Claw horn lesions in mid-lactation primiparous dairy cows under pasture-based systems: Association with behavioral and metabolic changes around calving

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ABSTRACT

The objectives of this study were to describe the lying behavior of primiparous dairy cows under pasture-based systems during the pre- and postcalving period and characterize the association of lying behavior and analytes related to energy metabolism during this period with claw horn disruption lesion development later in lactation. Our convenience sample included 39 primiparous Holstein cows from 3 commercial farms that were assessed for body condition score (BCS; 5-point scale, 0.25-point increments) and had blood collected at wk -3, -2, -1, 1, 2, and 3 relative to calving date. Blood samples were assayed for nonesterified fatty acids, β -hydroxybutyrate (BHB), and cholesterol concentrations. Electronic data loggers (HOBO Pendant G Acceleration, Onset Computer Corporation, Bourne, MA) recorded lying behavior at 1-min intervals from 3 wk before calving to 3 wk after calving. Starting at 4 wk after calving and until 16 wk after calving, cows were examined for claw lesions at approximately 4-wk intervals. Sole lesions and white line lesions were scored on a 0 to 10 scale. Of the 39 primiparous cows, 19 cows scored 0 at all exams during the entire study period and 20 cows had at least 1 severe lesion (score ≥ 4) between 8 and 16 wk after calving. Time spent lying before calving averaged 10.3 ± 0.3 h/d, but declined to 7.3 ± 0.3 h/d after calving (least squares means $\pm$ standard error). At calving, we noted an increase in the number of lying bouts (12.9 ± 0.45 bouts/d) compared with the pre- and postcalving averages of $11.6 (\pm 0.53)$ and $9.1 (\pm 0.47)$ bouts, respectively. Cows that developed claw lesions later in mid lactation spent less time lying down than cows without lesions during wk 3 after calving compared with healthy cows (7.29 ± 0.22 vs. 8.51 ± 0.16 h/d). Lesion cows had fewer lying bouts per

day, and these bouts were of longer duration than no-lesion cows after calving. Increased odds of lesion were found to be associated with shorter lying times and fewer number of lying bouts during wk 3 (odds ratio = 1.23). Nonesterified fatty acids (747 ± 58 vs. 990 ± 86.85 μ mol/L) and BHB (0.77 ± 0.06 vs. 0.60 ± 0.04 mmol/L) concentrations during wk 1 were greater in cows that developed claw lesions relative to cows that did not develop lesions. The BHB concentrations also remained higher in wk 2 for cows that developed claw lesions (0.63 ± 0.04 vs. 0.46 ± 0.03 mmol/L) compared with cows that did not develop any lesions. Cows that developed lesions experienced greater losses in BCS from wk -3 to 3 than cows without lesions (0.74 ± 0.01 and 0.61 ± 0.01 BCS change, respectively). In summary, changes in lying behavior and energy metabolic status after calving were associated with claw horn disruption lesions in mid-lactation primiparous cows under pasture-based systems.

Key words: lameness, negative energy balance, heifers, transition, grazing

INTRODUCTION

Lameness in dairy cattle is one of the most important economic and welfare issues facing the global dairy industry (Huxley, 2013). Lesions categorized as claw horn disruption lesions (**CHDL**; Hoblet and Weiss, 2001), including sole hemorrhage, sole ulcer, and white line disease, are common causes of lameness, and several studies have shown that these lesions are highly prevalent in first-lactation cows (Capon et al., 2009; Maxwell et al., 2015; Randall et al., 2016). For example, a study conducted in Denmark reported a prevalence of 56% for moderate to severe sole hemorrhage and 70% for white line lesions in primiparous cows (Capon et al., 2009). Moreover, studies have shown that previous CHDL lesions constitute a major risk factor for the future development of claw lesions (Foditsch et al., 2016; Newsome et al., 2017b). Thus, reducing the

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risk for claw lesion development in primiparous cows may greatly contribute to an overall reduction in lesion development and, consequently, lameness prevalence in dairy cows.

Some have argued that metabolic and behavioral changes during the transition period, from 3 wk before to 3 wk after calving, increase the risk for CHDL development. Cows with low BCS in the weeks around parturition had an increased risk for developing lameness in general (Hoedemaker et al., 2009), and lameness related to CHDL in particular (Green et al., 2014). Similarly, loss of body fat thickness (as measured by ultrasound) after calving was related to increased risk for sole hemorrhage and lesions (Newsome et al., 2017b). Loss of body fat and low BCS are typically a result of negative energy balance (NEB), as experienced by many cows at the onset of lactation. One theory is that loss of body fat also coincides with reduction of fat reserves of the digital cushion, which is mainly composed of adipose tissue (Räber et al., 2004; Räber et al., 2006). As a result, thinning of the digital cushion may then compromise its function of protecting the corium (Bicalho et al., 2009). The metabolites fatty acids, BHB, and cholesterol are common measures of NEB in transition animals (Herd, 2000) and may function as an earlier indicator of metabolic challenge than BCS or body fat loss. However, to our knowledge, no study has investigated the relationship of these metabolites during the transition period with claw horn lesion development further in lactation.

Changes in standing and lying behavior during the transition period may also predispose cows to the development of CHDL (Chapinal et al., 2009; Proudfoot et al., 2010; Dippel et al., 2011). Chapinal et al. (2009) reported that freestall-housed mid-lactation cows diagnosed with sole ulcers lay down less in the weeks before calving but increased their lying time at a faster rate in the weeks following parturition compared with cows that did not develop a sole ulcer. Similarly, Proudfoot et al. (2010) reported that freestall-housed multiparous cows diagnosed with CHDL 13 to 15 wk postpartum stood longer during the 2 wk before and 24 h after calving compared with their healthy counterparts. However, whether changes in standing and lying behavior similarly increase the risk for claw lesions in pasture-based systems is unknown. Given that healthy, nonlame cows housed in pasture-based systems differ in standing and lying behaviors compared to cows housed in confinement (Hernandez-Mendo et al., 2007; Legrand et al., 2009), further investigation of whether changes in these behaviors during transition are a risk factor for lesion development in dairy cows at pasture is needed.

To our knowledge, the majority of information available to date on lying behavior in transition cows has been carried out in a freestall barn environment. Thus, the aims of our study were to (1) characterize changes in lying behavior during the pre- and postcalving period of primiparous grazing dairy cows and, (2) determine if lying behavior and metabolic indicators of energy metabolism can be used to identify cows at risk for claw horn lesions later in lactation. We hypothesized that primiparous dairy cows with reduced lying time and metabolic changes suggesting NEB, both pre- and postcalving, would be at higher risk of developing claw lesions.

MATERIALS AND METHODS

Herd Selection and Management

This prospective observational study was conducted on 3 seasonally calving commercial dairy herds located in the S=southern province of Valdivia, Chile. Herds were selected as a convenience sample based on their proximity to the University Austral de Chile (Valdivia, Chile). Participating dairies met the following criteria: pasture housing during lactation and dry period, spring-calving regimen, more than 200 milking cows of predominantly Holstein breed, and willingness to participate in the study. Average herd size was 590 ± 310 ($\pm$ SD, ranging from 230 to 980) and average herd milk production was $9,125 \pm 1,430$ kg annually (ranging from 7,500 to 11,000) based on annual yield estimates. The study was approved by the University Austral de Chile Animal Ethics Committee (#202/2015).

In total, 105 pregnant heifers were enrolled in the study. On all farms, heifers were kept on pastures (mixture of grasses and legumes) until approximately 4 wk before expected calving date, at which time they were moved to a confined straw yard where they remained until calving. Upon moving into the straw yard, heifers were mixed with multiparous prepartum cows in 2 of the herds, whereas in the other herd heifers were maintained separately. On all farms, cows were provided with approximately 10 m²/cow of straw yard space. Access to feed was provided through a post-and-rail feed barrier system. While feeding, cows were required to stand on a concrete slatted floor that was positioned adjacent to the feed barrier. The prepartum diet was based on a ration consisting of ryegrass hay, corn or grass silage, and chopped straw. The typical composition of ryegrass hay consumed by cows under this system was 18 to 20% CP and 47 to 51% NDF, whereas consumed grass silage contained 14 to 18% CP and 50 to 55% NDF, and corn silage was 7 to 8% CP and 42 to 48% NDF. Cows were

offered 2 to 3 kg of commercial concentrates/d on an as-fed basis (86 to 88% DM) with an anionic mineral mix (Mg 4.0%, Cl 33.0%, S 2.6%, Ca 0.7%, K 0.3%, 2 mg of Na/kg, 1,050 mg of Cu/kg, 2,100 mg of Mn/kg, 3,500 mg of Zn/kg, 140 mg of I/kg, 13 mg of Co/kg, 10 mg of Se/kg) at a rate of 0.20 kg/cow per day.

After calving (0 or 1 DIM), all cows were moved to the mixed parity lactating group. Cows were maintained under pasture paddocks of 3 to 5 ha and managed in a daily rotational method. Stocking rate for the total area of pasture was approximately 8 to 10 cows/ha. The pasture consisted primarily of perennial ryegrass (*Lolium perenne*), which provided on average 70% of the daily energy intake. The typical composition of ryegrass consumed by cows during spring under grazing is 22 to 25% CP and 42 to 48% NDF. Cows were offered 4 to 6 kg of commercial concentrates per day on an as-fed basis (86 to 88% DM) during and immediately after each milking. A mineral mix (Ca 14.0%, P 10.0%, Mg 6.0%, Na 4.0%, S 0.2%, 5,000 mg of Zn/kg, 1,500 mg of Cu/kg, 200 mg of I/kg, 20 mg of Co/kg, 14 mg of Se/kg) was offered with the concentrate at a rate of 0.25 kg/cow per day. Cows did not receive glucogenic or Ca supplementation postpartum. All animals were milked twice daily between 0500 to 0800 and 1500 to 1800 h, depending on the farm management.

Data Collection

Two trained veterinarians performed all visits and data collection. Each week, cows that were 3 to 4 wk before their expected calving date and had already been moved to the straw pen were enrolled in the study based on the availability of data loggers used for the assessment of lying behavior. At enrollment, cows were locomotion scored as they walked at the concrete slatted floor using a 5-point numerical rating system (NRS), where 1 = sound and 5 = severely lame (Flower and Weary, 2006). Only cows with a NRS ≤ 2 were enrolled in the study.

Lying Behavior

Lying behavior was collected using electronic data-loggers (Hobo Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA) attached to the hind leg of each cow using a flexible bandage. Every 10 d, loggers were removed for data download and then reattached to the leg. Data from the day of logger download were discarded. The loggers were programmed to record the G-forces of the y- and z-

axes at 1-min intervals. Using the algorithm for SAS (SAS Inc., Cary, NC) developed by UBC Animal Welfare Program (2013) and a cutoff point validated by Ledgerwood et al. (2010), this information was used to determine whether the cow was standing or lying, and subsequently used to calculate daily lying time, number of lying bouts (i.e., frequency of transitions from lying to standing positions), and duration of lying bouts (i.e., calculated as a ratio of minutes lying to the number of lying bouts per day).

BCS, Serum Metabolites, and Clinical Disease

From the time of enrollment until 4 wk after parturition, the BCS of each cow was determined weekly by 2 trained observers based on visual observation and palpation, using a 5-point scale with 0.25 intervals (1 = lean, 5 = obese; Edmonson et al., 1989). Before the study, the observers scored 100 cows simultaneously and achieved high interobserver agreement (Cohen's Kappa = 0.89). At the same time as BCS assessment, blood samples were taken at approximately 0900 h during the prepartum period and approximately 1 h after morning milking during the postpartum period. Blood was collected from the coccygeal vein into 10-mL sterile tubes without anticoagulant and then transported to the laboratory within 5 h of collection. Serum was separated immediately upon arrival at the laboratory and stored at -20°C until analysis. Concentrations of BHB (Ranbut, Randox, Crumlin, United Kingdom), fatty acids (Randox), and total cholesterol (CHOD-PAP, Human, Wiesbaden, Germany) were measured by enzymatic analysis using an auto-analyzer (Metrolab 2300, Wiener Lab, Rosario, Argentina). Intra-assay coefficients of variation for the BHB, fatty acids, and cholesterol assays were 2.1, 3.6, and 4.2%, respectively.

Clinical diseases occurring during the first 3 wk after calving were recorded by farm staff, using established case definitions distributed and explained to the farm staff at the start of the experiment. Retained placenta was defined as failure to expel the placenta within 24 h after parturition. Clinical hypocalcemia was defined as any recumbent cow within 72 h after parturition exhibiting anorexia, nervous symptoms, staggering, varying degrees of unconsciousness, and good response to intravenously administered calcium. Clinical mastitis was characterized by the presence of abnormal milk or by signs of inflammation in 1 or more quarters. Puerperal metritis was characterized by a fetid watery red-brown vaginal discharge and rectal temperature $\geq 39.5^{\circ}\text{C}$. Presence of any other clinical health disorders such as digestive or respiratory problems were also recorded.

Table 1. Description of type and number of claw horn lesions in primiparous dairy cows housed primarily on pasture during the postcalving period and up to 2 and 4 mo of lactation (n = 105)

Type of lesion ¹	3 to 4 wk after calving			2 to 4 mo after calving		
	Herd 1	Herd 2	Herd 3	Herd 1	Herd 2	Herd 3
Sole lesion						
Score 1 to 3	20	12	6	15	12	7
Score 4 to 10	0	0	0	9	5	3
White line lesion						
Score 1 to 3	7	6	1	8	6	2
Score 4 to 10	0	0	0	2	2	1
Digital dermatitis						
Score 1 to 3	4	4	1	5	3	2
No visible lesion	12	15	17	4	9	10
Total cows	43	37	25	43	37	25

¹In cases where cows were identified as having both sole and white line lesions, the more severe of the 2 was counted and included.

Claw Lesion Recording

Starting at 3 to 4 wk after calving and until 16 wk after calving, cows were examined for claw lesions at approximately 4-wk intervals. At each examination, the sole surface of the hind hooves was cleaned with water, dried with paper towels, and then photographed. Thereafter, approximately 2 mm of sole horn were removed to allow visualization of any lesions present on the sole surface (see Capión et al., 2009) and a second photograph was taken. Hoof lesions were later diagnosed from the photographs by an experienced observer (see Miguel-Pacheco et al., 2017) that never visited the farm and was blind to cow. Each photograph was scored 2 times, with images presented in a random order. In case of disagreement on the diagnosis (lesion type and severity) between these 2 scorings, a third scoring was performed to allow for a final decision. At the end of the study, the intraobserver reliability for lesion identification was assessed by evaluating 20% of the photographs twice; the achieved intraobserver agreement was good (Cohen’s Kappa = 0.80).

Sole lesions and white line lesions were scored using a 1 to 10 scale as originally described by Offer et al. (2000) and adapted by Randall et al. (2016). Briefly, sole and white line lesions were classified as a score of 1 to 5 if sole or white line hemorrhage was observed, and 6 to 10 if ulcers of the sole or white line separation was identified. Sole lesions and white line lesions were classified as severe when scored as ≥4. In detail, we used scoring criteria for severe sole hemorrhages noting the presence of very dark red or purple coloration at any location on the sole; sole ulcers were from mild to severe sole ulcer, including disruption of sole surface, corium not exposed to penetration of sole surface with exposure of corium, or granulation tissue present;

severe white line hemorrhage noted presence of very dark red or purple coloration at any locations on the white line; and white line separation noted dark colored marks, fissures with the corium involved or purulent exudate, necrosis, granulation tissue, or separation of wall and sole at any location (Randall et al., 2016). In cases where cows were identified as having both sole and white line lesions, the more severe of the 2 was counted.

Digital dermatitis was classified using a 1 to 3 score (1 = mild lesion present in a small area; 2 = moderate larger lesion with slight exudate; 3 = severe deeper lesion with exudate reddening and swelling; Randall et al., 2016). See Table 1 for a detailed description of lesion distribution by farm.

Cow Inclusion Criteria

As lying behavior is altered in cows with lameness caused by claw lesions (Chapinal et al., 2009; Nechanitzky et al., 2016), we excluded cows identified as having CHDL at the first examination (3 to 4 wk after calving, n = 52); these cows may have been lame in the weeks beforehand when we assessed lying behaviors. Cows that showed signs of infectious hoof problems (digital dermatitis; n = 9) during the study period were excluded. Further, cows with other clinical disease during the postcalving period (i.e., retained placenta, metritis, or mastitis; n = 5) were also removed from the final data set.

For the remaining 39 cows, we retrospectively categorized them into 2 groups: 1) no lesion group (n = 19; score 0 at all exams during the entire study period) and, 2) lesion group (n = 20). The lesion group included cows that were at least once scored with a minimum of one severe lesion between 8 and 16 wk after calving (2

had sole ulcers scored as 6, 15 had sole hemorrhages scored as 4 or 5, and 3 had white line disease scored as 4).

Statistical Analysis

Statistical analysis were performed using SAS (version 9.3; SAS Institute Inc., Cary, NC) with cow as the experimental unit. To describe the lying behavior of healthy, nonlame transition dairy cows, we used data collected from cows identified as having no claw lesions at the first examination (3 to 4 wk after calving, $n = 39$). Lying behaviors measures were summarized for each day and then summarized by observational periods based on days relative to parturition: precalving (d -21 to d -2), calving (d -1 to 1), and postcalving (d 2 to 21). Data were analyzed using mixed models (PROC MIXED) with repeated measures over cow, considering farm as a random effect and period relative to calving and calving month (July, August, September, and November) as fixed effects. The covariance structure selected was compound symmetry based on the lowest Akaike's information criterion. To determine if these behaviors changed over time, the slopes from the pre- and postcalving periods were compared with 0.

To detect differences in lying behaviors, blood metabolite concentrations, and BCS during transition between cows with and without lesions in mid lactation, data were stratified by period (wk -3 = d -22 $\pm$ 2 to -15; wk -2 = d -14 to -8; wk -1 = d -7 to -2; wk 1 = d 3 to 7; wk 2 = d 8 to 14, wk 3 = d 15 to 22). Days around calving (d -1 to 1) were excluded for this analysis. The model considered farm as random effect, lesion group and calving month (July, August, September, and November) as fixed effects. Weeks were tested separately, using the ESTIMATE statement, because we had different predictions for each period. The covariance structures selected were compound symmetry and autoregressive based on the lowest Akaike's information criterion. Similarly, a mixed effect binomial logistic regression was performed to test if lying behavior, serum metabolites, and BCS were able to predict the risk of developing claw lesion later in lactation. The model included farm as random effect and behaviors, serum metabolites, and BCS were included as continuous explanatory variables. Odds ratios (OR) were used to describe the level of association between the variable of interest and claw health outcome (lesion and no lesion).

In all models, residuals were examined to verify normality and homogeneity of variances and to detect possible outliers and influential points. No observations were removed from the analyses. The significance level

was set at $P < 0.05$ and tendencies are reported when $P \leq 0.1$.

RESULTS

Lying Behavior During Transition

Lying behaviors as performed during transition by healthy primiparous cows without claw lesions are shown in Figure 1. The average lying time per day during the precalving period (d -21 to -2) was 10.3 ± 0.3 h/d. However, compared with the prepartum period, daily lying time declined about 3 h in the days around parturition (d -1 to 1, 7.4 ± 0.3 h/d; $P < 0.001$) and continued to remain low for the remainder of the transition period (d 2 to 21, 7.3 ± 0.3 h/d, $P < 0.001$). Within the prepartum period, lying time decreased in the days before calving (slope = -0.10 ± 0.01 ; $P < 0.001$) and gradually increased after parturition (slope = 0.05 ± 0.01 ; $P < 0.001$).

Primiparous cows divided their lying time into more bouts during the precalving and calving period (11.6 ± 0.53 and 12.9 ± 0.45 bouts/d, respectively) compared with the postpartum period (9.1 ± 0.47 bouts/d; $P < 0.001$), and this behavior increased as calving approached (slope = 0.08 ± 0.02 ; $P < 0.001$) and following parturition (slope = 0.12 ± 0.01 ; $P < 0.001$; Figure 1b). The duration of lying bouts was lower during the calving period (40.1 ± 2.22 min/bout; $P < 0.001$) compared with the precalving and postcalving periods (57.93 ± 2.62 and 53.30 ± 2.33 min/bout, respectively), which were not different from one another.

Lying Behavior and Claw Lesions

Cows that developed claw lesions later in lactation, spent less time lying ($P < 0.01$) during wk 3 after calving compared with healthy cows, but no difference was observed in any other period (Figure 2a). The frequency of lying bouts during all 3 wk after calving was lower for cows that were diagnosed with lesions than those without lesions (no lesion vs. lesion cows, wk 1 = 9.0 ± 0.3 vs. 8.0 ± 0.4 bouts/d, $P = 0.02$; wk 2 = 10.1 ± 0.2 vs. 9.1 ± 0.3 bouts/d, $P = 0.04$; wk 3 = 11.4 ± 0.3 vs. 8.6 ± 0.2 lying bouts/d, $P < 0.01$; Figure 2b). In wk 3, but no other period, cows that developed a lesion had longer lying bout durations than those without lesions (58 ± 1.5 vs. 48 ± 2.0 min/bout; $P < 0.01$). A decrease in lying time in wk 3 after calving was associated with lesion development (OR = 1.04; CI = 1.002–1.007; $P = 0.011$). Similarly, fewer lying bouts during wk 3 was associated with an increased risk of developing claw lesions (OR = 1.23; CI = 1.11–1.37; $P = 0.021$).

Figure 1. Pattern of daily lying time (min/d; A) and lying bouts (no./d; B) over the transition period (–21 to 21 d) for primiparous dairy cows housed primarily on pasture that did not have any hoof pathologies or clinical health problems during the first 4 wk of lactation ($n = 39$). Values are LSM $\pm$ SE.

Serum Metabolites, BCS, and Claw Lesions

Figure 3 shows analyte concentrations in serum during the transition period in primiparous cows by claw lesions status. Concentrations of fatty acids and BHB during wk 1 ($P = 0.04$ and $P = 0.03$, respectively) were greater in cows that developed claw lesions rela-

tive to cows that developed no lesion. Concentrations of BHB also remained greater in wk 2 ($P = 0.05$) for cows developing claw lesions. Cholesterol concentrations tended to be lower in wk 3 only ($P = 0.10$) for cows diagnosed with claw lesions compared with cows without lesions. Cow BCS did not differ between healthy cows or those that developed claw lesions during the

Figure 2. Pattern of daily lying time (min/d; A), number of lying bouts (no./d; B), and lying bouts duration (min; C) over the transition period in primiparous dairy cows housed primarily on pasture categorized according to claw lesions observed between 2 and 4 mo of lactation. Values are LSM $\pm$ SE.

Figure 3. Serum concentrations of fatty acids (μ mol/L; A), BHB (mmol/L; B), and cholesterol (mmol/L; C) over the transition period in primiparous dairy cows housed primarily on pasture categorized according to claw lesions observed between 2 and 4 mo of lactation. Values are LSM $\pm$ SE.

Figure 4. Body condition score (1 to 5 scale) over the transition period in primiparous dairy cows housed primarily on pasture categorized according to claw lesions observed between 2 and 4 mo of lactation. Values are LSM $\pm$ SE.

prepartum (3.78 ± 0.1 and 3.93 ± 0.1 , respectively) and postpartum periods (3.16 ± 0.1 and 3.18 ± 0.1 , respectively). However, cows that developed lesions experienced greater losses in BCS from wk -3 to 3 than cows without lesions (0.74 ± 0.01 and 0.61 ± 0.01 BCS change, respectively; $P = 0.03$; Figure 4). Logistic regression model was not able to identify any analyte or BCS as predictors of risk of developing claw lesions.

DISCUSSION

Lying Behavior During Transition

Lying and standing behaviors of dairy cows during the transition period from 3 wk before to 3 wk after calving has been the focus of several studies (Huzzey et al., 2005; Calderon and Cook, 2011; Kaufman et al., 2016; Neave et al., 2017). However, to our knowledge no work has described these behaviors in first-lactation cows managed in pasture-based systems.

Before calving, average daily lying time (10.3 h/d) was similar to previous results reported for primiparous prepartum cows housed in freestalls (10.4 h/d; Neave et al., 2017) or straw-bedded pack (10.5 h/d; Kaufman et

al., 2016), suggesting that lying times observed in the current study are similar to housed prepartum cows. In a Chilean pasture-based systems, where spring seasonal calving predominates, many farmers housed prepartum cows during late winter in paddocks that were muddy and wet. It has been well established that mid-lactation or nonlactating cows reduce their lying time when exposed to rain, wind, or muddy conditions (Tucker et al., 2007; Webster et al., 2008; Schütz et al., 2010); this response is magnified for multiparous cows compared with primiparous animals (Chen et al., 2017). Further research should focus on the effects of providing different environments and how they affect behaviors of prepartum cows under pasture-based systems.

Similar to cows in freestall and straw-bedded systems, primiparous cows in our study decreased time spent lying from before to after parturition. However, the average lying times recorded in our study after calving (7.3 h/d) were much lower than the values reported for postpartum primiparous cows housed in freestalls systems (9.7–9.0 h/d; Kaufman et al., 2016; Neave et al., 2017). The differences among precalving and post-calving periods were also greater in our study (3 h/d) compared with other studies using zero-grazing systems (1 to 1.5 h/d; Kaufman et al., 2016; Neave et al., 2017). Our findings may have been influenced by the fact that cows on pasture spend more time grazing and walking than cows in confinement (reviewed by Gregorini, 2012), behaviors that are affected by forage accessibility and distance to the milking parlor (Munksgaard et al., 2005). In addition, previous work reporting lying behavior in zero-grazing dairy cows was conducted in Canada (Kaufman et al., 2016; Neave et al., 2017), which may limit a comparison with our study given the difference in climate and production level.

In the current study, we reported a decrease in the number of transitions between standing and lying positions from the prepartum (12 bouts/d) to the postpartum (9 bouts/d) period, which is in contrast to other work (~ 11 bouts/d; Huzzey et al., 2005; Kaufman et al., 2016). The decrease in standing bouts may suggest that, under the conditions of our study, cows found the pasture to be a more comfortable surface for lying relative to the straw-bedded pack used in the prepartum period. This should, however, be viewed with some caution given that other work has reported that the number of lying bouts was greater in primiparous cows in the weeks after calving compared with the precalving period (16 and 12 bouts/d, respectively; (Neave et al., 2017). Regardless, the relative comfort of pasture versus indoor housing is no doubt highly context-dependent.

The increase in postural changes from lying to standing observed in the study during the calving period (d -1 to 1) compared with the days before and after

is similar with previous findings (Huzzey et al., 2005; Miedema et al., 2011b; Jensen, 2012), suggesting that cows were more restless, likely due to the discomfort (or pain) associated with calving (Huzzey et al., 2005). Proudfoot et al. (2009) demonstrated that cows with dystocia transitioned from standing to lying positions more frequently than cows without dystocia. As primiparous cows are more likely to have dystocia than multiparous cows (Mee, 2008), we can assume that at least part of our study animals had dystocia. Unfortunately, as calving ease was not recorded in the present study, we recommend further work to disentangle the effects of dystocia on lying behavior in cows housed primarily on pasture.

As reported by Huzzey et al. (2005) and Miedema et al. (2011a), multiparous cows housed under zero-grazing conditions decreased time spent lying during the calving period (1 to 2 h/d), but the depression in lying time was much greater in our study (3 h/d). The fact that our study group only included primiparous cows that were moved to the lactating group after calving (d 0 or 1), which also coincided with being mixed with multiparous cows, may explain the difference in lying times noted in our study (Sepúlveda-Varas et al., 2014; Kaufman et al., 2016; Neave et al., 2017). Moreover, cows were regrouped almost daily resulting in daily disruption of the herd.

Lying Behavior and Claw Lesions

Only 2 previous studies report lying or standing behavior differences during transition in cows that developed CHDL later in lactation and originate from intensively housed dairy systems (Chapinal et al., 2009; Proudfoot et al., 2010). Chapinal et al. (2009) showed that multiparous cows that later developed a sole ulcer had a decline in lying time from the wk -2 to 1 relative to calving compared with cows that remained healthy. Proudfoot et al. (2010) reported that multiparous cows that were diagnosed with sole hemorrhages and sole ulcers later in lactation stood longer per day during the 2 wk before calving and the first 24 h after calving. In the current study, differences in lying behaviors were seen only in wk 3, when primiparous cows that were diagnosed with claw lesions 2 to 4 mo after calving spent less time lying down compared with cows that remain healthy during the same period (7.3 and 8.5 h/d, respectively). In addition, cows with lesions had lower frequency of lying bouts after calving and the duration of the bouts was longer, similar to the results reported before calving by (Proudfoot et al., 2010). It is unclear if the lower lying bout number was merely a result of the lower lying time, or if the lower bout frequency also predisposed cows for claw lesions.

In the time around parturition, hormonal changes influence the integrity of the suspensory apparatus of the claw, making it more susceptible to environmental impacts (Tarlton et al., 2002; Knott et al., 2007; Newsome et al., 2017a). Hard flooring (Knott et al., 2007) in combination with increased standing times (Chapinal et al., 2009; Cook and Nordlund, 2009; Proudfoot et al., 2010) may be particularly important, as they may predispose the hoof for lesion development. In the current study, cows that developed claw lesions several weeks after calving were only housed on straw-bedded pack for approximately 3 wk before calving, possibly reducing the risk for trauma to the sole during that period (Knott et al., 2007). In addition, differences in standing behavior before calving in multiparous cows, as reported by Proudfoot et al. (2010), were driven by perching in a freestall cubicle (standing with only the 2 front feet in the stall), which in our study was not possible given the different housing system.

The lower lying times observed after calving in cows diagnosed with a lesion in mid lactation may be a consequence of prolonged times standing at milking for some primiparous cows. In dairy cattle, age and parity have been shown to be correlated with social dominance (e.g., Schein and Fohrman, 1955; Brantas, 1967). Low social rank was associated with lameness (Galindo and Broom, 2002) and increased standing time (Galindo and Broom, 2000), and thus may increase the risk of lesions. Position in the social hierarchy have been shown to affect the milking position of individual dairy cows (Rathore, 1982; Sauter-Louis et al., 2004). Although we did not monitor milking order, we expect that in our study some primiparous cows were further down the milking order, resulting in increased standing times in the holding area. Smoothness of flooring of holding area has been associated with increased risk for lameness in pasture-based systems (Ranjbar et al., 2016); thus, cows that spend more time standing in this environment may be even greater risk for lesion development.

Whereas pasture access has been associated with a decreased prevalence of lameness and the risk of claw disorders compared with systems with limited or no pasture access (Somers et al., 2003; Hernandez-Mendo et al., 2007; Olmos et al., 2009; Holzhauer et al., 2012), pasture-managed cows may have similar prevalences of lameness (i.e., 19% with a range from 4 to 44%; Ranjbar et al., 2016) to that reported in housed production systems (i.e., 22% with a range from 5 to 55%; Solano et al., 2016). In our study, we observed and omitted a high number of first-lactation cows that had sole and white line lesions at 4 wk after calving (52 of 105 animals), suggesting unknown factors already present likely affecting claw health before transition.

Our study had some limitations. Our results are specific to primiparous cows that were diagnosed using photographs of the plantar surface of the hoof. With this methodology, it was not feasible to assess the depth of these lesions, including their position relative to the corium and their extent within the thickness of the sole or white line, possibly resulting in underestimation of the number and severity of CHDL in the cows that we used. However, we based our scoring criteria on previous work that used appearance of the lesions rather than size or depth (Offer et al., 2000; Randall et al., 2016). In addition, Vanegas et al. (2006) reported good agreement ($r = 0.88$) between claw lesions scored in the field and claw lesions scored by the use of digital photographs.

Serum Metabolites, BCS, and Claw Lesions

Associations of metabolic markers (i.e., fatty acids, BHB, and cholesterol) and lipid mobilization (Ruegg et al., 1992; Kim and Suh, 2003; Ospina et al., 2013), lipid mobilization and BCS change (Roche et al., 2009; Barletta et al., 2017), and a positive correlation between BCS and the thickness of the digital cushion (Bicalho et al., 2009; Machado et al., 2011) have been well established. These associations suggest that metabolic energy balance around calving and hoof lesions are related to one another, given that a thin digital cushion (composed mostly of adipose tissue) is a strong predictor of lameness (Bicalho et al., 2009) and to CHDL due to reduced protective function (Bicalho et al., 2009; Newsome et al., 2017a).

Our aim was to establish a direct link between energy metabolic status during transition and claw horn lesion development. Our results partly supported this assumption, as primiparous cow that developed lesions had higher serum concentrations of fatty acids and BHB and lower serum concentrations of cholesterol after calving. Moreover, cows with and without lesions later in lactation did not differ in BCS before and after calving, but did differ in the rate of loss in BCS during the study period. These results suggest that the degree of NEB after calving, which can be identified by an increase in circulating concentration of fatty acids and BHB (Ospina et al., 2013), a decrease of cholesterol (Kim and Suh, 2003), and the extent of body condition loss after calving (Roche et al., 2007; Barletta et al., 2017), may collectively have detrimental effects on the hoof health of primiparous dairy cows. However, these variables were not associated with odds of claw horn lesion development later in lactation, suggesting that although NEB and lipid mobilization in primiparous cows may contribute to impair hoof health, they are

likely not useful predictors of claw horn lesion development.

To our knowledge, the association between NEB (as indicated by high fatty acids and BHB as well as low cholesterol around calving) and CHDL development later in lactation has not been documented in this way in previous studies. Others have, however, demonstrated that body condition loss preceded CHDL (Green et al., 2014). Bicalho et al. (2009) and Machado et al. (2011) also observed that, when BCS decreases, the digital cushion within the claw becomes thinner, compromising its shock-absorbing function. Given that the digital cushion contains adipose tissue (Räber et al., 2006), it could be predicted that lipid mobilization from the digital cushion occurs during periods of NEB. Additional evidence supporting this prediction comes from Newsome et al. (2017a), who recently reported that cows that developed claw horn lesions had a thinner digital cushion before the lesion occurrence. Given that we did not measure digital cushion thickness, it remains unclear if the elevated fatty acids (which is released from adipose tissue) in our study led to a decrease in a thinning of the digital cushion or if the metabolic status by some different mechanism influenced lesion development. Newsome et al. (2017a) suggested that a thin digital cushion might have to be present for a certain period for visible damage to the corium to occur, which may vary with environmental and management factors. Further investigation is required to determine more fully the potential influence of energy metabolism on claw disorders in pasture-based systems, where mobilization of body reserves is even more extreme compared with TMR-feeding systems (Kolver and Muller, 1998; Fulkerson et al., 2008; Pedernera et al., 2008).

CONCLUSIONS

Lying behavior and energy metabolic status during the transition period differed between cows in mid lactation diagnosed with or without CHDL. Cows that developed lesions spent less time lying down, had increased fatty acids and BHB concentrations, and presented greater losses in BCS after calving compared with cows without lesions. Our findings suggest a potential connection between lying behavior, energy metabolism, and development of CHDL.

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