study by Tsai et al. (2021) determined that hypocalcemic cows spent less time ruminating during the first 21 d of lactation. Neither of these authors assessed the effect of blood Ca status on DMI; however, Liboreiro et al. (2015) suggested that the reduced rumination times they observed in hypocalcemic cows could be caused by reduced DMI.

Several authors have speculated that these behavioral changes on the day of calving and postcalving in hypocalcemic cows may be evidence of impaired smooth (e.g., rumen and uterine; Murray et al., 2008) and skeletal muscle contractility due to low blood Ca (Jawor et al., 2012; Hendriks et al., 2020a). Lethargy and anorexia are common sickness behaviors, with increased lying and decreased activity well-known signs of clinical milk fever. However, cows may also spend more time resting postcalving to conserve energy to improve the likelihood of recovery from hypocalcemia (Hart, 1988; Dantzer and Kelley, 2007).

The behavioral differences in cows experiencing subclinical and clinical hypocalcemia indicate that behavior monitoring devices may have the potential to allow improved and early detection of hypocalcemia. It is also possible that wearable technology may be used to investigate behavior changes associated with hypocalcemia prevention strategies. In the following sections, I briefly review the literature around hypocalcemia prevalence, associations with other health disorders, risk factors, and prevention strategies. Furthermore, the known effects of zeolite supplementation before calving on transition cow behavior will be discussed.

2.5. Hypocalcemia

Calcium demand in the dairy cow increases quickly and markedly after calving, with a large amount of Ca drawn from the blood into the mammary gland to support the onset of lactation.

Parathyroid hormone (PTH) is released from the parathyroid glands in response to low concentrations of Ca in the blood. This hormone promotes the mobilization of Ca from bone reserves, as well as acting on the kidney to prevent the loss of Ca in the urine. The kidney also starts to produce the hormone 1,25-dihydroxyvitamin D, which acts on the small intestine to increase absorption of Ca from the diet (Horst et al., 1994; Goff, 2006; Goff, 2008). Hypocalcemia occurs when these homeostatic mechanisms fail to replenish blood Ca concentrations and the cow is unable to adapt to the increased physiological demand for Ca at calving.

Most dairy cows experience a decrease in blood Ca concentrations following calving (Littledike et al., 1981; Horst et al., 1994; Goff, 2008); the severity differs with the individual's ability to adapt to the change in Ca requirements within the first 1 to 2 d postcalving (Ramberg Jr et al., 1970; Barton et al., 1981). The concentrations of Ca in the blood are normally maintained between 2.1 and 2.5 mmol/L (Goff, 2006). Cows with a large decrease in blood Ca concentrations below 1.4 mmol/L are classified as clinically hypocalcemic (Lindsay and Pethick, 1983), which can be accompanied by periparturient paresis (i.e., milk fever). Cows with subclinical hypocalcemia have sub-normal blood Ca concentrations; however, the definition of subclinical hypocalcemia differs between studies based on the Ca threshold, the number of blood samples, and the timing of blood sampling. Subclinical hypocalcemia is often classified using a single blood Ca threshold (below between 1.8 – 2.2 mmol/L), with one or more samples taken within the first 4 DIM (e.g., Jawor et al., 2012; Rodríguez et al., 2017; Roberts and McDougall, 2019; Seely et al., 2021). However, by using multiple blood Ca thresholds for multiple samples within the first 4 DIM, researchers have recently associated the severity, timing of onset, and duration of hypocalcemia with, for example, differences in milk production (Seely et al., 2021) and postpartum health events (McArt and Neves, 2020). These studies indicate that multiparous cows experiencing transient subclinical hypocalcemia have increased DMI and milk production (Seely et al., 2021) and are at a slightly higher risk for an adverse health event (McArt and Neves, 2020), whereas those experiencing delayed or persistent subclinical hypocalcemia have depressed DMI, lower milk yields (Seely et al., 2021), and are at a greater risk of an adverse health event compared with normocalcemic cows (McArt and Neves, 2020).

The weakened muscle contractility (Goff, 2008) and immune suppression (Ducusin et al., 2003; Kimura et al., 2006; Martinez et al., 2012) associated with clinical and subclinical hypocalcemia have been associated with mastitis (Goff, 2008), metritis (Martinez et al., 2012; Rodríguez et al., 2017), retained placenta (Neves et al., 2017; Rodríguez et al., 2017), displaced abomasum (Rodríguez et al., 2017), and have resulted in early removal from the herd (McArt and Neves, 2020) in studies undertaken in housed cows. Other researchers, however, have found no associations between blood Ca immediately following calving and metritis (Chamberlin et al., 2013; Neves et al., 2018), displaced abomasum (LeBlanc et al., 2006; Chamberlin et al., 2013), or the risk of culling (Martinez et al., 2012), but this may be due to differences among studies in the severity of hypocalcemia and the timing of blood sampling.

Between 2 and 5% of housed (Reinhardt et al., 2011) and grazing (Roche, 2003; Roberts and McDougall, 2019) dairy cows experience clinical milk fever. However, the incidence of subclinical hypocalcemia is much greater in both systems: reportedly between 30 to 40% in grazing dairy cows (Roche, 2003; Roberts and McDougall, 2019) and between 25 to 54 % in housed dairy cows (Reinhardt et al., 2011) using a threshold of blood Ca < 2.0 mmol/L (Roche, 2003; Roberts and McDougall, 2019), and 52% of grazing dairy cows using a threshold of < 2.15 mmol/L (Roche, 2003; Roberts and McDougall, 2019). Due to the significant prevalence of subclinical hypocalcemia and its relationship with other periparturient disorders, it is important that strategies to prevent hypocalcemia be explored.

2.5.1. Hypocalcemia risk factors

There are several risk factors associated with an increased likelihood of a cow experiencing subclinical or clinical hypocalcemia. For instance, increasing age and parity have been identified as major cow-level risk factors for hypocalcemia. Multiparous cows reportedly have lower blood Ca concentrations than primiparous cows immediately following calving (Reinhardt et al., 2011; Rodríguez et al., 2017), and have a greater risk of developing hypocalcemia (Roche and Berry, 2006; Neves et al., 2017; Roberts and McDougall, 2019). It has been suggested that the ability for a cow to correct her blood Ca concentrations at calving becomes more difficult with increasing age (Hernández-Castellano et al., 2020), as older cows are less efficient at releasing Ca from bone (Iwama et al., 2004) and absorb less Ca in the intestine (Horst et al., 1994). Older cows also produce more milk than younger cows, which further contributes to their greater risk.

Other cow-level risk factors include, but are not limited to, breed, previous lactation yield, and previous history of milk fever. It is well noted that Jersey cows have a greater incidence of hypocalcemia than Holstein-Friesian (Roche and Berry, 2006) or Holstein-Jersey crossbred cows (Saborío-Montero et al., 2017), and it had been suggested that this could be related to fewer 1,25-dihydroxyvitamin D receptors on the small intestine, leading to lower uptake of dietary Ca or due to greater Ca concentrations in colostrum (Saborío-Montero et al., 2017). Cows with greater milk yields also have an increased incidence of hypocalcemia (Saborío-Montero et al., 2017), possibly due to greater colostrum production and, therefore, a greater loss of Ca from the blood (National Research Council, 2001). Additionally, the incidence of hypocalcemia is greater in cows that have experienced hypocalcemia in the previous lactation (Roche and Berry, 2006).

In addition to the cow-level factors highlighted above, a few studies have noted an association between adverse weather conditions (especially cold and wet conditions, which are commonly experienced by spring-calving dairy cows at pasture) and an increased risk of hypocalcemia and paresis in both dairy (Simesen, 1974; Schnier et al., 2002; Roche and Berry, 2006) and beef (Moisan, 1994) cows. The reasons explaining this association have yet to be elucidated, but authors have suggested the effects of temperature, in particular, may relate to decreases in DMI and Ca intake (Roche and Berry, 2006) or changes to mineral metabolism (e.g., Shiga et al., 1985) in response to adverse weather.

2.5.2. Hypocalcemia prevention

A range of strategies have been researched to provide practical tools for farmers to prevent hypocalcemia; however, the practicality and effectiveness of these techniques differ between grazing and housed systems. The supplementation of magnesium (Mg) before calving is a common technique to prevent hypocalcemia in grazing dairy cows (Roche, 2003; Roche and Berry, 2006). This is either dusted on pasture (magnesium oxide), given as an oral drench (magnesium chloride or magnesium sulfate), top-dressed or mixed into supplementary feed (magnesium oxide), or provided in the water supply (magnesium chloride or magnesium sulfate) (Roche, 2003). As discussed in section 2.3.2, adverse weather can limit feed intake (Schütz et al., 2010a), leading to a reduction in Mg intake. Furthermore, the relatively greater levels of potassium (K; Metson and Saunders, 1978a) and nitrogen (Metson and Saunders, 1978b) in the pasture during early spring (around the time of calving) can increase the pH of the rumen, resulting in poor absorption of dietary Mg (reviewed by Martens et al., 2018). Cows do not have readily available stores of Mg, therefore, Mg is typically supplemented from 2 to 3 weeks pre-calving and through early to mid-lactation. Magnesium is important for the release of PTH when blood Ca concentrations are low and it facilitates the response to PTH in the bones and kidneys (Goff, 2006; Goff, 2008) preserving the mechanisms involved in Ca homeostasis and preventing hypomagnesemia. Grazing cows are also supplemented with Limeflour, a source of Ca, during the colostral period to increase the amount of Ca present in the immediate postpartum diet (Roche, 2003). Nevertheless, despite these strategies substantially reducing the risk of milk fever in New Zealand dairy systems, subclinical hypocalcemia remains highly prevalent as demonstrated by Roberts and McDougall (2019) and clinical milk fever can still be an issue on many farms.

Dietary cation-anion difference (DCAD) formulation is a technique commonly used to prevent hypocalcemia in housed cows fed TMR. The DCAD describes the balance of cations [sodium (Na^+), K^+, Ca^{2+}, and Mg^{2+}] to anions [chloride (Cl^-), sulfate (SO_4^{2-}), and phosphate (PO_4^{3-})] in the diet (Goff, 2006). These ions are subsequently absorbed into the blood. To maintain electroneutrality, the blood gains hydroxide ions and loses hydrogen ions (as carbonic acid) when a greater number of cations to anions are absorbed (Goff, 2000, 2006). As a result, the pH of the blood increases (metabolic alkalosis). Conversely, increasing the number of anions in the diet and reducing the number of cations, achieved by adding anionic salts of chloride and sulfate to the diet and feeding low-K feeds such as maize silage (Roche, 2003; Roche et al., 2003), reduces DCAD and induces a partially compensated metabolic acidosis that can increase the sensitivity of the PTH receptor on surface of the bone and kidney cells, thereby reducing the incidence of milk fever in dairy cows (Block, 1984; Goff, 2008; Santos et al., 2019). However, it is impractical to implement in grazing systems due to the high DCAD in the pasture, which is driven by a high K content (Roche, 2003).

A further strategy used to prevent hypocalcemia involves promoting a negative Ca balance prepartum. This stimulates the activation of the Ca homeostatic mechanisms before the onset of lactation, resulting in a better response to the high Ca demand after calving (Thilsing-Hansen and Jørgensen, 2001; Goff, 2008). Although adjusting the prepartum diet to be low in Ca (<20 g/d of absorbable Ca) was found to be effective in inducing a negative Ca balance and preventing hypocalcemia in housed cows fed TMR (Boda and Cole, 1954; Thilsing-Hansen and Jørgensen, 2001; Goff, 2008), it is difficult to implement in grazing systems and has been largely abandoned in favor of DCAD strategies in housed systems. Instead, reducing the prepartum feed intake can help limit the amount of Ca and K ingested, and, therefore, improve blood Ca status at calving (Roche et al., 2013). Conversely, cows that are offered large amounts of feed precalving may have lower postcalving feed intake, and consequently lower Ca intake, resulting in lower blood Ca concentrations and an increased risk of parturient hypocalcemia (Roche et al., 2005; Roche, 2007).

Supplementation with synthetic zeolite A (sodium aluminosilicate) during the pre-calving period has been investigated as an alternative strategy to promote negative Ca balance prepartum. Zeolite is a microporous mineral that can bind dietary cations (Ca and Mg) and some anions (e.g., phosphorus, P) in the digestive tract (Mumpton, 1999; Thilsing-Hansen et al., 2002), reducing the absorption of these ions into the blood and, consequently, influencing Ca metabolism. Furthermore, prepartum feed intake is reportedly lower in zeolite-fed cows (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009; Kerwin et al., 2019), further leading to less dietary Ca absorbed by the cow. Supplementation of between 500 and 1000 g/d zeolite for 2 to 4 weeks prepartum has been demonstrated to improve the postcalving blood Ca concentrations in both housed (Thilsing-Hansen and Jørgensen, 2001; Thilsing-Hansen et al., 2002; Kerwin et al., 2019) and grazing cows (Roche et al., 2018; Crookenden et al., 2020), thereby reducing the risk of hypocalcemia.

2.5.3. The effect of zeolite supplementation on cow behavior

To our knowledge, the effect of the pre-partum supplementation of zeolite on the eating time, lying behaviors, or activity of dairy cows during the transition period has not been studied. However, feed intake is reportedly lower in cows fed zeolite (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009; Kerwin et al., 2019), with greater reductions in feed intake observed at higher doses of zeolite (Grabherr et al., 2009). This effect could be related to the poor palatability of zeolite (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009) and altered rumination and digestion patterns (Kerwin et al., 2019). Furthermore, deficiencies in P are associated with reduced DMI (Kincaid et al., 1981), and zeolite reduces P concentrations in the blood (Thilsing-Hansen et al., 2002; Grabherr et al., 2009; Kerwin et al., 2019), leading researchers to suggest zeolite-induced hypophosphatemia as an alternative explanation to the observed reductions in feed intake (Thilsing-Hansen et al., 2002; Grabherr et al., 2009). Supporting this is the observation that zeolite-fed cows not supplemented with P had higher refusals, and consequently lower DMI, than those supplemented with P (Thilsing et al., 2007). Therefore, while the effect of zeolite supplementation on blood mineral status has been demonstrated, the effects on feeding intakes and rumination times demonstrated in housed cows indicate that feeding zeolite may have other physiological effects that are linked to behavior.

Hence, other behaviors such as lying and activity must be investigated in zeolite-fed cows to determine the wider implications of this strategy. Given that lying times are increasingly being used in animal welfare criteria internationally and that hypocalcemia is known to affect lying and activity behaviors in both housed (Jawor et al., 2012; Barraclough et al., 2020b; Tsai et al., 2021) and grazing cows (Hendriks et al., 2020a), an investigation into the effects of zeolite on these behaviors is warranted.

2.6. Aims and objective

The existing literature regarding prepartum zeolite supplementation in housed cows indicates zeolite has an anorexic effect during the supplementation period, which may be mediated by changes in feeding behavior. Furthermore, the improved Ca status at calving due to precalving zeolite supplementation could affect lying and activity behavior, which may reflect a positive effect of zeolite on cow health and comfort around the time of calving. Therefore, I hypothesize that: (1) grazing cows supplemented with synthetic zeolite A during the prepartum period will spend less time eating than non-supplemented control cows, and (2) the peripartum lying times will be shorter, and activity greater, in cows supplemented with zeolite precalving, particularly in older cows who are at greater risk of developing hypocalcemia.

The main objective of this Thesis is to determine the effects of parity and prepartum supplementation of synthetic zeolite A on the eating, lying, and activity behaviors in grazing dairy cows during the transition period.

CHAPTER 3. MATERIALS AND METHODS

3.1. Animal handling and experimental design

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations (RAEC #13871), as per the New Zealand Animal Welfare Act (Ministry of Primary Industries, 1999). This experiment was conducted at Lye Farm, Hamilton, New Zealand (37°46'S, 175°18'E) between June 21 and August 16, 2016. The experimental design was described by Roche et al. (2018), with additional detail provided in Crookenden et al. (2020). Briefly, 50 late-gestation cows of mixed age (mean ± standard deviation (SD) 5.2 ± 1.8 years) and breed (Holstein-Friesian, n = 45; Holstein-Friesian x Jersey, n = 5) that were nearing their second or greater parity were randomly allocated to 1 of 2 treatment groups (Control, n = 25; or Zeolite, n = 25). The two groups were assessed to ensure they were balanced for expected calving date (range: 5 to 12 July 2016; actual calving date range: 30 June to 20 July 2016), age, previous milk production, and genetic merit within a balanced selection index (i.e., New Zealand Breeding Worth; NZAEL, 2016), as well as body condition score (BCS) and body weight (BW) before enrollment. Cows were managed to achieve a target BCS of 5.0 (mean BCS ± SD = 4.7 ± 0.4 units; 10-point scale, where 1 is emaciated and 10 obese; Roche et al., 2004) at the start of the experimental period (~2 weeks before expected calving date).

Precalving, all cows grazed a mixture of fresh pasture daily (82 ± 7.4% perennial ryegrass, *Lolium perenne*; 9 ± 7.7% white clover, *Trifolium repens;* 6 ± 2.3% weeds and other grasses; 3 ± 0.9% dead material; mean ± SD). Cows from both treatments were grazed together, with grazing areas adjusted daily to provide a common allowance that targeted pasture intakes of 9 kg DM/cow per d. Pasture quality variables averaged: 11 ± 0.2 MJ ME/kg DM; DM 13 ± 2.7% fresh weight; CP 22.0 ± 2.29% DM; NDF 39.3 ± 0.85% DM; ADF 19.7± 0.08% DM; Lipid 4.3 ± 0.32% DM; soluble sugars and starch (SSS) 13.3 ± 3.89% DM; and Ash 9.6 ± 0.50 % DM. During the precalving treatment period, all animals were brought to a covered facility between approximately 0700 – 1000 h for sampling and measurements and then individually fed 5 kg (wet weight) of maize silage (10 ± 0.2 MJ ME/kg DM; DM 36 ± 2.1% fresh; CP 8.1 ± 0.32% DM; NDF 34.5 ± 1.18% DM; ADF 28.8 ± 1.80% DM; Lipid 4.6 ± 0.19% DM; SSS 39.5 ± 2.10% DM; Ash 4.5 ± 0.21% DM; Ammonia-N 127.9 ± 15.05 mg/100 g DM) before a fresh allocation of pasture grazed *in situ*. Control cows received maize silage only and cows allocated to the Zeolite treatment group received 500 g/cow per d of zeolite A (80% sodium aluminosilicate, synthetic embedded in starch; XZelit, Optimate MF+, Blue Pacific Minerals, Tokoroa, New Zealand) mixed into the maize silage (Crookenden et al., 2020). Consistent with standard farm practice, cows were supplemented with magnesium by dusting pasture with magnesium oxide daily [Causmag (Causmag International, Young, NSW, Australia); 55% magnesium] to target an application rate of 80-100 g/cow per d.

The start of the treatment period relative to the actual day of calving varied between cows, as it was based on their expected calving date. Cows were separated into two cohorts, with staggered treatment start dates (either 21 or 24 June 2016) to achieve a target treatment duration of 14 d before their expected calving date (mean ± SD treatment duration for Zeolite = 18.2 ± 3.6 d and Control = 20.6 ± 4.1 d). Supplementation with zeolite stopped at calving. Postcalving, lactating cows from both precalving treatments grazed together on fresh perennial ryegrass and white clover pasture (88 ± 6.6% perennial ryegrass, *Lolium perenne*; 3 ± 1.6% white clover, *Trifolium repens;* 6 ± 1.9% weeds and other grasses; 3 ± 0.9% dead material). A daily allowance of >35 kg DM to ground level/cow per d was offered to achieve target residuals of 1500 to 1600 kg DM/ha per pasture break following a 24-h grazing period. Pasture quality attributes during the postcalving experimental period averaged: 11 ± 0.0 MJ ME/kg DM; DM 16 ± 1.7% fresh weight; CP 21.1 ± 0.16% DM; NDF 37.0 ± 0.11% DM; ADF 19.0 ± 0.11% DM; Lipid 4.5 ± 0.03% DM; SSS 15.8 ± 0.55% DM; and Ash 9.5 ± 0.78% DM. Maize silage was offered to the lactating herd once daily at an average of 5 kg (wet weight)/cow per d. The cows were supplemented with pasture silage if target grazing residuals could not be met due to insufficient pasture. Cows also received 200 g/cow per d of ground limestone (calcium carbonate) for the first 4 d after parturition (colostrum period). Samples of feeds offered were collected weekly for the determination of DM content (all feeds) and botanical composition (pasture only). The nutritional quality of feeds offered was determined monthly by pooling the samples collected weekly for each feed type. The monthly pooled samples were submitted for NIRS analysis at the Nutrition Laboratory, Riddet Innovation, Massey University, New Zealand.

3.2. Milk production, BW, and BCS

Cows were milked twice daily following parturition and were away from pasture between approximately 0645 – 0830 h and 1445 – 1600 h. Individual milk yields were recorded at each milking (Westfalia Surge Metatron Milk Meter; GEA Farm Technologies, Cambridge, New Zealand). Milk samples (1.25% of total volume) were collected once weekly using in-line milk meters at consecutive p.m. and a.m. milkings and combined to give one daily sample per cow. Composite milk fat, total protein, and lactose concentrations were determined by an infrared milk analyzer (Milkso-Scan FT1; Foss Electric A/S, Hillerod, Denmark). Milk component data were verified by reference techniques for a subset of milk samples [milk fat by the Rose-Gottlieb method (IDF, 1987); CP by the Kjeldahl technique (Barbano et al., 1991); lactose by the chloramine-T method (Amin et al., 1982)]. Energy-corrected milk (**ECM**) yield was calculated using the following equation (Nielsen et al., 2009):

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

Body weight and BCS were measured weekly for all cows from before treatment started until 4 weeks postpartum. Cows were assigned a BCS on a 10-point scale (Roche et al., 2004) by a trained assessor.

3.3. Blood sampling and analyses

Blood samples (~7 mL) were collected from each cow by coccygeal venipuncture into lithium heparin vacutainers (BD Bioscience, Plymouth, UK) before the start of treatment at 14 to 16 d before the expected calving date (mean ± SD 19.4 ± 4.0 d before actual calving date), and then at least weekly to target d −7 precalving relative to the expected calving date, the day of calving (**Cday 0**), and d 1, 2, 3, 4, 7, 14, 21, and 28 (± 1) postcalving. A subset of 10 cows per treatment had additional blood samples collected using lithium heparin vacutainers daily precalving from the start of treatment to 10 d postcalving, as well as on d 18 and 24 postcalving. For all blood samples, tubes were immediately inverted after collection and placed on ice; within 30 min, the tubes were centrifuged at 1,500 x *g* for 12 min at 4˚C, the plasma was aspirated, and aliquots were stored at -20˚C until assayed.

Plasma was analyzed by Gribbles Veterinary Pathology Ltd. (Hamilton, New Zealand) using colorimetric techniques at 37˚C with a Hitachi Modular P800 analyzer (Roche Diagnostics, Indianapolis, IN) and Roche reagent kits to determine the concentrations of calcium (Ca; mmol/L; 5-nitro-5′-methyl-(1,2-bis(o-aminophenoxy)ethan-*N*,*N*,*N′*,*N*′-tetraacetic acid (NM-BAPTA) method; CA2 Kit), Mg (mmol/L; xylidyl blue reaction Kit), inorganic P (mmol/L; reactivity with ammonium molybdate to form ammonium phosphomolybdate without reduction in presence of sulfuric acid; PHOS2 Kit), and β-hydroxybutyrate (BHB; mmol/L; reduction of NAD^+ to NADH during oxidation of d-3-hydroxybutryate to acetoacetate; BOH Kit). Plasma non-esterified fatty acid (NEFA; mmol/L) concentration was measured using the acyl Co-A synthetase, acyl-CoA oxidase (ACS-ACOD) colorimetric method (NEFA C Kit; Wako Chemicals, Osaka, Japan). The inter- and intra-assay coefficients of variation were between 0.8 and 5.3% and between 0.5 and 15%, respectively.

3.4. Behavioral measurements

Lying and activity behaviors. Lying and activity behavior data, recorded using triaxial accelerometers, were available for analysis of the experimental period [–21 to +28 d relative to the day of calving (Cday 0)]. The IceQube devices (IceRobotics Ltd., Edinburgh, Scotland) used in this study have been described in detail by Hendriks et al. (2019). Briefly, each cow was fitted with a device just above the fetlock joint of a hind leg before the start of the experimental period. The devices were housed in a plastic case and recorded the position and movement of the leg through the three-dimensional space (i.e. x-, y- and z-axes). Lying time was recorded when the device was horizontal, based on the orientation of the hind leg, and a lying bout was defined as the period between the device moving from a vertical position to horizontal and back to vertical. The device measured step count as the number of times a cow lifted her leg and placed it back down (IceRobotics, 2020).

Data were stored on the device's memory and then downloaded using the IceManager 2010 software (IceRobotics Ltd.) at the end of the experimental period. One summary file containing information on the time spent lying (s) and standing (s), as well as the step count, in 15-min intervals was generated per device. An additional file contained data for the start and end times for individual lying bouts within day. The hourly lying time (min/h), total daily lying time (h/d), daily number of lying bouts (no./d), mean lying bout duration (min/bout), hourly step count (steps/h), and daily step count (steps/d) were calculated for each individual cow from these two files. The use of IceQube devices to record daily lying times has been validated previously against visual observations (Mattachini et al., 2013; Borchers et al., 2016).

Eating behavior. Cows were fitted with CowScout neck sensors (GEA, New Zealand; manufactured as Smarttag neck sensors by Nedap Livestock Management, Groenlo, The Netherlands) to measure eating time. The sensor was housed in a plastic casing attached to a neck collar (Nedap, 2017), and recorded the position and movement of the head through the three-dimensional space. Data were stored on the device until it came in proximity to the base receiver (at least once daily), where it was automatically assigned a timestamp aligned to the nearest 15 min (either 14, 29, 44, or 59 min past the hour) and uploaded to the server (Dela Rue et al., 2020). The time spent eating (min) was determined for every 15-min interval by a proprietary algorithm and this data file was kindly made available by the sensor manufacturers for each individual cow to calculate both hourly eating time (min/h) and total daily eating time (h/d). The use of CowScout neck sensors for measuring eating time has been validated for grazing cows using visual observations (Dela Rue et al., 2020).

3.5. Weather

Data for daily rainfall (mm; 24-h period), minimum air temperature at 0900 h (°C), and maximum daily wind speed (m/s) were retrieved from The National Climate Database (NIWA, 2021) for the duration of the experiment. Data were retrieved from the nearest weather station ~3 km from the study site (agent number 26,117; 37.8°, 175.3°E). The mean ± SD for daily rainfall, minimum air temperature at 0900 h, and maximum daily wind speed during the experimental period were 4.5 ± 6.59 mm (range = 0 to 30.8 mm), 5.4 ± 3.75˚C (range = -3.3 to 14.6 °C), and 2.2 ± 1.73 m/s (range = 0.3 to 8.3 m/s), respectively.

3.6. Data editing

For ease of data interpretation relative to the day of calving, the sampling dates for all data were assigned a day relative to the actual calving date (**Cday**), where Cday 0 was equal to the day of calving, which was the basis of subsequent analyses. An alternative approach for the interpretation of data relative to treatment start is presented in Appendix 7.

Of the 50 cows enrolled, 43 cows were included in the final analysis of lying and activity behaviors derived from the IceQube dataset. Six cows were removed from analyses due to inaccessible files or incomplete data, defined by Hendriks et al. (2020) as >10 d of data missing between Cday –5 to +10, and one cow was removed due to missing treatment before calving. In addition, data from one cow were excluded from her recorded day of calving onwards due to inadvertently receiving 350 ml Calform Plus (≥10 to ≤50% calcium diformate; Bayer New Zealand Ltd., Auckland, NZ) for milk fever prevention without exhibiting clinical signs, which could influence her subsequent data. A further two cows in the CowScout dataset were excluded from the eating time behavior analyses, as they pretreatment lacked data needed as a covariate. In addition, the following criteria were used to remove data on particular days for individual cows in the IceQube dataset: 1) when data were incomplete (n = 41 observations), as indicated by the daily sum of time spent lying and standing being <24 h; and 2) on the day a device fell off (n = 1 observation). Lying bouts were also removed from the raw data if they were <33 s (Hendriks et al., 2020). In the CowScout dataset, data were removed on particular days for individual cows if eating time was equal to 0 h (n = 6 observations), as it is unlikely a cow will not eat within a 24-h period.

Adjustment of calving day. Calf collection occurred once daily at ~0900 h and the day of calving (Cday 0) for each cow was recorded by farm staff as the same day her calf was collected from the paddock and the cow was brought to the parlor for her first milking in the afternoon.

However, the cow could have calved anytime within 24 h before calf collection. Previous authors have reported that the lying bout number increases on the day of calving (Huzzey et al., 2005; Borchers et al., 2017). The calving day was reassigned using the criterion described by Hendriks et al. (2020), where if the number of lying bouts was >14 on the day before the recorded calving date, it was assumed that the cow calved on the previous day (Cday –1), and her data were adjusted accordingly. Otherwise, the recorded day of calving was assumed to be correct. The calving day was reassigned for 21 of 43 cows (49%).

Identification of possible outliers. Behavior data were categorized into three periods across the transition period: **PRE** (–21 to –3 d precalving), **PERI** [–2 to +2 d relative to the day of calving (Cday 0)], and **POST** (3 to 28 d postcalving). The SHEWHART procedure in SAS 9.4 (SAS Institute Inc., Cary, NC) was used to identify a list of possible outliers using a 3-d moving average and upper and lower control limits of 3 SD from the mean for all behavior measures. Individual charts for eating time, lying time, step count, number of lying bouts, and lying bout duration were created for each individual cow during the PRE and POST periods (Supplemental Figure 1) and data points outside the critical limits (i.e., > 3 SD from the mean) were identified (lying time, n = 3; the number of lying bouts, n = 12; average duration of lying bouts, n = 10; step count, n = 77; eating time, n = 18). However, no outliers were outside the realms of possibility. It was, therefore, decided that there was no justifiable case to exclude any further data points from the analysis.

3.7. Statistical analyses

Statistical analyses were performed using SAS 9.4. The cohort treatment start date (categorical: cohort 1 or cohort 2) and parity (categorical: Parity 2 to 3 or 4+) were included in all analyses. The htype=1 option was specified in all repeated measure ANOVA to include the fixed effects sequentially. The repeated measures models were pairwise comparison adjusted using Tukey-Kramer. Variables were checked for multicollinearity; however, no variables were highly correlated, had tolerance values less than 0.1, or variance inflation factors greater than 10. Significant effects were declared at $P < 0.05$. Least squares mean, standard error of the difference (**SED**) are presented in the text, tables, and figures, and Tukey-adjusted P-values are presented in the text and tables.

Milk production, BW, and BCS. Data for BW and BCS of the 43 cows used in the final behavior analysis precalving, and of the 42 cows used in the final behavior analysis postcalving were summarized into 7 weeks, relative to the adjusted day of calving (Cday 0) [week –3 (d –21 to –15), week –2 (d –14 to –8), week –1 (d –7 to –1), week 1 (d 0 to d 6), week 2 (d 7 to 13), week 3 (d 14 to 20), week 4 (d 21 to 27)]. Milk production measurements (yields of milk, fat, protein, lactose, and ECM; milk, fat, protein, and lactose percentages) of the 42 cows used in the final behavior analysis postcalving were summarized for weeks 2, 3, and 4 postcalving. Milk production, BW, and BCS were analyzed using repeated-measures ANOVA (PROC MIXED) to investigate the effects of treatment, parity, week relative to the day of calving, and their 2- and 3-way interactions. Week was defined as the repeated measure, cow as a random effect and treatment, parity, week, and their interactions as fixed effects. Cohort and treatment duration (calculated as the days from the treatment start date to calving date) were also included as fixed effects in all models. Body weight and BCS measured during the week pre-treatment were also included as fixed effects as covariates in the models corresponding to the outcome of interest for the analyses of BW and BCS. Compound symmetry covariance structure was used for all models.

Plasma minerals and metabolites. After adjustment of calving day, it was determined that, of the 43 cows used in the final behavior analysis precalving and of the 42 cows used in the final behavior analysis postcalving, all cows had a pretreatment covariate blood sample (mean ± SD: 19.4 ± 4.0 d before actual calving date) and most cows had blood samples occurring on Cday -17 (± 3 d), -10 (± 3 d), the day of calving (Cday 0), and Cday 1, 4 (± 2 d), 7 (± 2 d), 14 (± 2 d), 21 (± 2 d), and 28 (± 2 d). Plasma mineral (Ca, Mg and P) and metabolite (BHB and NEFA) concentrations in the nearest sample to the day of interest were analyzed using repeated-measures ANOVA to investigate the effects of treatment, parity, week relative to the day of calving, and their 2- and 3-way interactions. Cday was defined as the repeated measure, cow as a random effect and treatment, parity, week, and their interactions as fixed effects. The pretreatment covariate corresponding to the outcome variable, cohort, and treatment duration (the number of days from the treatment start date to calving date) were also included as fixed effects in all models. Compound symmetry covariance structure was used for all models.

Daily eating, lying, and activity pretreatment measures. The eating behavior data collected during the 2 d before treatment start date (overall mean ± SD = –21.4 ± 4.6 d relative to calving) and lying and activity behavior data collected during the 3 d before treatment start date (overall mean ± SD = –21.6 ± 4.8 d relative to calving) were averaged (PROC MEANS) for each individual cow to create a pretreatment covariate for each corresponding behavior of interest.

Behavior analyses during the transition period relative to the day of calving. Each period (PRE, PERI, and POST) was analyzed separately using a repeated-measures ANOVA to investigate the main effects of treatment, parity, experimental day relative to the day of calving (Cday), and their 2- and 3-way interactions for all five behavior measures (daily eating time, lying time, number of lying bouts, mean lying bout duration, and number of steps taken). The Cday was defined as the repeated measure, cow as a random effect and treatment, parity, Cday, and their interactions as fixed effects. The pretreatment covariate behavior corresponding to the outcome variable, cohort, daily rainfall (continuous: mm), minimum air temperature at 0900 h (continuous: ˚C), and maximum daily wind speed (continuous: m/s) were also included as fixed effects in all models to account for their possible effects on behavior. Large within- and across-day variation in behavior was present between individual animals. After comparing different models with raw daily data or 3-d moving averages, it was determined that a 3-d moving average for both weather and behavior variables during the PRE and POST periods should be used when examining differences between treatment and parity groups. However, raw daily data were used for the PERI period due to the parturition event. Autoregressive and compound symmetry covariance structures were tested, and the lowest Akaike's Information Criterion (**AIC**) value was used to identify compound symmetry as the appropriate covariance structure. An alternative approach for the interpretation of daily eating time, lying time, the number and duration of lying bouts, and step count data relative to treatment start is presented in Appendix 7.

***Within-day eating, lying, and activity measures relative to calving*.** The hourly eating, lying and activity data within a 24-h period were summarized into six 4-h intervals (0200 to 0559 h, 0600 to 0959 h, 1000 to 1359 h, 1400 to 1759 h, 1800 to 2159 h, and 2200 to 0159 h). The eating behavior data collected 2 d before the treatment start date and lying and activity behavior data collected 3 d before treatment start date were averaged (PROC MEANS) for each interval for individual cows to create a pretreatment covariate for the corresponding behavior of interest.

The main effects of treatment, parity, interval, and all their interactions for eating time, lying time, and step count were analyzed separately for the PRE period, d –2 and d –1 precalving, the day of calving (Cday 0), d 1 and d 2 postcalving, and the POST period using a repeated-measures ANOVA. Interval was defined as the repeated measure, cow as the random effect, and treatment, parity, interval, and their interactions as fixed effects. The pretreatment covariate behavior corresponding to the outcome variable, and cohort were included as fixed effects for all periods, while daily rainfall, minimum air temperature at 0900 h, and maximum daily wind speed were included as fixed effects for d –2 and d –1 precalving, Cday 0, and d 1 and d 2 postcalving only. Heterogenous Toeplitz was identified as the appropriate covariance structure. An alternative approach for the interpretation of within-day eating time, lying time, and step count data relative to treatment start is presented in Appendix 7.

CHAPTER 4. RESULTS

4.1. Descriptive statistics

Descriptive statistics for pretreatment covariate BCS, breeding worth, ECM yield, and age, along with the number of animals within each parity and breed group for cows included in the final analysis of behavior data are presented in Table 4.1. The mean daily eating time, daily lying time, the number of lying bouts, lying bout duration, and step count during the pretreatment period are also presented in Table 4.1. Treatment groups were balanced (within 1 SD) for age and breeding worth. There were no differences between the treatment, parity, and treatment x parity groups for pretreatment behaviors and BCS.

The mean ± SD for daily eating time and lying time across all cows for the entire experimental period (–21 to +28 d relative to calving) were 7.4 ± 1.39 h/d (range: 1.0 to 12.1 h/d) and 8.5 ± 2.19 h/d (range: 1.9 to 16.9 h/d), respectively. On average, cows engaged in 7.5 ± 3.18 lying bouts/d, with a mean lying bout duration of 74.6 ± 25.7 min/bout. The number and duration of lying bouts varied from 2 to 33 bouts/d and 9.27 to 193 min/bout, respectively. Cows took, on average, 3,821 ± 1,136 steps/d (range: 1,227 to 9,515 steps/d) across this period.

Table 4.1. Descriptive data for all cows by treatment (Control vs. Zeolite), parity (2–3 vs. 4+), and by treatment x parity groups (Control 2–3; Control 4+; Zeolite 2–3; Zeolite 4+). Number of cows (n) in each group and by breed, and the mean ± standard deviation for pretreatment covariate body condition score (Cov BCS), breeding worth (BrW), energy-corrected milk (ECM) yield, age, length of the precalving treatment period (treatment length), and pretreatment covariate (Cov) daily eating and lying time, number of lying bouts (LB), LB duration, and step count.

Parameter	Control		Zeolite		Overall Treatment		Overall Parity	
	2–3	4+	2–3	4+	Control	Zeolite	2–3	4+
n (cows)	11	11	8	13	22	21	19	24
Holstein Friesian (HF)	10	9	8	12	19	20	18	21
HF x Jersey	1	2	0	1	3	1	1	3
Cov BCS[1], units	4.5 ± 0.29	4.8 ± 0.39	4.6 ± 0.33	4.7 ± 0.41	4.6 ± 0.35	4.7 ± 0.38	4.6 ± 0.30	4.7 ± 0.39
BrW, $/5 t DM	153 ± 39.1	130 ± 35.1	135 ± 35.4	109 ± 43.4	141 ± 38.3	119 ± 41.6	146 ± 37.7	119 ± 40.3
BrW reliability, %	48.4 ± 1.63	55.4 ± 7.27	50.3 ± 1.67	55.7 ± 7.66	51.9 ± 6.27	53.6 ± 6.60	49.2 ± 1.86	55.5 ± 7.32
ECM yield[2], kg/d	22.9 ± 2.58	25.0 ± 3.36	22.8 ± 3.38	24.4 ± 3.12	23.8 ± 3.29	23.9 ± 3.13	22.8 ± 2.91	24.7 ± 3.21
Age, yrs	3.4 ± 0.50	6.5 ± 1.51	3.6 ± 0.52	6.2 ± 1.42	5.0 ± 1.96	5.2 ± 1.73	3.5 ± 0.51	6.4 ± 1.44
Treatment length[3], d	19.3 ± 5.0	18.7 ± 3.8	15.6 ± 2.7	16.5 ± 3.5	19.0 ± 4.5	15.9 ± 3.1	17.7 ± 4.5	17.5 ± 3.7
Cov daily eating time[4], h/d	7.2 ± 1.23	7.0 ± 0.79	7.3 ± 1.61	6.8 ± 1.136	7.1 ± 1.02	6.9 ± 1.30	7.2 ± 1.36	6.9 ± 0.99
Cov daily lying time[5], h/d	10.6 ± 3.39	10.7 ± 2.25	10.2 ± 2.26	10.1 ± 3.01	10.6 ± 2.86	10.1 ± 2.73	10.4 ± 2.95	10.4 ± 2.68
Cov number of LB[5], no./d	9.5 ± 2.90	8.7 ± 2.20	10.8 ± 5.22	9.9 ± 3.75	9.1 ± 2.61	10.3 ± 4.36	10.1 ± 4.07	9.3 ± 3.18
Cov LB duration[5], min/bout	72.9 ± 25.98	76.6 ± 17.30	66.5 ± 23.31	67.2 ± 24.27	74.7 ± 21.98	66.9 ± 23.72	70.2 ± 24.88	71.5 ± 21.73
Cov step count[5], steps/d	2,939 ± 1,475.0	3,021 ± 1,401.2	3,065 ± 1,405.7	3,027 ± 1,287.1	2,980 ± 1,428.1	3,042 ± 1,322.4	2,992 ± 1,434.8	3,024 ± 1,331.0

[1] Measured on a 10-point scale (1 is emaciated and 10 obese; Roche et al., 2004) at an average of –20.4 ± 4.8 d before calving (across all cows).

[2] Calculated as an average of 7 to 28 d of lactation.

[3] Precalving treatment length based on adjusted calving day. Calving day was adjusted for 21 cows.

[4] Measured at an average of -21.4 ± 4.6 d before calving (across all cows).

[5] Measured at an average of -21.9 ± 4.8 d before calving (across all cows).

4.2. Milk production, BW, and BCS

Least squares means and mean SED for the main effects of treatment and parity on BCS, BW, and milk production parameters are presented in Table 4.2, along with the *P*-values for the effect of treatment, parity, week, and their 2- and 3-way interactions. The *P*-values for pretreatment covariate, cohort, and treatment duration are presented in Supplemental Table 1. Week affected ($P \leq 0.05$) all milk production parameters and tended to affect ($P \leq 0.10$) ECM yield and milk fat yield but did not affect lactose percentage (Table 4.2). Milk yield and lactose yield increased, but ECM yield, milk fat percentage and yield, and protein percentage and yield decreased across weeks 2 to 4 postcalving (data not presented).

Parity 4+ cows had greater mean ECM and milk fat yields, with a tendency for greater milk ($P = 0.08$), protein ($P = 0.06$), and lactose ($P = 0.09$) yields than Parity 2−3 cows; milk fat, protein, and lactose percentages were not different between parities. There was a parity x week interaction for protein yield: Parity 4+ cows produced more protein during weeks 2 ($P = 0.02$) and 3 ($P = 0.03$) postcalving but were not different from the Parity 2–3 cows during week 4 postcalving ($P = 0.30$). A further parity x week interaction was detected for protein percentage; however, Tukey-pairwise comparisons within week indicated the two parity groups were not different. No further main or interactive effects of treatment or parity were observed for milk production parameters.

A significant parity x week interaction was detected for BW (Table 4.2): Parity 2–3 cows were heavier than the Parity 4+ cows during the first 4 weeks postcalving but were not different precalving (Figure 4.1a). Treatment did not affect mean BW during the transition period, nor were there any main or interactive effects of treatment or parity on BCS (Table 4.2; Figure 4.1b).

Table 4.2. The effect of feeding zeolite precalving and parity on body condition, body weight, and milk production parameters. Least squares mean and standard error of the difference (SED) for the main effects of treatment (Control, Zeolite) and parity (2−3, 4+) on body condition score (BCS) and body weight (BW) during the 3 weeks before and 4 weeks after calving, and milk production parameters during weeks 2 to 4 postcalving. *P*-values for the effects of treatment (Treat), parity, and week relative to calving (Week), and their 2- and 3-way interactions are also presented.

Parameter	Treatment			Parity			*P*-values						
	Control	Zeolite	SED	2-3	4+	SED	Treat	Parity	Treat x Parity	Week	Treat x Week	Parity x Week	Treat x Parity x Week
BCS[1], units	4.5	4.6	0.05	4.6	4.5	0.05	0.38	0.72	0.64	<0.001	0.84	0.56	0.74
BW, kg	517.6	512.4	4.59	520.0	510.0	5.63	0.19	0.09	0.12	<0.001	0.74	<0.001	0.63
Milk yield, kg/d	24.0	23.5	1.07	22.8	24.6	0.99	0.86	0.08	0.85	<0.001	0.92	0.82	0.22
ECM yield[2], kg/d	25.4	24.6	1.12	23.8	26.2	1.03	0.78	0.02	0.99	0.10	0.86	0.64	0.50
Milk fat yield, kg/d	1.06	1.01	0.055	0.97	1.10	0.050	0.66	0.02	0.91	0.09	0.90	0.84	0.83
Milk fat, %	4.42	4.32	0.176	4.26	4.48	0.162	0.76	0.19	0.78	<0.001	0.91	0.86	0.91
Milk protein yield, kg/d	0.85	0.84	0.034	0.81	0.87	0.032	0.92	0.06	0.91	<0.001	0.49	0.04	0.18
Milk protein, %	3.54	3.59	0.074	3.57	3.56	0.069	0.49	0.92	0.96	<0.001	0.24	<0.01	0.45
Milk lactose yield, kg/d	1.16	1.14	0.053	1.11	1.20	0.049	0.95	0.09	0.93	<0.001	0.73	0.82	0.42
Milk lactose, %	4.85	4.88	0.042	4.87	4.86	0.039	0.47	0.84	0.72	0.97	0.24	0.75	0.64

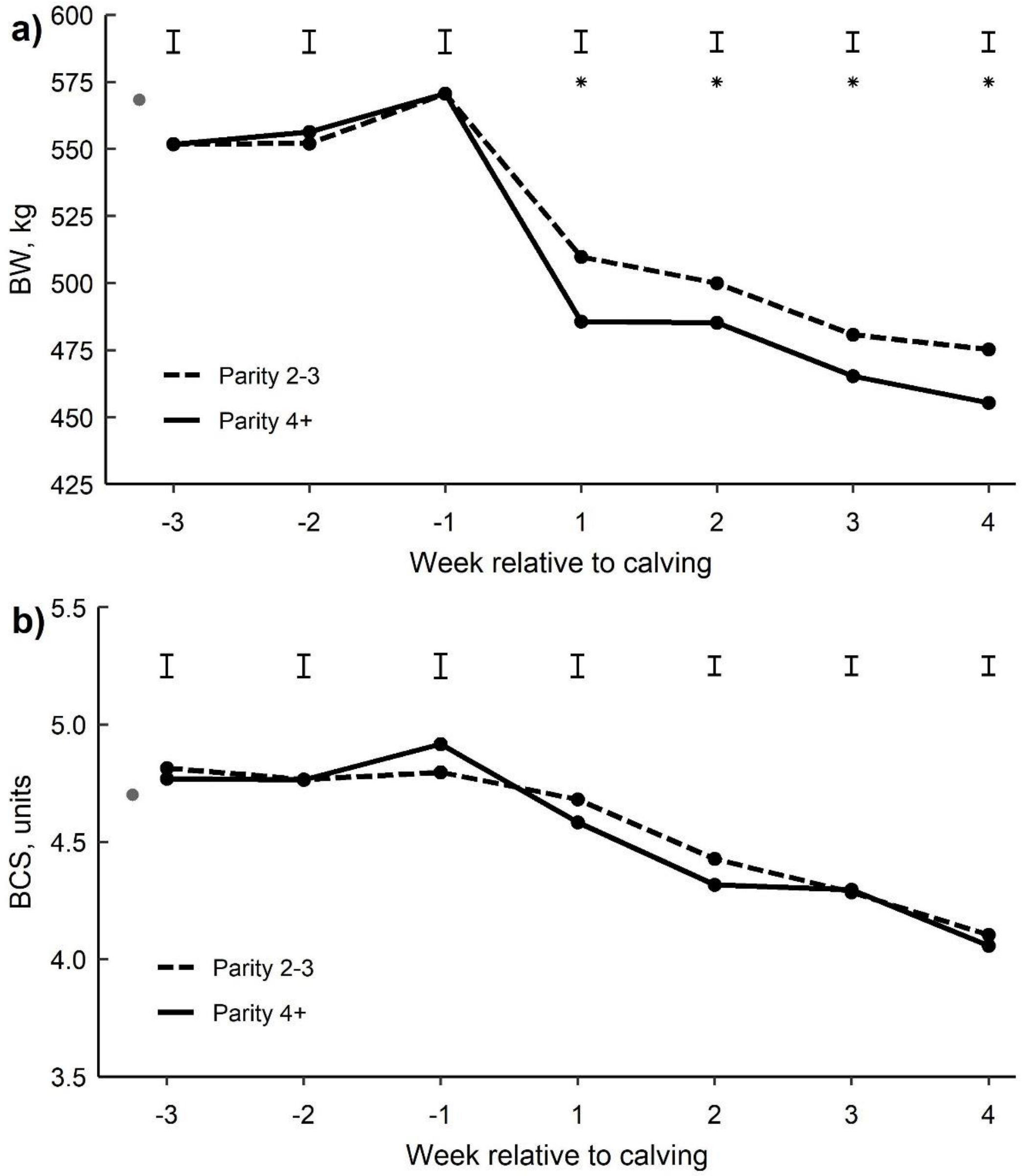

Figure 4.1. The effect of parity on body weight and body condition score during the transition period. Body weight [BW; a); kg] and body condition score [BCS; b); units (10-point scale)] of the two parity groups (Parity 2–3 and Parity 4+) across weeks –3 to 4 relative to the week of calving (week 1) are presented. The average pretreatment BW and BCS for all cows is presented as gray-filled circles. Error bars represent 1 x standard error of the difference. Parity effects on individual Cdays are represented by * ($P < 0.05$) when the overall interaction with Cday was significant.

4.3. Plasma minerals and metabolites

Means and SED for the main effects of treatment and parity on blood minerals and metabolites are presented in Table 4.3, along with the *P*-values for the effect of treatment, parity, Cday, and their 2- and 3-way interactions. The *P*-values for pretreatment covariate, cohort, and treatment duration are presented in Supplemental Table 2.

The 3-way interactive effects of treatment, parity, and Cday on plasma Ca concentrations are presented in Figure 4.2. Zeolite-fed cows had greater ($P < 0.001$) plasma Ca concentrations on the day of calving compared with Controls (2.3 vs. 2.0 mmol/L, respectively). Within both treatment groups, Parity 2−3 cows had greater blood Ca concentrations than Parity 4+ cows on the day of calving. Plasma Ca concentrations remained significantly lower on the day after calving in the Control 4+ cows; however, the Zeolite 2–3, Zeolite 4+, and Control 2–3 cows did not differ from one another. The prevalence of subclinical hypocalcemia using a threshold of Ca < 2.0 mmol/L on Cday 0 or 1 was 10%, 60%, 0%, and 0% for the Control 2–3, Control 4+, Zeolite 2–3, and Zeolite 4+ groups, respectively; whereas, the prevalence was 70%, 80%, 38%, and 33%, respectively, using a threshold of Ca ≤ 2.15 mmol/L on Cday 0 or 1. The prevalence of clinical hypocalcemia without paresis (Ca < 1.4 mmol/L on Cday 0 or 1) was 20% in the Control 4+ group but 0% in the other three groups, and there were no recorded cases of clinical milk fever.

The treatment x Cday interactions for plasma Mg and P are presented in Figure 4.3 a and b. Zeolite cows had lower ($P < 0.01$) Mg concentrations than Controls on the day of calving, the day after calving, and on Cday 14, and tended to have lower Mg on Cday 21 ($P = 0.05$) and 28 ($P = 0.12$). Zeolite cows also had lower ($P < 0.001$) plasma P concentrations on Cday −17, −10, 0, and 1, but had greater ($P < 0.001$) P concentrations on Cday 7 and tended ($P = 0.12$) to have greater blood P concentrations on Cday 21. Parity also affected plasma P concentrations (Table 4.3), whereby the Parity 4+ cows had lower P concentrations, on average, during the transition period.

The parity x Cday interaction for plasma NEFA concentrations is presented in Figure 4.4. Except for greater NEFA concentrations on Cday −17 ($P < 0.01$), plasma NEFA concentrations were lower in the Parity 2–3 cows on Cday 0 ($P < 0.01$), 4 ($P < 0.05$), and 21 ($P < 0.05$). No further main or interactive effects of treatment or parity on the concentrations of blood minerals or metabolites were detected (Table 4.3).

Table 4.3. The effect of feeding zeolite precalving and parity on blood minerals and metabolites. Least squares mean and standard error of the difference (SED) for the main effects of treatment (Control, Zeolite) and parity (2−3, 4+) on plasma calcium (Ca), magnesium (Mg), phosphorus (P), β-hydroxybutyrate (BHB), and non-esterified fatty acid (NEFA) concentrations during the −21 to 28 d relative to the day of calving (Cday 0). *P*-values for the effects of treatment (Treat), parity, and day relative to calving (Cday), and their 2- and 3-way interactions are also presented.

Parameter	Treatment			Parity			*P*-values						
	Control	Zeolite	SED	2-3	4+	SED	Treat	Parity	Treat x Parity	Cday	Treat x Cday	Parity x Cday	Treat x Parity x Cday
Ca, mmol/L	2.26	2.33	0.029	2.31	2.28	0.026	0.02	0.34	0.21	<0.001	<0.001	<0.01	0.01
Mg, mmol/L	0.92	0.87	0.020	0.89	0.90	0.019	<0.01	0.97	0.34	<0.001	0.03	0.47	1.00
P, mmol/L	1.53	1.19	0.056	1.45	1.27	0.050	<0.001	<0.01	0.54	<0.001	<0.001	0.93	0.39
BHB, mmol/L	0.60	0.62	0.038	0.59	0.63	0.035	0.55	0.23	0.73	<0.001	0.71	0.18	0.86
NEFA, mmol/L	0.98	0.96	0.069	0.91	1.03	0.071	0.99	0.05	0.47	<0.001	0.54	<0.01	0.58

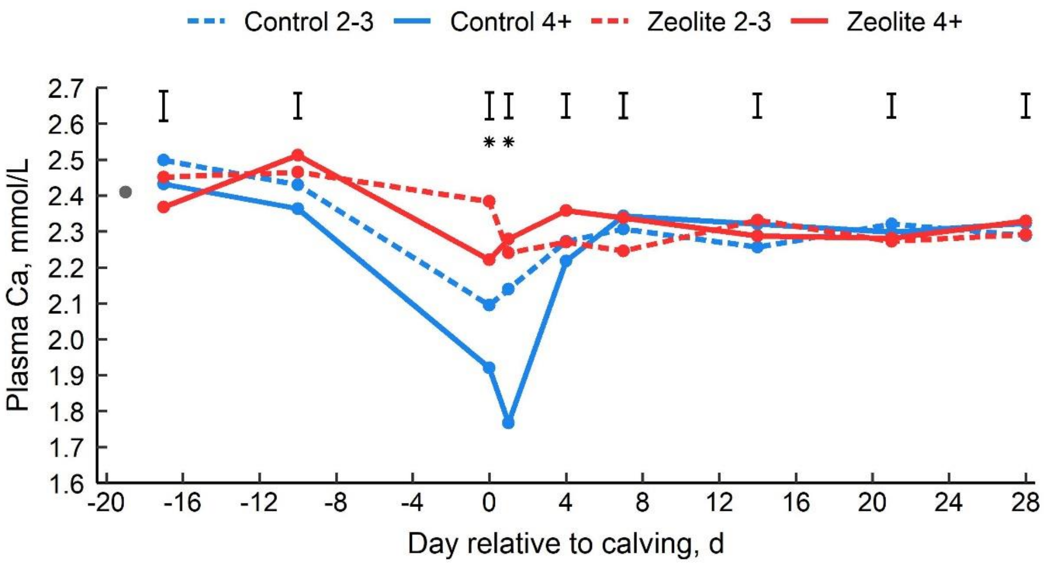

Figure 4.2. The effect of feeding zeolite precalving and parity on plasma calcium concentrations during the transition period. Plasma calcium (Ca) concentrations (mmol/L) of the four treatment x parity groups (Control 2–3; Control 4+; Zeolite 2–3; Zeolite 4+) across –17 to 28 d relative to the day of calving (Cday 0) are presented. The average pretreatment plasma Ca concentration for all cows (gray-filled circle at d –19) was measured between 14 and 16 d before the expected calving date (overall mean ± SD: –19.4 ± 4.0 d relative to the actual day of calving). Error bars represent 1 x mean standard error of the difference. Treatment x parity interactions on individual Cday are represented by * ($P < 0.05$) when the overall treatment x parity x Cday interaction was significant.

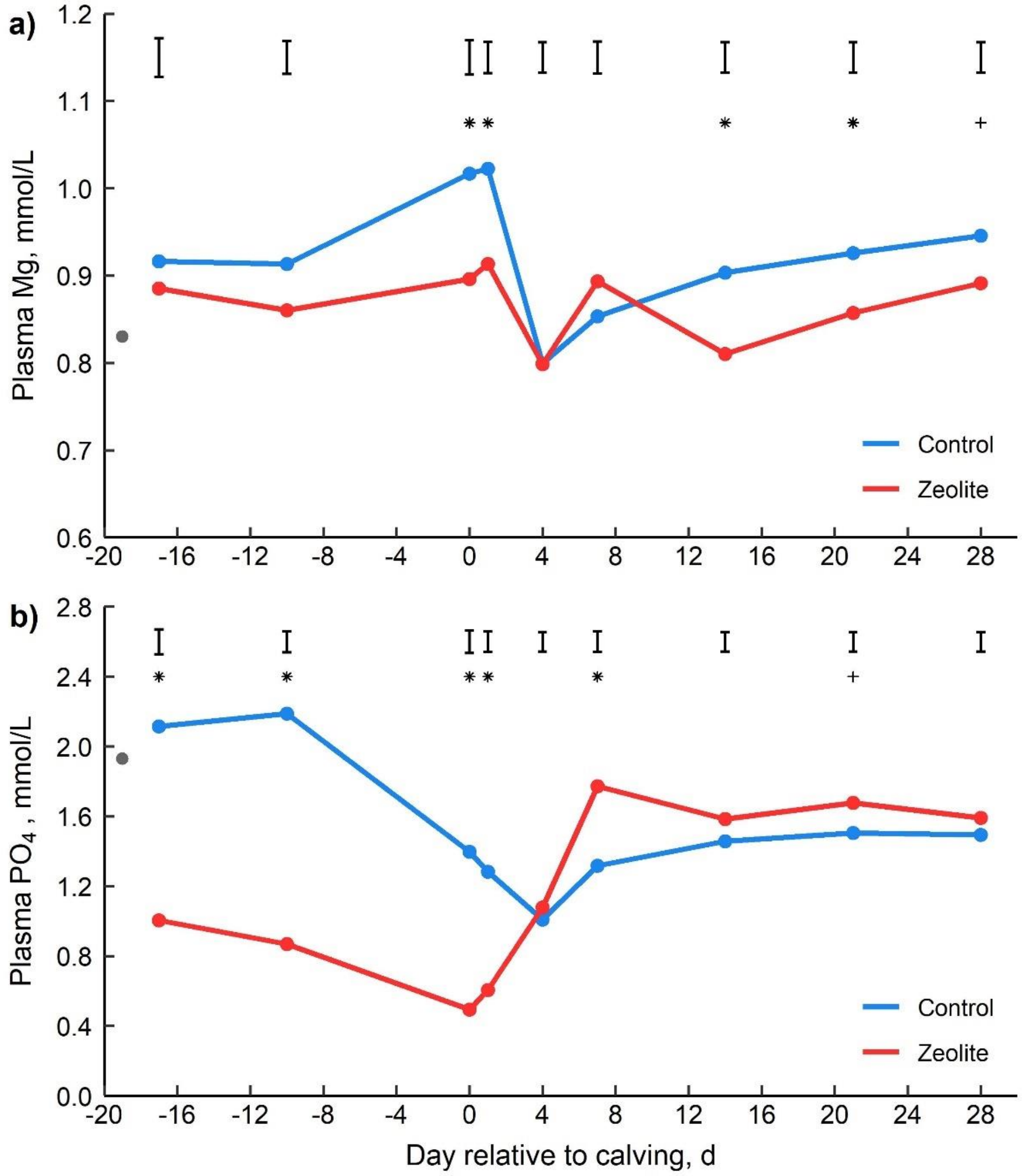

Figure 4.3. The effect of feeding zeolite precalving on plasma magnesium and phosphorous concentrations during the transition period. Plasma Magnesium (Mg) [(a); mmol/L] and phosphorous (P) concentrations [(b); mmol/L] of the two treatment groups (Control and Zeolite) across d –17 to 28 relative to the day of calving (Cday 0) are presented. The average pretreatment plasma Mg or P concentration for all cows (gray-filled circles at d –19) was measured between 14 and 16 d before the expected calving date (overall mean ± SD: –19.4 ± 4.0 d relative to the actual day of calving). Error bars represent 1 x standard error of the difference. Treatment effects on individual Cday are represented by * ($P < 0.05$) and + ($P < 0.15$) when the overall interaction with Cday was significant.

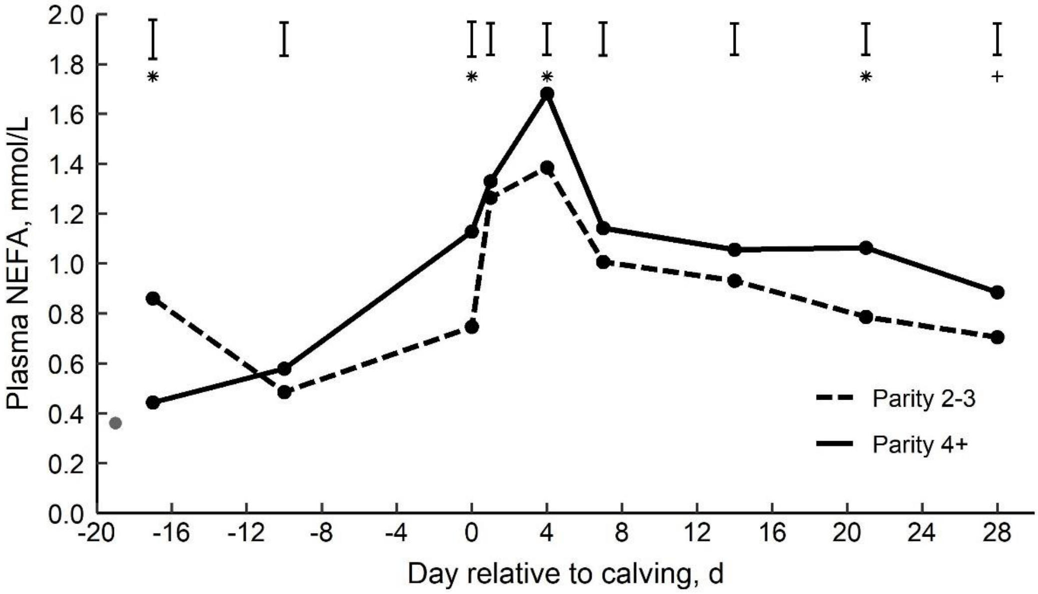

Figure 4.4. The effect of parity on plasma NEFA concentrations during the transition period. Plasma non-esterified fatty acids (NEFA) concentrations (mmol/L) of the two parity groups (Parity 2–3 and Parity 4+) across d –17 to 28 relative to the day of calving (Cday 0) are presented. The average pretreatment plasma NEFA concentration for all cows (filled circle at d –19) was measured between 14 and 16 d before the expected calving date (overall mean ± SD: –19.4 ± 4.0 d relative to the actual day of calving). Error bars represent 1 x standard error of the difference. Parity effects on individual Cday are represented by * ($P < 0.05$) and + ($P < 0.15$) when the overall interaction with Cday was significant.

4.4. Behavior during the transition period relative to the day of calving

Means and SED for the main effects of treatment and parity on eating, lying, and activity behaviors during the PRE, PERI, and POST periods are presented in Table 4.4, as well as the *P*-values for the effect of treatment, parity, Cday, and their 2- and 3-way interactions. The *P*-values for the three weather variables, pretreatment covariate behavior corresponding to the outcome variable, and cohort that were also included in the models are presented in Supplemental Table 3. There was a significant effect of Cday across all behavior measures and periods, with the exceptions of eating time PERI and lying time POST (Table 4.4).

Eating behavior. During the PRE period, the Parity 2–3 cows spent, on average, 48 mins longer eating/d than the Parity 4+ cows; however, there were also significant interactions for treatment x Cday, parity x Cday, and treatment x parity x Cday (Table 4.4). The 3-way interactive effects of treatment, parity, and Cday on daily eating time across d –14 to 28 relative to calving are presented in Figure 4.5 and indicate that the Parity 4+ Control and Zeolite cows spent a similar amount of time eating each Cday (Cday –14 to –2) during the PRE period (mean 6.9 h/d (range: 6.5 to 7.3 h/d) vs 6.9 h/d (range: 6.4 to 7.6 h/d). The Parity 2–3 Control cows were generally consistent in their greater daily eating time (mean 7.9 h/d, range: 7.4 to 8.5 h/d) during the PRE period, whereas daily eating time fluctuated across Cday in the Parity 2–3 Zeolite cows (mean 7.6 h/d, range: 6.7 to 9.3 h/d) (Figure 4.5).

During the PERI period, daily eating time was, on average, 24 min shorter/d in the Zeolite compared with Control cows and 36 min longer/d in the Parity 2–3 compared with the Parity 4+ cows, with no interactive effects (Table 4.4). There were no treatment effects on daily eating time during the POST period, but Parity 2–3 cows tended (*P* = 0.11) to spend, on average, 24 mins longer eating/d than Parity 4+ cows (Table 4.4).

Lying and activity behavior. The 2-way interactive effects of treatment and Cday and parity and Cday on daily lying time, step count, and the number and duration of lying bouts across d –14 to 28 relative to calving are presented in Figure 4.6 a – d and 4.7 a – d, respectively. There were no treatment effects on lying behavior during the PRE period (Table 4.4). There was a significant treatment x Cday interaction for step count (Table 4.4); however, comparisons within Cday indicated that the only significant difference between Control and Zeolite cows occurred at Cday –16 (3185 vs 2819 steps/d; *P* < 0.05), with no further treatment differences detected at any Cday (all *P* ≥ 0.12) during the PRE period (Figure 4.6c).

Parity affected lying and activity behaviors during the PRE period, as Parity 4+ cows were consistently less active, averaging 338 fewer steps/d and lying for 30 mins longer/d than Parity 2–3 cows (Table 4.4). A tendency ($P = 0.07$) for a 3-way interaction between treatment, parity, and Cday for lying time was evident during the PRE period (Table 4.4); however, Tukey-pairwise comparisons within Cday indicated the only significant differences ($P < 0.05$) between treatment and parity groups occurred on Cday –16 and d –5 precalving when Parity 2–3 Zeolite cows differed from Parity 2–3 Control cows and Parity 4+ Zeolite cows, respectively, but did not differ from Parity 4+ Control cows. No further consistent effects were detected (results not presented).

During the PERI period, mean daily lying time, the duration of lying bouts, and step count were not affected by treatment (Table 4.4). There was, however, a treatment x Cday interaction ($P = 0.05$) for the number of lying bouts (Figure 4.7a and Table 4.4): Zeolite cows engaged in 1.8 more bouts of lying than Control cows ($P = 0.03$) on the day after calving but were not different ($P \geq 0.23$) from Control cows during the 2 days before calving, on the day of calving, or Cday 2. Parity did affect lying time and step count during the PERI period and tended ($P = 0.08$) to affect lying bout duration, but with no parity effect on the number of lying bouts recorded (Table 4.4). Mean daily differences in lying behavior and activity between parity groups were greater than those recorded during the PRE period; Parity 4+ cows spent, on average, 48 min longer lying/d with 5.5 min longer bouts of lying; they also took 672 fewer steps/d than Parity 2–3 cows during the PERI period. There were no significant interactions between parity and Cday for lying and activity behaviors during the PERI period (Table 4.4).

During the POST period, Zeolite cows lay for 30 min longer/d than Control cows and spent, on average, 7.8 min longer lying during each bout (Table 4.4). There was also a significant treatment x parity x Cday interaction for the number of lying bouts (Table 4.4); however, Tukey-pairwise comparisons indicated there were no consistent significant differences within Cday between groups across the POST period (data not presented). There was a tendency ($P = 0.09$) for a treatment x Cday interaction during the POST period (Figure 4.6c and Table 4.4). The Zeolite cows took between 534 and 701 more steps/d than Control cows on Cday 13 to 15 postcalving (all $P < 0.05$), but there were no other significant differences within Cday between the treatment groups during the POST period (Figure 4.6c). While there were no effects of parity on lying behaviors POST (Table 4.4), Parity 2–3 cows tended ($P = 0.13$) to be more active than Parity 4+ cows, averaging 200 more steps/d during the POST period (Table 4.4).

Table 4.4. The effect of feeding zeolite precalving and parity on eating, lying, and activity behaviors during the transition period. Least squares mean and standard error of the difference (SED) for the main effects of treatment (Control vs. Zeolite) and parity (2–3 vs. 4+) within the PRE (–21 to –3 d), PERI (–2 to 2 d), and POST (3 to 28 d) periods relative to the day of calving (Cday 0) are presented along with P-values for the effects of treatment (Treat), parity and day relative to calving (Cday), and their 2- and 3-way interactions.

Behavior	Period	Treatment			Parity			*P*-values[1]						
		Control	Zeolite	SED	2–3	4+	SED	Treat	Parity	Cday	Treat x Parity	Treat x Cday	Parity x Cday	Treat x Parity x Cday
Eating time, h/d	PRE	7.4	7.3	0.23	7.7	6.9	0.23	0.21	<0.01	<0.001	0.54	<0.01	<0.001	0.01
	PERI	7.3	6.9	0.26	7.4	6.8	0.26	0.05	0.02	0.27	0.55	0.32	0.87	0.27
	POST	7.4	7.5	0.21	7.7	7.3	0.22	0.88	0.11	<0.001	0.55	1.00	0.62	0.99
Lying time, h/d	PRE	9.8	10.0	0.24	9.6	10.1	0.24	0.44	0.02	<0.001	0.65	0.45	0.58	0.07
	PERI	7.3	7.8	0.39	7.2	8.0	0.38	0.19	0.04	<0.001	0.99	0.88	0.83	0.74
	POST	7.5	8.0	0.24	7.7	7.8	0.24	0.03	0.57	0.79	0.72	0.46	0.90	0.44
Number of LB[2], no./d	PRE	7.1	6.7	0.41	6.8	6.9	0.40	0.22	0.84	<0.001	0.76	0.78	0.14	0.54
	PERI	11.0	10.9	0.57	10.8	11.1	0.54	0.83	0.53	<0.001	0.91	0.05	0.58	0.17
	POST	7.1	6.6	0.35	6.9	6.8	0.35	0.12	0.67	<0.001	0.26	0.90	0.48	<0.01
Mean LB duration, min/bout	PRE	88.8	95.0	4.03	89.3	94.5	3.89	0.14	0.14	<0.001	0.74	0.87	0.31	0.26
	PERI	48.2	50.8	3.09	46.7	52.2	2.94	0.20	0.08	<0.001	0.91	0.18	0.88	0.87
	POST	67.3	75.1	3.15	69.3	73.1	3.05	0.01	0.22	<0.001	0.14	0.73	0.18	0.34
Steps, steps/d	PRE	3,047	2,995	100.1	3,190	2,852	100.0	0.47	<0.01	0.03	0.26	0.03	0.99	0.44
	PERI	4,052	4,057	225.9	4,390	3,719	223.4	0.87	<0.01	<0.001	0.40	0.53	0.38	0.37
	POST	4,236	4,321	131.4	4,379	4,178	131.8	0.60	0.13	<0.001	0.21	0.09	1.00	0.66

[1] *P*-values for weather variables (daily rainfall, minimum air temperature at 0900 h, and maximum daily wind speed), cohort, and pretreatment covariate included as fixed effects in the models for each of the behavioral parameters are presented in Supplemental Table 1.

[2] LB = lying bout.

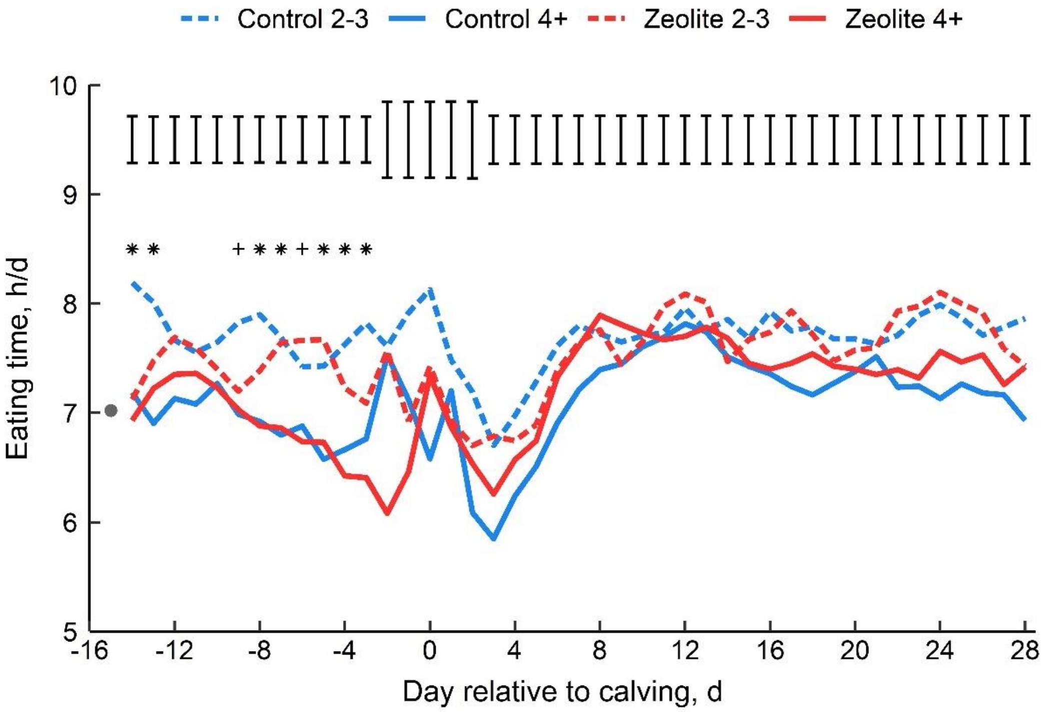

Figure 4.5. The effect of feeding zeolite precalving and parity on daily eating time during the transition period. Daily eating times (h/d) of the four treatment x parity groups (Control 2–3; Control 4+; Zeolite 2–3; Zeolite 4+) across –14 to 28 d relative to the day of calving (Cday 0) are presented. Days –21 to –15 precalving are not reported in the figure due to low cow numbers (≤ 10 per group) at these time points. The average pretreatment daily eating time for all cows (filled circle at d –15) was measured during the 2 d before treatment start (overall mean ± SD: –21.4 ± 4.6 d relative to calving). Error bars represent 1 x mean standard error of the difference. The overall treatment x parity x Cday interaction was significant during the PRE period (*P* = 0.01), and days when there was a treatment x parity interaction are represented by * (P < 0.05) and + (P < 0.15).

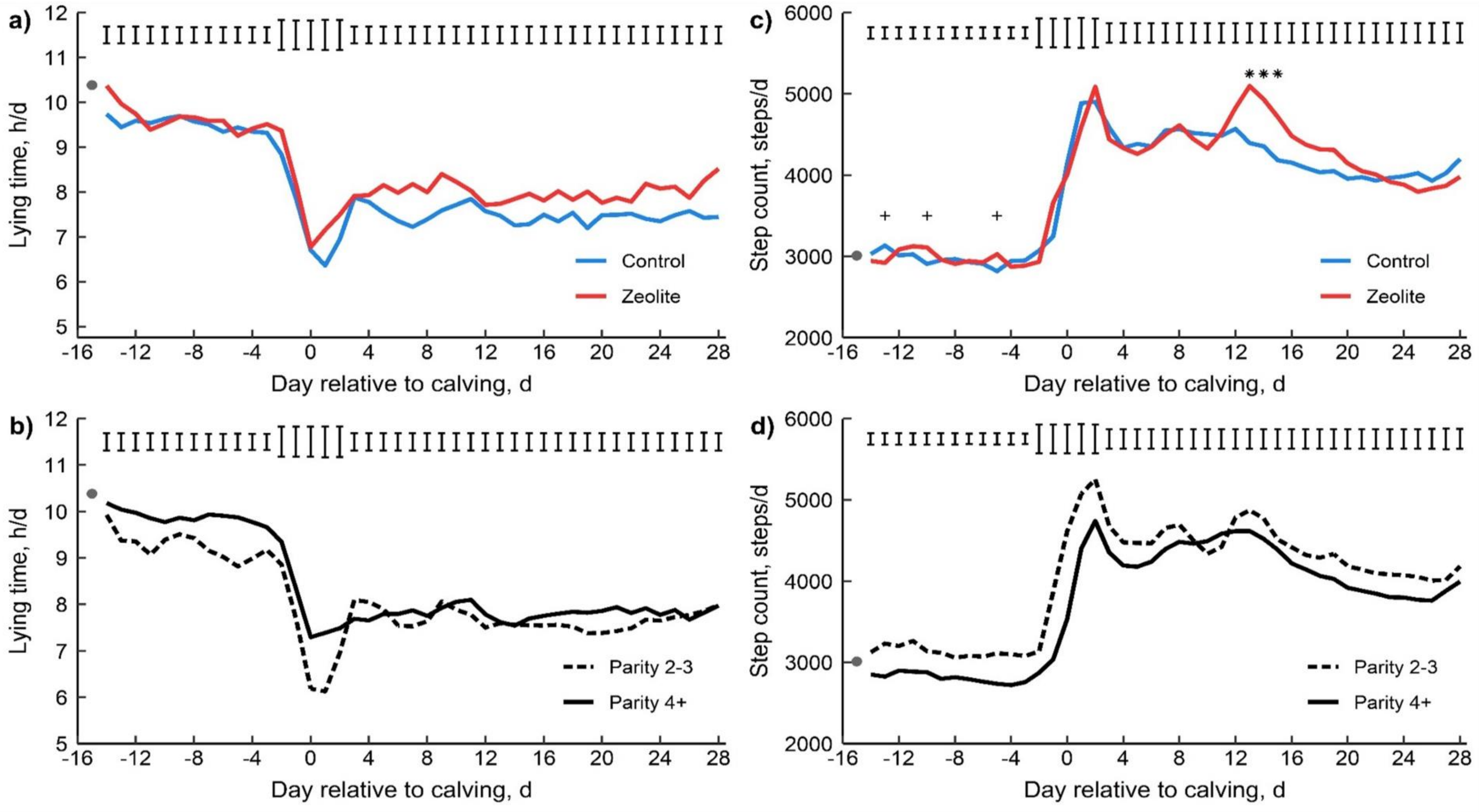

Figure 4.6. The effect of feeding zeolite precalving or parity on daily lying time and activity during the transition period.

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Figure 4.6 (continued). The effect of feeding zeolite precalving or parity on daily lying time and activity during the transition period. Daily lying times [(a) and (b); h/d], and step counts [(c) and (d); steps/d)] of the two treatment groups [(top); Control vs Zeolite] and two parity groups (bottom; Parity 2−3 vs 4+) across –14 to 28 d relative to the day of calving (Cday 0) are presented. Days –21 to –15 precalving are not reported in the figure due to low cow numbers (≤ 10 per group) at these time points. The average pretreatment covariates for all cows (filled circles at d –15) were measured during the 3 d before treatment start (–¬21.6 ± 4.8 d relative to calving, overall mean ± SD). Error bars represent 1 x standard error of the difference. Treatment or parity effects on individual Cdays are represented by * (P < 0.05) and + (P < 0.15) when the overall interaction with Cday was significant.

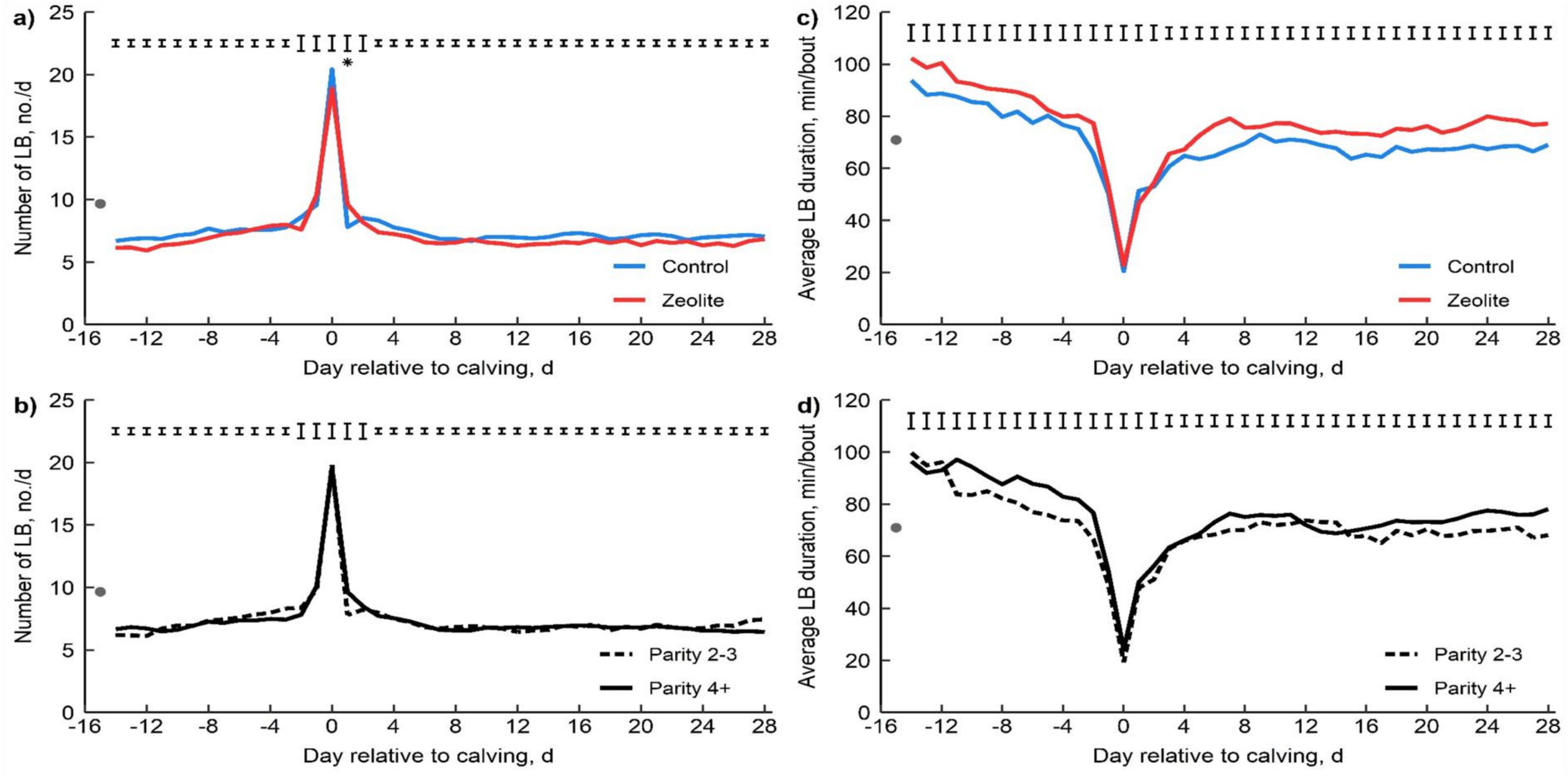

Figure 4.7. The effect of feeding zeolite precalving or parity on lying bouts during the transition period.

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Figure 4.7 (continued). The effect of feeding zeolite precalving or parity on lying bouts during the transition period. The daily number of lying bouts [LB; (a) and (b); no./d] and average LB duration [(c) and (d); min/bout] of the two treatment groups [(top); Control vs Zeolite] and two parity groups (bottom; Parity 2–3 vs 4+) across –14 to 28 d relative to the day of calving (Cday 0) are presented. Days –21 to –15 precalving are not reported in the figure due to low cow numbers (≤ 10 per group) at these time points. The average pretreatment covariates for all cows (filled circles at d –15) were measured during the 3 d before treatment start (– 21.6 ± 4.8 d relative to calving, overall mean ± SD). Error bars represent 1 x standard error of the difference. Treatment or parity effects on individual Cdays are represented by * ($P < 0.05$) when the overall interaction with Cday was significant.

4.5. Within-day behavior during the transition period relative to the day of calving.

Mean and SED for eating time (min/h), lying time (min/h), and activity (steps/h) during each 4-hourly interval within day relative to the day of calving are presented in Table 4.5, with the main effects of treatment and parity within day and their 2-way interactions with interval presented in Table 4.6 and Table 4.7, respectively. The *P*-values for the three weather variables, pretreatment covariate behavior corresponding to the outcome variable, and cohort that were also included in the models are presented in Supplemental Table 4. The average sunrise and sunset times across the period –21 to 28 d were 0730 h and 1719 h, respectively (Timeanddate.com, 2021).

Eating behavior. The temporal patterns of eating time within day during the PRE period, the day of calving, and the POST period are presented on an hourly basis as raw means for all cows in Figure 4.8 a – c. There was a significant effect of interval across all Cday periods (Table 4.5). Eating time was lowest during the early morning (0200 – 0559 h) and was greatest during the middle of the day (1000 – 1359 h) and mid to late afternoon (1400 – 1759 h) (Table 4.5). The pattern of eating time changed from PRE to POST periods, reflecting two pronounced bouts of eating following the morning and afternoon milking when cows returned to pasture (at approximately 0830 h and 1600 h), as well as more time spent eating in the evening during the POST period.

There were significant parity x interval interactions for eating time during both PRE and POST periods (Table 4.7). The temporal patterns of within-day eating time for the two parity groups during the PRE and POST periods are presented on an hourly basis as raw means in Figure 4.9 a and c, respectively. During the PRE period, the Parity 2–3 cows spent longer eating at night (all $P < 0.01$) than the Parity 4+ cows, especially between 2200 – 0159 h. They also tended to spend more time eating during the day between 1000 – 1359 h ($P = 0.08$) and 1400 – 1759 h ($P = 0.07$). During the POST period, the Parity 2–3 cows again spent more time eating at night between 1800 – 2159 h ($P = 0.02$) and tended to eat for longer between 2200 – 0159 h ($P = 0.05$).

There was a trend for a treatment x interval interaction ($P = 0.05$) for eating time on Cday –2, which indicated a tendency ($P = 0.09$) for shorter eating times in the Zeolite cows (30 mins/h) than in the Control cows (35 mins/h) during 1400 to 1759 h ($P = 0.01$). A trend for a treatment x parity x interval interaction was also evident on Cday 2 ($P = 0.06$); however, Tukey-pairwise comparisons within interval indicated there were no consistent significant differences between groups (results not shown). There were no further interactions for treatment or parity with interval for eating time (Table 4.6 and Table 4.7).

Lying and activity behavior. The temporal patterns of lying time and activity within day during the PRE period, the day of calving, and the POST period are presented on an hourly basis as raw means for all cows in Figure 4.8 a – c and least squares mean for the effects of interval on lying time and activity are presented in Table 4.5. There was a significant effect of interval across all Cday periods (Table 4.5). Cows were less active during the night (1800 – 0559 h) and most active during the day (0600 – 1759 h), with the longest lying times per h and the fewest number of steps per h occurring between 0200 – 0559 h (Table 4.5). The pattern of lying and activity changed from PRE to POST periods, reflecting more time spent lying in the middle of the day between bouts of activity around milking times during the POST period.

The temporal patterns of lying time within day for the two parity groups during the PRE and POST periods are presented on an hourly basis as raw means in Figure 4.9 b and d, respectively. During the PRE period, there was a parity x interval interaction for lying time (Table 4.7), whereby the Parity 2–3 cows lay down less at night compared with Parity 4+ cows, particularly during 2200 and 0159 h ($P < 0.001$) and tended to have shorter hourly lying times during 0200 – 0559 ($P = 0.07$) and 1800 – 2159 ($P = 0.11$). During the PERI period, there were treatment x parity x interval interactions for lying time on Cday –2 ($P = 0.09$) and for activity on Cday 2 ($P < 0.05$); however, there were no significant differences between groups within any interval for either behavioral parameter (results not shown). No further significant interactions between treatment, parity, and interval for lying and activity behavior were detected (Table 4.6 and Table 4.7).

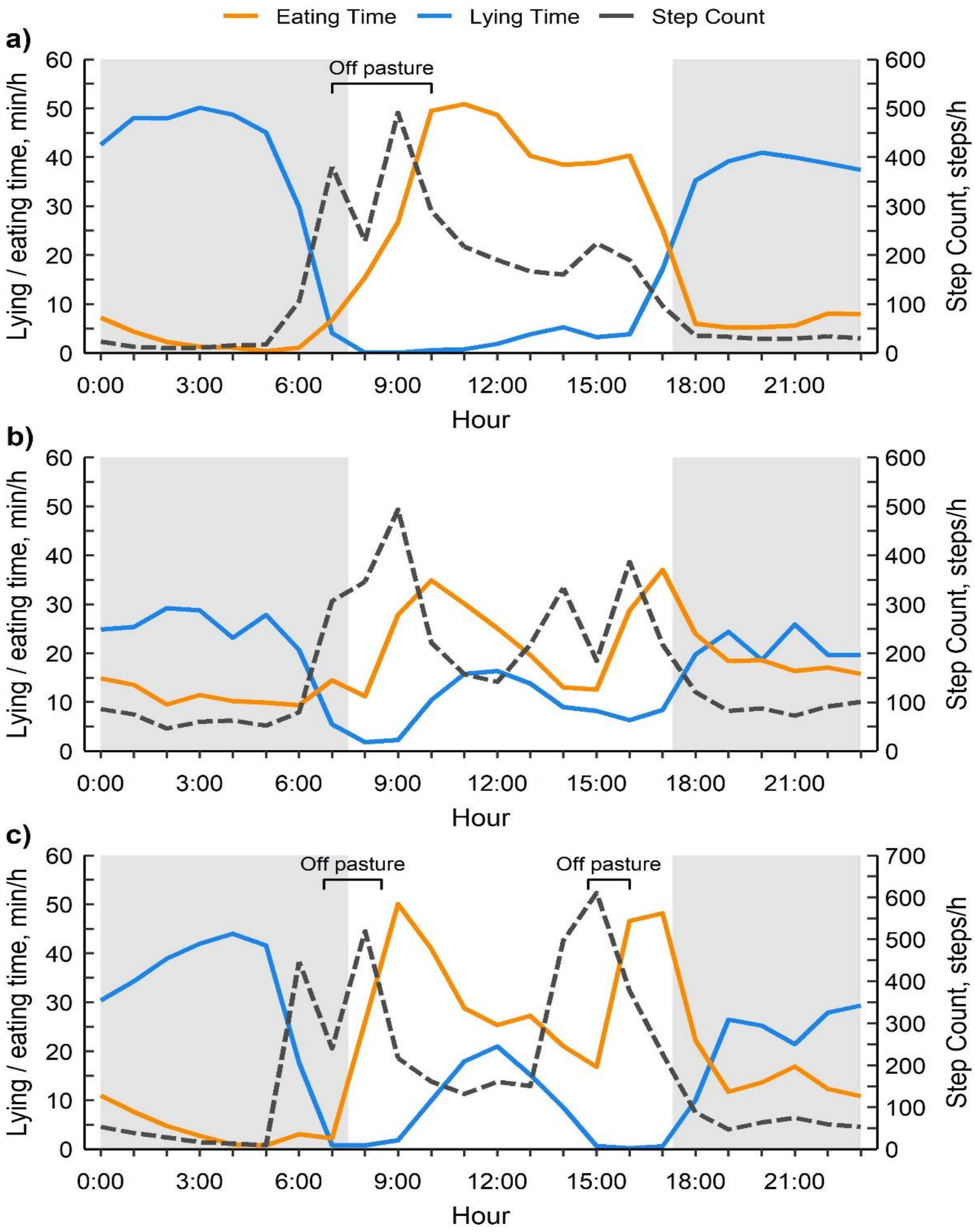

Figure 4.8. The pattern of behavior within day during the transition period in grazing dairy cows. The temporal pattern of eating time (min/h), lying time (min/h), and step count (steps/h) within day during (a) the PRE period (–21 to –3 d), (b) on the day of calving (Cday 0), and (c) the POST period (3 to 28 d). The shaded areas represent the hours of darkness (sunrise = 0730 h, sunset = 1719 h), and brackets represent the average time spent off pasture for sampling/measurements and feeding PRE (approximately 0700 – 1000 h) and for morning sampling/measurements and the morning and afternoon milkings POST (approximately 0645 – 0830 h and 1445 – 1600 h).

Table 4.5. Behavior changes across time intervals within day during the transition period. Least squares mean and mean standard error of the difference (SED) for eating time (mins/h), lying time (mins/h), and step count (steps/h) across 4-hourly time intervals for all cows during the PRE period (–21 to –3 d), d –2 and d –1 precalving, the day of calving (d 0), d 1 and d 2 postcalving, and the POST period (3 to 28 d), along with *P*-values for the effects of interval.

Behavior	Period	Interval, h[1]						SED	*P*-value
		02-05	06-09	10-13	14-17	18-21	22-01		
Eating time, min/h	PRE	4.1	10.4	44.6	33.2	8.1	9.2	0.93	<0.001
	d -2	3.3	9.7	46.1	32.4	8.8	8.9	1.37	<0.001
	d -1	5.0	7.5	40.6	24.4	15.4	13.9	2.01	<0.001
	d 0	11.7	15.0	25.9	21.9	20.1	15.5	2.46	<0.001
	d 1	5.9	17.3	26.3	23.0	18.8	14.1	1.84	<0.001
	d 2	3.7	16.5	26.2	23.6	18.8	9.6	1.52	<0.001
	POST	3.1	19.7	30.0	32.4	17.0	11.0	1.01	<0.001
Lying time, min/h	PRE	47.1	8.1	2.9	8.0	38.5	40.8	1.10	<0.001
	d -2	44.6	12.1	8.0	5.3	31.6	36.1	2.53	<0.001
	d -1	47.6	10.8	0.0	6.1	25.7	32.4	2.63	<0.001
	d 0	28.5	6.7	11.1	7.6	22.9	23.3	3.29	<0.001
	d 1	33.5	9.1	10.8	5.1	21.0	21.9	3.10	<0.001
	d 2	39.9	5.5	10.9	3.0	23.7	25.5	2.95	<0.001
	POST	41.9	5.2	15.7	2.5	21.0	30.7	1.11	<0.001
Steps, steps/h	PRE	24	293	196	164	41	34	8.6	<0.001
	d -2	24	277	197	159	55	42	18.5	<0.001
	d -1	46	282	158	182	116	84	22.1	<0.001
	d 0	57	310	185	284	94	91	30.4	<0.001
	d 1	56	314	223	350	125	120	33.1	<0.001
	d 2	43	334	248	375	128	136	32.7	<0.001
	POST	21	353	141	427	72	55	8.8	<0.001

[1] Interval is inclusive of the hour (i.e., 02-05 is 0200 h to 0559 h)

Table 4.6. The effect of feeding zeolite on eating, lying, and activity behaviors within day during the transition period. Least squares mean and standard error of the difference (SED) for the main effects of treatment (Control vs. Zeolite) on eating time (min/h), lying time (min/h), and step count (steps/h) across the whole day (Average) and across the 4-hourly time intervals during the PRE period (–21 to –3 d), on the day of calving (Cday 0), and during the POST period (3 to 28 d), along with the *P*-values for the treatment x interval (Treat x Interval) interaction.

Behavior	Period[1]	Effect	Average	Interval, h[2]						Treat x Interval *P*-value
				02-05	06-09	10-13	14-17	18-21	22-01	
Eating time, min/h	PRE	Control	18.6	4.0	10.4	44.9	34.6	8.4	9.3	0.29
		Zeolite	17.9	4.1	10.4	44.3	31.7	7.8	9.1	
		SED	0.66	0.25	1.03	1.07	1.56	0.66	1.03	
	Cday 0	Control	18.3	9.1	16.5	26.7	22.0	20.8	14.9	0.55
		Zeolite	18.4	14.4	13.5	25.1	21.9	19.4	16.1	
		SED	1.75	3.77	3.12	4.32	3.06	3.70	3.03	
	POST	Control	18.8	2.8	19.9	29.8	32.6	17.0	10.9	0.81
		Zeolite	18.9	3.5	19.5	30.3	32.3	16.9	11.0	
		SED	0.72	0.49	0.85	1.52	0.92	1.34	1.05	
Lying time, min/h	PRE	Control	24.3	47.0	9.0	3.1	7.9	38.0	41.2	0.75
		Zeolite	24.1	47.3	7.3	2.7	8.1	39.0	40.4	
		SED	0.77	1.52	1.28	0.52	2.04	1.51	1.10	
	Cday 0	Control	17.0	30.5	6.5	12.3	7.1	20.6	24.8	0.47
		Zeolite	16.4	26.5	6.8	9.9	8.1	25.2	21.9	
		SED	1.69	5.32	2.08	3.47	2.81	3.47	3.77	
	POST	Control	19.1	39.8	5.6	15.5	2.6	21.0	29.8	0.13
		Zeolite	19.9	44.0	4.7	15.9	2.3	21.1	31.6	
		SED	0.81	1.86	0.95	1.54	0.57	1.68	1.45	
Steps, steps/h	PRE	Control	128	28	293	196	170	44	34	0.66
		Zeolite	123	19	292	196	159	38	34	
		SED	5.7	10.6	12.6	13.0	10.8	3.0	3.0	
	Cday 0	Control	173	66	334	182	293	84	79	0.17
		Zeolite	167	48	285	188	275	103	102	
		SED	15.5	15.6	33.2	24.6	50.1	21.2	18.6	
	POST	Control	176	24	345	139	418	73	56	0.62
		Zeolite	180	19	360	143	436	71	53	
		SED	6.0	6.6	12.5	9.5	14.5	6.5	8.6	

[1] Cday -2, -1, 1, and 2 aren't presented for brevity.

[2] Interval is inclusive of the hour (i.e., 02-05 is 0200 h to 0559 h).

Table 4.7. The effect of parity on eating, lying, and activity behaviors within day during the transition period. Least squares mean and standard error of the difference (SED) for the main effects of parity (2–3 vs. 4+) on eating time (min/h), lying time (min/h), and step count (steps/h) across the whole day (Average) and across 4-hourly time intervals during the PRE period (−21 to −3 d), on the day of calving (Cday 0), and during the POST period (3 to 28 d), along with the *P*-values for the parity x interval interaction.

Behavior	Period[1]	Effect	Average	Interval, h[2]						Parity x Interval *P*-value
				02-05	06-09	10-13	14-17	18-21	22-01	
Eating time, min/h	PRE	2–3	19.4^{a}	4.6^{a}	10.6^{a}	45.5^{a}	34.6^{a}	9.1^{a}	11.7^{a}	<0.001
		4+	17.1^{b}	3.5^{b}	10.2^{a}	43.7^{a}	31.7^{a}	7.1^{b}	6.7^{b}	
		SED	0.66	0.25	1.03	1.08	1.58	0.66	1.03	
	Cday 0	2–3	19.6	13.2	15.9	27.0	23.8	22.4	15.6	0.90
		4+	17.1	10.3	14.2	24.8	20.0	17.8	15.4	
		SED	1.75	3.77	3.12	4.33	3.15	3.70	3.05	
	POST	2–3	19.5^{a}	3.4^{a}	19.6^{a}	31.1^{a}	32.4^{a}	18.6^{a}	12.0^{a}	<0.05
		4+	18.2^{a}	2.8^{a}	19.8^{a}	29.0^{a}	32.5^{a}	15.4^{b}	9.9^{a}	
		SED	0.72	0.49	0.85	1.52	0.93	1.34	1.05	
Lying time, min/h	PRE	2–3	23.4^{b}	45.7^{a}	7.6^{a}	2.8^{a}	8.8^{a}	37.3^{a}	38.5^{b}	<0.01
		4+	25.0^{a}	48.5^{a}	8.7^{a}	3.0^{a}	7.2^{a}	39.7^{a}	43.2^{a}	
		SED	0.77	1.52	1.28	0.53	2.05	1.51	1.10	
	Cday 0	2–3	15.3	27.5	6.4	11.5	5.6	17.6	22.8	0.15
		4+	18.1	29.5	6.9	10.7	9.6	28.1	23.9	
		SED	1.69	5.32	2.09	3.49	2.83	3.48	3.77	
	POST	2–3	19.2	40.6	5.1	15.9	2.5	20.9	30.5	0.58
		4+	19.7	43.2	5.3	15.5	2.5	21.2	30.9	
		SED	0.8	1.9	0.9	1.5	0.6	1.7	1.5	
Steps, steps/h	PRE	2–3	132^{a}	29	303	207	168	44	39	0.34
		4+	119^{b}	18	282	185	161	38	29	
		SED	5.7	10.6	12.6	13.0	10.9	3.0	3.0	
	Cday 0	2–3	192^{a}	76	346	180	332	113	108	0.14
		4+	148^{b}	39	273	190	236	75	73	
		SED	15.5	15.6	33.3	24.9	50.2	21.1	18.7	
	POST	2–3	182	25	353	147	428	78	61	0.51
		4+	174	17	352	136	426	66	49	
		SED	6.1	6.6	12.5	9.6	14.6	6.5	8.6	

[1] Cdays -2, -1, 1, and 2 aren’t presented for brevity.

[2] Interval is inclusive of the hour (i.e., 02 – 05 is 0200 h to 0559 h)

[a – b] Means within a column differ significantly ($P < 0.05$)

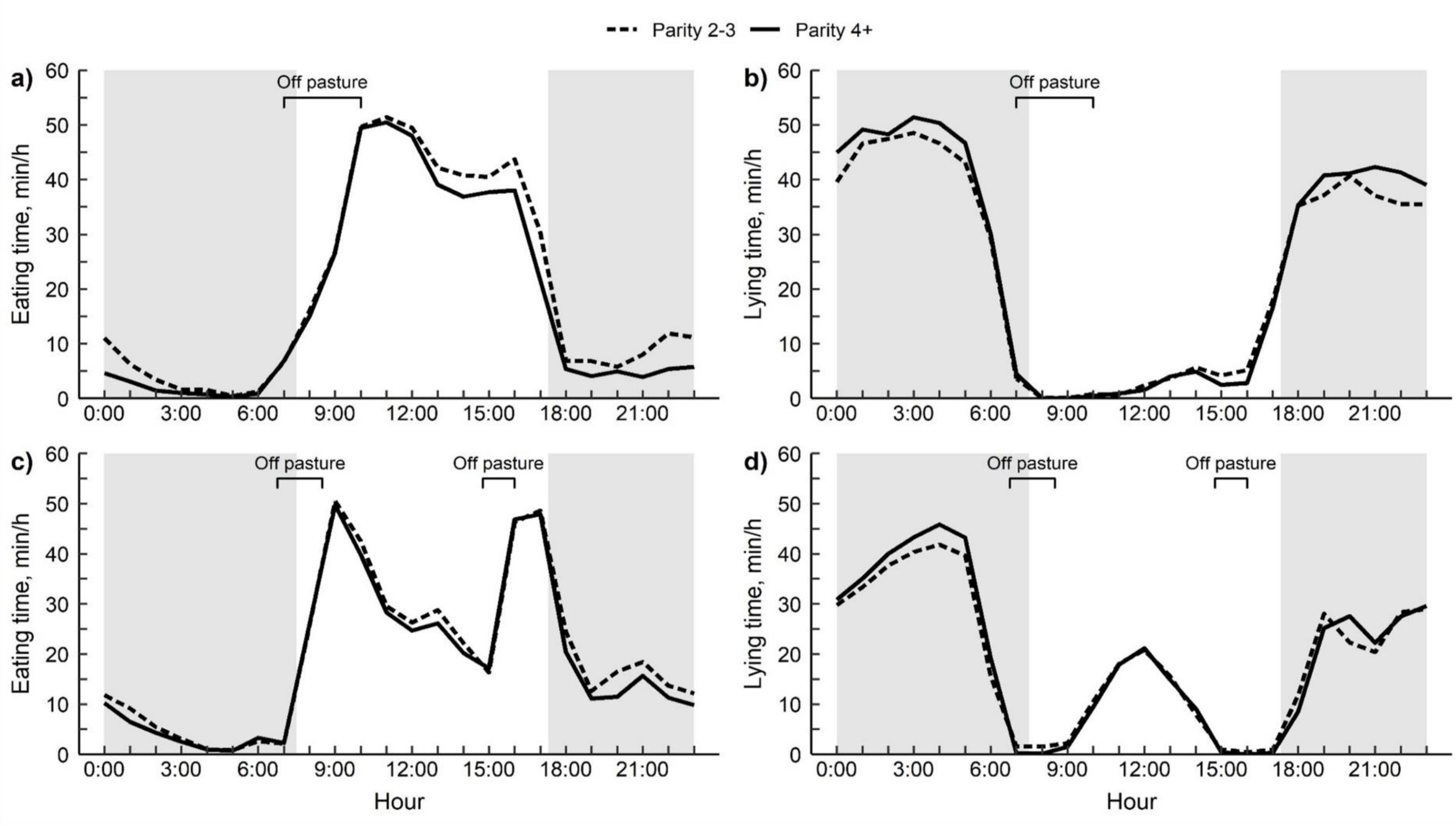

Figure 4.9. The effect of parity on hourly profiles of eating and lying times with day during the PRE and POST periods.

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Figure 4.9 (continued). The effect of parity on hourly profiles of eating and lying times with day during the PRE and POST periods. The temporal pattern of eating time [(a) and (c); min/h] and lying time [(b) and (d); min/h] within day for the two parity groups (Parity 2–3 vs 4+) during the PRE period [top; –21 to –3 d] and the POST period [bottom; 3 to 28 d]. The shaded areas represent the hours of darkness (sunrise = 0730 h, sunset = 1719 h). The average time spent off pasture for sampling, measurements, and feeding PRE was approximately between 0700 – 1000 h, and for morning sampling/measurements and the morning and afternoon milkings POST was approximately between 0645 – 0830 h and 1445 – 1600 h, respectively.

CHAPTER 5. DISCUSSION

Feeding synthetic zeolite A prepartum has been demonstrated to improve blood Ca status at calving and reduce the risk of hypocalcemia in both housed (Thilsing-Hansen and Jørgensen, 2001; Grabherr et al., 2009; Kerwin et al., 2019) and grazing cows (Roche et al., 2018; Crookenden et al., 2020). The current study sought to investigate the wider effects of zeolite on animal behaviors in multiparous dairy cows managed under a pasture-based grazing system. Based upon evidence from housed systems that zeolite reduces DMI (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009; Kerwin et al., 2019) and rumination time (Kerwin et al., 2019) during the period of prepartum supplementation, I hypothesized that eating time would be reduced in grazing cows. I further hypothesized that, due to the stabilizing effects of zeolite on blood Ca around calving, the lying times would be shorter and activity greater in the zeolite-supplemented cows during the peripartum period, particularly in older multiparous cows who are at greater risk of developing hypocalcemia.

5.1. The interactive effect of treatment and parity on eating time

In support of my first hypothesis, supplementing zeolite precalving altered eating time in grazing dairy cows, but these effects were only minor and differed with parity. The older (Parity 4+) cows spent a similar amount of time eating each day during the PRE period irrespective of whether they were fed zeolite; the eating time of younger (Parity 2−3) cows fed zeolite varied precalving but was generally less than Controls. It is unclear why this interaction with parity occurred. Nevertheless, all zeolite-supplemented cows had shorter eating times averaged across the PERI period when feeding behaviors varied widely and were disrupted by the calving process, in agreement with other studies (e.g., Schirmann et al., 2013; Neave et al., 2017). The effects of treatment on eating time did not extend into early lactation once the supplementation of zeolite stopped at calving, consistent with previous studies in TMR-fed housed cows (Kerwin et al., 2019). The slightly shorter eating times precalving may indicate a possible anorexic effect of zeolite in grazing cows that could result in small reductions in DMI. Unfortunately, this effect cannot be confirmed as DMI was not measured in the current study due to the complexities of obtaining accurate intakes for individual cows in a grazing system. Nevertheless, the lack of any treatment effects on BCS, BW, plasma concentrations of NEFA or BHB, or milk production parameters indicates that if reductions in prepartum DMI did occur due to zeolite supplementation they were relatively minor. An understanding of the effect of zeolite on the feeding rate of grazing dairy cows, which is also related to DMI and feeding time, may also provide more evidence towards the effects of zeolite on the feeding behaviors in grazing dairy cows and could be an area of future research.

The exact cause for reductions in eating time, rumination time, and DMI in zeolite-supplemented cows (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009; Kerwin et al., 2019) is currently unknown; however, previous authors suggested that reduced DMI may be related to zeolite's suppressive effect on circulating concentrations of P. Zeolite is capable of binding P at pH levels similar to those found in the abomasum and small intestine (Thilsing et al., 2006), which prevents P absorption into the blood. Consistent with previous studies (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009; Kerwin et al., 2019), the zeolite-supplemented cows in the current study had considerably lower circulating P concentrations prepartum, which recovered quickly after calving. Thilsing et al. (2007) reported that zeolite-fed cows not supplemented with P prepartum had higher feed refusals, and consequently lower intakes, than those supplemented with P suggesting that zeolite-induced hypophosphatemia leads to the observed reductions in feed intake. Deficiencies in P are also associated with lower feed intakes in both dairy cows (Kincaid et al., 1981) and beef heifers (Gartner et al., 1982); however, these effects are only seen after periods of longer than 4 months. Further, reductions in precalving DMI are not observed in housed cows fed low-P diets during the dry period (Keanthao et al., 2021), which indicates that the large zeolite-induced P deficiencies of only two to four weeks prepartum might not be sufficient to cause a significant reduction in DMI alone.

Additional explanations for reduced DMI in zeolite-fed cows are related to palatability issues or a reduction in digestion rate. The poor palatability of zeolite, especially at higher dose rates, has been suggested to negatively influence DMI in TMR-fed cows by several authors (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009). Whereas the housed cows in those studies had zeolite added to the entire daily ration of feed with no alternative feeds offered, the effect in grazing cows might be somewhat mitigated by the ability of the cow to access fresh pasture for the rest of the day after supplementation of zeolite in the maize silage in the morning. Another reason for reduced DMI was provided by Kerwin et al. (2019), who speculated that zeolite supplementation reduces the rate of digestion. Reducing the availability of Ca through feeding zeolite precalving may lead to reduced rumination times because of increased PTH, which has a relaxing effect on ruminal contractions (Kerwin et al., 2019); however, rumination time was not measured in the current study and further work is required to determine if this effect occurs in grazing cows and is associated with decreased eating time and lower DMI.

5.2. Parity effects on eating time

To my knowledge, no studies have previously investigated the effects of parity on eating time in multiparous grazing dairy cows, but the data from this study indicate that longer feeding times are characteristic of younger cows during the transition period. On average, the younger (Parity 2–3) cows spent between 30 and 48 mins longer eating each day and, consequently, were more active than the older (Parity 4+) cows. These differences were more pronounced PRE, with smaller, but significant, differences occurring during the PERI and POST periods. Further, these differences in eating time are consistent with research in prepartum beef cows grazing pasture, which determined that 3-year-old cows grazed for 42 mins longer/d than 5- and 7-year-old cows (Dunn et al., 1988). Studies in housed dairy systems have also indicated a negative association between age and time spent feeding prepartum (Proudfoot et al., 2009b; Neave et al., 2017) but differ from the current study in that this relationship changed to a positive association during early lactation (Neave et al., 2017; Henriksen et al., 2019; Munksgaard et al., 2020). In my study, the grazing areas offered to the cows during the PRE, PERI, and POST periods were allocated to match their energy requirements, which were lower during the PRE period when cows were non-lactating. Interestingly, however, an increase in eating time as cows transitioned from late gestation PRE to early lactation POST was evident only in the older cows. The younger cows spent a similar amount of time grazing PRE compared with POST, despite lower feed requirements and a smaller grazing area PRE. This suggests that they had to work harder to meet their energy requirements relative to older cows, particularly during the PRE period. Therefore, the differences between parity groups may have been influenced by competition for specific grazing sites.

Variation in within-day profiles of eating time between younger (Parity 2- 3) and older (Parity 4+) cows also support a role for grazing competition. The parity-driven differences in total daily eating time during both the PRE and POST periods were mainly due to the younger cows eating for longer at night (1700 h – 0559 h). Given that most eating behaviors occurred during daylight hours, this parity effect may reflect that the older cows had greater feed conversion, better grazing efficiency, and were more experienced (Dunn et al., 1988; Gregorini et al., 2013; Gregorini et al., 2015), or that the younger cows were avoiding competition with older cows by grazing at different times. More dominant grazing dairy cows are generally older than submissive cows (Phillips and Rind, 2002) and spend less time grazing (Phillips and Rind, 2002; Ungerfeld et al., 2014). It is possible that by being more dominant, the older cows had better access to preferred grazing sites and, therefore, needed to do less work to fulfill their requirements. It is thought that grazing during the nighttime is unfavorable, as the ability to distinguish between pasture swards may be lower and the evolutionary risk of predation, and therefore, the need to be vigilant, is greater (Rook et al., 1994; Gregorini et al., 2006a) than during the daytime. Further, as pasture is progressively grazed, the surface height of the sward is reduced and the preferred parts of the pasture are likely eaten, meaning the food available to graze at nighttime is likely of lower quantity and quality. These factors may exacerbate the already long eating times in the younger, more submissive cows by increasing the amount of work they need to do to achieve their daily requirements.

5.3. Effects of parity on lying time

The lying behavior of cattle grazing pasture was also influenced by parity. During the PRE and PERI periods, the younger cows spent less time lying down (~30 mins/d) and were more active (338 more steps/d) than older cows. These parity differences in daily totals were driven by hourly differences in lying time during the night. However, an inverse relationship with eating time indicated that the younger cows were eating instead of lying and resting like their older herdmates. The precalving lying time of the Parity 4+ cows was similar (10.1 h/d) to the 'typical' precalving lying times (10.3 h/d) reported in both grazing cows (Hendriks et al., 2019a) and TMR-supplemented cows at pasture (Rice et al., 2017). However, the mean lying time of the Parity 2–3 cows in the current study was lower (9.6 h/d). This result contrasts with earlier findings by Hendriks et al. (2019a), who did not observe parity differences in the precalving lying times of multiparous grazing dairy cows, which may reflect differences in management between studies. However, it is not possible to determine the cause-and-effect interactions between lying behavior and parity in my study. The differences in daily lying time and step count between parities were more prominent during the PERI period, whereby the younger cows lay for 48 fewer mins/d and took 671 more steps/d compared with the older cows. Shorter lying times PRE and PERI in younger cows could mostly be explained by their greater eating times in the current study but may also reflect the influence of comfort and welfare on the time budgets and motivation to lie in dairy cows (Munksgaard and Simonsen, 1996; Hendriks et al., 2019a). This could particularly be the case during the PERI period, due to increased restlessness in younger cows during calving and increased difficulty coping with the physiological changes during this time (Wehrend et al., 2006). Other factors influencing lying behavior during the PRE and PERI periods may include interactions with other behaviors (e.g., rumination time and social interactions) as well as individual traits (e.g., genetics, personality, other underlying conditions).

5.4. Effects of zeolite on lying time

I hypothesized that zeolite-supplemented cows would have shorter lying times and be more active during the peripartum period because of improved blood Ca status and its association with muscle function (Goff, 2008). Consistent with the literature (Thilsing-Hansen and Jørgensen, 2001; Grabherr et al., 2009; Kerwin et al., 2019; Crookenden et al., 2020), zeolite improved Ca concentrations around the time of calving and markedly reduced the risk of hypocalcemia; however, my results indicate that there were few effects of treatment on lying and activity behaviors. The zeolite-fed cows had more lying bouts on the day after calving, which could be a result of improved skeletal muscle contractility due to improved Ca balance immediately postpartum. However, the number of lying bouts did not differ enough to significantly alter the total daily lying time, nor was step count affected by treatment. Differences in lying time or activity in the periparturient period may only be distinguished between groups with more divergent blood Ca concentrations. For example, longer lying times and reduced activity on the day before and the day of calving were previously reported in cases of clinical hypocalcemia (Ca ≤ 1.4 mmol/L) without paresis in dairy cows grazing pasture; however, the lying time and activity of subclinically hypocalcemic (Ca < 2.0 mmol/L) and normocalcemic (Ca ≥ 2.0 mmol/L) cows did not differ (Hendriks et al., 2020a). Similarly, subclinically hypocalcemic housed cows had shorter standing times (i.e. longer lying times) on the day after calving when lower Ca thresholds were used (Ca ≤ 1.8 mmol/L; Jawor et al., 2012), but no differences in lying time (Ca < 2.0 mmol/L; Piñeiro et al., 2019) or activity (Ca < 2.13 mmol/L; Liboreiro et al., 2015) during the peripartum period are observed when using greater Ca thresholds to define subclinical hypocalcemia. In the current study, although there was a high prevalence of subclinical hypocalcemia in the Control groups, there were only two cases of clinical hypocalcemia without paresis (Ca < 1.4 mmol/L on d 0 or 1 postcalving), and no cows experienced clinical hypocalcemia with paresis at calving (i.e., milk fever). Greater numbers of cows presenting clinical hypocalcemia with and without paresis may be required to examine the effects of zeolite on lying and activity behaviors.

Interestingly, supplementing cows with zeolite prepartum affected lying behaviors after calving. The zeolite-fed cows consistently spent about 30 min longer lying each day in the POST period, which was driven by longer lying bout durations. This is an interesting response considering a previous study in grazing dairy cows indicated that the effects of blood Ca status at calving on lying behavior were short-lived, with no differences observed beyond 3 d postcalving (Hendriks et al., 2020a). Other studies in housed cows have reported longer lying times (between 0.6 and 2.1 h/d) in clinically and subclinically hypocalcemic cows during the first

21 DIM (Barraclough et al., 2020b; Tsai et al., 2021). Therefore, shorter lying times in the zeolite-fed cows are expected due to their improved Ca status. In the current study, the lack of differences in eating time and activity between treatment groups indicates that the Control cows were likely spending more time standing idle, grooming, or ruminating while standing; however, it is difficult to disentangle the reasons for these differences between the Control and Zeolite cows. Lying down is a biologically important behavior in cows (Munksgaard and Simonsen, 1996), and may become more important as the time available to lie down is limited due to postcalving management. The lying times of the Control cows in the current study (7.5 h/d) were at the lower end of the range of what is reported as 'typical' postcalving lying times in grazing cows (range: 7.50 to 8.58 h/d; Sepúlveda-Varas et al., 2014; Hendriks et al., 2019a), and were also lower than the lying times of healthy housed cows (7.9 – 8.6 h/d) in the studies by Barraclough et al. (2020b) and Tsai et al. (2021). It is possible that, because of the already very short lying times in this study, an improved Ca status at calving due to zeolite supplementation may encourage recovery and rest leading to longer lying times and better cow comfort postcalving.

5.5. Additional limitations and areas for future research

This study only had two behavior monitoring technologies (IceRobotics leg-attached accelerometers and CowScout neck collars), and was, therefore, limited to the range of eating time, lying, and activity behaviors measured. Future work in this area could use different behavior monitoring technologies validated in grazing dairy cows to capture a broader range of behaviors, which would improve our understanding of the effects of precalving zeolite supplementation on the feeding behaviors of grazing dairy cows, as well as the range of 'typical' behaviors of grazing dairy cows during the transition period. For example, rumination time (measured using the AfiCollar; Iqbal et al., 2021) might provide insights into the possible relationship between zeolite supplementation, Ca status, and rumination in grazing dairy cows. Further research into accurate estimations of individual feed intake could also be used to confirm whether there is a subtle anorexic effect of zeolite supplementation in grazing dairy cows. Furthermore, including measurements regarding the social relationships between individual cows may improve understanding of how competition influences the feeding strategies and time budgets of different parity groups during the transition period in the context of animal welfare. Measuring these behaviors would likely involve visual observation (e.g., Ungerfeld et al., 2014), however, this is time-consuming and requires trained assessors, making it often impractical to implement in large grazing dairy herds. Further development of devices that could reliably measure interactions between individual cows in a grazing herd without the need for human observers would, therefore, be of benefit to understanding the effects of social interactions on grazing dairy cow behavior.

In the current study, the time of calving in a grazing system could occur anywhere in the 24 h before calf collection. The number of lying bouts, which reportedly increases on the day of calving (Huzzey et al., 2005; Borchers et al., 2017), was used to reassign the calving date of individual cows based on the criteria previously used by Hendriks et al. (2020a). Although this methodology is a pragmatic approach to correcting data that are analyzed relative to the day of calving, it has not been validated and is a limitation of the current study. Future research is required to validate this approach, which also has potential on-farm application in detecting calving cows for earlier intervention if required, and in the correct drafting of newly calved cows for inspection and milking.

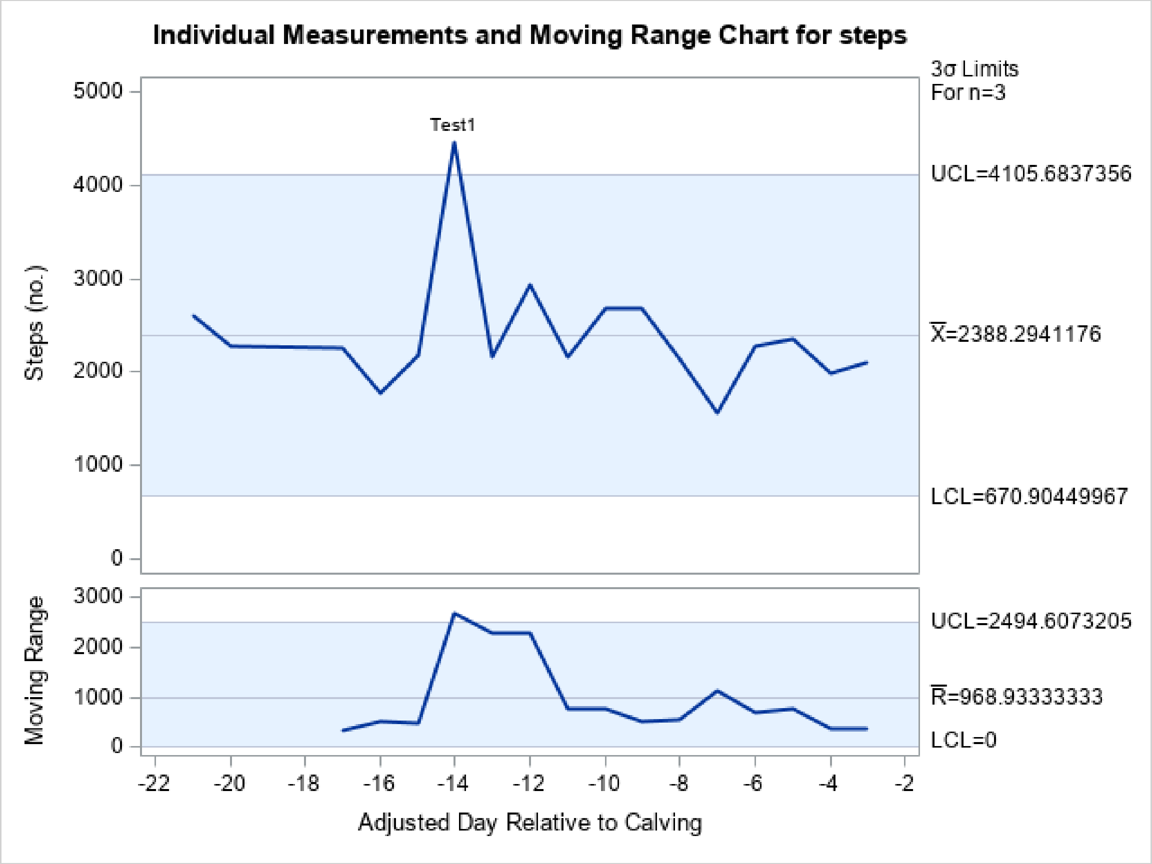
A few aspects of this study differ from conditions typical of a commercial New Zealand dairy farm, including the intensive sampling and measurements cows were subjected to throughout the transition period, and the relatively small herd size compared with the average herd size of commercial dairy herds in New Zealand (440 cows; LIC & DairyNZ Ltd., 2020). These factors could influence the time cows spent off pasture, social competition, and access to resources throughout the transition period, which may result in differences in the daily and within-day behaviors expressed by cows in commercial versus research herds. Furthermore, the social dynamics between the herd in the current study and others may differ due to the smaller herd size and the lack of primiparous animals in the current study. Nevertheless, this study provides strong evidence towards the effects of prepartum zeolite supplementation and parity on the eating, lying, and activity behaviors of grazing dairy cows during the transition period.

CHAPTER 6. CONCLUSIONS

Prepartum supplementation of dairy cows with synthetic zeolite A reduced the daily eating time of younger multiparous grazing dairy cows, indicating that it had a subtle anorexic effect during the time of supplementation. Zeolite also increased the daily lying time postpartum across all multiparous age groups, which is potentially associated with improved cow comfort and welfare during early lactation. Irrespective of prepartum management, my study indicates that cows in their second and third parity spend more time eating than older multiparous cows, especially during the nighttime instead of lying, which may be driven by competitive interactions between these parity groups. The current study provides further evidence that the effects of parity should be considered when investigating the eating, lying, and activity behavior of grazing dairy cows.

APPENDICES

APPENDIX 1. SUPPLEMENTAL FIGURE 1.



Supplemental Figure 1. Identification of possible outliers using the SHEWHART procedure. An example of an "individual measurements and moving range chart" for the step count of cow 8112 during the PRE period (d –21 to –3 relative to the day of calving (Cday 0)). Individual charts for eating time, lying time, step count, and the number and duration of lying bouts were created for each cow during the PRE and POST (d –3 to 28 relative to calving) periods. The procedure uses a 3-d moving average, and upper and lower control limits calculated as three standard deviations from the mean. Data points outside of these critical limits were identified as possible outliers (labeled as "Test 1" in this example).

APPENDIX 2. SUPPLEMENTAL TABLE 1.

Supplemental Table 1. *P*-values for additional fixed effects used in the models investigating the effects of zeolite and parity on pre- and postcalving BCS and BW, and postcalving milk production. *P*-values for pretreatment covariate (Cov.) corresponding to the outcome variable, cohort, and treatment duration, which were included as fixed effects in the model to investigate the main and interactive effects of treatment, parity, and day relative to calving (d 0) on BCS and BW during the three weeks before and four weeks after calving, as well as the *P*-values for cohort and treatment duration used in the analysis of milk production parameters (milk yield, energy corrected milk (ECM) yield, fat percentage, fat yield, protein percentage, protein yield, lactose percentage, and lactose yield) during weeks 2 to 4 postcalving.

Parameter	*P*-values		
	Cov.	Cohort	Treat Duration
BCS[1], units	<0.001	0.68	0.03
BW, kg	<0.001	0.91	0.02
Milk yield, kg/d	.	0.30	0.80
ECM[2], kg/d	.	0.13	0.50
Fat yield, kg/d	.	0.15	0.36
Fat percentage, %	.	0.50	0.37
Protein yield, kg/d	.	0.10	0.74
Protein percentage, %	.	0.33	1.00
Lactose yield, kg/d	.	0.16	0.72
Lactose percentage, %	.	0.02	0.51

[1] Measured on a 10-point scale (1 is emaciated and 10 obese; Roche et al., 2004) at an average of –20.4 ± 4.8 d before calving (across all cows).

[2] ECM = energy-corrected milk yield

APPENDIX 3. SUPPLEMENTAL TABLE 2.

Supplemental Table 2. *P*-values for additional fixed effects used in the models investigating the effects of zeolite and parity on blood mineral and metabolite concentrations during the transition period. P-values for pretreatment covariate (Cov.) corresponding to the outcome variable (Cov), cohort, and treatment duration, which were included as fixed effects in the model to investigate the main and interactive effects of treatment, parity, and day relative to calving (Cday 0) on plasma calcium (Ca), magnesium (Mg), phosphate (PO4), β-hydroxybutyrate (BHB), and non-esterified fatty acid (NEFA) concentrations during the transition period (d −21 to 28 d relative to the day of calving).

Parameter	*P*-values		
	Cov.	Cohort	Treat Duration
Ca, mmol/L	0.13	0.47	0.07
Mg, mmol/L	<0.001	0.59	0.37
PO_4, mmol/L	0.16	0.13	0.78
BHB, mmol/L	0.04	0.11	0.77
NEFA, mmol/L	0.49	0.91	0.75

APPENDIX 4. SUPPLEMENTAL TABLE 3.

Supplemental Table 3. *P*-values for additional fixed effects used in the models investigating the effects of zeolite and parity on eating, lying, and activity behaviors during the transition period. *P*-values for average daily rainfall (Rain), minimum air temperature at 0900 h (Min. Temp.), maximum daily wind speed, pretreatment covariate behavior corresponding to the outcome variable (Cov.), and cohort, which were included as fixed effects in the model to investigate the main and interactive effects of treatment, parity, and day relative to calving (Cday 0) on eating, lying and activity behaviors during the PRE (–21 to –3 d), PERI (–2 to 2 d), and POST (3 to 28 d) periods.

		P-values				
Behavior	Period	Rain	Min. Temp.	Wind Speed	Cov.	Cohort
Eating time, h/d	PRE	<0.001	<0.001	<0.001	<0.001	0.35
	PERI	0.39	0.89	0.29	<0.001	0.97
	POST	<0.01	0.29	<0.001	<0.001	0.95
Lying time, h/d	PRE	<0.001	<0.001	<0.001	<0.001	0.68
	PERI	0.09	0.08	0.07	<0.001	0.85
	POST	<0.001	0.04	0.51	<0.001	0.82
Number of LB[1], no./d	PRE	<0.001	<0.001	<0.01	<0.001	0.25
	PERI	0.90	0.34	0.10	<0.001	1.00
	POST	<0.001	0.43	<0.01	<0.001	0.83
Mean LB[1] duration, min/bout	PRE	<0.001	<0.01	0.10	<0.01	0.59
	PERI	0.31	0.07	0.61	0.05	0.85
	POST	<0.001	0.01	<0.001	0.06	0.78
Steps, steps/d	PRE	<0.001	<0.001	0.02	<0.001	0.20
	PERI	<0.01	0.36	<0.01	0.24	0.74
	POST	<0.001	0.81	<0.01	<0.01	0.85

[1] LB = lying bout

APPENDIX 5. SUPPLEMENTAL TABLE 4.

Supplemental Table 4. P-values for additional fixed effects used in the models investigating the effects of zeolite and parity across time intervals within day during the transition period. *P*-values for pretreatment covariate behavior corresponding to the outcome variable and cohort, which were included as fixed effects in all models to investigate the main and interactive effects of treatment, parity, and interval on within-day eating, lying, and activity behaviors during the PRE period (–21 to –3 d), individual days of the PERI period (d –2 and –1 precalving, the day of calving (Cday 0), and d 1 and 2 postcalving), and the POST period (3 to 28 d) relative to the day of calving (Cday 0). Average daily rainfall (Rain), minimum air temperature at 0900 h (Min. Temp.), maximum daily wind speed (Wind Speed), were also included in the models for d –2 and –1 precalving, the day of calving (Cday 0), and d 1 and 2 postcalving.

Behavior	Period	*P*-values				
		Rain	Min. Temp.	Wind Speed	Cov.	Cohort
Eating time, min/h	PRE	.	.	.	<0.001	0.87
	d –2	0.88	0.27	0.02	<0.001	0.46
	d –1	0.31	0.19	0.93	<0.001	0.52
	d 0	0.77	0.49	0.54	<0.001	0.40
	d 1	0.68	0.12	0.00	<0.001	0.98
	d 2	0.65	0.16	0.06	<0.001	0.03
	POST	.	.	.	<0.001	0.88
Lying time, min/h	PRE	.	.	.	<0.001	0.02
	d –2	0.42	0.21	0.97	<0.001	0.23
	d –1	0.10	0.69	0.10	<0.001	0.32
	d 0	0.41	0.34	0.05	<0.001	0.73
	d 1	0.17	0.38	0.01	<0.001	0.84
	d 2	0.28	0.53	0.25	<0.001	0.80
	POST	.	.	.	<0.001	0.87
Steps, steps/h	PRE	.	.	.	<0.001	0.08
	d –2	0.03	0.22	0.60	<0.001	0.70
	d –1	0.22	0.05	0.17	<0.001	0.05
	d 0	0.54	0.28	0.12	<0.001	0.37
	d 1	0.99	0.08	0.61	<0.001	0.03
	d 2	0.36	0.43	0.97	<0.001	0.06
	POST	.	.	.	<0.001	0.32

APPENDIX 6. ADDITIONAL ANALYSIS OF BEHAVIOR DURING THE PRECALVING TREATMENT PERIOD RELATIVE TO TREATMENT START DATE.

Eating, lying, and activity data were also analyzed during the precalving treatment period relative to the start of treatments. Results reported in this section are very similar to those reported during the PRE (Cdays –21 to –3) and PERI (Cdays −2 to 2) periods, when the data were analyzed relative to the actual calving date (Cday 0), likely because the treatment period encompasses the PRE and some of the PERI period. This analysis is not intended for publication and is, therefore, not presented in Chapter 3.

Appendix 6.1 Statistical analyses

For ease of interpretation, the sampling dates for all data were assigned a day relative to the treatment start date (**Tday**), where Tday 0 was equal to the first treatment day. This transformed dataset is the basis of the subsequent analysis. The eating behavior data collected during the 2 d before treatment start date (overall mean ± SD = –21.4 ± 4.6 d relative to calving) and lying and activity behavior data collected during the 3 d before treatment start date (overall mean ± SD = –21.6 ± 4.8 d relative to calving) were averaged (PROC MEANS) for each individual cow to create a pretreatment covariate for each corresponding behavior of interest. The htype=1 option was specified in all repeated measure ANOVA to include the fixed effects sequentially. The repeated measures models were pairwise comparison adjusted using Tukey-Kramer. Variables were checked for multicollinearity; however, no variables were highly correlated, had tolerance values less than 0.1, or variance inflation factors greater than 10. Significant effects were declared at $P < 0.05$. Least squares mean, standard error of the difference (**SED**) are presented in the text, tables, and figures, and Tukey-adjusted *P*-values are presented in the text and tables.

Behavior during the precalving period relative to treatment start. Data from each cow from the day of treatment start (Tday 0) until the day of calving, or until d 17 of treatment for the later-calving cows, were analyzed using a repeated-measures ANOVA to determine the main effects of treatment, parity, and experimental day relative to the treatment start date (Tday), and their 2- and 3-way interactions for all five behavior measures. A 3-d moving average was calculated for the behavior variables during the precalving treatment period (Tday 0 up to Tday 17). The Tday was defined as the repeated measure, cow as a random effect and treatment, parity, Tday, and their interactions as fixed effects. The pretreatment covariate behavior corresponding to the outcome variable, cohort, and the number of days from the treatment start date to calving date were included as fixed effects in all models. Weather variables were not included in these analyses as the calendar date of treatment start (Tday 0) was identical within cohort, and, therefore, differences in weather were already accounted for by cohort. Autoregressive and compound symmetry covariance structures were tested, and the lowest AIC value was used to identify compound symmetry as the appropriate covariance structure.

Within-day eating, lying, and activity measures relative to treatment start. The hourly eating, lying, and activity data were divided into six 4-hourly intervals (0200 to 0559 h, 0600 to 0959 h, 1000 to 1359 h, 1400 to 1759 h, 1800 to 2159 h, and 2200 to 0159 h) and averaged into 3-d blocks (d 0 to 2, 3 to 5, etc.) up to and including d 17 relative to the day of treatment start (Tday 0). The pretreatment covariate used in the analyses within day relative to calving was also used in this analysis within day relative to treatment start. The associations between treatment, parity, interval, and their 3-way interactions for eating time, lying time, and step count were analyzed for each 3-d block relative to treatment start (Tday 0) separately using a repeated-measures ANOVA. Data from each cow was included from the day of treatment start (d 0) until the day of calving, or until day 17 of treatment for the later-calving cows. Interval was defined as the repeated measure, cow as the random effect, and treatment, hour, parity, and their interactions as fixed effects. The pretreatment covariate behavior corresponding to the outcome variable, and cohort were included as fixed effects. Heterogenous Toeplitz covariance structure was used for all models.

Appendix 6.2 Results.

Behavior during the precalving treatment period relative to treatment start date

Overall means and SED for the main effects of treatment and parity on eating, lying, and activity behaviors during the treatment period are presented in Supplemental Table 5, as well as the *P*-values for the effect of treatment, parity, Tday, and their 2- and 3-way interactions, and the *P*-values for cohort, and treatment to calving interval that were also included in the models. There was an effect ($P < 0.001$) of Tday across all behavior measures, with behavior varying across days during the treatment period (Supplemental Table 5).

Eating behavior. The daily eating times of the two treatment and two parity groups across the precalving treatment period are presented in Supplemental Figure 2 a and b, respectively. While there was no treatment x Tday interaction during the treatment period, Zeolite supplementation tended ($P = 0.09$) to reduce eating time by 18 mins/d, on average, during the treatment period (Supplemental Table 5 and Supplemental Figure 2a). Parity also affected eating time during the treatment period, with the Parity 4+ cows spending, on average, 42 mins less time eating/d compared with the Parity 2-3 cows (Supplemental Table 5). Furthermore, there was a parity x Tday interaction (Supplemental Table 5), where daily eating times were more variable across Tday in the Parity 4+ cows compared with the Parity 2-3 cows (Supplemental Figure 2b). There were no significant treatment x parity or treatment x parity x Tday interactions for daily eating time (Supplemental Table 5).

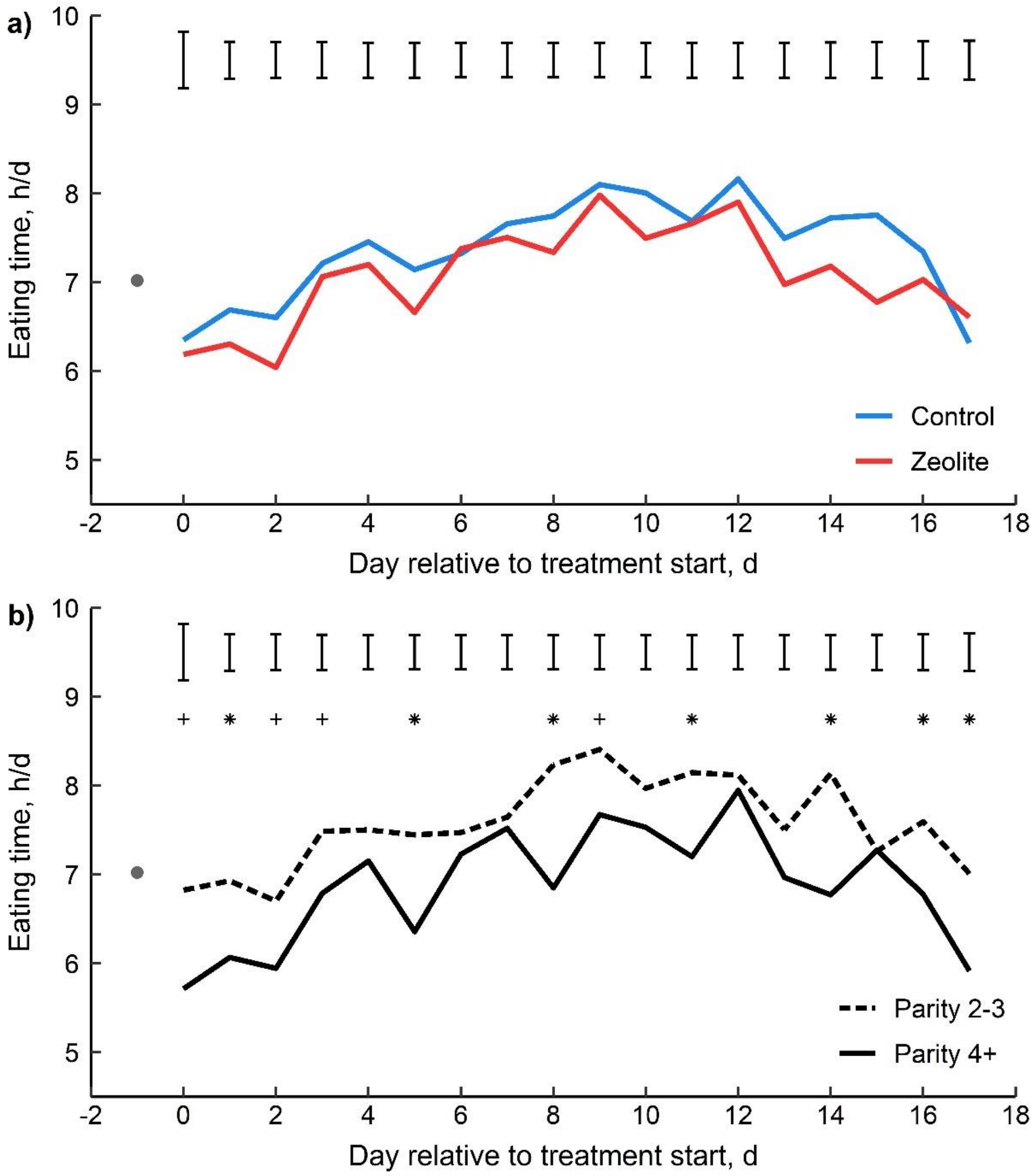
Lying and activity behavior. While there were no 2-way interactions between treatment x Tday or parity x Tday for lying time and step count, there were significant overall effects of both parity and Tday on these variables (Supplemental Table 5); therefore, the daily lying times and step counts of the two parity groups across the precalving period are presented in Supplemental Figure 3 a and b to demonstrate temporal trends. Daily lying time decreased in the 2 d following treatment start but had increased by 4 d and remained relatively constant thereafter (Supplemental Figure 3a). Parity 4+ cows were less active during the treatment period, lying for 36 mins longer and taking 297 fewer steps/d, on average, compared with the Parity 2-3 cows (Supplemental Table 5). Treatment groups did not significantly differ in their mean daily lying time, the number of lying bouts, or step count, but there was a trend (P = 0.09) for Zeolite cows to have longer lying bouts (6.4 min/bout), on average, compared with Control cows (Supplemental Table 5). Further, a treatment x parity x Tday interaction for lying bout duration was evident Supplemental Table 5); however, the Tukey-pairwise comparisons indicated no significant differences between the four groups within Tday (Supplemental Figure 4). There were no significant parity effects on the number of lying bouts or mean LB duration (Supplemental Table 5); although, there was a Tday effect whereby lying bouts gradually increased across the treatment period from an overall daily mean of 6.7 lying bouts/d on the treatment start date to 8.7 lying bouts/d at the end of the treatment period (figure not shown).

Supplemental Table 5. The effect of feeding zeolite precalving and parity on eating, lying, and activity behaviors during the treatment period. Least squares mean and standard error of the difference (SED) for the main effects of treatment (Control vs. Zeolite) and parity (2–3 vs. 4+) during the treatment period, the *P*-values for the effects of treatment (Treat), parity, and day relative to treatment start date (Tday), and their 2- and 3-way interactions, and the *P*-values for cohort and treatment to calving interval (Treat to Calving) are presented.

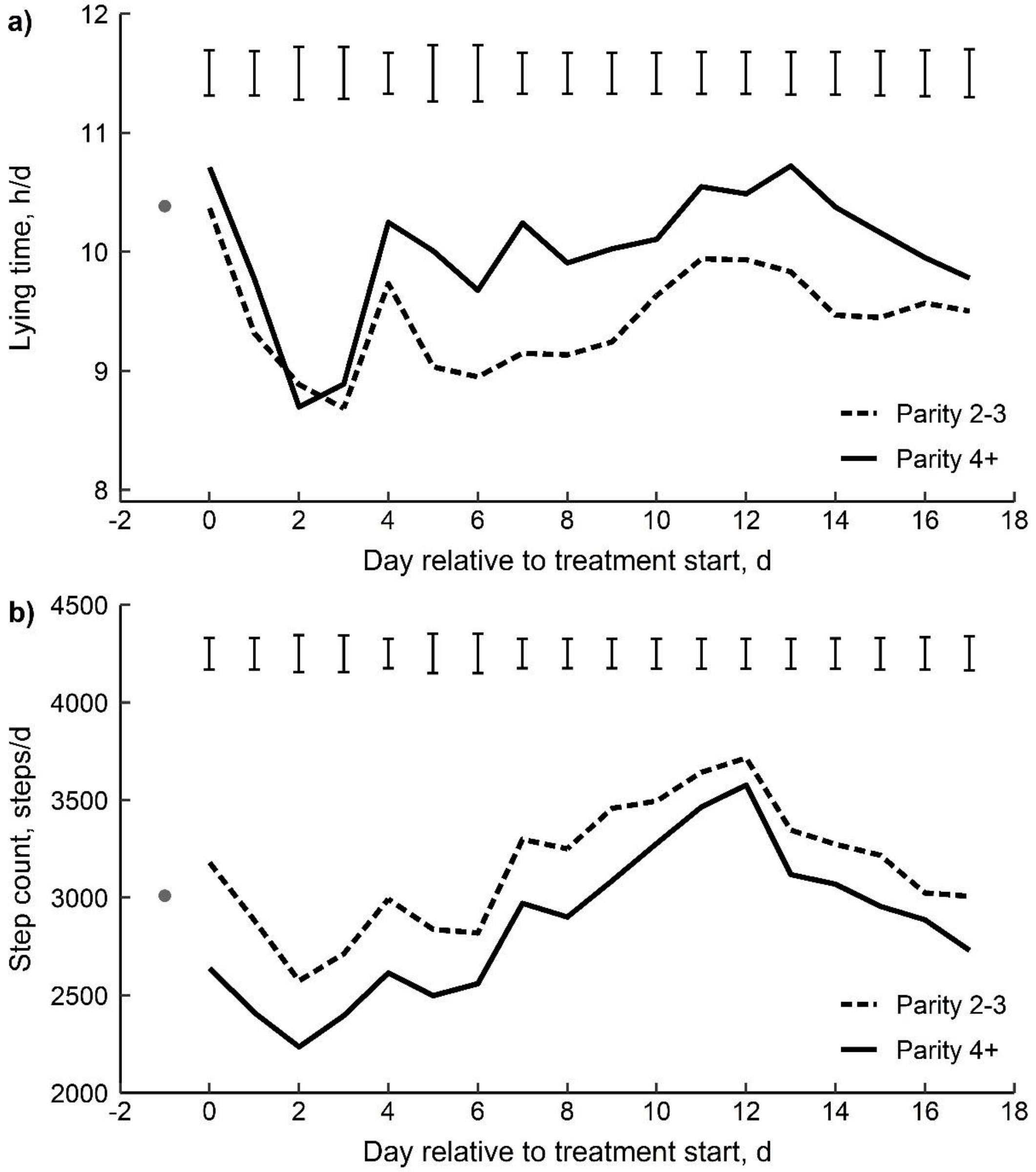
Behavior	Treatment			Parity			P-values[1]								
	Control	Zeolite	SED	2–3	4+	SED	Cohort	Treat to Calving	Treat	Parity	Tday	Treat x Parity	Treat x Tday	Parity x Tday	Treat x Parity x Tday
Eating time, h/d	7.4	7.1	0.25	7.6	6.9	0.24	0.32	0.30	0.09	0.01	<0.001	0.64	0.62	0.05	0.89
Lying time, h/d	9.7	9.8	0.24	9.4	10.0	0.22	0.21	0.72	0.57	0.01	<0.001	0.63	0.86	0.47	0.33
Number of LB[2], no./d	7.2	6.8	0.5	7.1	7.0	0.4	0.86	0.81	0.17	0.89	<0.001	0.46	0.78	0.23	0.16
Mean LB duration, min/bout	86.1	92.5	4.2	86.7	91.9	3.8	0.89	0.52	0.09	0.17	<0.001	0.56	0.46	0.31	0.02
Steps, steps/d	3051	2956	111	3152	2855	103	0.01	0.38	0.33	0.01	<0.001	0.42	1.00	0.82	1.00

[1] *P*-values for pretreatment covariate behavior are < 0.001 for all behavioral parameters.

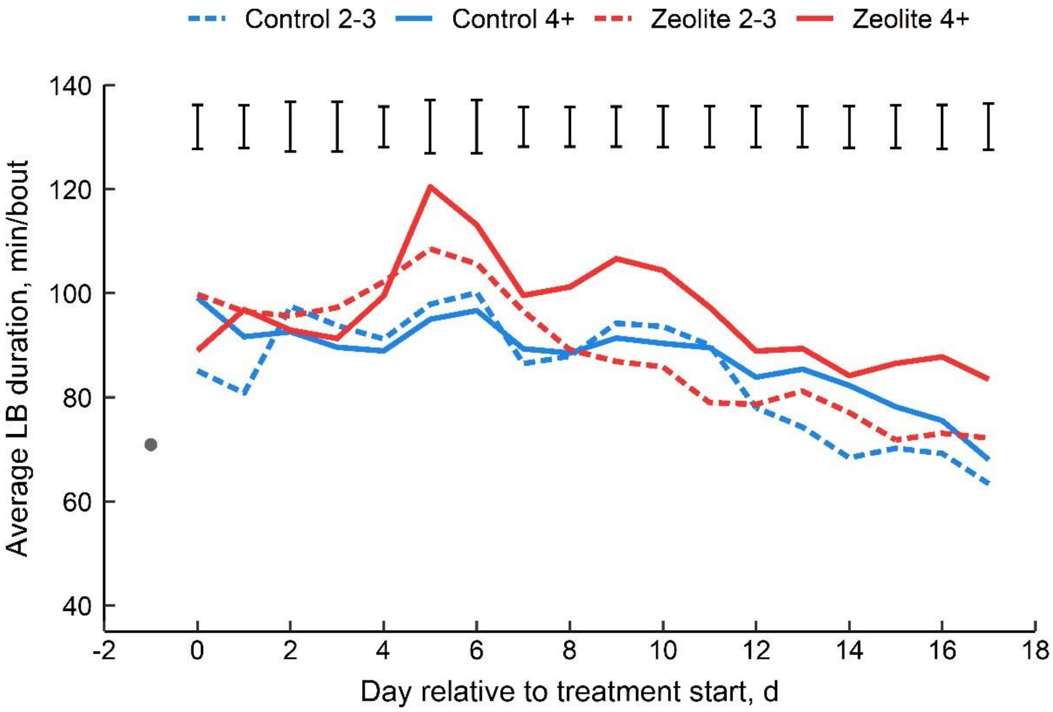
[2] LB = lying bout.



Supplemental Figure 2. The effect of feeding zeolite precalving or parity on daily eating time during the treatment period. Daily eating times (h/d) of the two treatment groups (a; Control and Zeolite) and two parity groups (b; Parity 2–3 and Parity 4+) are presented relative to the treatment start date (Tday 0). The average pretreatment eating time for all cows (filled circle at d –1) was measured during the 2 d before treatment start (–21.4 ± 4.6 d relative to calving, overall mean ± SD). Error bars represent 1 x standard error of the difference. Parity effects on individual Tdays are represented by * ($P < 0.05$) and + ($P < 0.15$) as the overall parity x Tday interaction was significant ($P < 0.05$). No overall treatment x Tday interaction was observed ($P = 0.62$).



Supplemental Figure 3. The effect of parity on daily lying time and activity during the precalving treatment period. Daily lying times [(a); h/d], and step counts [(b); steps/d)] of the two parity groups (Parity 2–3 and Parity 4+) are presented relative to the treatment start date (Tday 0). The average pretreatment lying time and step count for all cows (filled circles at d –1) was measured during the 3 d before treatment start (–21.6 ± 4.8 d relative to calving, overall mean ± SD). Error bars represent 1 x standard error of the difference. No overall parity x Tday interactions were detected for either daily lying time or step count (P ≥ 0.47).



Supplemental Figure 4. The effect of feeding zeolite precalving and parity on lying bout duration during the treatment period. Average lying bout (LB) durations of the four treatment x parity groups (Control 2–3; Control 4+; Zeolite 2–3; Zeolite 4+) are presented relative to the treatment start date (Tday 0). The average pretreatment LB duration for all cows (filled circles at d –1) was measured during the 3 d before treatment start (–21.6 ± 4.8 d relative to calving, overall mean ± SD). Error bars represent 1 x mean standard error of the difference. An overall treatment x parity x Tday interaction was detected ($P < 0.05$), but there were no differences ($P < 0.05$) between treatment x parity groups within Tday.

Within-day behavior during the precalving treatment period relative to treatment start date

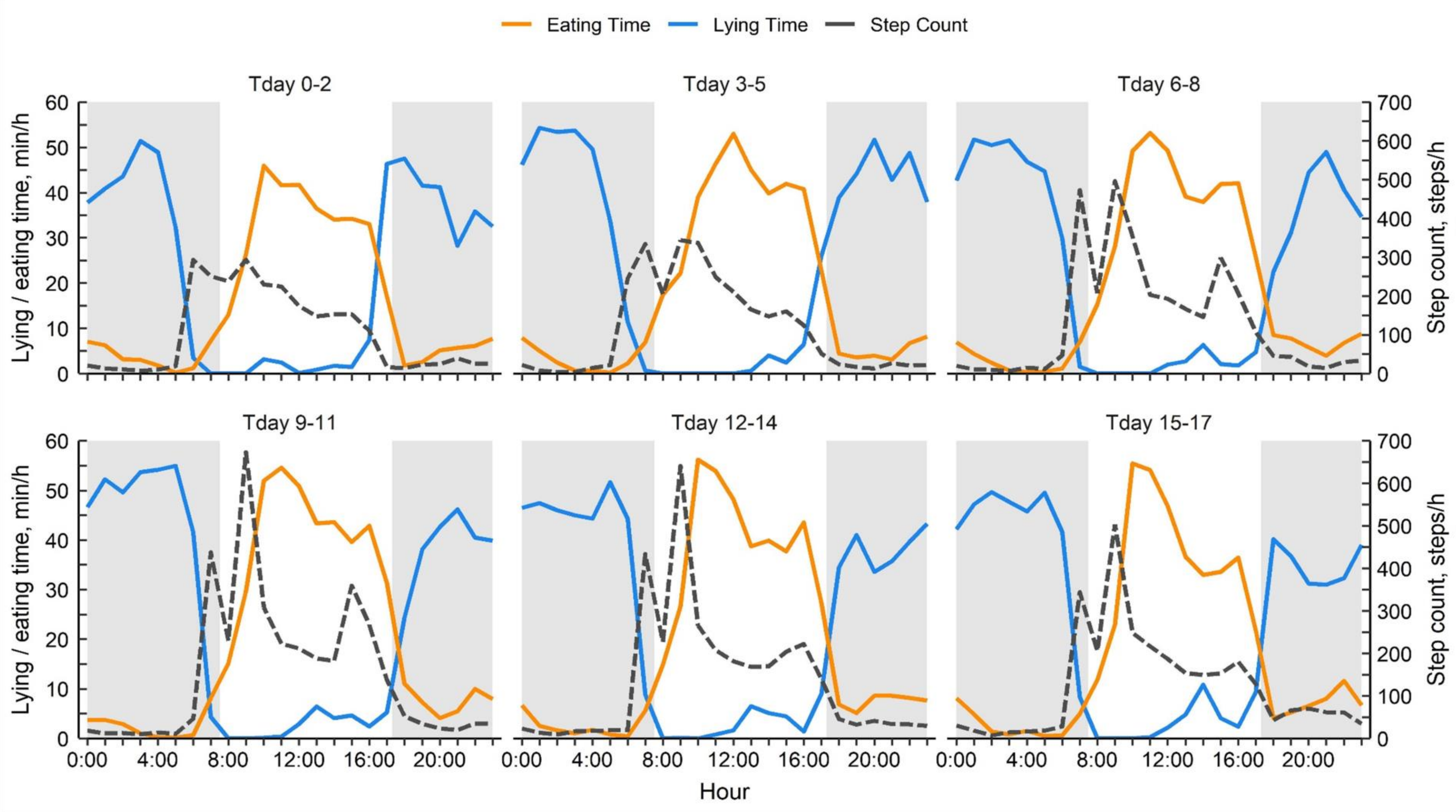
The overall means and SED for eating time (min/h), lying time (min/h), and activity (steps/h) during each 4-hourly interval within day relative to treatment start date are presented in Supplemental Table 6, with the main effects of treatment and parity within day and their 2-way interactions with interval presented in Supplemental Table 6 to 10. The *P*-values for the pretreatment covariate behavior corresponding to the outcome variable, cohort, and treatment to calving interval that were also included in the models are presented in Supplemental Table 11.

Eating time. The temporal patterns of eating time within day during the precalving treatment period are presented on an hourly basis in Supplemental Figure 5. There was a significant effect of interval across all Tday periods (Supplemental Table 6). Eating time peaked during 1000 – 1359 h (mean range: 29.0 to 41.9 min/h) and was generally lowest during 0200 – 0600 h (mean range: 5.5 to 8.5 mins/h), except for Tday 0 – 2, where it was lowest during 0600 – 0959 h (9.0 min/h).

There was a tendency for a treatment x interval interaction on Tday 3 – 5 ($P = 0.05$), where Zeolite cows ate for 5.0 min/h less than Control cows between 1400 – 1759 h ($P < 0.01$; Supplemental Table 7). A further treatment x interval interaction was observed on Tday 6 – 8 (Supplemental Table 7), but there were no differences between treatments within interval ($P > 0.10$). Parity x interval interactions for eating time were observed on Tday 3 – 5 and 12 – 14, as well as a tendency for an interaction on Tday 9 – 11 ($P = 0.08$; Supplemental Table 9). These differences were driven by Parity 2–3 cows eating for longer during the night (Supplemental Table 9).

Lying and activity behavior. The temporal patterns of lying time and activity within day during the precalving treatment period are presented on an hourly basis in Figure 13. There was a significant effect of interval across all Tday periods (Supplemental Table 6). Cows lay down for longer and took fewer steps at night, particularly during 0200 – 0559 h (mean range: 40.2 to 50.2 min/h; 30 to 48 steps/h, respectively). In contrast, activity was greatest during the morning, particularly between 0700 – 0900 h (mean range: 256 to 331 steps/h).

There was a tendency for a 3-way interaction between treatment, parity, and interval on lying time during the first three days of treatment (Tday 0 – 2, $P = 0.11$); however, Tukey-pairwise comparisons within interval indicated there were no consistent significant differences between groups (results not shown). No significant 2-way treatment x interval interactions for lying and activity behaviors during the treatment period were detected (Supplemental Table 8). A parity x interval interaction for lying time was observed between Tday 0 – 2 (Supplemental Table 10), where the Parity 4+ cows lay down for longer during 0600 – 0959 h ($P = 0.01$) and tended to lie down for longer ($P = 0.05$) during 1800 – 2159 h. Further parity x interval interactions for both lying time and activity were observed during Tday 3 – 5 (Supplemental Table 10). The Parity 4+ cows tended to lie down for longer during 0200 – 0559 ($P = 0.06$) and lay down for longer during 2200 – 0159 h ($P < 0.001$), and were less active ($P < 0.05$) during all intervals except between 0200 – 0559 h and 1400 – 1759 h. There was also a trend for a parity x interval interaction during Tday 9 – 11 ($P = 0.08$), where the Parity 4+ cows lay down for longer during 2200 – 0159 h ($P < 0.01$).



Supplemental Figure 5. Temporal pattern of behavior during the precalving treatment period in grazing dairy cows.

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Supplemental Figure 5 (continued). Temporal pattern of behavior during the precalving treatment period in grazing dairy cows. The temporal pattern of eating time (min/h), lying time (min/h), and step count (steps/h) for all cows during each 3-day block during the precalving treatment period relative to treatment start date (Tday 0). The shaded areas represent the hours of darkness (sunrise = 0730 h, sunset = 1719 h).

Supplemental Table 6. Behavior changes across time intervals within day during the precalving treatment period. Least squares mean and mean standard error of the difference (SED) for eating time (mins/h), lying time (mins/h), and step count (steps/h) across 4-hourly intervals for all cows during each 3-day block during the precalving treatment period relative to treatment start date (Tday 0), along with P-values for the effect of interval.

Behavior	Tday Period	Interval, h[1]						SED	*P*-value
		02-05	06-09	10-13	14-17	18-21	22-01		
Eating time, min/h	d 0 – 2	11.4	9.0	29.0	19.3	12.7	13.5	1.30	<0.001
	d 3 – 5	5.5	10.6	40.5	31.9	7.7	10.7	1.00	<0.001
	d 6 – 8	7.5	10.4	39.5	30.2	12.6	11.9	1.17	<0.001
	d 9 – 11	8.5	10.8	40.4	31.6	14.0	12.1	1.01	<0.001
	d 12 – 14	6.4	10.6	41.9	32.1	12.0	10.1	1.01	<0.001
	d 15 – 17	8.1	10.1	35.4	24.3	14.3	13.3	1.11	<0.001
Lying time, min/h	d 0 – 2	42.2	3.5	4.2	15.0	36.4	35.3	1.62	<0.001
	d 3 – 5	47.0	3.5	0.4	9.9	44.1	46.2	1.33	<0.001
	d 6 – 8	46.5	8.8	2.4	4.3	36.4	41.2	1.70	<0.001
	d 9 – 11	50.2	13.6	5.2	5.5	34.6	41.8	1.88	<0.001
	d 12 – 14	42.8	16.7	7.4	6.8	32.2	39.6	1.92	<0.001
	d 15 – 17	40.2	15.7	8.2	8.1	28.7	34.2	2.37	<0.001
Steps, steps/h	d 0 – 2	48	256	142	98	63	55	13.7	<0.001
	d 3 – 5	44	259	185	108	54	50	10.0	<0.001
	d 6 – 8	39	276	178	167	59	52	13.5	<0.001
	d 9 – 11	38	331	191	229	62	52	18.0	<0.001
	d 12 – 14	30	319	187	196	53	44	19.9	<0.001
	d 15 – 17	42	270	155	181	92	66	20.8	<0.001

[1] Interval is inclusive of the hour (i.e., 02 – 05 is 0200 h to 0559 h)

Supplemental Table 7. The effect of treatment on eating behavior within day during the precalving treatment period. Least squares mean and standard error of the difference (SED) for the main effects of treatment (Control vs. Zeolite) on eating time (min/h) across the whole day (Average) and across 4-hourly time intervals during each 3-day block during the precalving treatment period relative to treatment start date (Tday 0), with the P-values for the parity x interval interaction.

Behavior	Tday period	Effect	Average	Interval, h[1]						Treat x Interval *P*-value
				02-05	06-09	10-13	14-17	18-21	22-01	
Eating time, min/h	d 0 – 2	Control	16.3	12.0	8.9	28.6	21.2	13.7	13.4	0.63
		Zeolite	15.3	10.8	9.1	29.3	17.4	11.6	13.6	
		SED	0.92	0.83	1.09	2.72	2.26	1.04	1.54	
	d 3 – 5	Control	18.2^{a}	5.2^{a}	10.4^{a}	40.8^{a}	34.4^{a}	7.9^{a}	10.5^{a}	0.05
		Zeolite	17.4^{a}	5.7^{a}	10.7^{a}	40.2^{a}	29.4^{b}	7.6^{a}	10.8^{a}	
		SED	0.71	0.41	1.00	1.34	1.75	0.62	1.37	
	d 6 – 8	Control	18.8^{a}	7.7^{a}	11.2^{a}	38.9^{a}	31.4^{a}	12.3^{a}	11.1^{a}	0.02
		Zeolite	18.6^{a}	7.4^{a}	9.7^{a}	40.0^{a}	29.1^{a}	12.8^{a}	12.7^{a}	
		SED	0.84	0.36	0.99	1.99	1.73	1.33	1.35	
	d 9 – 11	Control	19.9	8.3	11.3	39.5	32.9	14.6	13.0	0.20
		Zeolite	19.2	8.6	10.2	41.4	30.3	13.5	11.2	
		SED	0.73	0.53	1.05	2.11	2.06	1.36	1.56	
	d 12 – 14	Control	19.4	6.5	11.1	41.0	34.0	12.9	10.6	0.20
		Zeolite	18.3	6.3	10.0	42.7	30.3	11.0	9.5	
		SED	0.74	0.75	1.17	2.22	2.07	1.21	1.45	
	d 15 – 17	Control	18.3	8.2	10.5	37.3	25.6	13.8	14.2	0.76
		Zeolite	16.9	7.9	9.7	33.5	23.0	14.7	12.4	
		SED	0.80	0.71	1.59	3.65	1.92	1.56	1.69	

[1] Interval is inclusive of the hour (i.e., 02 – 05 is 0200 h to 0559 h)

[a – b] Means within a column differ significantly between treatments ($P < 0.05$)

Supplemental Table 8. The effect of treatment on lying and activity behaviors within day during the precalving treatment period. Least squares mean and standard error of the difference (SED) for the main effects of treatment (Control vs. Zeolite) on lying time (min/h) and activity (steps/h) across the whole day (Average) and across 4-hourly time intervals during each 3-day block during the precalving treatment period relative to treatment start date (Tday 0), with the P-values for the parity x interval interaction.

Behavior	Tday period	Effect	Average	Interval, h[1]						Treat x Interval P-value
				02-05	06-09	10-13	14-17	18-21	22-01	
Lying time, min/h	d 0 – 2	Control	21.9	40.7	3.4	4.3	14.3	34.7	33.8	0.50
		Zeolite	23.7	43.8	3.6	4.0	15.7	38.2	36.7	
		SED	1.21	2.20	0.46	0.66	2.06	2.95	3.30	
	d 3 – 5	Control	25.2	47.2	3.4	0.4	10.3	43.5	46.3	1.00
		Zeolite	25.2	46.9	3.6	0.4	9.6	44.7	46.0	
		SED	0.82	2.27	1.32	0.25	2.27	2.27	1.76	
	d 6 – 8	Control	23.5	45.5	9.5	2.6	4.5	36.8	42.3	0.51
		Zeolite	23.0	47.6	8.2	2.3	4.2	35.9	40.1	
		SED	0.91	2.27	1.33	0.81	1.57	2.72	2.43	
	d 9 – 11	Control	25.3	49.4	13.3	5.5	5.8	35.4	42.6	0.89
		Zeolite	25.0	51.1	14.0	5.0	5.2	33.9	41.0	
		SED	0.90	2.16	1.30	1.34	2.09	2.50	2.17	
	d 12 – 14	Control	24.6	42.4	16.6	7.3	7.3	32.9	41.0	0.89
		Zeolite	23.9	43.2	16.7	7.4	6.3	31.5	38.3	
		SED	0.87	2.65	1.53	1.36	1.82	2.31	2.31	
	d 15 – 17	Control	22.2	38.9	15.6	6.6	8.8	28.9	34.5	0.60
		Zeolite	22.8	41.4	15.7	9.8	7.4	28.5	33.9	
		SED	1.02	3.31	1.72	1.87	2.61	2.71	2.88	

[1] Interval is inclusive of the hour (i.e., 02 – 05 is 0200 h to 0559 h)

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Supplemental Table 8 (continued). The effect of treatment on lying and activity behaviors within day during the precalving treatment period.

Behavior	Tday period	Effect	Average	Interval, h[1]						Treat x Interval P-value
				02-05	06-09	10-13	14-17	18-21	22-01	
Steps, steps/h	d 0 – 2	Control	112	53	255	139	103	64	58	0.75
		Zeolite	108	43	256	145	93	61	52	
		SED	6.0	9.0	16.6	18.4	8.7	3.9	4.4	
	d 3 – 5	Control	118	47	263	182	113	55	48	0.37
		Zeolite	115	41	256	187	103	52	51	
		SED	4.2	8.0	8.4	11.9	7.5	2.9	4.1	
	d 6 – 8	Control	130	45	278	181	169	59	49	0.60
		Zeolite	126	32	273	175	165	59	54	
		SED	7.0	8.8	13.4	13.6	22.1	4.1	4.4	
	d 9 – 11	Control	152	45	337	183	230	65	54	0.68
		Zeolite	149	30	325	198	228	60	50	
		SED	8.0	11.9	20.0	18.2	14.7	5.9	6.3	
	d 12 – 14	Control	140	39	324	179	198	57	44	0.50
		Zeolite	136	21	314	196	194	49	43	
		SED	8.4	15.0	18.4	19.2	22.8	4.9	6.8	
	d 15 – 17	Control	134	49	269	162	172	87	62	0.62
		Zeolite	135	35	271	149	191	97	71	
		SED	8.6	11.9	14.4	20.6	24.9	18.1	8.7	

[1] Interval is inclusive of the hour (i.e., 02 – 05 is 0200 h to 0559 h)

Supplemental Table 9. The effect of parity on eating behavior within day during the precalving treatment period. Least squares mean and standard error of the difference (SED) for the main effects of parity (2–3 vs. 4+) on eating time (min/h) across the whole day (Average) and across 4-hourly time intervals during each 3-day block during the precalving treatment period relative to treatment start date (Tday 0), with the P-values for the parity x interval interaction.

Behavior	Tday period	Effect	Average	Interval, h[1]						Parity x Interval *P*-value
				02-05	06-09	10-13	14-17	18-21	22-01	
Eating time, min/h	d 0 – 2	2–3	16.8	12.0	10.3	31.3	20.0	13.1	14.4	0.74
		4+	14.8	10.9	7.7	26.7	18.6	12.2	12.6	
		SED	0.92	0.82	1.09	2.72	2.29	1.03	1.56	
	d 3 – 5	2–3	18.7^{a}	6.0^{a}	11.2^{a}	41.3^{a}	33.2^{a}	7.8^{a}	13.1^{a}	0.03
		4+	16.9^{b}	5.0^{b}	9.9^{a}	39.8^{a}	30.6^{a}	7.7^{a}	8.3^{b}	
		SED	0.71	0.40	1.00	1.35	1.77	0.61	1.37	
	d 6 – 8	2–3	19.5	7.9	11.0	40.9	31.7	12.6	13.2	0.40
		4+	17.8	7.1	9.9	38.1	28.8	12.5	10.6	
		SED	0.83	0.34	0.99	1.99	1.75	1.33	1.36	
	d 9 – 11	2–3	20.5^{a}	9.0^{a}	10.6^{a}	41.8^{a}	32.1^{a}	15.7^{a}	13.9^{a}	0.08
		4+	18.6^{a}	7.9^{b}	11.0^{a}	39.1^{a}	31.0^{a}	12.4^{b}	10.3^{b}	
		SED	0.71	0.51	1.05	2.11	2.09	1.35	1.57	
	d 12 – 14	2–3	19.8	6.5^{a}	10.4^{a}	43.0^{a}	33.7^{a}	13.4^{a}	11.6^{a}	0.03
		4+	17.9	6.3^{a}	10.7^{a}	40.7^{a}	30.6^{a}	10.5^{b}	8.5^{b}	
		SED	0.71	0.72	1.16	2.21	2.09	1.20	1.46	
	d 15 – 17	2–3	18.4	8.9	10.3	34.9	25.3	16.0	15.3	0.47
		4+	16.7	7.3	9.9	35.8	23.3	12.6	11.3	
		SED	0.79	0.69	1.59	3.65	1.96	1.55	1.71	

[1] Interval is inclusive of the hour (i.e., 02 – 05 is 0200 h to 0559 h)

$^{a-b}$ Means within a column differ significantly between parities ($P < 0.05$)

Supplemental Table 10. The effect of parity on lying and activity behaviors within day during the precalving treatment period. Least squares mean and standard error of the difference (SED) for the main effects of parity (2–3 vs. 4+) on lying time (min/h) and activity (steps/h) across the whole day (Average) and across 4-hourly time intervals during each 3-day block during the precalving treatment period relative to treatment start date (Tday 0), along with the P-values for the parity x interval interaction.

Behavior	Tday period	Effect	Average	Interval, h[1]						Parity x Interval *P*-value
				02-05	06-09	10-13	14-17	18-21	22-01	
Lying time, min/h	d 0 – 2	2–3	22.4[a]	42.7[a]	2.9[b]	4.0[a]	16.6[a]	33.6[a]	34.5[a]	0.01
		4+	23.1[a]	41.7[a]	4.1[a]	4.4[a]	13.4[a]	39.3[a]	36.0[a]	
		SED	1.21	2.20	0.46	0.66	2.06	2.95	3.30	
	d 3 – 5	2–3	24.1[b]	44.9[a]	4.2[a]	0.5[a]	9.9[a]	42.6[a]	42.8[b]	<0.01
		4+	26.3[a]	49.2[a]	2.9[a]	0.4[a]	10.0[a]	45.6[a]	49.6[a]	
		SED	0.82	2.26	1.32	0.24	2.26	2.27	1.76	
	d 6 – 8	2–3	22.3[b]	44.1	8.3	2.8	4.0	35.2	39.4	0.11
		4+	24.3[a]	48.9	9.4	2.0	4.7	37.5	43.1	
		SED	0.87	2.26	1.32	0.77	1.55	2.71	2.42	
	d 9 – 11	2–3	24.2[b]	49.0[a]	12.7[a]	4.6[a]	6.9[a]	33.3[a]	38.8[b]	0.08
		4+	26.1[a]	51.5[a]	14.6[a]	5.8[a]	4.1[a]	36.0[a]	44.8[a]	
		SED	0.85	2.14	1.28	1.31	2.06	2.48	2.15	
	d 12 – 14	2–3	23.2[b]	41.4	15.7	7.4	6.9	30.7	37.0	0.50
		4+	25.3[a]	44.2	17.6	7.4	6.7	33.7	42.3	
		SED	0.82	2.63	1.52	1.34	1.79	2.29	2.30	
	d 15 – 17	2–3	21.6	39.2	14.1	7.6	8.5	27.8	32.7	0.89
		4+	23.4	41.2	17.3	8.8	7.7	29.6	35.7	
		SED	0.96	3.29	1.70	1.85	2.58	2.69	2.87	

[1] Interval is inclusive of the hour (i.e., 02 – 05 is 0200 h to 0559 h)

[a – b] Means within a column differ significantly between parities (*P* < 0.05)

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Supplemental Table 10 (continued). The effect of parity on lying and activity behaviors within day during the precalving treatment period.

Behavior	Tday period	Effect	Average	Interval, h[1]						Parity x Interval *P*-value
				02-05	06-09	10-13	14-17	18-21	22-01	
Steps, steps/h	d 0 – 2	2–3	119^a	52	278	158	103	66	59	0.19
		4+	101^b	44	233	126	94	59	51	
		SED	6.0	9.0	16.6	18.4	8.7	3.9	4.4	
	d 3 – 5	2–3	123^a	47^a	270^a	200^a	110^a	57^a	56^a	0.03
		4+	110^b	41^a	248^b	169^b	106^a	50^b	44^b	
		SED	4.1	8.0	8.4	12.0	7.5	2.8	4.0	
	d 6 – 8	2–3	136^a	42	288	190	180	61	55	0.39
		4+	121^b	35	264	166	154	57	48	
		SED	0.02	0.44	0.08	0.08	0.24	0.27	0.11	
	d 9 – 11	2–3	154	42	347	194	224	63	57	0.39
		4+	147	34	315	187	234	62	48	
		SED	7.8	11.7	19.9	18.2	14.6	5.6	6.0	
	d 12 – 14	2–3	142	35	326	189	200	55	51	0.73
		4+	134	25	313	186	191	52	36	
		SED	8.2	15.0	18.3	19.3	22.7	4.6	6.6	
	d 15 – 17	2–3	145^a	49	284	168	192	103	74	0.96
		4+	124^b	35	255	143	170	81	59	
		SED	8.2	11.7	14.2	20.5	24.8	17.9	8.3	

[1] Interval is inclusive of the hour (i.e., 02 – 05 is 0200 h to 0559 h)

[a – b] Means within a column differ significantly between parities ($P < 0.05$)

Supplemental Table 11. *P*-values for additional fixed effects used in the models investigating the effects of zeolite and parity across time intervals within day during the precalving treatment period. *P*-values for pretreatment covariate behavior corresponding to the outcome variable (Cov.), cohort, and treatment to calving interval, which were included as fixed effects in the model to investigate the main and interactive effects of treatment, parity, and interval on within-day eating time (min/h), lying time (min/h), and activity (steps/h) relative to treatment start date (Tday 0).

Behavior	Tday, d	*P*-values		
		Cov.	Cohort	Treat to Calving Interval
Eating time, min/h	0 – 2	<0.001	<0.01	0.47
	3 – 5	<0.001	0.01	0.57
	6 – 8	<0.001	0.69	0.02
	9 – 11	<0.001	0.44	0.82
	12 – 14	<0.001	0.52	0.85
	15 – 17	<0.001	<0.01	<0.01
Lying time, min/h	0 – 2	<0.001	0.18	0.09
	3 – 5	<0.001	0.48	<0.01
	6 – 8	<0.001	0.05	0.26
	9 – 11	<0.001	<0.01	0.70
	12 – 14	<0.001	0.05	0.65
	15 – 17	<0.001	0.01	0.59
Steps, steps/h	0 – 2	<0.001	0.83	0.82
	3 – 5	<0.001	0.19	0.07
	6 – 8	<0.001	<0.001	0.38
	9 – 11	<0.001	0.05	0.01
	12 – 14	<0.001	0.59	0.05
	15 – 17	<0.001	<0.01	0.07

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