The behavior of dairy cattle in the transition period: Effects of blood calcium status

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ABSTRACT

The aim of this study was to use an automated behavior-monitoring system to objectively assess the association between lying and activity behavior in the precalving, calving, and postcalving periods between multiparous and primiparous cows with (1) normocalcemia, (2) subclinical hypocalcemia, or (3) clinical hypocalcemia at calving. Behavioral data and blood serum samples were collected from 51 multiparous and 21 primiparous Holstein dairy cattle. Blood samples from the coccygeal vein were taken within 24 h of calving, and serum was analyzed to measure total calcium concentration. Cows were classified into one of 3 categories: normocalcemia (serum calcium concentration ≥ 2.0 mmol/L), subclinical hypocalcemia (serum calcium concentration < 2.0 mmol/L, absence of clinical signs), and clinical hypocalcemia (clinical signs and successful treatment). An activity sensor was fitted to the right hind leg of cows 3 wk before their expected calving date. Data for lying time, standing time, number of steps, and the total number of standing and lying bouts (postural transitions) were automatically collected and summed into 15-min blocks. Behavioral variables were summarized into 2-h and 24-h periods before analyses. Mixed effect models were used to analyze cow behavior in the entire 14 d before calving (d -14 to -1), on the day of calving, and the entire 21 d postcalving (d 1 to 21). In the precalving period, multiparous cows with normocalcemia had fewer postural transitions (18.5 ± 6.9 no./d) compared with cows with subclinical hypocalcemia (23.5 ± 8.0 no./d) and clinical hypocalcemia (23.5 ± 8.6 no./d). However, there was no association

between blood calcium status on lying time (min/d) or step count (no./d) for multiparous cows. For primiparous cows, the step count of cows with subclinical hypocalcemia remained constant across the period, and the step count of cows with normocalcemia decreased from 842.8 steps/d on d -14 to 427.5 steps/d on d -1 . Postpartum cows with clinical hypocalcemia were less active (fewer steps) and spent 88 min/d (1.5 h) and 125 min/d (2.1 h) more time lying down compared with cows with subclinical hypocalcemia and normocalcemia, respectively. This shows that clinical hypocalcemia is associated with significant long-lasting behavioral effects on cows during the critical postpartum period.

Key words: hypocalcemia, transition period, dairy cow, calving

INTRODUCTION

Hypocalcemia (or milk fever) is a metabolic disease caused by low blood calcium concentration (Goff, 2008). The onset of lactation results in a sudden demand for calcium and, as calcium homeostasis adapts to meet the challenge, it is reported that most cows have some level of hypocalcemia at calving (Horst et al., 1994). Severe hypocalcemia occurs when cows are unable to maintain blood calcium concentrations and display clinical signs such as lethargy, excitability, prolonged recumbency and, if left untreated, death (Oetzel, 2011).

The annual incidence of clinical hypocalcemia in the United States is 5% (Goff, 2008). Reinhardt et al. (2011) reported the prevalence of subclinical hypocalcemia (blood Ca concentration < 2.0 mmol/L) to be 41 to 54% in cows of second lactation or greater, and 25% for first lactation cows. Hypocalcemia affects farm profitability, and the total cost of a nonfatal case of hypocalcemia has been estimated to be US\$246.23 $\pm$ 52.25 (Liang et al., 2017). This figure rises to NZD\$1,854 for a case where the cow is culled or dies on farm (US\$1,192.40

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at a conversion rate of NZD\$1.00/US\$0.64; Kerslake et al., (2018)).

Blood calcium concentrations in healthy dairy cows are reported to be between 2.1 and 2.5 mmol/L (Goff, 2008). Clinical hypocalcemia is normally diagnosed through symptoms such as sternal recumbency, muscle tremors, or weakness and depression (Oetzel, 2011). Subclinical hypocalcemia is diagnosed after a blood test; however, the threshold used to categorize cows as having subclinical hypocalcemia varies. Most studies use blood calcium concentrations of ≤ 2.0 mmol/L to categorize cows as having subclinical hypocalcemia (Reinhardt et al., 2011; Sepúlveda-Varas et al., 2015; Wilhelm et al., 2017). However, thresholds ranging from 1.8 mmol/L (Jawor et al., 2012) to 2.3 mmol/L (Seifi et al., 2011) have been applied in various research studies.

Hypocalcemia is considered a gateway to other diseases of the transition cow, and is associated with other disorders such as metritis (Neves et al., 2018), displaced abomasum (Neves et al., 2018), retained fetal membranes (Rodríguez et al., 2017), and ketosis (Rodríguez et al., 2017). Cows with blood calcium concentrations ≤ 2.1 or ≤ 2.2 mmol/L in the first week after calving have a higher odds ratio of developing displaced abomasum and an increased culling risk in the first 60 DIM (Chapinal et al., 2012; Roberts et al., 2012). Hypocalcemia presents farmers with an animal health and welfare concern, and the early identification of hypocalcemia could improve the ability of farmers to treat and manage their transition cows.

Studies have indicated that changes in cow behavior could be used to identify cows at risk of clinical ketosis (Itle et al., 2015), lameness (Weigle et al., 2018), and metritis (Neave et al., 2018). However, few studies have reported the relationship between hypocalcemia and cow behavior during the transition period. Jawor et al. (2012) reported that during the 24-h period before calving, cows with subclinical hypocalcemia (blood Ca concentration < 1.8 mmol/L) stood for 2.6 h longer than cows that had no subclinical hypocalcemia. Cows diagnosed with subclinical hypocalcemia at calving were reported to consume an average of 1.7 kg of DM more per day during wk 2 and wk 1 before calving compared with control cows.

The study of behavior in cows diagnosed with normocalcemia, subclinical hypocalcemia, and clinical hypocalcemia could be used to develop indicators of hypocalcemia in dairy cattle during the prepartum period or on the day of calving before she calves. The aims of this study were to use automated behavioral monitoring under commercial farm conditions to describe

the association between hypocalcemia and behavior in primiparous and multiparous cows within the (1) prepartum period, (2) the day of calving, and (3) the postpartum period.

MATERIALS AND METHODS

Animals and Housing

The study was conducted at the University of Edinburgh Langhill Farm (Roslin, Midlothian, UK) between November 2016 and April 2018. The farm had a milking herd of approximately 240 Holstein cows. The calving environment was a straw-bedded shed (11 m $\times$ 18.4 m), which was kept at a stocking rate of 5 to 15 heifers and cows. Cows were fed a TMR consisting of wholecrop, grass silage, concentrate, molasses, and dry cow mineral once per day. The ration had a 4:1 forage:concentrate ratio. Cows had access to self-filling water troughs that supplied municipal water. The experimental work was approved by The Royal Dick School of Veterinary Studies Veterinary Ethical Review Committee (Ref 82-16) and Home Office Project License 70/8105.

Serum Analysis

Within 24 h of calving, blood was taken from each cow from the coccygeal vein into a 6-mL serum vacutainer (BD Vacutainer Serum Tubes; Becton Dickinson, Franklin Lakes, NJ). Blood was centrifuged at $1,300 \times g$ for 10 min. Serum samples were stored at -20°C before analyses at the Dairy Herd Health Productivity Service Laboratory (The Royal Dick School of Veterinary Studies, Edinburgh, Scotland). An automated chemistry analyzer (Beckman Coulter AU480; Beckman Coulter Inc., Brea, CA) was used to measure total calcium concentration (OSR60117; Beckman Coulter Inc.).

Classification of Normocalcemia, Subclinical Hypocalcemia, and Clinical Hypocalcemia on the Day of Calving

Cows were classed as having clinical hypocalcemia when clinical signs indicative of stage I to stage III were observed by farm staff (Oetzel, 2011). All cows with clinical hypocalcemia were recumbent and subsequently successfully treated with a 400-mL solution of calcium gluconate and calcium borogluconate, which provided 11.88 g of calcium (Calciject 40; Norbrook Laboratories Limited, Newry, Northern Ireland). All cows with clinical hypocalcemia recovered fully within 24 h. A cow was classified as having normal blood cal-

cium concentrations when serum calcium concentration was ≥ 2.0 mmol/L (Reinhardt et al., 2011). Cows were classified as having subclinical hypocalcemia when their serum calcium concentration was below 2.0 mmol/L but showed no clinical signs of hypocalcemia.

The original data set included 106 cows. However, 32 multiparous cows and 2 primiparous cows were observed but excluded from the analysis for various reasons including incomplete data sets caused by cow death or data gaps ($n = 20$), severe lameness ($n = 2$; mobility score 2 and 3 using 0–3 point scale; AHDB, 2018), no recorded time of calving ($n = 7$), not bled within 24 h ($n = 3$), or had twins ($n = 2$). The final data set included 21 primiparous and 51 multiparous cows. Of the 21 primiparous cows sampled, 10 primiparous cows were identified as having normal calcium concentrations (2.13 ± 0.13 mmol/L), and 11 primiparous cows were identified as having subclinical hypocalcemia (1.68 ± 0.20 mmol/L). Of the 51 multiparous cows sampled, 15 multiparous cows were identified as having clinical hypocalcemia (0.77 ± 0.17 mmol/L), 30 cows were identified as having subclinical hypocalcemia (1.42 ± 0.08 mmol/L), and 6 cows were identified as having normal blood calcium concentration (2.16 ± 0.06 mmol/L). Seven cows with clinical hypocalcemia were not blood sampled because farm staff judged their clinical signs to be consistent with a clinical diagnosis and did not blood sample them before treatment. However, their activity data were included in the analysis of the clinical hypocalcemia group because their clinical symptoms and successful treatment justified inclusion in this group.

Behavioral Measurements

Approximately 3 wk before their predicted calving date, an IceQube (IceRobotics, South Queensferry, Scotland) was attached to the hind leg of each cow. The IceQube sampled cow behavior at 4 Hz (equivalent to 4 samples/s), before summarizing behavior into 15-min blocks for 5 behavioral measures: standing times, lying times, the number of postural transitions, motion index, and the number of steps (Elischer et al., 2013; Borchers et al., 2016). Observations for time of calving were made when cows were housed in the straw-yard calving shed. One webcam (AXIS P5414-E PTZ Network Camera, Axis Communications, Lund, Sweden) was installed in an overhead position that gave a good overview of the calving shed. A timestamp (hour:min:sec) and a date (year:month:day) were visible on all video files. The exact time of calving (to the nearest minute) was ascertained by retrospective analysis of video recordings.

Statistical Analysis

To investigate the behavior of dairy cattle with differing blood calcium status, the number of postural transitions (total number of lying and standing bouts), the duration of lying time, and the number of steps were summarized from the time of calving into 2 data sets: behavior in 2-h and 24-h periods (behavior per day). The bihourly data set was used for the analyses of cow behavior on the day of calving, and data were analyzed in 2-h periods from -24 h to 0 h (the time of calving). The data set containing cow behavior per day was used to create 2 experimental periods based on the time relative to calving: pre-calving (d -14 to -1) and postcalving (d 1 to d 21). The experimental period was chosen to reflect the transition period, described as the 3 wk before and after parturition (Drackley, 1999). However, to maximize the number of animals included in the study, d -14 to d 21 were selected. In this study, the number of lying and standing bouts had been combined to provide a value for the number of postural transitions within a period. The data sets were summed from the time relative to calving (i.e., calving was used as time 0), which ensured all cows followed the same timeline.

The presence of hypocalcemia is associated with other disorders of the transition cow. Therefore, animals that developed a clinical disease postcalving (other than clinical hypocalcemia) were not excluded from the study, and disease was controlled for as a fixed effect (diseased and not diseased). Disease was diagnosed after observation of clinical signs by farm staff or a veterinarian. Of the 21 primiparous cows, 4 cows developed disease (normocalcemia, $n = 2$; subclinical hypocalcemia, $n = 2$) at 11 ± 0.2 d postcalving diagnosed as lameness ($n = 2$), mastitis ($n = 1$), and metritis ($n = 1$). Of the 51 multiparous cows, 6 cows developed disease (clinical hypocalcemia, $n = 4$; subclinical hypocalcemia, $n = 2$) at 6.8 ± 1.4 d postcalving diagnosed as displaced abomasum ($n = 1$), metritis ($n = 2$), and retained fetal membranes ($n = 3$). Animals that had an assisted calving were included in the study, and assistance at calving was controlled for as a fixed effect. Four primiparous cows and 8 multiparous cows were assisted at calving.

All analyses and data manipulations were carried out using RStudio (version 3.4.4; R Foundation for Statistical Computing, Vienna, Austria). Residual plots, histogram plots, and data normality tests were examined to assess data assumptions of normality. Mixed effect analyses, as outlined below, were carried out using the lmerTest package. Residual plots were examined to ascertain the best model fit for the data. Statistical

significance was taken as $P \leq 0.05$. Nonsignificant ($P > 0.05$) interactions were removed from models. Degrees of freedom (df) were calculated using the Satterthwaite approximation for linear models and polynomial regression models, and Laplace Approximation was used to calculate degrees of freedom for generalized piecewise regression models. Differences between the 3 levels of hypocalcemia (normocalcemia, subclinical hypocalcemia, and clinical hypocalcemia) were determined using a Tukey HSD test. The F -values were not reported by the Tukey HSD test.

Pre- and Postcalving Analysis. There was an effect of blood calcium status $\times$ parity (primiparous and multiparous) on all behavioral variables across the study period (d -14 to d 21). In addition, there were no clinical hypocalcemia cases in primiparous cows. As a result, we decided to analyze data for primiparous cows and multiparous cows separately.

Mixed effects models were used to explore (1) the behavioral differences between primiparous cows with subclinical hypocalcemia ($n = 11$) and normocalcemia ($n = 10$) and (2) the behavioral differences between multiparous cows with clinical hypocalcemia ($n = 15$), subclinical hypocalcemia ($n = 30$), and normocalcemia ($n = 6$) in the period relative to calving, both precalving (d -14 to -1) and postcalving (d 1 to d 21).

To analyze the differences in lying time behavior and step count, linear mixed effects models were used. The step count was $\log_{10}$ transformed to meet normal residual data distribution assumptions. To analyze the differences in postural transitions, generalized linear mixed effects models with a Poisson error distribution were used. Blood calcium status (normocalcemia, subclinical hypocalcemia, or clinical hypocalcemia), days from calving, and the interaction between day $\times$ blood calcium status were included as fixed effects. Disease postcalving (diseased and not diseased) and assistance level at calving (assisted and nonassisted) were included as covariates. Cow identification was included as a random effect to account for repeated behavioral measurements per cow. The primiparous precalving analysis contained 294 data points and the multiparous precalving analysis contained 714 data points. Each animal had 14 repeated observations. The primiparous postcalving analysis contained 441 data points and the multiparous postcalving analysis contained 1,071 data points. Each animal had 21 repeated observations.

The Last 24 h Before Calving. To investigate whether blood calcium status affected the pattern of behavior exhibited on the day of calving, a set of mixed effect models with different temporal relationships were

fitted to behavioral variables contained within the bi-hourly data set. Data for primiparous and multiparous cows were analyzed separately over 12 (2-h) periods from -24 h to 0 h (the time of calving).

Generalized piecewise mixed effect regression models (or “broken-stick” models—where a step change in behavior at a certain time point is observed; Das et al., 2016), polynomial mixed effect regression models (where there is either a decrease and then increase, or increase and then decrease in behaviors; Ostertagová, 2012), and linear models (Bangdiwala, 2018) were fitted to each behavioral variable. Residual plots were examined to ascertain the best model fit for the data. A piecewise mixed effect regression model provided the best description of the change in the number of postural transitions for primiparous cows, while a generalized mixed effect linear model (Poisson error distribution) best described the change in postural transitions for multiparous cows. A polynomial mixed effect regression model best described the change in lying time. Linear mixed effect models best described the change in log-transformed step count. Blood calcium status (normocalcemia, subclinical hypocalcemia, or clinical hypocalcemia), hours from calving, and the interaction between hour $\times$ blood calcium status were included as fixed effects. Disease postcalving (diseased and not diseased) and assistance level at calving (assisted and nonassisted) were included as covariates. Cow identification was included as a random effect to account for repeated behavioral measurements per cow. The primiparous cow day-of-calving analysis contained 252 data points, and the multiparous cow day-of-calving analysis contained 612 data points. Each cow had 12 repeated observations.

RESULTS

Precalving Analysis: Primiparous Cows

During d -14 to d -1 , there was no interaction between blood calcium status and days from calving on lying time (min/d; Figure 1a), nor on the number of postural transitions (no./d; Figure 1b). There was an interaction between blood calcium status and days from calving on step count (no./d; Figure 1c). The data showed that although the step count of cows with subclinical hypocalcemia remained constant across the period ($F_{1,142} = 0.9$, $P = 0.35$), the step count of cows with normocalcemia decreased, and back transformation of log steps showed that steps decreased from 842.8 steps/d on d -14 to 427.5 steps/d on d -1 ($F_{1,129} = 37.9$, $P < 0.001$).

Figure 1. Behavioral variables for primiparous cows with normocalcemia (solid line; $n = 10$) and primiparous cows with subclinical hypocalcemia (dashed line; $n = 11$) summarized into 24-h periods $\pm$ SE in the -14 d to 21 d around calving for (a) lying time duration (min/d), (b) number of postural transitions (no./d), and (c) step count (no./d).

Day of Calving: Primiparous Cows

On the day of calving, there was no interaction between hours to calving and blood calcium status on the lying time duration (min/2h), the number of postural transitions (no./2 h), and the number of steps (no./2 h; $P > 0.05$). In addition, there was no association between blood calcium status and the lying time duration, the number of postural transitions, or the number of steps ($P > 0.05$).

Postcalving Analysis: Primiparous Cows

During d 1 to d 21, there was no interaction between blood calcium status and days from calving on lying time (min/d; Figure 1a) or the number of postural transitions (no/d; Figure 1b; $P > 0.05$). However, there was an interaction between blood calcium status and days from calving on step count (no/d; Figure 1c).

Back transformation of log steps shows that step count of cows with normocalcemia decreased from 1,351.6 steps/d on d 1 to 912.9 steps/d on d 21, and the step count of cows with subclinical hypocalcemia decreased from 2022.1 steps/d on d 1 to 651.9 steps/d on d 21 ($F_{1,432} = 22.2$, $P < 0.001$). There was no association between blood calcium status on lying time, the number of postural transitions, or step count ($P > 0.05$).

Precalving Analysis: Multiparous Cows

During d -14 to d -1 , there was no interaction between blood calcium status and days from calving on lying time (min/d), the number of postural transitions (no./d), or step count (no./d; Figure 2a–c; $P > 0.05$). Cows with normocalcemia had fewer postural transitions in the prepartum period (18.5 ± 6.9 no./d) compared with cows with subclinical hypocalcemia (23.5 ± 8.0 no./d; $P = 0.036$) and clinical hypocalcemia ($23.5 \pm$

8.6 no./d; $P = 0.031$). Cows with clinical hypocalcemia tended to have fewer steps across the period compared with cows with normocalcemia and subclinical hypocalcemia ($F_{2,46} = 3.1$, $P = 0.052$). There was no difference in step count between cows with normocalcemia and cows with subclinical hypocalcemia, nor an association between hypocalcemia and lying time duration ($P > 0.05$).

Day of Calving: Multiparous Cows

On the day of calving, there was no interaction between hours to calving and blood calcium status, nor an association between blood calcium status on the lying time duration (min/2 h), the number of postural

transitions (no./2 h), or the number of steps (no./2 h; $P > 0.05$).

Postcalving Analysis: Multiparous Cows

There was an interaction between blood calcium status and days from calving on lying time, the number of postural transitions, and step count (Figure 2a–c). The lying time of cows diagnosed with clinical hypocalcemia decreased by 5.6 ± 1.3 min/d across the period from 780.6 ± 30.3 min/d on d 1 to 661.2 ± 32.6 min/d on d 21 ($F_{1,299} = 19.1$, $P < 0.001$). In contrast, the lying time of cows diagnosed with subclinical hypocalcemia increased by 2.6 ± 0.8 min/d from 474.9 ± 52.4 min/d on d 1 to 585.3 ± 20.2 min/d on d 21 ($F_{1,599} = 11.8$,

Figure 2. Behavioral variables for multiparous cows with normocalcemia (solid line; n = 6), multiparous cows with subclinical hypocalcemia (dashed line; n = 30), and multiparous cows with clinical hypocalcemia (dashed-dot line; n = 15) summarized into 24-h periods $\pm$ SE in the -14d to 21d around calving for (a) lying time duration (min/d), (b) number of postural transitions (no./d), and (c) step count (no./d).

$P < 0.001$). The lying time of cows with clinical hypocalcemia was higher in the postcalving period (643.7 ± 10.3 min/d) compared with cows with subclinical hypocalcemia (555.3 ± 5.8 min/d) and normocalcemia (518.3 ± 11.5 min/d; $F_{2,59} = 15.1$, $P < 0.001$). There was no difference in lying time between cows with normocalcemia and cows with subclinical hypocalcemia ($df = 1,34$, $P > 0.05$).

For cows with clinical hypocalcemia, the average number of postural transitions decreased from 30.9 ± 2.2 /d on d 1 to 23.6 ± 2.6 /d on d 21 ($z_{1,299} = -5.9$, $P < 0.001$), and the average number of postural transitions for subclinical hypocalcemia cows also decreased from 32.5 ± 1.7 no./d on d 1 to 22.4 ± 1.4 no./d on d 21 ($z_{1,599} = -6.5$, $P < 0.001$). Cows with normocalcemia had fewer daily postural transitions (18.4 ± 0.5 no./d; $z_{2,59} = -2.6$, $P = 0.01$) compared with cows with clinical hypocalcemia (22.0 ± 0.5 no./d). There was no difference in the average postural transitions between cows with subclinical hypocalcemia (21.1 ± 0.3 no./d) and cows with normocalcemia, nor any difference in the number of postural transitions between cows with subclinical hypocalcemia and cows with clinical hypocalcemia ($P > 0.05$).

Back transformation of log-transformed steps shows the step count of cows with clinical hypocalcemia decreased from 620.0 steps/d on d 1 to 462.0 steps/d on d 21 ($F_{1,299} = 7.02$, $P = 0.008$), and the step count of cows with subclinical hypocalcemia decreased from 1,391.0 steps/d on d 1 to 672.0 steps/d on d 21 ($F_{1,599} = 233.6$, $P < 0.001$). For cows with normocalcemia, step count decreased from 1,442.6 steps/d on d 1 to 801.8 steps/d on d 21 ($F_{1,119} = 27.4$, $P < 0.001$). Over the period, cows with clinical hypocalcemia had an average of 503.4 steps/d compared with cows with normocalcemia that had 948.0 steps/d and cows with subclinical hypocalcemia that had 774.5 steps/d ($F_{2,59} = 14.8$, $P < 0.001$). There was no difference in step count between cows with normocalcemia and cows with subclinical hypocalcemia ($df = 1,34$, $P > 0.05$).

DISCUSSION

To our knowledge, this is the first study to investigate behavioral differences associated with normocalcemia, subclinical hypocalcemia, and clinical hypocalcemia in primiparous and multiparous transition cows. We found behavioral differences in the precalving period for both primiparous and multiparous cows. These behavioral differences could be developed to help categorize animals as having clinical hypocalcemia, subclinical hypocalcemia, and normocalcemia before calving. We also found notable associations between clinical hypocalcemia and lying and activity behaviors in multiparous

cows within 21 d postcalving. This finding illustrates that the effects of lowered blood calcium status at calving can be significant and prolonged, even in cows that had successful treatment (clinical hypocalcemia).

In the precalving period (d -14 to d -1), we found that multiparous cows with normocalcemia had fewer lying and standing bouts compared with cows that developed subclinical and clinical hypocalcemia. Our findings contradict those of Jawor et al. (2012) that found no difference in standing bout behavior in the 7 d before calving when cows with subclinical hypocalcemia ($n = 15$) and normocalcemia ($n = 15$) were compared. Differences in the 2 study findings could be explained by categorization of subclinical hypocalcemia and study size. Jawor et al. (2012) classed cows as having subclinical hypocalcemia when serum calcium concentration was ≤ 1.8 mmol/L, whereas the threshold in this study was higher (2.0 mmol/L). Our study had fewer cows categorized as normocalcemia ($n = 6$); however, we had an additional category (clinical hypocalcemia, $n = 15$) and more cows categorized as having subclinical hypocalcemia ($n = 30$). Due to the limited sample size of cows categorized with normocalcemia, our results should be treated with caution. Future work should look to increase the number of cows within the study population, which will increase the sample size of cows within each category.

Within the precalving period (d -14 to d -1), the step count of primiparous cows with normocalcemia decreased. There was no interaction between day to calving and step count for primiparous cows diagnosed with subclinical hypocalcemia; however, their step count was initially lower. Jawor et al. (2012) did not observe activity behaviors. Primiparous cows had never experienced the precalving environment or management, a time of stress due to dietary changes and regrouping. Neave et al. (2017) reported multiple differences in the behavior of healthy primiparous and multiparous cows in the transition period. For example, primiparous cows spent more time exploring their feeding environment and visited the feeder more often than multiparous cows. Primiparous cows were displaced at the feeder more frequently than multiparous cows, ate more slowly, and spent a longer duration feeding. It could be theorized that in this study, primiparous cows with normocalcemia were exploring and adapting to their new environment before settling within the precalving environment. However, there was no difference observed in lying time duration or the number of lying and standing bouts. This study set out to identify if normocalcemia, subclinical hypocalcemia, and clinical hypocalcemia were associated with behavioral differences in the precalving period. We found differences in precalving behavior for primiparous and multiparous

cows associated with blood calcium status, which suggests precalving behavior could be used to categorize animals as having normocalcemia, subclinical hypocalcemia, or clinical hypocalcemia before calving. Further research is required to develop these findings, and to see if behavior can be used to classify cows on commercial dairy farms.

This study identified no association between blood calcium status and lying and activity behaviors on the day of calving (−24 h to 0 h) for both primiparous and multiparous cows. Hypocalcemia is caused by a failure of calcium homeostasis to adapt to the sudden demand for calcium at the onset of lactation (Goff, 2008). Cows diagnosed with subclinical or clinical hypocalcemia are less able to regulate calcium and are likely to have lower circulating concentration of calcium during parturition. Calcium is required for skeletal and smooth muscle contraction (Wilkens et al., 2020), and it was theorized that hypocalcemia would affect how a cow behaved during parturition. Previous literature has reported behavior on the day of calving to be vastly unlike any other day (Miedema et al., 2011), and perhaps subtle changes in behavior caused by reduced calcium concentration may not manifest itself in a period that is typically characterized by pain and exhaustion of the cow (Barrier et al., 2012). Our findings contrast with those of Jawor et al. (2012) that reported cows with subclinical hypocalcemia spent 3 h longer standing during the 24-h period before calving compared with control cows. It is unclear why the findings of these 2 studies differ. However, differences in methodology could be an attributing factor. Jawor et al. (2012) pair-matched 15 cows with subclinical hypocalcemia and 15 cows with normocalcemia based on parity and, when possible, health disorders such as metritis, mastitis, and fever. We were unable to pair-match multiparous cows due to the limited sample size of cows diagnosed with normocalcemia.

In the postcalving period (d 1 to d 21), there was an association of blood calcium status with all behavioral variables for multiparous cows. Multiparous cows with clinical hypocalcemia were less active, spending more time lying down compared with cows with subclinical hypocalcemia and normocalcemia. Over the period, cows with clinical hypocalcemia had fewer average steps per day compared with cows with normocalcemia and subclinical hypocalcemia. A possible explanation for our findings is that cows with clinical hypocalcemia display sickness behavior in the postcalving period. Sick cows are reported to alter their daily behavioral patterns (Dittrich et al., 2019), and previous literature has reported behavioral changes in dairy cattle during sickness. For example, lame cows reduce their activity and increase their lying time (Weigelt et al., 2018), and

primiparous cows with more than one clinical disease (not including lameness) are reported to have longer lying bouts and spend more time lying compared with healthy animals (Sepúlveda-Varas et al., 2014). In light of these findings, management of cattle with clinical hypocalcemia could be altered. Farmers could allow cows to spend more time in the sick pen, typically a shed with a soft surface (e.g., straw bedded), that would enable cows to lie down in greater comfort (Thomsen et al., 2019).

Although cows diagnosed with clinical hypocalcemia recovered posttreatment, the significant postcalving behavioral differences indicated that hypocalcemia has long-lasting effects, and prevention of hypocalcemia should have high priority on dairy farms. Recent studies (Caixeta et al., 2017; Rodríguez et al., 2017; Neves et al., 2018) reported that hypocalcemia at calving leads to impaired reproductive function and an increased risk of health disorders, such as ketosis, retained placenta, and metritis, which further emphasizes the importance of preventing hypocalcemia to improve cattle health and welfare.

Jawor et al. (2012) and the current study did not monitor rumination or the duration of lying bouts. Previous literature has reported that in the week before calving, rumination times of cows with subclinical ketosis (plus another health problem) were lower compared with their healthy counterparts (Kaufman et al., 2016). Investigation of rumination and lying bout behavior before calving could provide an early indicator of calcium status on the day of calving, allowing farmers to intervene and to administer prompt treatment. Future work should look at the inclusion of rumination and the duration of lying bouts.

A limitation of this study was that only a small number of multiparous cows were categorized as having normocalcemia ($n = 6$). A small study population of normocalcemic cows has occurred in other behavioral studies ($n = 15$; Jawor et al., 2012). It is theorized that this study contains fewer normocalcemic cows due to a higher serum calcium threshold specified within the categorization of cows (≥ 2.0 mmol/L vs. ≥ 1.8 mmol/L). Another limitation of the current study was that some cows were categorized as having clinical hypocalcemia based on clinical signs alone, without the back-up of clinical biochemistry. This was allowed to increase the number of cows in the study population with clinical hypocalcemia. In addition, this study included animals that developed clinical disease postcalving. These limitations could have affected the results of this study, and thus the reader must be cautioned on the interpretation of these findings that are based on a relatively small population size. In addition, it was not possible to investigate the behavioral changes associated with

normocalcemia, subclinical hypocalcemia, and clinical hypocalcemia on subsequent animal health, as only a small number of animals ($n = 10$) developed a health issue postcalving.

CONCLUSIONS

In the precalving period, multiparous cows with normocalcemia had fewer postural transitions compared with multiparous cows with subclinical and clinical hypocalcemia. In addition, there was a decrease in step count across the precalving period for normocalcemic primiparous cows. Blood calcium status at calving was associated with differences in lying time duration, the number of postural transitions, and step count in the postcalving period for multiparous cows. Although these findings must be treated with caution due to the limited population size, they suggest that behavioral differences could be developed to help categorize animals as having clinical hypocalcemia, subclinical hypocalcemia, and normocalcemia before calving, and also illustrate the profound and long-lasting effects of clinical hypocalcemia on the cow.

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