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Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in freestall barns

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ABSTRACT

Lying behavior is an important measure of comfort and well-being in dairy cattle, and changes in lying behavior are potential indicators and predictors of lameness. Our objectives were to determine individual and herd-level risk factors associated with measures of lying behavior, and to evaluate whether automated measures of lying behavior can be used to detect lameness. A purposive sample of 40 Holstein cows was selected from each of 141 dairy farms in Alberta, Ontario, and Québec. Lying behavior of 5,135 cows between 10 and 120 d in milk was automatically and continuously recorded using accelerometers over 4 d. Data on factors hypothesized to influence lying behavior were collected, including information on individual cows, management practices, and facility design. Associations between predictor variables and measures of lying behavior were assessed using generalized linear mixed models, including farm and province as random and fixed effects, respectively. Logistic regression models were used to determine whether lying behavior was associated with lameness. At the cow-level, daily lying time increased with increasing days in milk, but this effect interacted with parity; primiparous cows had more frequent but shorter lying bouts in early lactation, changing to mature-cow patterns of lying behavior (fewer and longer lying bouts) in late lactation. In barns with stall curbs >22 cm high, the use of sand or >2 cm of bedding was associated with an increased average daily lying time of 1.44 and 0.06 h/d, respectively. Feed alleys \geq 350 cm wide or stalls \geq 114 cm wide were associated with increased daily lying time of 0.39 and 0.33 h/d,

respectively, whereas rubber flooring in the feed alley was associated with 0.47 h/d lower average lying time. Lamé cows had longer lying times, with fewer, longer, and more variable duration of bouts compared with nonlamé cows. In that regard, cows with lying time \geq 14 h/d, \leq 5 lying bouts per day, bout duration \geq 110 min/bout, or standard deviations of bout duration over 4 d \geq 70 min had 3.7, 1.7, 2.5, and 3.0 higher odds of being lame, respectively. Factors related to comfort of lying and standing surfaces significantly affected lying behavior. Finally, we inferred that automated measures of lying behavior could contribute to lameness detection, especially when interpreted in the context of other factors known to affect lying behavior, including those associated with the individual cow (e.g., parity and stage of lactation) or environment (e.g., stall surface).

Key words: lying time, automated measures, lameness detection, dairy cattle, welfare

INTRODUCTION

Adequate rest has been positively associated with productivity, health, and welfare of dairy cattle. When access to stalls is restricted, cows prioritize lying down over feeding (Munksgaard et al., 2005), and preventing cows from lying down induces stress (Cooper et al., 2008). As a consequence, measures of lying behavior, such as the daily duration and the frequency and duration of lying bouts, is a measure of cow comfort (Haley et al., 2001; Rushen et al., 2008). Furthermore, changes in lying behavior can be associated with pain and malaise, enabling the use of lying behavior not only as an indicator of present illness, but also as a tool to predict cattle at risk of becoming ill (Weary et al., 2009). These findings contributed to development of automated systems to measure lying time that are less time-consuming than live or video-based observations

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and that provide a useful measure of health, welfare, and comfort (Rushen et al., 2008; Bewley et al., 2010).

In freestall systems, lactating cows commonly lie down for approximately 11 h/d (Bewley et al., 2010; von Keyserlingk et al., 2012). However, lying duration varies considerably among dairy systems, with the shortest duration often in pasture systems (6.7 h/d; Botheras, 2006; 8 h/d; Sepúlveda-Varas et al., 2014) and the longest usually in tiestalls (12.5 h/d; Charlton et al., 2015). Typically, cows have 6 to 13 lying bouts daily, averaging 55 to 90 min each (EFSA, 2009). However, lying behavior is influenced by several factors, including housing system (Hernandez-Mendo et al., 2007; von Keyserlingk et al., 2012), stall dimensions (Tucker et al., 2004), stall surface (Cook et al., 2008), stocking density (Fregonesi et al., 2007), flooring (Haley et al., 2001), parity, stage of lactation (Vasseur et al., 2012), lameness (Ito et al., 2010; Thomsen et al., 2012), and heat stress (Cook et al., 2007). Understanding dynamics of lying behavior provides insight into how cows interact with their environment and what management practices may modify this behavior (Rushen et al., 2008).

Diseased animals often exhibit abnormal or reduced activity; therefore, changes in lying behavior have been used in dairy cattle as potential indicators and predictors of health issues, including dystocia (Proudfoot et al., 2009), postpartum disorders (i.e., metritis and retained placenta; Sepúlveda-Varas et al., 2014), and lameness (Ito et al., 2010; Blackie et al., 2011; Alsaad et al., 2012). The latter is one of the most important welfare and productivity problems in the dairy industry. That it causes pain (Rushen et al., 2007) and reduces both milk yield (Green et al., 2002) and reproductive performance (Hernandez et al., 2001) makes it extremely costly (Ettema and Ostergaard, 2006). Early recognition and treatment of lameness is fundamental to mitigate its negative effects. Therefore, changes in measures of lying behavior have been identified as a potential behavioral indicator of lameness, based on differences in lying responses of lame and nonlame cows (Ito et al., 2010). However, changes in lying time can be both a risk factor for and a consequence of lameness, as lameness can be preceded by reduced duration of lying, and once clinically lame, cows tend to have longer lying bouts and longer total lying time per day (Chapinal et al., 2009; Ito et al., 2010). Reports on lame cows' lying behavior vary among studies. For example, some authors reported that the length and variability of lying bouts were greater in lame cows compared with nonlame cows (Chapinal et al., 2009; Ito et al., 2010), whereas others reported no difference in bout duration between lame and nonlame cows (Gomez and Cook, 2010). Furthermore, there were interactions of certain

stall design features (e.g., stall surface) with the severity of lameness, relative to lying behavior (Cook et al., 2008). Hence, it is expected that lying behavior and its association with lameness are related to housing conditions, as well as management and cow factors.

Lameness detection is a challenge for dairy producers; therefore, its prevalence is often underestimated (Espejo et al., 2006). Automated detection systems based on changes in lying behavior could alert the farmer of the onset of lameness or a high probability of the presence of lameness and would be of great benefit to farm productivity and cow well-being (de Mol et al., 2013). Although lying behavior has potential as an indicator of lameness, automated technologies that provide real-time lameness detection based on changes in lying behavior have not proven to be highly accurate (Alsaad et al., 2012; de Mol et al., 2013). Unfortunately, most research on lying behavior has been conducted with limited sample sizes, on experimental dairy farms, or focused on limited individual (e.g., DIM, parity) or management factors (e.g., stall surface; Bewley et al., 2010; Gomez and Cook, 2010; Ito et al., 2010). Therefore, the objectives of our study were to determine (1) individual and herd-level risk factors associated with measures of lying behavior and (2) associations between lying behavior and lameness; doing so allowed us to determine whether measures of lying behavior can be used to detect lameness.

MATERIALS AND METHODS

Farms

A total of 141 Canadian freestall dairy farms were enrolled as part of a larger study characterizing dairy cow comfort and longevity (Charlton et al., 2014; Vasseur et al., 2015). Farms were located in 3 Canadian provinces: Alberta [(**AB**); $n = 81$], Ontario [(**ON**); $n = 40$], and Québec [(**QC**); $n = 20$]. Data were collected between May 2011 and July 2012 by 6 trained graduate students and research assistants. Three of the observers were from the University of Calgary (Calgary, AB, Canada), 2 from University of Guelph (Guelph, ON, Canada), and 1 from Université Laval (Québec City, QC, Canada). All methods were approved by the Animal Care Committees and Research Ethics Boards of each participating academic institution.

The farm selection process has been described in detail (Zaffino Heyerhoff et al., 2014; Solano et al., 2015). In short, eligible farmers from all 3 provinces were recruited via mail and participation was voluntary. In AB, farms already enrolled in a collaborative study, the Alberta Dairy Hoof Health Project (Alberta Milk, 2013), were invited to participate ($n = 158$). The sub-

population of farms enrolled in the Alberta Dairy Hoof Health Project was representative of the average AB dairy farm in terms of herd size, breed, type of dairy barn, and longevity (Zaffino Heyerhoff et al., 2014). In ON and QC, farms invited to participate were selected on the basis of representative strata of longevity and having mean milk production $\geq 7,000$ kg/cow per year (Vasseur et al., 2015), which was estimated to be within Canada's lower end of the normal range of milk production per cow per year. Farmers who indicated that they were willing to participate were then contacted by telephone, after which it was determined whether they met the study criteria. To ensure that participating farms were representative of the majority of freestall herds in Canada, farms had to be enrolled in an organized milk-recording system, provided by CanWest DHI (Guelph, ON, Canada) or Valacta Inc. (Sainte-Anne-de-Bellevue, QC, Canada), and have a herd size ≥ 40 Holstein-Friesian lactating cows. Farms were excluded if lactating cows were subjected to management practices not commonly used in Canada (e.g., access to an outdoor exercise area or pasture for >2 h/d).

Cow Selection

Based on a validation study (Vasseur et al., 2012), a purposive sample of 40 lactating Holstein-Friesian cows between 10 and 120 DIM was selected on each farm. The 10 to 120 DIM interval was chosen as it was characterized by a higher incidence of lameness than in later lactation (Green et al., 2002). If >40 cows between 10 and 120 DIM were present on a farm, the sample of study cows was balanced to reflect the proportion of primiparous and multiparous cows in the herd and cows were then randomly selected.

Lying Behavior

Lying behavior was recorded using electronic data loggers (HOBO Pendant G Acceleration Data Loggers, Onset Computer Corp., Pocasset, MA), validated for recording lying and standing positions (Ito et al., 2009; Ledgerwood et al., 2010). Data loggers were attached with bandaging wrap (CoFlex, Andover Coated Products Inc., Salisbury, MA) to the cow's hind leg during milking and were programmed to record the position of the cow at 1-min intervals for 4 consecutive 24-h periods (Charlton et al., 2014). Lying data based on 4 d of continuous sampling was sufficient to obtain a representative herd mean lying time estimate (Ito et al., 2009; Vasseur et al., 2012). Each individual farm was visited twice within a 5- to 10-d period. During the second farm visit, data loggers were removed and data

downloaded. The total duration of lying and the duration and frequency of individual lying bouts were computed using Excel macros (Microsoft Corp., Redmond, WA) for the 4-d period (Vasseur et al., 2012), from which daily lying time (h/d), bout frequency (bout/d), and bout duration (min/bout) were calculated for each cow. In addition, variation in duration of lying bouts within cow over 4 d was calculated from the standard deviation of bout duration, previously identified as being associated with lameness (Ito et al., 2010)

Animal-Based Measures

Cows were video recorded while returning from the milking parlor. Lameness was assessed independently by 1 observer per farm using a binomial (yes/no) simplified version of a numerical gait scoring system (Flower and Weary, 2006) that had been previously validated (Chapinal et al., 2009; Ito et al., 2010). This scoring system aimed to identify cows that were reluctant to bear weight on at least 1 limb (i.e., walked with a limp). A cow was defined as lame if limping was present (i.e., reluctance to bear weight on at least 1 limb), which was equivalent to a score of ≥ 3 on the 5-point scale numerical rating score developed by Sprecher et al. (1997). Locomotion was not assessed if the video quality was poor, the cow was trotting or running, or <2 complete strides were recorded ($n = 370$). Only cows with complete lameness assessment were included in the analyses.

Standard operating procedures were developed and tested to score animal-based measures. Cows were scored (<2 trained observers per farm) for the presence of hock and knee injuries using standard operating procedures. These injuries were scored in the milking parlor, in headlocks, or where the cows were free to move, as described previously (Gibbons et al., 2012; Zaffino Heyerhoff et al., 2014). In short, conditions of the lateral surface of the left and right tarsal joints (hock assessment) and carpal joints (knee assessment) were recorded using a 4-point scale: 0 = no swelling, no hair missing; 1 = bald area with no swelling or swelling <1 cm; 2 = medium swelling (1 to 2.5 cm) or lesion on bald area; and 3 = major swelling (>2.5 cm; Gibbons et al., 2012). Individual cow data on parity, DIM, and test-day milk production (measured at the most recent milk recording after data collection) were obtained from CanWest DHI and Valacta Inc. The average interval between data collection and milk recording was 17 d (range = 0 to 51 d).

Training of observers for lameness assessment and other animal-based measures have been described in detail (Gibbons et al., 2012; Solano et al., 2015).

Briefly, the 6 observers were trained during an intensive 2-wk program. A refresher course and midway check (3 to 4 wk and 5 to 15 wk after initial training, respectively) were done to ensure and maintain a high level of agreement [weighted Kappa statistic (K_w) ≥ 0.6]. Video recording cows on farm for lameness assessment allowed for interobserver repeatability checks. Of all videos recorded, 20% were reanalyzed by the trainers. The percentage exact agreement was calculated as (the number of exact agreements/total number of observations) $\times 100$. Exact agreement between the 2 trainers for locomotion scoring was $\geq 82\%$ throughout the study period, whereas exact agreement for all 6 observers across provinces was 94% ($K_w \geq 0.8$) for lame versus not lame (Solano et al., 2015). Lameness scores of reanalyzed videos remained unchanged due to the high interobserver agreement.

General Management

A questionnaire on management practices (<https://www.dairyresearch.ca/cow-comfort.php#self>) was conducted on every farm (Solano et al., 2015; Vasseur et al., 2015). Data for our study were collected using closed-ended questions related to the timing of feeding relative to milking and the frequency of milking, feeding, feed push-up, alley scraping, stall bedding, stall cleaning, and so on. Data on other management practices related to feed management (e.g., if 90% of cows had access to feed when checked 4 times with at least 1 h between observations) and milking duration were collected by direct observation. Milking duration was defined as the time from when the first cow was taken out of the pen for milking and the last cow returned to the pen after milking.

Facility Design

Pen Features. Environmental measures were collected from all pens where the 40 study cows were housed on the day of the visit. Pen length and width were measured, as well as other pen features such as the flooring in the feed alley (categorized as solid or slatted and concrete or rubber) and width (measured from the feedbunk to the stall curb), length of water access (i.e., length or diameter was measured if the drinker was rectangular or circular, respectively), and feedbunk type and length.

Stall Management. Each pen where the study cows were housed was assessed for stocking density, stall dimensions, stall base, stall bedding type, cleanliness, quantity, and dryness. Information on stocking density was obtained as described previously (Charlton

et al., 2014) and estimated as the number of cows per usable stalls. Data on 8 dimensions per stall, bedding cleanliness, quantity, and dryness were estimated as described previously (Zaffino Heyerhoff et al., 2014; Vasseur et al., 2015). Briefly, stall dimensions were measured at the end stalls of 3 representative rows in each pen ($n = 2$ stalls per row). If the pen had < 3 rows, stall dimensions were measured from all rows. As end stalls are often narrower or wider than average, stall width was measured in the middle of each row (minimum 6 stalls per farm) as the average width of 3 adjacent stalls (Solano et al., 2015). Lunge space was considered adequate if no obstruction was present ≤ 76 cm forward from the brisket board. If no brisket board was present, this measure was taken from the point of the neck rail and 10 cm above the stall surface. Bedding quantity was evaluated as the bedding depth (in cm) after the stall was raked evenly, and was evaluated as ≤ 2 cm (equivalent to 1 kg of chopped straw) or > 2 cm. Type of stall base and bedding were also recorded. If different types of stall bases were present in the same pen, the predominant stall base type was considered.

Statistical Analyses

Statistical analyses were performed using STATA 13.1 (StataCorp, College Station, TX). A P -value < 0.05 was considered statistically significant. If the lactating cows were housed in ≥ 2 pens and these pens differed in flooring or stall characteristics, the pen with the highest number of study cows was selected for analysis. If an equal number of cows was housed in each pen, 1 of the 2 pens was randomly selected. Unusual stall bases, bedding types, and floorings that did not justify a category in analysis due to a low sample size (≤ 4 farms) were considered in a category as “other” (Zaffino Heyerhoff et al., 2014). If 2 predictors were highly correlated ($|r| \geq 0.7$), the one with the strongest association with the outcome (or the one with the least missing observations) was chosen.

Daily variation of each measure of lying behavior was tested using repeated-measures ANOVA. As no significant differences among days when data loggers were recording were observed, outcomes were averaged over the 4-d period. Lying behavior data of cows that only had 3 d of recording lying behavior ($n = 50$) were included in the analyses, as no significant differences were detected if 3 d were randomly selected from a 4-d period. However, cows with < 3 d ($n = 46$) of recording lying behavior were excluded from analyses. The mean of daily lying time (h/d), bout frequency (bout/d), bout duration (min/bout), and standard deviation of bout duration were calculated for each cow. Correla-

tions (Spearman coefficients) among the 4 lying behavior outcomes were determined. Natural logarithmic transformation was applied to bout frequency, bout duration, and standard deviation of bout duration as they showed a positive skew. Extreme values (outliers 3 times the interquartile range from the first and third quartile: $n = 1$ for lying duration; $n = 25$ for bout frequency; $n = 24$ for bout duration; $n = 34$ for SD of bout duration) were carefully examined and analyses were performed with and without their presence. Including extreme values did not affect the association or significance of any variables included in the analyses; therefore, all were retained.

The model-building process to assess cow- and herd-level risk factors associated with variations in lying behavior involved 2 steps. The outcome of interest was each measure of lying behavior measured on a continuous scale (lying duration, log bout frequency, log bout duration, log SD of bout duration), using a separate model for each lying behavior. First, univariable analyses were performed to assess associations between each outcome and predictor variable. Outcomes were assessed at the herd and cow levels, using farm and cow, respectively, as the experimental unit. Predictors with a univariable association with $P \leq 0.10$ were considered for the next step of multivariable modeling. In the second step, predictors for each of the 4 outcomes were screened in separate generalized linear mixed models (GLMM) that included cow- (e.g., DIM, parity) and herd-level variables (e.g., stall management, flooring characteristics, holding time, feed and water access). The cow was considered the experimental unit and a backward elimination process was performed. Cow- and herd-level variables significant at $P \leq 0.05$ were retained in the final model for each outcome. Additionally, if confounding was present (i.e., removal of any variable resulted in a 30% change in the estimate of any other significant predictor) that variable was also retained in the final model. Two-way interactions (e.g., bedding quantity and type of bedding; bedding quantity and stall curb height; stall width and bed length, type of flooring, and floor cleanliness; parity and DIM; lameness and milk production) were tested among the significant predictors in the main effects model. Akaike's information criterion (Akaike, 1973) was used to compare models within each of the 4 lying behavior outcomes, and the model with the lowest estimate was considered the best model. Farm was included as a random effect and province was forced as a fixed effect into all models.

Logistic regression models were performed to determine whether measures of lying behavior were associated with lameness. The outcome of interest was the presence of lameness, considering cow as the unit of

interest and using a separate model for each measure of lying behavior. Interaction terms between different measures of lying behavior (e.g., lying duration and bout frequency, lying duration and bout duration, SD of bout duration and bout frequency) were tested, but none was retained due to collinearity. The nonlinear relationship between the log odds of lameness and lying behavior was visually inspected using scatter plots. Characteristics of lying behavior as a diagnostic test (sensitivity, specificity, positive and negative predictive values) were examined, using cut-off points to define extreme lying behavior based on the log odds of lameness graphs of each lying variable. Parity and DIM were forced into all models, as they are known to influence lying behavior (Vasseur et al., 2012).

RESULTS

The study population had an average herd size of 124 lactating cows. The average cow in the study was in its second parity producing 36 kg of milk daily. Farms were representative of the overall respective provincial cow population housed in freestalls, in terms of parity, but slightly higher in milk production and herd size (Table 1).

Lying Behavior Within and Among Herds

Of 5,634 cows in the study, usable lying behavior data were obtained from 5,135 cows (2,920, 1,516, and 699 cows for AB, ON, and QC, respectively). In total, 4,790 cows had complete observations on parity, DIM, and milk production. Cows had a median lying bout duration of 63 ± 28 min, with 10.2 ± 4.7 bouts during a standard deviation of bout duration of 38 ± 18 , for a mean total daily lying time of 10.6 ± 2.3 h/d (Table 1). Bout duration was correlated with bout frequency ($r = 0.71$; $P < 0.001$) and SD of bout duration ($r = 0.76$; $P < 0.001$). Mean herd-level daily lying time ranged from 8.2 to 13.2 h/d (Figure 1) and individual daily lying time for cows ranged from 1.3 to 22.1 h/d.

Cow and Herd Factors Associated with Lying Behavior

Lying behavior was associated with parity and DIM of the cows. Daily duration of lying increased with DIM among all parities. Bout frequency decreased in primiparous cows ($P < 0.001$) throughout lactation. In multiparous cows, bout frequency remained similar until the seventh month of lactation ($P > 0.13$), but decreased ($P < 0.04$) when cows reached ≥ 8 mo of lactation (Figure 2). Bout duration increased with DIM regardless of parity, but primiparous cows had the

Table 1. Characteristics (mean \pm SD) of 141 freestall dairy farms and the average freestall farm in 3 Canadian provinces

Herd characteristic	Average freestall farm ¹			Study farms		
	Alberta (n = 347)	Ontario (n = 788)	Québec (n = 306)	Alberta (n = 81)	Ontario (n = 40)	Québec (n = 20)
Herd size (no. of milking cows)	123	105	91	157 \pm 76	122 \pm 75	93 \pm 57
Parity	2.3	2.2	2.4	2.3 \pm 1.4	2.3 \pm 1.4	2.5 \pm 1.6
Daily milk yield (kg)	32	30	30	38 \pm 9	36 \pm 9	34 \pm 8
305-d milk yield (\times 1,000 kg)	10.0	9.6	9.2	10.4 \pm 2.0	10.2 \pm 1.9	9.5 \pm 1.7
DIM ²	—	—	—	76 \pm 66	83 \pm 87	102 \pm 103
Lying time (h/d)	—	—	—	10.3 \pm 2.2	11.0 \pm 2.3	11.2 \pm 2.4
Bout frequency (no./d) ²	—	—	—	10.5 \pm 4.7	10 \pm 4.5	9.2 \pm 4
Bout duration (min/bout) ²	—	—	—	59 \pm 26	66 \pm 26	73 \pm 32
SD of bout duration (min) ²	—	—	—	36 \pm 6	41 \pm 19	47 \pm 21
Milking duration (h/d) ²	—	—	—	3.1 \pm 1.9	2.3 \pm 1.6	1.7 \pm 1.0
Lameness prevalence (%)	—	—	—	19.6 \pm 12	21.9 \pm 13.7	24.2 \pm 11.2

¹Data from 2012. Source: CanWest DHI (Guelph, ON, Canada).

²Median \pm interquartile range were calculated.

highest increase ($P < 0.001$) in relation to multiparous cows. Bout duration of multiparous cows remained similar in the first 3 mo of lactation ($P > 0.07$), but increased ($P < 0.001$) when cows reached ≥ 8 mo of lactation (Figure 3).

Several correlations were found among the independent variables. Milk production was negatively correlated with DIM ($r = -0.40$; $P < 0.001$); however, milk production was included in the multivariable analysis, as it is known to influence lying time (Fregonesi and Leaver, 2002). Pen area, feeder length, and herd size were correlated with each other ($r > 0.33$; $P < 0.001$). Linear water space increased as the pen area and feeder length increased ($r > 0.55$; $P < 0.001$). The timing of

feeding relative to milking was correlated with feed push-up frequency ($r = -0.42$; $P < 0.001$) because most of the farms that fed cows around milking time also pushed up feed ≥ 2 times/d.

Barn design and management practices varied greatly among farms (Table 2). Farms with stocking density of ≤ 1 cow/stall, pen area of >9 m²/cow, or with linear water space of >9 cm/cow tended to have longer daily lying time than farms with >1 cow/stall, pen area of <6 m²/cow, and linear water space of <4 cm/cow, respectively ($0.10 > P > 0.05$). However, all measures of lying behavior varied widely and were significantly affected by the stall lying surface and management ($P < 0.05$). For example, mean herd daily lying time and

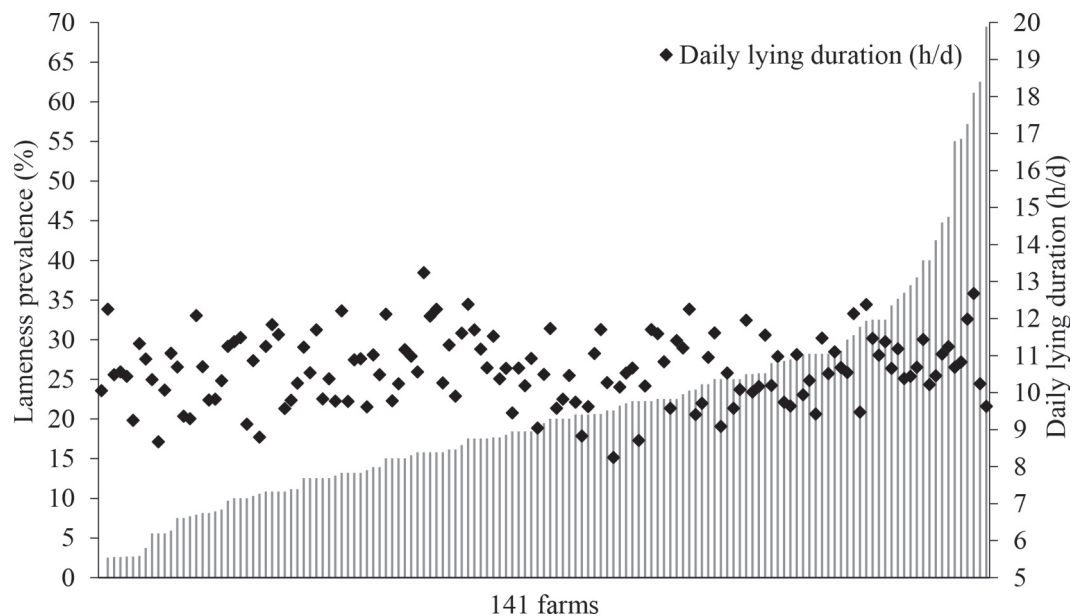


Figure 1. Mean lameness prevalence (bars) and mean daily lying time (h/d) on each of 141 farms.

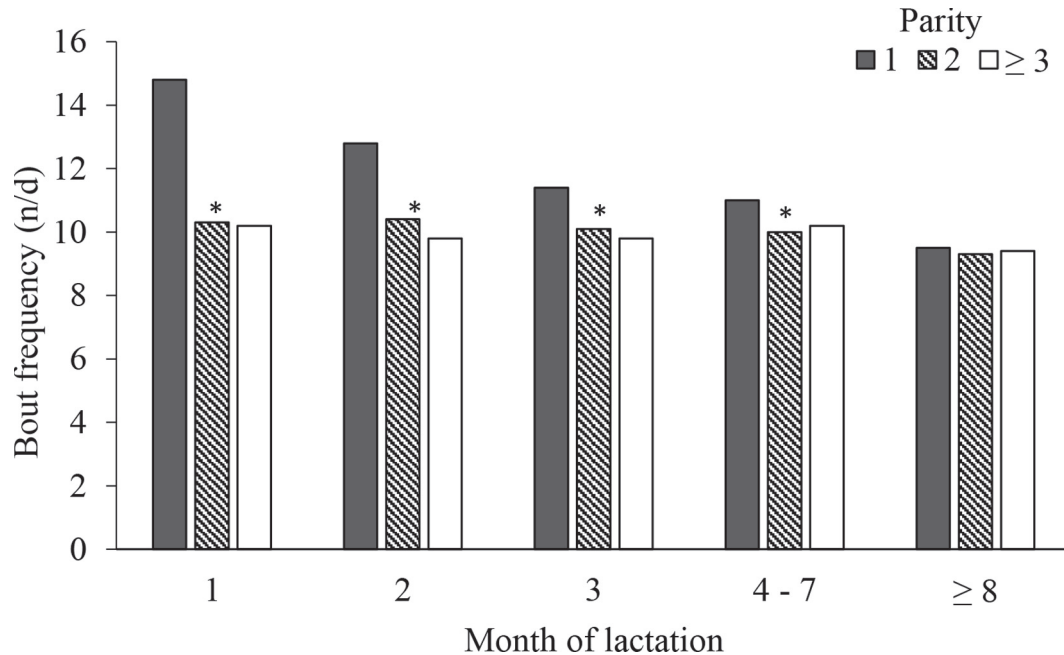


Figure 2. Mean lying bouts (no./d) per parity and month of lactation. An asterisk (*) represents the difference ($P < 0.05$) between primiparous and multiparous cows.

bout duration ranged from an average of 9.7 h/d and 61 min/bout for farms with waterbeds to an average of 11.3 h/d and 76 min/bout for farms with sand or dirt as the stall base (Table 2).

Multivariable Analysis

Based on descriptive herd-level (Table 2) and multivariable analyses, daily lying time was associated with

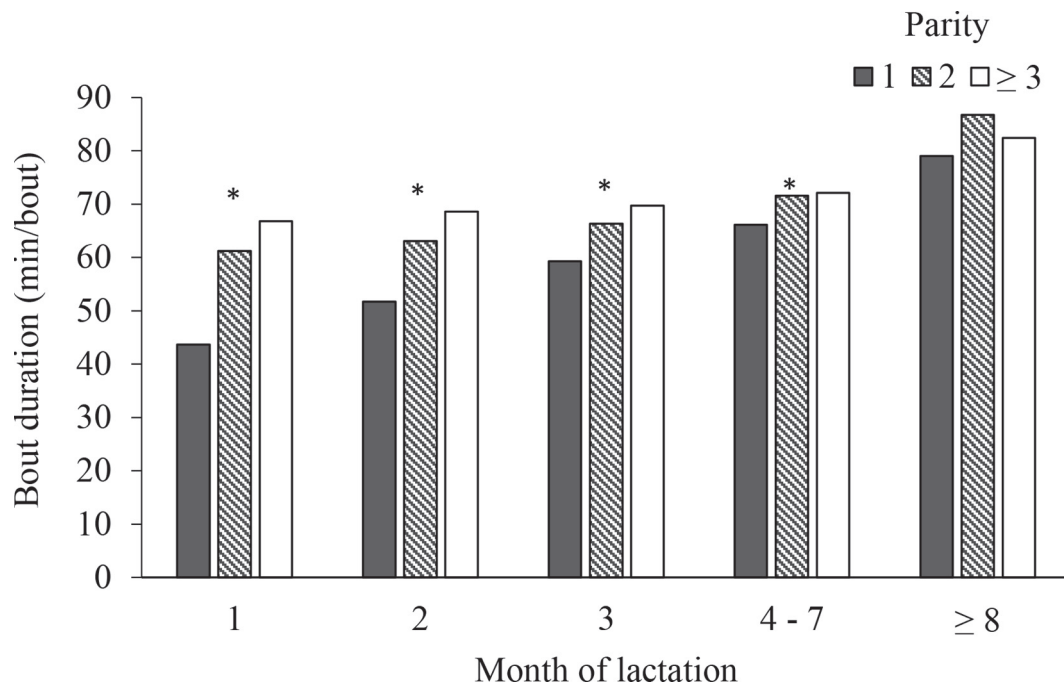


Figure 3. Mean lying bout duration (min/d) per parity and month of lactation. An asterisk (*) represents the difference ($P < 0.05$) between primiparous and multiparous cows.

the same risk factors as the other measures of lying behavior. Explicitly, daily lying time represents information that bout frequency, bout duration, and standard deviation of bout duration provided independently. Therefore, to simplify the presentation of results, we reported only results related to our daily lying time model (Table 3). Results of the hierarchical models for bout frequency, bout duration, and standard deviation of bout duration are included as an appendix (Table A1). At the cow level, bout duration was shorter for

cows with injured hocks. Increasing parity and DIM were associated with decreased bout frequency but increased bout duration and standard deviation of bout duration. Furthermore, at the herd level, bout duration increased in farms with stalls with >2 cm of bedding and feed alley width >450 cm.

At the cow level, daily lying time was higher for lame cows and increased with increasing parity and DIM, but this effect interacted with parity; in that regard, primiparous cows had more frequent but shorter lying

Table 2. Distribution (mean \pm SD) of lying behavior for 141 dairies assessed using 40 early lactation cows on each farm

Herd variable	Proportion of farms (%)	Lying duration (h/d)	Bout frequency (n/d)	Bout duration (min/bout)	SD of bout duration (min)
Pen area (m ² /cow)					
<6	11	10.3 \pm 1.0 ^a	10.8 \pm 1.9	63 \pm 10 ^a	41 \pm 8
6–9	58	10.6 \pm 1.0 ^{a,b}	10.8 \pm 1.4	65 \pm 10 ^{a,b}	41 \pm 7
>9	31	10.8 \pm 0.8 ^b	10.5 \pm 1.3	68 \pm 10 ^b	43 \pm 7
Stocking density (cows/stall)					
\leq 1	71	10.7 \pm 0.9 ^a	10.6 \pm 1.3	67 \pm 11	42 \pm 8 ^a
>1	29	10.4 \pm 0.9 ^b	10.8 \pm 1.6	64 \pm 8	40 \pm 5 ^b
Feed alley flooring					
Solid concrete	64	10.7 \pm 0.9 ^a	10.9 \pm 1.5 ^c	65 \pm 10 ^c	41 \pm 7 ^c
Slatted concrete	17	10.3 \pm 1.1 ^b	10.8 \pm 1.2 ^c	63 \pm 7 ^c	39 \pm 6 ^c
Solid rubber	19	10.7 \pm 1.1 ^a	10.0 \pm 1.1 ^d	70 \pm 10 ^d	46 \pm 8 ^d
Feeding frequency (times/d)					
>1	43	10.6 \pm 0.9	10.5 \pm 1.3	67 \pm 11	42 \pm 8
1	57	10.8 \pm 0.9	10.9 \pm 1.4	66 \pm 9	41 \pm 6
Feed availability					
Feed for 90% of cows	91	10.7 \pm 0.9	10.6 \pm 1.3	67 \pm 9	42 \pm 7
Feed for <90% of cows	9	11.0 \pm 0.6	11.2 \pm 2.1	67 \pm 16	40 \pm 10
Feeder type					
Post and rail	38	10.9 \pm 1.0 ^c	10.5 \pm 1.2 ^c	68 \pm 11 ^c	43 \pm 8 ^c
Headlocks	53	10.5 \pm 0.8 ^d	11.0 \pm 1.5 ^d	63 \pm 8 ^d	40 \pm 6 ^d
Diagonal bars	6	10.9 \pm 1.1 ^c	9.7 \pm 1.0 ^c	73 \pm 12 ^c	47 \pm 10 ^c
Bunk or troughs	3	9.3 \pm 0.6 ^d	9.5 \pm 0.2 ^c	63 \pm 4	40 \pm 2
Linear water space (cm/cow)					
<4	17	10.3 \pm 1.0 ^a	10.5 \pm 1.5	65 \pm 9 ^a	42 \pm 7
4–9	69	10.8 \pm 0.9 ^b	10.8 \pm 1.4 ^a	67 \pm 11	41 \pm 8 ^a
>9	14	10.9 \pm 0.8 ^b	10.2 \pm 1.1 ^b	70 \pm 6 ^b	45 \pm 6 ^b
Milking frequency (times/d)					
2	86	10.6 \pm 2.3	10.7 \pm 4.0	66.2 \pm 23	41.7 \pm 16
3	14	10.5 \pm 2.1	10.8 \pm 3.8	63.2 \pm 19	39 \pm 16
Stall base					
Concrete	11	10.4 \pm 0.7 ^c	9.8 \pm 1.5 ^c	70 \pm 11	43 \pm 9
Rubber mattress	11	10.7 \pm 0.8	11.4 \pm 1.3 ^d	62 \pm 9 ^c	40 \pm 6 ^c
Geotextile mattress	59	10.6 \pm 0.8	11.0 \pm 1.3 ^d	64 \pm 9 ^c	40 \pm 7 ^c
Sand or dirt	11	11.3 \pm 1.2 ^c	9.8 \pm 1.3 ^c	76 \pm 11 ^d	47 \pm 8 ^d
Waterbed	5	9.7 \pm 0.4 ^d	10.5 \pm 0.6	61 \pm 4 ^c	38 \pm 4 ^c
Other	3	10.9 \pm 1.1	10.3 \pm 0.7	67 \pm 6	44 \pm 4
Stall bedding					
Straw	23	10.4 \pm 1.0 ^c	10.4 \pm 1.1	66 \pm 8 ^c	41 \pm 6 ^c
Sawdust	23	10.6 \pm 0.8 ^c	10.9 \pm 1.4 ^c	65 \pm 10 ^c	41 \pm 7 ^c
Wood shavings	40	10.5 \pm 0.9 ^c	10.9 \pm 1.4 ^c	64 \pm 9 ^c	40 \pm 7 ^c
Sand	8	11.7 \pm 1.0 ^d	9.8 \pm 1.5 ^d	77 \pm 11 ^d	48 \pm 9 ^d
Other	6	10.8 \pm 0.7	10.9 \pm 1.9 ^c	67 \pm 14	42 \pm 11
Bedding depth (cm)					
\leq 2	61	10.5 \pm 0.8 ^c	10.9 \pm 1.4 ^a	64 \pm 10 ^c	41 \pm 7
>2	55	10.8 \pm 1.0 ^d	10.5 \pm 1.3 ^b	68 \pm 10 ^d	42 \pm 7
Bedding dryness					
Dry	80	10.6 \pm 0.9	10.7 \pm 1.4	65 \pm 10	41 \pm 7
Wet	20	10.8 \pm 0.9	10.6 \pm 1.4	67 \pm 9	42 \pm 7

^{a,b}Within a column and category, means without a common superscript differed ($P < 0.10$).

^{c,d}Within a column and category, means without a common superscript differed ($P < 0.05$).

bouts in early lactation, whereas mature cows had fewer and longer lying bouts in late lactation. In addition, lying time decreased with increasing milk yield (Table 3). At the herd level, cows housed in stalls with sand had an increased average daily lying time of 1.44 h/d compared with cows housed in stalls with wood shavings. In barns with stall curbs >22 cm high, the use of >2 cm of

bedding was associated with an increased average daily lying time of 0.06 h/d. Furthermore, bedding quantity was confounded by stall base, presumably because bedding management practices are associated with the use of certain stall bases. Feed alleys ≥ 350 cm wide or stalls ≥ 114 cm wide were associated with increased daily lying time of 0.39 and 0.33 h/d, respectively, whereas

Table 3. Final generalized linear mixed model for mean daily lying time (h/d) with cow- and herd-level factors in 141 Canadian dairy herds, considering cow (n = 4,790) within herd as the experimental unit

Predictor	Estimate	95% CI	P-value
Intercept	9.06	8.15 to 9.97	
Lame			
No	Ref. ¹		
Yes	0.55	0.40 to 0.70	<0.001
Parity			
1	Ref.		
2	0.18	-0.13 to 0.49	0.253
≥ 3	0.98	0.68 to 1.28	<0.001
DIM			
Fresh (1–44)	Ref.		
Early (45–99)	0.48	0.23 to 0.74	<0.001
Mid (100–199)	1.29	1.02 to 1.55	<0.001
Late (≥ 200)	1.50	1.05 to 1.95	<0.001
Parity \times DIM			
First parity, fresh DIM	Ref.		
Second parity, early DIM	0.02	-0.35 to 0.39	0.921
Second parity, mid DIM	-0.15	-0.55 to 0.25	0.459
Second parity, late DIM	0.10	-0.58 to 0.77	0.781
\geq Third parity, early DIM	-0.41	-0.76 to -0.05	0.024
\geq Third parity, mid DIM	-0.83	-1.21 to -0.45	<0.001
\geq Third parity, late DIM	-0.74	-1.39 to -0.10	0.023
Daily milk yield (kg)	-0.03	-0.04 to -0.03	<0.001
Stall base ²			
Concrete	Ref.		
Rubber mattress	0.04	-0.46 to 0.54	0.877
Geotextile mattress	0.20	-0.20 to 0.61	0.330
Sand or dirt	-0.48	-1.22 to 0.28	0.223
Waterbed	-0.12	-0.80 to 0.55	0.716
Other	-0.05	-0.79 to 0.69	0.887
Stall bedding			
Wood shavings	Ref.		
Straw	-0.15	-0.46 to 0.15	0.326
Sawdust	0.08	-0.21 to 0.38	0.593
Sand	1.44	0.62 to 2.26	0.001
Other	0.38	-0.14 to 0.90	0.154
Bedding depth (cm)			
≤ 2	Ref.		
> 2	-1.12	-2.38 to 0.15	0.083
Stall curb height >22 cm (per 1 cm increase)	0.04	0.02 to 0.07	0.001
Bedding depth \times stall curb height			
≤ 2 cm bedding, stall curb height increase	Ref.		
> 2 cm bedding, stall curb height increase	0.06	0.01 to 0.12	0.028
Stall width (cm)			
< 114	Ref.		
≥ 114	0.33	0.06 to 0.60	0.016
Feed alley flooring			
Solid concrete	Ref.		
Slatted concrete	-0.05	-0.39 to 0.28	0.758
Solid rubber	-0.47	-0.83 to -0.11	0.010
Feed alley width (cm)			
< 350	Ref.		
≥ 350	0.39	0.12 to 0.67	0.005

¹Referent.

²Confounds bedding quantity.

rubber flooring in the feed alley was associated with 0.47 h/d lower average lying time (Table 3).

Lying Behavior as a Detection Tool for Lameness

Lying behavior differed between nonlame and lame cows, and among herds with low, medium, or high lameness prevalence (Table 4). On average, lame cows had longer lying times, and fewer, longer, and more variable lying bouts compared with nonlame cows. Similarly, herds with high lameness prevalence had longer mean daily lying time, bout duration, and higher standard deviation of bout duration (Table 4).

Several thresholds in the measures of lying behavior were associated with increased risk of being lame (Table 5; $P < 0.001$). Daily lying time and bout frequency had a nonlinear relationship with lameness, which allowed for meaningful cut-off points to be identified. Cows with lying time ≥ 14 h/d, bout frequency ≤ 5 times/d, bout duration ≥ 110 min/bout, or standard deviation of bout duration ≥ 70 min had 3.7, 1.7, 2.5, and 3.0 higher odds of being lame, respectively. All thresholds analyzed provided low sensitivity (54–64%) and specificity (60–69%) and positive predictive values (29–32%), but high negative predictive values (85–87%) for the presence of lameness at the cow level (Table 5).

DISCUSSION

Ours was the largest study conducted to investigate lying behavior and associated risk factors in dairy cows on commercial farms in Canada. Regarding risk factors associated with measures of lying behavior, some individual factors (i.e., parity and DIM) and herd-level factors related to stall dimensions or flooring surface consistently affected measures of lying behavior. Regarding associations between lying behavior and lameness, cows with lying time ≥ 14 h/d, ≤ 5 lying bouts/d, or with daily mean bout duration ≥ 110 min/bout, were at higher risk of being lame. Therefore, identifying lying behaviors beyond these thresholds has potential for automated detection of lameness; however, this must be interpreted in the context of individual and management factors identified in the first objective.

In our study, all measures of lying behavior varied considerably among farms. Mean daily lying time (10.6 h/d) was comparable to findings in 42 Danish farms (10.7 h/d; Thomsen et al., 2012) and 121 commercial farms in British Columbia, California, and the north-eastern United States (11.0, 10.4, and 10.6 h/d, respectively; von Keyserlingk et al., 2012). However, mean daily lying time in the present study seemed lower than findings on 16 farms in Wisconsin (11.9 h/d, Gomez and Cook, 2010) and 1 farm in the United Kingdom

Table 4. Distribution of cow-level [median \pm interquartile range, IQR (range)] and herd-level [mean \pm SD (range)] explanatory variables of 5,135 cows from 141 dairy herds with a low ($\leq 10\%$), medium (10–30%), or high ($\geq 30\%$) lameness prevalence

Variable	Cow-level			Herd-level lameness prevalence			
	Nonlame ¹ (n = 4,062)	Lame ¹ (n = 1,073)	Total	Low (n = 24)	Medium (n = 94)	High (n = 23)	Total
Parity	2 \pm 2	3 \pm 2	2 \pm 2 (1–11)	2.4 \pm 0.5	2.3 \pm 0.3	2.3 \pm 0.3	2.3 \pm 0.4 (1.4–3.5)
DIM	81 \pm 70	83 \pm 79	81 \pm 72 (1–486)	85 \pm 33	91 \pm 29	101 \pm 34	91 \pm 31 (29–188)
Milk yield (kg/d)	37 \pm 9 ²	36 \pm 10 ²	37 \pm 9 (4–69) ²	39.1 \pm 4.8 ³	37.5 \pm 4.6	34.4 \pm 5.2	37.2 \pm 4.9 (24.0–48.8)
Lying time (h/d)	10.5 \pm 2.7 ²	11.1 \pm 2.8 ²	10.6 \pm 2.3 (1.3–19.3) ²	10.4 \pm 0.9 ³	10.6 \pm 0.9	11.0 \pm 0.8	10.6 \pm 0.9 (8.2–13.2)
Bout frequency (n/d)	10.2 \pm 4.5	9.7 \pm 4.7	10.2 \pm 4.7 (1.7–48.7)	10.8 \pm 1.3	10.7 \pm 1.2	10.7 \pm 2.1	10.7 \pm 1.4 (6.7–13.9)
Bout duration (min/bout)	61 \pm 27	69 \pm 32	63 \pm 28 (7–219)	63 \pm 8	65 \pm 9	70 \pm 15	66 \pm 10 (46–106)
SD of bout duration (min)	38 \pm 17	42 \pm 22	39 \pm 18 (8–243)	39 \pm 6 ³	41 \pm 6	45 \pm 11	41 \pm 7 (27–66)
Size of adult herd	—	—	—	167 \pm 94	135 \pm 72	122 \pm 73	138 \pm 77 (40–470)

¹Mean of nonlame differed ($P \leq 0.05$) from mean of lame cows for every variable.

²Mean \pm SD was calculated.

³Mean was different ($P \leq 0.05$) from high-lameness prevalence herds.

Table 5. Use of lying behavior as independent predictors of lameness estimated by logistic regression models including parity and days in milk as covariates in 141 farms¹

Lying behavior	Odds ratio (95% CI)	Se ²	Sp ³	PPV ⁴	NPV ⁵
Lying time (h/d)					
≤7	1.57 (1.20 to 2.07)	55.3	66.2	30.2	84.9
≥12	1.90 (1.63 to 2.20)	63.9	60.4	29.9	86.4
≥13	2.62 (2.20 to 3.13)	62.9	63.3	31.1	86.6
≥14	3.72 (2.97 to 4.67)	59.0	67.5	32.4	86.2
≥15	4.56 (3.27 to 6.36)	55.7	68.5	31.8	85.4
Bout frequency (n/d)					
≤4	1.78 (1.12 to 2.82)	54.4	67.3	30.5	84.8
≤5	1.69 (1.24 to 2.29)	55.5	66.5	30.4	85.0
≤6	1.55 (1.24 to 1.93)	56.7	65.5	30.3	85.2
≤7	1.51 (1.27 to 1.80)	58.0	64.1	29.9	85.2
Bout duration (min/bout)					
≥90	1.93 (1.65 to 2.26)	60.7	63.2	30.3	85.9
≥100	2.24 (1.78 to 2.82)	58.0	66.3	31.2	85.7
≥110	2.51 (1.84 to 3.42)	54.9	67.6	30.9	85.0
≥120	2.64 (1.79 to 3.89)	54.3	67.9	30.9	84.9
SD of bout duration (min)					
≥50	1.89 (1.61 to 2.20)	63.9	59.5	29.4	86.2
≥60	2.09 (1.77 to 2.55)	59.8	63.8	30.4	85.7
≥70	2.96 (2.26 to 3.88)	56.2	67.2	31.1	85.3
≥80	4.50 (3.01 to 6.74)	54.0	68.4	31.1	84.9

¹All associations between lameness and lying behaviors were significant ($P \leq 0.01$).

²Sensitivity = proportion of lame cows identified by the threshold of lying behavior.

³Specificity = proportion of nonlame cows correctly classified by the threshold of lying behavior.

⁴Positive predictive value = probability that being above the given threshold of lying behavior, the cow is lame.

⁵Negative predictive value = probability that being below the given threshold of lying behavior, the cow is not lame.

(11.7 h/d, Blackie et al., 2011). In the present data, cows had on average 10 lying bouts/d, lasting 63 min each. These results seemed comparable to other reports where frequency and duration of lying bouts ranged from 11 to 13 bouts/d, with a duration of 62 to 72 min (Bewley et al., 2010; Gomez and Cook, 2010; Thomsen et al., 2012), but were higher and shorter, respectively, than reported by Ito et al. (2009; 9 bouts of 88 min) and Watters et al. (2013; 9 bouts of 85 min). Apart from facility design and management factors, apparent differences in lying behavior among regions could be explained by several factors; for instance, the method used to record behavior (e.g., video analysis vs. automated recording systems), cow selection criteria (e.g., studies in the United States assessed cows with higher mean DIM which are known to have longer daily lying times), herd selection (e.g., experimental farms with controlled environments vs. commercial farms), and the proportion of lame cows.

When assessing cow comfort, most attention has focused on daily lying time. Sufficient amounts of lying time have been suggested for confined dairy cattle. For example, the Canadian Dairy Code of Practice recommends that stalls should allow cows to lay comfortably for at least 12 h/d (Dairy Farmers of Canada, 2009); therefore, by this standard, on the majority of farms

(91.5%) in our study, cows were not getting sufficient lying time, indicating a potential welfare issue. However, this recommendation was based on small-scale studies (Cook et al., 2004) or under conditions with a small sample size (Jensen et al., 2005; Munksgaard et al., 2005). In addition, based on results from the present and previous studies (Ito et al., 2010), although associated, no large effect of herd-average daily lying time on the prevalence of lameness was noted. Moreover, all recommendations about optimum lying times must take into account individual factors of the cow (i.e., parity and DIM), instead of merely focusing on an average number for all cows.

Daily lying time increased with increasing DIM, due to decreased bout frequency and increased bout duration, although it varied among parities. These results were consistent with those of Vasseur et al. (2012) and Sepúlveda-Varas et al. (2014), but differed from those of Endres and Barberg (2007), who reported increased bout frequency with DIM in compost barn-housed cows. Given that frequent changes of positions between lying and standing may be attributed to increased comfort (Haley et al., 2001), the change in lying behavior that cows exhibited throughout lactation could have been a response to physical and metabolic adaptations related to comfort. Primiparous cows exhibited the greatest

differences in lying bout frequency and duration based on DIM. In the first month after calving, primiparous cows had a high frequency of short-duration lying bouts, whereas later in lactation they decreased the frequency and increased duration of bouts and their lying behavior became more similar to older cows. This change in behavior could be an indicator of restlessness due to the stress related to calving for the first time and to adapting to a social structure in a new environment (Blowey, 2005). In addition, primiparous cows have a higher prevalence and more severe udder edema resulting in udder distension (Melendez et al., 2006), which could contribute to observed differences in lying time in early lactation and first parity (Vasseur et al., 2012). We inferred that much of the variation in a herd's lying behavior was due to parity and DIM; therefore, these factors must be considered when assessing lying behavior at individual and herd levels.

The use of sand or >2 cm depth of bedding in stalls with high curbs was associated with increased daily lying time. These findings were in agreement with previous studies conducted on commercial farms (Gomez and Cook, 2010; Ito et al., 2014) and the Canadian industry standard (Dairy Farmers of Canada, 2009). We inferred that sand and greater bedding quantity promoted lying behavior, perhaps due to greater cushion (Gomez and Cook, 2010). In the present study, bedding quantity interacted with stall curb height, but this was affected by stall base. This relationship probably occurred because a high rear curb not only allows for a greater quantity of bedding (i.e., stalls with ≤ 2 and > 2 cm of bedding had mean higher rear curb of 21 and 23 cm, respectively), but may also help maintain bedding in stalls. In addition, bedding management practices are associated with the presence of certain stall bases. For example, 87% of the farms that used sand as a stall base also used >2 cm of bedding. Conversely, 100% of the farms that used waterbeds as a stall base used ≤ 2 cm of bedding.

In regards to stall dimensions, cows spent more time lying down in farms with stalls ≥ 114 cm wide. This was in agreement with an experimental study which reported cows lying down for 72 min (1.2 h/d) longer in wider stalls (112 compared with 132 cm wide, Tucker et al., 2004). To our knowledge, ours is the first large-scale study to provide evidence of an association between stall width and lying behavior on commercial farms.

Cows in pens with rubber flooring in the feed alley spent an average of 29 min/d less lying compared with cows on solid concrete. Perhaps cows may sometimes lie down to avoid standing on an uncomfortable floor, or may choose to spend longer time standing on softer floors (Tucker et al., 2006). However, the present data did not allow us to distinguish the extent to which

this extra standing time may have been spent in other activities (e.g., eating). Only 9 of the 27 farms with rubberized flooring had >2 cm of bedding in stalls, suggesting that rubber flooring was not necessarily combined with, presumably, more comfortable stalls. Experimental studies demonstrated that, when given a preference, cows stood on soft rubber compared with solid concrete (Telezhenko et al., 2007). This preference could affect the cows' time budgets, as when exposed to a soft standing surface cows may reduce their requirement for rest (Cook and Nordlund, 2009). However, evidence exists that reduced time spent lying down precedes development of claw lesions (Chapinal et al., 2009). Therefore, in agreement with Cook and Nordlund (2009), the use of rubber should be combined with comfortable stalls to avoid an increased risk for lameness.

In the present study, cows exposed to feed alleys ≥ 350 cm wide had increased daily lying time. The effect of feed alley space on daily lying time could be related to the display of aggressive or competitive behavior among cows when feeding space is reduced (DeVries et al., 2004). Adequate feed alley space could also ease cow traffic flow, as cows lying in the stalls facing the feed alley would not necessarily be forced by their herd mates to exit their stall.

Overstocking and milking duration and frequency are likely to affect lying time. In contrast to previous small-scale studies where overstocking (Fregonesi et al., 2007) or time away from the pen for milking (Gomez and Cook, 2010) reduced lying time for cows, no evidence of these associations was observed in the present study. As described by Charlton et al. (2014), the lack of association with stocking density may be due to good management practices, as the majority of farms (98%) met or bettered the recommendation from the Canadian Dairy Code of Practice that stocking density must not exceed 1.2 cows/stall (Dairy Farmers of Canada, 2009). In regards to milking practices, AB was the province with the longest milking duration, which could be attributed to the herd size, but, interestingly, it is also the province with the shortest average lying time. The lack of an association with duration and frequency of milking may be due to failure to fully capture the data. First, the measurement did not necessarily reflect individual cows' time away from the pen. Second, the variation of barn design and milking management practices biased our observation. Some farms had a holding pen where all lactating cows were moved in 1 group or in subgroups. In the absence of a holding pen, other farms allowed their cows to flow freely from stalls to the milking parlor, providing access to a lying space, water, and food while waiting to be milked. Therefore, we suggest that measuring the individual cow's time away from

pen instead of a herd's milking duration would provide a better estimate.

In agreement with other reports, lame cows differed in their lying behavior from nonlame cows and lame cows had slightly fewer but longer bouts, resulting in longer daily lying time compared with nonlame cows (Ito et al., 2010; Blackie et al., 2011; Sepúlveda-Varas et al., 2014). The locomotion scoring system in the present study did not capture different lameness severities. However, it is expected that severely lame cows will present even longer daily lying time and lying bouts than moderately lame cows, as demonstrated by Ito et al. (2010). In agreement with Gomez and Cook (2010) and Ito et al. (2010), lame cows had both extremely high and low lying times, suggesting that the effect of lameness on lying behavior may be 2-way. Cows lie down longer because rising is challenging, or they may compromise resting because lying down is challenging; in turn, this may be confounded by stage of lactation, parity, and lying surface among other variables. When assessing lameness and lying behavior at the herd level, the results in our study indicated that farms with high lameness prevalence also had longer mean lying time and higher mean SD of bout duration. Therefore, the proportion of lame cows in a farm influenced lying data. Thus, caution must be exercised when evaluating farms based on average daily lying duration and bout duration estimates.

The odds of lameness increased as bout frequency decreased and daily lying time, bout duration, and standard deviation of bout duration increased. Regardless, the use of lying behavior was not optimal to diagnose lameness. With no true gold standard available to detect lameness and the wide variability of lying behavior regardless of lameness, poor test accuracy was expected. These results were in agreement with Ito et al. (2010), where lying behavior was not a sensitive diagnostic tool for severely lame cows (<70%); however, careful attention should be paid to cows with extreme lying behaviors. In the present study, low sensitivity and specificity resulted in a low positive predictive value ($\leq 32\%$), leading to a high proportion of false-positive cows that could result in extra treatments. Lying behavior as a screening test only provided moderate negative predictive value ($>84\%$). A moderate negative predictive value alone is not an extremely useful result from a practical standpoint, as it solely gives high confidence that a cow that does not exhibit extreme lying behavior is truly nonlame. However, results related to predictive values could aid in improving automated detection of lameness, and the sensitivity and specificity may increase if additional individual and management factors are included through serial testing (Dohoo et al., 2009).

CONCLUSIONS

Lying behavior was associated with individual factors such as parity and stage of lactation and herd-level factors related to comfort of lying and standing surfaces. Daily lying time was a good measure that summarized herd-level risk factors associated with lying behavior. In addition, lame cows differed in their lying behavior from nonlame cows, and they exhibited extreme lying behaviors more often. Finally, automated measures of lying behavior may improve lameness detection, especially when interpreted in the context of other factors known to affect lying behavior, including those associated with the individual cow (e.g., parity and stage of lactation) or environment (e.g., stall surface).

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REFERENCES

- Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pages 267–281 in Proc. 2nd Int. Symp. Inf. Theory. Akademiai Kiado, Budapest, Hungary.
- Alberta Milk. 2013. Dairy cattle hoof health. Accessed Aug. 3, 2015. <http://dairyhoofhealth.info/lesions/hoof-lesion-prevalence-in-3-canadian-provinces/>.
- Alsaad, M., C. Römer, J. Kleinmanns, K. Hendriksen, S. Rose-Meierhöfer, L. Plümer, and W. Büscher. 2012. Electronic detection of lameness in dairy cows through measuring pedometric activity and lying behavior. *Appl. Anim. Behav. Sci.* 142:134–141.
- Bewley, J. M., R. E. Boyce, J. Hockin, L. Munksgaard, S. D. Eicher, M. E. Einstein, and M. M. Schutz. 2010. Influence of milk yield, stage of lactation, and body condition on dairy cattle lying behav-

- four measured using an automated activity monitoring sensor. *J. Dairy Res.* 77:1–6.
- Blackie, N., J. Amory, E. Bleach, and J. Scaife. 2011. The effect of lameness on lying behaviour of zero grazed Holstein dairy cattle. *Appl. Anim. Behav. Sci.* 134:85–91.
- Blowey, R. 2005. Factors associated with lameness in dairy cattle. In *Pract.* 27:154–162.
- Botheras, N. A. 2006. The behaviour and welfare of grazing dairy cows (*Bos taurus*): Effects of time away from pasture and position in the milking order. Vol. PhD Thesis. University of Melbourne, Melbourne, Australia.
- Chapinal, N., A. M. de Passillé, D. M. Weary, M. A. G. von Keyserlingk, and J. Rushen. 2009. Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. *J. Dairy Sci.* 92:4365–4374.
- Charlton, G. L., V. Bouffard, J. Gibbons, E. Vasseur, D. B. Haley, D. Pellerin, J. Rushen, and A. M. de Passillé. 2015. Can automated measures of lying time help assess lameness and leg lesions on tie-stall dairy farms? *Appl. Anim. Behav. Sci.* In press.
- Charlton, G. L., D. B. Haley, J. Rushen, and A. M. de Passillé. 2014. Stocking density, milking duration, and lying times of lactating cows on Canadian freestall dairy farms. *J. Dairy Sci.* 97:2694–2700.
- Cook, N. B., T. B. Bennett, and K. V. Nordlund. 2004. Effect of free stall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. *J. Dairy Sci.* 87:2912–2922.
- Cook, N. B., M. J. Marin, R. L. Mentink, T. B. Bennett, and M. J. Schaefer. 2008. Comfort zone-design free stalls: Do they influence the stall use behavior of lame cows? *J. Dairy Sci.* 91:4673–4678.
- Cook, N. B., R. L. Mentink, T. B. Bennett, and K. Burgi. 2007. The effect of heat stress and lameness on time budgets of lactating dairy cows. *J. Dairy Sci.* 90:1674–1682.
- Cook, N. B., and K. V. Nordlund. 2009. The influence of the environment on dairy cow behavior, claw health and herd lameness dynamics. *Vet. J.* 179:360–369.
- Cooper, M. D., D. R. Arney, and C. J. C. Phillips. 2008. The effect of temporary deprivation of lying and feeding on the behaviour and production of lactating dairy cows. *Animal* 2:275–283.
- Dairy Farmers of Canada. 2009. Code of practices for the care and handling of dairy cattle. Dairy Farmers of Canada, Ottawa, ON, Canada.
- de Mol, R. M., G. André, E. J. B. Bleumer, J. T. N. van der Werf, Y. de Haas, and C. G. van Reenen. 2013. Applicability of day-to-day variation in behavior for the automated detection of lameness in dairy cows. *J. Dairy Sci.* 96:3703–3712.
- DeVries, T. J., M. A. G. von Keyserlingk, and D. M. Weary. 2004. Effect of feeding space on the inter-cow distance, aggression, and feeding behavior of free-stall housed lactating dairy cows. *J. Dairy Sci.* 87:1432–1438.
- Dohoo, I., W. Martin, and H. Stryhn. 2009. *Veterinary Epidemiology Research*. 2nd ed. VER Inc., Charlottetown, PEI, Canada.
- EFSA. 2009. Scientific report of EFSA prepared by the Animal Health and Animal Welfare Unit on the effects of farming systems on dairy cow welfare and disease. Annex to the EFSA J. 1143:1–284.
- Endres, M. I., and A. E. Barberg. 2007. Behavior of dairy cows in an alternative bedded-pack housing system. *J. Dairy Sci.* 90:4192–4200.
- Espejo, L. A., M. I. Endres, and J. A. Salfer. 2006. Prevalence of lameness in high-producing Holstein cows housed in freestall barns in Minnesota. *J. Dairy Sci.* 89:3052–3058.
- Ettema, J. F., and S. Ostergaard. 2006. Economic decision making on prevention and control of clinical lameness in Danish dairy herds. *Livest. Sci.* 102:92–106.
- Flower, F. C., and D. M. Weary. 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. *J. Dairy Sci.* 89:139–146.
- Fregonesi, J. A., and J. D. Leaver. 2002. Influence of space allowance and milk yield level on behaviour, performance and health of dairy cows housed in strawyard and cubicle systems. *Livest. Prod. Sci.* 78:245–257.
- Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007. Overstocking reduces lying time in dairy cows. *J. Dairy Sci.* 90:3349–3354.
- Gibbons, J., E. Vasseur, J. Rushen, and A. M. de Passillé. 2012. A training programme to ensure high repeatability of injury scoring of dairy cows. *Anim. Welf.* 21:379–388.
- Gomez, A., and N. B. Cook. 2010. Time budgets of lactating dairy cattle in commercial freestall herds. *J. Dairy Sci.* 93:5772–5781.
- Green, L. E., V. J. Hedges, Y. H. Schukken, R. W. Blowey, and A. J. Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. *J. Dairy Sci.* 85:2250–2256.
- Haley, D. B., A. M. de Passille, and J. Rushen. 2001. Assessing cow comfort: Effects of two floor types and two tie stall designs on the behaviour of lactating dairy cows. *Appl. Anim. Behav. Sci.* 71:105–117.
- Hernandez, J., J. K. Shearer, and D. W. Webb. 2001. Effect of lameness on the calving-to-conception interval in dairy cows. *J. Am. Vet. Med. Assoc.* 218:1611–1614.
- Hernandez-Mendo, O., M. A. G. von Keyserlingk, D. M. Veira, and D. M. Weary. 2007. Effects of pasture on lameness in dairy cows. *J. Dairy Sci.* 90:1209–1214.
- Ito, K., N. Chapinal, D. M. Weary, and M. A. G. von Keyserlingk. 2014. Associations between herd-level factors and lying behavior of freestall-housed dairy cows. *J. Dairy Sci.* 97:2081–2089.
- Ito, K., M. A. G. von Keyserlingk, S. J. LeBlanc, and D. M. Weary. 2010. Lying behavior as an indicator of lameness in dairy cows. *J. Dairy Sci.* 93:3553–3560.
- Ito, K., D. M. Weary, and M. A. G. von Keyserlingk. 2009. Lying behavior: Assessing within- and between-herd variation in free-stall housed dairy cows. *J. Dairy Sci.* 92:4412–4420.
- Jensen, M. B., L. J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. *Appl. Anim. Behav. Sci.* 90:207–217.
- Ledgerwood, D. N., C. Winckler, and C. B. Tucker. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. *J. Dairy Sci.* 93:5129–5139.
- Melendez, P., C. C. Hofer, and G. A. Donovan. 2006. Risk factors for udder edema and its association with lactation performance on primiparous Holstein cows in a large Florida herd, U.S.A. *Prev. Vet. Med.* 76:211–221.
- Munksgaard, L., M. B. Jensen, L. J. Pedersen, S. W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities—Effects of time constraints on behaviour of dairy cows, *Bos taurus*. *Appl. Anim. Behav. Sci.* 92:3–14.
- Proudfoot, K. L., J. M. Huzzey, and M. A. G. von Keyserlingk. 2009. The effect of dystocia on the dry matter intake and behavior of Holstein cows. *J. Dairy Sci.* 92:4937–4944.
- Rushen, J., A. M. de Passillé, M. A. G. von Keyserlingk, and D. M. Weary. 2008. *The Welfare of Cattle*. Vol. 5. Springer, Dordrecht, the Netherlands.
- Rushen, J., E. Pombourcq, and A. M. de Passillé. 2007. Validation of two measures of lameness in dairy cows. *Appl. Anim. Behav. Sci.* 106:173–177.
- Sepúlveda-Varas, P., D. M. Weary, and M. A. G. von Keyserlingk. 2014. Lying behavior and postpartum health status in grazing dairy cows. *J. Dairy Sci.* 97:6334–6343.
- Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, J. C. Zaffino Heyerhoff, C. G. R. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Passillé, E. Vasseur, and K. Orsel. 2015. Prevalence of lameness and associated risk factors in Canadian Holstein-Friesian cows housed in freestall barns. *J. Dairy Sci.* 98:6978–6991.
- Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology* 47:1179–1187.
- Telezhenko, E., L. Lidfors, and C. Bergsten. 2007. Dairy cow preferences for soft or hard flooring when standing or walking. *J. Dairy Sci.* 90:3716–3724.
- Thomsen, P. T., L. Munksgaard, and J. T. Sørensen. 2012. Locomotion scores and lying behaviour are indicators of hoof lesions in dairy cows. *Vet. J.* 193:644–647.
- Tucker, C. B., D. M. Weary, A. M. De Passille, B. Campbell, and J. Rushen. 2006. Flooring in front of the feed bunk affects feed-

ing behavior and use of freestalls by dairy cows. *J. Dairy Sci.* 89:2065–2071.

Tucker, C. B., D. M. Weary, and D. Fraser. 2004. Free-stall dimensions: Effects on preference and stall usage. *J. Dairy Sci.* 87:1208–1216.

Vasseur, E., J. Gibbons, J. Rushen, D. Pellerin, E. Pajor, D. Lefebvre, and A. M. de Passillé. 2015. An assessment tool to help producers improve cow comfort on their farms. *J. Dairy Sci.* 98:698–708.

Vasseur, E., J. Rushen, D. B. Haley, and A. M. de Passillé. 2012. Sampling cows to assess lying time for on-farm animal welfare assessment. *J. Dairy Sci.* 95:4968–4977.

von Keyserlingk, M. A. G., A. Barrientos, K. Ito, E. Galo, and D. M. Weary. 2012. Benchmarking cow comfort on North American freestall dairies: Lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. *J. Dairy Sci.* 95:7399–7408.

Watters, M. E. A., K. M. A. Meijer, H. W. Barkema, K. E. Leslie, M. A. G. von Keyserlingk, and T. J. DeVries. 2013. Associations of herd- and cow-level factors, cow lying behavior, and risk of elevated somatic cell count in free-stall housed lactating dairy cows. *Prev. Vet. Med.* 111:245–255.

Weary, D. M., J. M. Huzzey, and M. A. G. von Keyserlingk. 2009. Using behavior to predict and identify ill health in animals. *J. Anim. Sci.* 87:770–777.

Zaffino Heyerhoff, J. C., S. J. LeBlanc, T. J. DeVries, C. G. R. Nash, J. Gibbons, K. Orsel, H. W. Barkema, L. Solano, J. Rushen, A. M. de Passillé, and D. B. Haley. 2014. Prevalence of and factors associated with hock, knee, and neck injuries on dairy cows in freestall housing in Canada. *J. Dairy Sci.* 97:173–184.

Appendix

Table A1. Final generalized linear mixed model for 3 measures of lying behavior with cow and herd-level factors in 141 Canadian dairy herds, considering cow (n = 4,790) as the experimental unit

Predictor	Log bout frequency (n/d)		Log bout duration (min/bout)		Log SD of bout duration (min)	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Intercept	2.43 (2.34 to 2.52)		3.92 (3.84 to 4.00)		3.46 (3.36 to 3.56)	
Lame						
No	Ref. ¹		Ref.		Ref.	
Yes	-0.04 (-0.06 to -0.01)	0.003	0.07 (0.05 to 0.09)	<0.001	0.08 (0.06 to 0.10)	<0.001
Hock injury						
Not injured (score 0-1)						
Injured (score 2-3)						
Knee injury ²						
Not injured (score 0-1)						
Injured (score 2-3)	0.01 (-0.01 to 0.04)	0.393	-0.03 (-0.05 to -0.02)	<0.001	—	—
Parity						
1	Ref.		Ref.		Ref.	
2	-0.32 (-0.37 to -0.26)	<0.001	0.34 (0.30 to 0.39)	<0.001	0.23 (0.18 to 0.28)	<0.001
≥3	-0.36 (-0.41 to -0.31)	<0.001	0.45 (0.40 to 0.49)	<0.001	0.27 (0.22 to 0.32)	<0.001

Continued

Table A1 (Continued). Final generalized linear mixed model for 3 measures of lying behavior with cow and herd-level factors in 141 Canadian dairy herds, considering cow ($n = 4,790$) as the experimental unit

Predictor	Log bout frequency (n/d)		Log bout duration (min/bout)		Log SD of bout duration (min)	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
DIM						
Fresh (1–44)	Ref.	<0.001	Ref.	<0.001	Ref.	<0.001
Early (45–99)	-0.18 (-0.22 to -0.14)	<0.001	0.24 (0.20 to 0.27)	<0.001	0.24 (0.20 to 0.28)	<0.001
Mid (100–199)	-0.23 (-0.28 to -0.19)	<0.001	0.37 (0.33 to 0.41)	<0.001	0.33 (0.29 to 0.38)	<0.001
Late (≥ 200)	-0.29 (-0.37 to -0.21)	<0.001	0.45 (0.39 to 0.52)	<0.001	0.42 (0.35 to 0.49)	<0.001
Parity \times DIM						
First parity, fresh DIM	Ref.	<0.001	Ref.	<0.001	Ref.	<0.001
Second parity, early DIM	0.17 (0.11 to 0.23)	<0.001	-0.18 (-0.23 to -0.13)	<0.001	-0.15 (-0.21 to -0.09)	<0.001
Second parity, mid DIM	0.19 (0.12 to 0.26)	<0.001	-0.23 (-0.28 to -0.17)	<0.001	-0.17 (-0.23 to -0.10)	<0.001
Second parity, late DIM	0.26 (0.15 to 0.38)	<0.001	-0.27 (-0.37 to -0.18)	<0.001	-0.19 (-0.30 to -0.09)	<0.001
\geq Third parity, early DIM	0.16 (0.10 to 0.22)	<0.001	-0.21 (-0.26 to -0.15)	<0.001	-0.14 (-0.20 to -0.08)	<0.001
\geq Third parity, mid DIM	0.26 (0.19 to 0.32)	<0.001	-0.35 (-0.41 to -0.30)	<0.001	-0.24 (-0.30 to -0.18)	<0.001
\geq Third parity, late DIM	0.39 (0.28 to 0.50)	<0.001	-0.45 (-0.55 to -0.36)	<0.001	-0.30 (-0.40 to -0.20)	<0.001
Stall base						
Concrete	Ref.	0.003	Ref.	0.025	Ref.	0.062
Rubber mattress	0.13 (0.04 to 0.21)	0.001	-0.08 (-0.16 to -0.01)	0.075	-0.08 (-0.17 to 0.00)	0.004
Geotextile mattress	0.11 (0.04 to 0.17)	0.553	-0.06 (-0.12 to 0.01)	0.393	-0.10 (-0.16 to -0.03)	0.616
Sand or dirt	0.03 (-0.06 to 0.12)	0.344	0.03 (-0.04 to 0.11)	0.540	0.02 (-0.07 to 0.11)	0.410
Waterbed	0.05 (-0.05 to 0.16)	0.380	-0.03 (-0.14 to -0.07)	0.363	-0.05 (-0.16 to 0.05)	0.885
Other	0.06 (-0.08 to 0.21)		-0.05 (-0.17 to 0.06)		-0.01 (-0.14 to 0.12)	
Bedding depth (cm)						
≤ 2	—		Ref.		—	
> 2	—		0.05 (0.01 to 0.09)	0.023	—	
Feed alley flooring						
Solid concrete	Ref.	0.725	—		Ref.	0.343
Slatted concrete	0.01 (-0.06 to 0.08)	0.020	—		-0.23 (-0.08 to 0.03)	0.044
Solid rubber	-0.07 (-0.13 to -0.01)		—		0.06 (0.01 to 0.13)	
Scrape frequency (times/d)						
≤ 2	Ref.	0.387	—		—	
2–9	0.03 (-0.04 to 0.09)	0.020	—		—	
≥ 10	0.08 (0.01 to 0.14)		—		—	
Feed alley width (cm)						
< 350	—		Ref.		—	
350–450	—		-0.02 (-0.06 to 0.02)	0.406	—	
> 450	—		0.14 (0.03 to 0.24)	0.014	—	
Milking duration (30 min increase)						
					0.01 (0.00 to 0.02)	0.033

¹Referent.²Scrape frequency confounded by knee injury.