Activity behaviors and relative changes in activity patterns recorded by precision technology were associated with diarrhea status in individually housed calves

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ABSTRACT

The objective of this case-control study was to quantify if there was an association of daily activity behaviors and relative changes in activity patterns (lying time, lying bouts, step count, activity index) with diarrhea status in preweaned dairy calves. Individually housed calves sourced from auction were health scored daily for signs of diarrhea (fecal consistency loose or watery for 2 consecutive d) for the 28 d after arrival. Calves with diarrhea were pair matched to healthy controls (n = 13 matched by arrival date, arrival weight, and diagnosis d to diarrheic calves). Mixed linear regression models were used to evaluate the association of diarrhea status, and the diarrhea status by day interaction with activity behaviors (d −3 to d 4) and relative changes in activity patterns (d −3 to d 4) relative to diagnosis of a diarrhea bout. The serum Brix % at arrival and daily THI from the calf barn were explored as quantitative covariates, with d as a repeated measure. The baseline for relative changes in activity patterns was set at 100% on d 0. Diarrheic calves were less active; they averaged less steps (119.1 ± 18.81 steps/d) than healthy calves (227.4 ± 18.81 steps/d, LSM ± SEM). Diarrheic calves also averaged lower activity indices (827.34 ± 93.092 daily index) than healthy calves (1396.32 ± 93.092 daily index). There was also a diarrhea status by day interaction on d −3, with diarrheic calves spending more time lying (20.80 ± 0.300 h/d) than healthy calves (19.25 ± 0.300 h/d). For relative changes in activity patterns, there was a diarrhea status by day interaction for lying time on d −3, with diarrheic calves spending more time lying (20.80 ± 0.300 h/d) than healthy calves (19.25 ± 0.300 h/d). These results show that diarrheic calves were more lethargic, and they had relative changes in activity patterns −2 d before clinical signs of diarrhea. Future research should explore the potential of an activity alert which positively indicates an individually housed calf at-risk for a diarrhea bout using deviations from relative changes in individual calf activity patterns.

KEYWORDS: precision livestock farming, technology, sickness behavior, scours

INTRODUCTION

Diarrhea is the leading cause of dairy calf mortality, accounting for 56% of producer reported deaths of preweaned calves in the United States (USDA, 2018). While diarrhea can be characterized through observation of feces with a loose or watery consistency (Renaud et al., 2020), these calves also often have compromised milk intake (Conboy et al., 2022) and reduced activity (Goharshahi et al., 2021) compared with healthy calves. These sickness behaviors are problematic because they exacerbate a calf’s risk for dehydration, metabolic acidosis, and hypoglycemia, which are leading risk factors for diarrhea-associated mortality in calves (Trefz et al., 2017). However, using activity patterns to indicate disease status could provide valuable information for producers. For example, machine learning techniques which used informatics from precision technology sensors (e.g., pedometers and automated feeders) were highly accurate at labeling calves with Bovine Respiratory Disease up to 6 d before clinical signs of disease (Cantor et al., 2022a). In addition, for the detection of diarrhea, machine learning techniques accurately labeled diarrheic calves as sick with feeding behavior data (Ghaffari et al., 2022), though this has yet to be assessed using activity data.

Diarrhea status in calves has been associated with activity behaviors recorded by accelerometers (Studds et al., 2018; Swartz et al., 2020; Goharshahi et al., 2021). Specifically, individually housed diarrheic calves wearing ear-based accelerometers had longer lying times and reduced activeness compared with healthy...
could develop an alert with diagnostic accuracy which period of individual housing. Ideally, future research patterns are associated with diarrheal bouts during a weeks of life (Urie et al., 2018), it would be beneficial to better understand if relative changes in calf activity behavior before diarrhea bout diagnosis (Lowe et al., 2019). There are, however, some challenges within the forementioned studies because the age at diarrhea onset varies among the diarrheic calves. In addition, external factors, such as temperature humidity index which alters activity behavior in heat stressed dairy cattle (Becker et al., 2020) should be also explored. Moreover, a calf with a lower serum IgG status at 48 h of age has a higher likelihood of diarrhea at a younger age and decreased odds of survival (Lora et al., 2018). Therefore, a study is needed to determine if activity behavior is associated with diarrhea status in calves if the day that diarrhea occurs, and the duration of diarrhea are similar in the calves in consideration of external covariates which may affect calf activity.

No study to our knowledge has quantified if individual dairy calves have relative changes in their activity patterns relative to the diagnosis of a diarrhea bout. However, it is known that dairy calves have relative changes in their activity patterns before the diagnosis of respiratory disease bouts (Cantor and Costa, 2022), and before the re-diagnosis of relapsed cases of respiratory disease compared with calves who recovered from infection (Cantor et al., 2022). It is important to quantify if individual calves change their activity patterns before a diarrhea bout because precision technology software uses relative changes from baseline behavior to alert for dairy cattle at-risk for digestive disorders (Stangaferro et al., 2016,a). This exploratory research is required to determine if there is the potential to use relative changes in activity patterns for algorithm development that have diagnostic accuracy to alert for calves at-risk for diarrhea bouts in activity monitoring systems.

Most dairy calves in North America are still housed individually or in pairs (USDA, 2016). Furthermore, many dairy farms house calves individually before introduction to a calf feeder (Medrano-Galarza et al., 2017). Since dairy calves often incur diarrhea at 2 weeks of life (Urie et al., 2018), it would be beneficial to better understand if relative changes in calf activity patterns are associated with diarrheal bouts during a period of individual housing. Ideally, future research could develop an alert with diagnostic accuracy which uses activity patterns recorded by precision technology to identify calves at-risk for diarrhea bouts very close to when the calf experiences clinical signs of diarrhea. This could provide the producer with a list of calves who may require monitoring and supportive care relative to diagnosis of a diarrhea bout. However, there are some behaviors such as step counts, and activity index, that may be restricted in individually housed calves, which would make detecting differences in activity patterns before the diagnosis of the diarrhea unrealistic. For example, individually housed calves have less locomotory play behavior bouts compared with group housed calves (Valničková et al., 2015), suggesting that a smaller pen and lack of social interaction in individual pens may restrict the opportunity for locomotory play. There is also the possibility that individual housing restricts total step counts in calves, and it has not been explored if step counts decrease surrounding the diagnosis of a diarrhea bout in individually housed calves. Activity index, which is based on total activeness, has been associated with play bouts in calves (Gladden et al., 2020). It is worthwhile to evaluate if activity index is associated with diarrhea bouts as calves were observed to have relative changes in their activity index before the diagnosis of bovine respiratory disease complex (Cantor and Costa, 2022). However, it has not been explored if activity index was associated with diarrhea status in calves and this should be investigated.

The objective of this case-control study was to quantify if there was an association of daily activity and relative changes in activity (lying time, lying bouts, step count, activity index), recorded by pedometers (IceQube, Ice Robotics, Scotland), with diarrhea status in preweaned dairy calves relative to diagnosis of a diarrhea bout (d −3 to d 4 after diarrhea diagnosis) when all diarrheic calves experienced their diarrhea bout around the same time, and for a similar duration. It was expected that there would be an interaction between day and diarrhea status for activity behaviors, with diarrheic calves being less active for −2 to d 0 relative to diarrhea diagnosis as a reflection of sickness behavior based on relative changes in activity observed in calves at this time before the diagnosis of bovine respiratory disease.

**MATERIALS AND METHODS**

This study was conducted at the Grober Young Animal Development Centre in Woodstock, ON, Canada. Two consecutive groups of calves were observed, and data were collected from May 4, 2022, to August 1, 2022. All calves enrolled were approved by the Animal Care Committee at the University of Guelph (AUP 4745). This study and manuscript were conducted following the quality standards of Strengthening the
Health exams

Calves were health scored daily in the morning by the research staff from the day of arrival until study completion, at 28 d after arrival. There were 2 researchers in charge of health exams. During the acclimation period of this study, these researchers’ health scored both blocks of 60 calves twice (e.g., in May and July) to ensure observer agreement using Cohen’s kappa (κ = 0.90). Observer agreement was consistent between tests, and this was done to ensure that the detection of diarrhea in the calves was adequately represented in the testing. Calves were assessed for signs of bloat by visually evaluating the paralumbar fossa. If the paralumbar fossa was extended beyond the rib cage, bloat was diagnosed; however, no bloat was detected in this study. Daily, researchers recorded each calf’s attitude for depression, and appetite including refusal (0) pauses (1) or strong willingness to suckle (2), fecal consistency and signs of respiratory disease. If a calf was positive for any sign of morbidity rectal temperature was taken to assess for fever (≥39.5°C). All calves on the Calf Health Scoring app (UW Madison, WI, USA) were deemed healthy if the calf on the health exam had bright attitude, good appetite, no fever, no umbilical inflammation, no signs of respiratory disease, and a normal fecal consistency score. Fecal consistency scoring was based on the following scale: (0 normal; 1 semi formed, pasty; 2 loose, stays on top of bedding; or 3 watery, sifts through bedding; Renaud et al., 2020). Diarrhea was defined as a fecal score of ≥2 for 2 consecutive days (Villot et al., 2019). Calves with a diarrhea bout received electrolytes by bottle once daily in between feedings on d 0 of diarrhea diagnosis until fecal consistency was normal (≤1 score). Calves were also scored daily for signs of Bovine Respiratory Disease in the app and all enrolled calves were negative; this included an assessment for spontaneous cough, abnormal nasal discharge, ocular discharge, a rectal temperature, and ear position, scored on a scale from 0 (normal) to 3 (very abnormal).

Additional data collection

Transfer of passive immunity. Upon arrival to the facility, calves were tested for passive immunity status. Blood samples were taken via jugular venipuncture, allowed to clot at room temperature, then placed on ice when being transported to the laboratory at the University of Guelph. All samples were centrifuged at room temperature for 15 min at 1500 g, and serum was separated and measured for Brix % using a digital refractometer (MISCO Palm Abee; MISCO, Solon, OH, USA).

Temperature and humidity. Two environmental temperature and humidity monitors (Kestrel DROP D2AG; Kestrel Instruments, Boothwyn, PA, USA) were placed in the center of the calf barn. The temperature humidity index (THI) (NRC, 2001), temperature, and relative humidity were stored on the device every 15 min for the duration of the study. The monitors were downloaded wirelessly to the cloud weekly. The daily barn THI was summarized into daily measures for both monitors and the daily average of THI from the 2 monitors was calculated.

Accelerometers. Each calf was assigned an IceQube pedometer (IceRobotics, Edinburgh, Scotland), which was attached on the rear left leg upon arrival using a Velcro band. Accelerometer activity was recorded at 4 Hz. Activity behavior summaries were logged by the pedometers for lying time, lying bouts, total step count, and activity index (IceQube; IceRobotics, Edinburgh, Scotland) every 15 min. The activity index has been previously described and is based on both total step count and the average daily rate of acceleration of the pedometer (Cantor and Costa, 2022). Twice weekly, the activity data were retrieved from the pedometer.
The calves in this study were part of a separate concurrent, randomized double blind clinical control trial to evaluate the effectiveness of an essential oil on ADG: a low dose of oregano (1g/d), a high dose of oregano (2 g/d), or a control (none) in the milk replacer. We did not expect behavioral differences by essential oil, or dosage surrounding diarrheal bouts. However, we wanted to ensure that we controlled for this in case there was a positive effect of the essential oil to improve a calf’s feed intake, which may have affected the severity of the diarrheal bout. To account for essential oils assigned to the calves, diarrheic calves were pair matched to healthy calves by dosage. We enrolled 6 control pairs, 4 low dose oregano pairs, and 3 high dose oregano pairs.

**Study population and power analysis.** This study was powered based on detecting a difference between mean lying time behavior for healthy and diarrheic calves (healthy 17.6 h/d vs sick 16.7 h/d; Duthie et al., 2021). We assumed a maximum variation of 0.8 h/d among the calves. Therefore, for 80% power and 95% confidence level, we needed a minimum of 12 pairs or 24 calves to detect a difference between healthy and diarrheic calves.

**Statistical analysis.**

All statistical analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Data were verified for normality and checked for outliers using univariate measures and probability distribution plots. The activity behaviors were summarized into daily values by calf relative to the first d of diarrhea diagnosis (d −3 to d 4).

For all linear mixed regression models in this study, multivariable models were reduced by manual stepwise backward elimination, Brix % at arrival, and the daily calf barn THI were tested as quantitative covariates and removed if non-significant. The daily calf barn THI, while not significant (P = 0.10), was retained in the total steps model due to its effect size on the model. Since this was a case-control study, the case-control matching was a fixed effect, the day relative to diarrhea diagnosis was used as a repeated measure (d −3 to d 4), and calf was the subject. The covariance structure was selected based on the lowest AIC from the model and residual fit. Compound symmetry, Toepplitz, first order autoregressive and unstructured covariance structures were assessed, and Toepplitz covariance structure was selected for all models. Visual examination of the residuals from the linear mixed models were completed to evaluate for outliers. Any suspect visual outliers (modeled residuals greater than 3 SD) were tested for model leverage and Cook’s Distance was calculated. Four outliers were identified, but these outliers did not have model...
leverage and remained in the models. Model fit was determined by evaluating homoscedasticity of the best linear unbiased predictions using standardized residuals. Statistical significance was declared at $P \leq 0.05$.

**Association of diarrhea status with average calf behavior.** Linear mixed regression models were used to investigate the association of diarrhea status (sick or healthy) with activity behavior (lying time, lying bouts, step count, activity indices) in preweaned calves (d $-3$ to d 4). The diarrhea status by day interactions were also explored for all models at $P \leq 0.05$ and daily least squares means differences between diarrhea status by day were adjusted with Tukey corrections.

**Association of diarrhea status with relative changes in activity patterns.** Linear mixed regression models were used to investigate the association of diarrhea status with relative changes in activity patterns using the modeling structure described above. The diarrhea status by day interactions were also explored for all models at $P \leq 0.05$ and daily least squares means differences between diarrhea status by day were adjusted with Tukey corrections. As this was an exploratory analysis, we set the behavioral baseline for relative change on the day of diarrhea diagnosis (d 0). This d was selected to determine if sick calves had significantly different activity patterns from healthy calves relative to the d of clinical disease diagnosis. Relative change was calculated by dividing every calf’s individual behavior on every daily measure ($d -3$, $d -2$, $d -1$, d 1, d 2) by every calf’s behavioral baseline at d 0. The equation was:

$$\text{Relative change activity patterns} = \left( \frac{\text{Current day behavior}}{\text{Baseline day 0}} \right) \times 100.$$

**RESULTS**

**Demographics and enrollment**

A participant flowchart for this study is in Figure 1. Of the 104 calves fitted with pedometers, 87.50% (91/104) experienced a diarrhea bout during the study. During the study, the mortality rate was 04.16% (5/120: 4/5 diarrhea, 1/5 pneumonia), all of which occurred within 7 d after arrival. In total, we enrolled 13 diarrheic calves and 13 control calves who met enrollment criteria, had complete data, had a diarrhea bout at 5.2 ± 0.32 d after arrival, and the duration of the diarrhea bout was similar among diarrheic calves 3.2 ± 0.28 d. The calves had a mean arrival serum Brix % of 8.7 ± 0.06%, and an arrival body weight of 45.5 ± 0.30 kg. The microclimate in the calf barn for the duration of this study was a temperature of 15.5 ± 0.30°C, a humidity of 49.7 ± 0.70%, and a THI 58.4 ± 0.42.

**Association of diarrhea status with average calf behavior**

The association of diarrhea status with activity behaviors are in Table 1, and diarrheic calves were generally less active. Specifically, diarrheic calves took less steps (119.1 ± 18.8 steps/d) than healthy calves (227.4 ± 18.8 steps/d, LSM ± SEM, $F_{1,12} = 15.03, P = 0.001$). Diarrheic calves also had lower activity indices (827.34 ± 93.09 daily index) than healthy calves (1396.32 ± 93.09 daily index, $F_{1,12} = 16.98, P = 0.001$). There was a diarrhea status by day interaction for lying time on d $-3$ ($P = 0.05$), with sick calves spending more time lying (20.80 ± 0.30 h/d) than healthy calves (19.25 ± 0.30 h/d, $P = 0.05$, Figure 1). There was no diarrhea status by day interaction for step counts, activity index, or lying bouts ($P > 0.05$), and these activity behaviors (mean ± SE) relative to diagnosis of a diarrhea bout are in Figure 2. Calf arrival serum Brix % was associated with activity index ($P = 0.01$) and total step counts ($P = 0.01$).

**Association of diarrhea status with relative changes in activity patterns**

The association of diarrhea status with relative changes in activity patterns are in Table 2. There was a diarrhea status by day interaction ($P < 0.001$) for relative changes in step counts: on d $-2$ diarrheic calves had greater relative changes in step counts (634.85 ± 87.58%) than healthy calves (216.51 ± 87.58%, $P = 0.05$, Figure 3 A). There was also a diarrhea status by day interaction ($P < 0.001$) for relative changes in activity index: on d $-2$ diarrheic calves had greater relative changes in activity index (316.83 ± 35.69%) than healthy calves (150.68 ± 35.69%, $P = 0.05$, Figure 3 B). Relative changes in lying time and relative changes in lying bouts were not associated with diarrhea status ($P > 0.05$). No quantitative covariates were associated with relative changes in activity patterns ($P > 0.05$).

**DISCUSSION**

The objective of this case-control study was to investigate the association of diarrhea status with activity behavior and relative changes in activity patterns (lying time, lying bouts, step count, and activity index) for the day relative to diagnosis of a diarrhea bout in individually housed calves. We observed that diarrheic calves were generally more lethargic than healthy calves. This was expressed by lower step counts and...
lower activity indices from d −3 to d 4 relative to diagnosis of a diarrhea bout in the diarrheic calves in this study. There was also a diarrhea status by day interaction on d −3, the diarrheic calves increased their lying time compared with healthy calves. What makes this work unique when compared with others is that we observed that diarrheic, individually housed calves had significant changes in their own activity patterns relative to diarrhea diagnosis when compared with healthy calves. Specifically, diarrheic calves had greater relative changes in activity patterns (e.g., step counts and activity index) compared with healthy calves on d −2 relative to diarrhea diagnosis. Our work suggests that activity data may be useful for alert development using precision technology to detect diarrhea bouts in individually housed calves. Researchers should investigate the potential of using relative changes in activity patterns to be used for alert development to detect a diarrheal bout in individually housed calves.

In this study, there was evidence of lethargic behavior in the diarrheic calves. Specifically, diarrheic calves had reduced step counts and lower activity indices compared with healthy calves. Many studies agree with the study findings, that activity behaviors are associated with diarrhea status in calves (Studds et al., 2018; Swartz et al., 2020; Goharshahi et al., 2021). However, one
study observed no association of diarrhea status with activity behavior in group housed calves (Sutherland et al., 2018). Others who socially housed calves observed that diarrheic calves spent less time lying than healthy calves (Studds et al., 2018). Socially housed diarrheic calves also spent less time lying and had increased lying bouts when compared with healthy calves relative to diagnosis of a diarrhea bout (Swartz et al., 2020), which disagreed with our findings. Differences in our study among others is possibly due to individual housing. Indeed, our study findings agreed with Goharshahi et al. (2021), who also individually housed their calves and observed that diarrheic calves spent more time lying on d −1 and d 0 relative to diarrhea diagnosis. Diarrheic group housed calves may be disturbed by healthy calves, affecting their lying behaviors. Indeed, group housed calves who experienced a pathogen challenge changed their social network and were less likely to initiate social contact compared with saline controls (Burke et al., 2022). This suggests that group housed calves may be motivated to self-isolate while experiencing sickness behavior, but group housing may prevent them from doing so. Future research should investigate if social housing impacts a calf’s motivation to socially isolate during a diarrhea bout.

This study is unique to others in that there was an enrollment criterion for the case calves to have diarrhea bouts for a similar duration and at a similar time after arrival. It was important to limit severe disease in this study as calves with severe diarrhea would likely bias the data to detecting a difference from the mean. Indeed, lengthy diarrhea bouts are a common criterion for antimicrobial therapy in diarrheic calves (Habing et al., 2016). Furthermore, one of the challenges of using precision technology data for disease detection in calves is the daily variability of a calf’s own activity behavior, particularly as calves experience “ontogenetic motor development” during the first weeks of life (Kerr et al., 1987). Therefore, it was important to limit when calves experienced diarrhea in this study as age likely affects the activity patterns of calves. Specifically postnatal calves had increased their activeness when housed individually or when housed with the dam as they aged (Kerr et al., 1987). We suggest that future work that uses case control studies with precision technology to detect disease in calves should consider the severity of disease, and the age at onset as enrollment criterion.

For this study, sick calves had decreased step counts, and decreased their own activity index on d −2 relative to the healthy calves. Table 1. The association of diarrhea status with average activity behavior (LSM ± SEM) for pair matched calves (13 pairs sick to healthy) wearing a pedometer relative to diagnosis of a diarrhea bout (−3 relative to diarrhea diagnosis to d 4) from mixed linear regression models.

<table>
<thead>
<tr>
<th>Baseline Relative change</th>
<th>Disease status</th>
<th>LSM ± SEM</th>
<th>F-value_ df²</th>
<th>Diarrhea status P-value²</th>
<th>Diarrhea status × day P-value³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying time h/d</td>
<td>Healthy</td>
<td>19.77 ± 0.181</td>
<td>10.11_1,12</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>20.56 ± 0.181</td>
<td>2.61_1,12</td>
<td>0.13</td>
<td>0.99</td>
</tr>
<tr>
<td>Lying bouts /d</td>
<td>Healthy</td>
<td>22.69 ± 0.733</td>
<td>2.61_1,12</td>
<td>0.13</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>21.05 ± 0.733</td>
<td>2.61_1,12</td>
<td>0.13</td>
<td>0.99</td>
</tr>
<tr>
<td>Step counts /d</td>
<td>Healthy</td>
<td>227.35 ± 18.832</td>
<td>15.03_1,12</td>
<td>0.001</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>119.06 ± 18.832</td>
<td>15.03_1,12</td>
<td>0.001</td>
<td>0.16</td>
</tr>
<tr>
<td>Activity index⁵</td>
<td>Healthy</td>
<td>1296.32 ± 93.091</td>
<td>16.98_1,12</td>
<td>0.001</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>827.34 ± 93.091</td>
<td>16.98_1,12</td>
<td>0.001</td>
<td>0.25</td>
</tr>
</tbody>
</table>

1 Pair matching criteria based on age at onset, arrival date to facility, and essential oil from a concurrent trial (control, oregano oil at 1 or 2 g/d in milk replacer).
2 Degrees of freedom and F-values fixed effects from mixed linear regression models.
3 Statistical significance P ≤ 0.05.
4 Significant diarrhea status × day interactions (P ≤ 0.05) adjusted with Tukey’s method.
5 Activity index metric from commercial software (IceRobotics, Scotland) generated from average daily acceleration rate of accelerometer unit and step count.
to the diagnosis of the diarrhea bout, which is likely a sickness behavior repertoire. The sickness behavior repertoire is a common phenomenon in mammals, cytokine feedback from the immune system and activation of the HPA immune axis are metabolically costly (Silverman et al., 2005). We suggest that calves had relative changes in their behavior relative to diarrhea diagnosis because calves were primed to conserve energy by becoming overall less active before the expression of clinical signs of the disease which has also been observed in LPS challenged calves (Borderas et al., 2008).

It is also possible that while the diarrheic calves were less active overall, these calves had lying bout behavior that was not different from their healthy peers because of an intrinsic motivation to consume liquids to stay hydrated. However, this is speculation as water intake was not measured in this study. Alternatively, lying behavior patterns are also highly variable among healthy individual cattle (Ito et al., 2009), so it is possible that lying bout behavior in young calves is not yet consistent. However, there is evidence that overall activeness decreases before calves have respiratory disease (Cantor et al., 2022a). We suggest that the next exploratory work, as a day that occurs once calves have clinical disease is not a useful baseline measure to detect relative changes in behavior before the diagnosis of a diarrhea bout. However, this was a proof of concept and suggests that activity index is an impactful metric to use relative to diagnosis of a diarrhea bout in dairy calves. Researchers who used machine learning techniques have also observed that changes in activity behaviors improved the algorithm accuracy for the diagnosis of respiratory disease in calves (Bowen et al., 2021; Cantor et al., 2022a). We suggest that the next

### Table 2. The association of diarrhea status with baseline relative changes in activity patterns (LSM ± SEM) for pair matched calves (13 pairs sick to healthy) wearing a pedometer relative to diagnosis of a diarrhea bout (−3 relative to diarrhea diagnosis to d 2) from mixed linear regression models

<table>
<thead>
<tr>
<th>Baseline Relative change</th>
<th>Disease status</th>
<th>LSM ± SEM</th>
<th>F-value$^3$</th>
<th>Diarrhea status P-value$^4$</th>
<th>Diarrhea status × day P-value$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying time h/d</td>
<td>Healthy</td>
<td>98.51 ± 1.462</td>
<td>0.031,12</td>
<td>0.87</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>98.06 ± 1.462</td>
<td>0.241,12</td>
<td>0.63</td>
<td>0.50</td>
</tr>
<tr>
<td>Lying bouts /d</td>
<td>Healthy</td>
<td>105.76 ± 5.691</td>
<td>3.951,12</td>
<td>0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>109.15 ± 5.691</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step counts /d</td>
<td>Healthy</td>
<td>167.06 ± 64.862</td>
<td>4.871,12</td>
<td>0.05</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>349.41 ± 64.862</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity index$^5$</td>
<td>Healthy</td>
<td>131.50 ± 25.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>209.52 ± 25.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Relative changes in behavior were calculated by measuring the difference from the behavioral baseline set at d 0. D 0 was selected as a baseline to observe when calf activity patterns were different from behavior on the d of clinical diarrhea diagnosis.

2 Pair matching criteria based on age at onset, arrival date to facility, and essential oil from a concurrent trial (control, oregano oil at 1 or 2 g/d in milk replacer).

3 Degrees of freedom and F-values fixed effects from mixed linear regression models.

4 **Statistical significance** $P \leq 0.05$.

5 Activity index metric from commercial software (IceRobotics, Scotland) generated from average daily acceleration rate of accelerometer unit and step count.
step for this research is to develop an alert and investigate a baseline to use for activity patterns to positively indicate calves at-risk for a diarrhea bout in individual housing.

There was a lack of association of diarrhea status with relative changes in lying time in this study. However, there was a diarrhea status by day interaction for lying time, suggesting that the best baseline to use for relative change in lying patterns is before d −3 relative to diarrhea diagnosis. Indeed, one study which used sick calves as their own controls found lying behavior was different before d −3 relative to disease diagnosis (Lowe et al., 2019). Deviations from baseline activity patterns also occurred several d before diarrhea diagnosis in calves when machine learning techniques were used with feeding behavior data (Ghaffari et al., 2022). Thus, this was a limitation in this study because calves were sourced by auction and calves could not be followed from birth. Diarrhea often occurs within the first 3 wk of life, and peaks at 14 d (Urie et al., 2018). Surplus bull calves are often a few days of age before being sold on a dairy (Shivley et al., 2019). Thus, behaviors before d −3 relative to diarrhea diagnosis could not be explored because of the acclimation period that was provided to the calves after their arrival to the facility. Future research should explore the potential of deviations from relative changes in activity patterns using individual behavioral baselines relative to diarrhea bout diagnosis.

CONCLUSION

Diarrheic calves in this study were lethargic relative to diagnosis of a diarrhea bout (d −3 to d 4). Specifically, diarrheic calves exhibited fewer steps, had a reduced activity index, and there was an interaction

Figure 3. A) The activity behavior (mean ± SEM) of 13 case-control pairs relative to diagnosis of a diarrhea bout (d −3 to d 4) for lying bouts. B) The activity behavior (mean ± SEM) of 13 case-control pairs relative to diagnosis of a diarrhea bout (d −3 to d 4) for step counts (Figure 2B). C) The activity behavior (mean ± SEM) of 13 case-control pairs relative to diagnosis of a diarrhea bout for an activity index (Figure 2C).
with disease status and day for lying time, where sick calves increased their lying times on d-3 compared with healthy calves. Furthermore, diarrhea status by day interactions were observed on d -2 and there were relative changes in activity index and greater relative changes in step counts compared with healthy calves. These results suggest that diarrheic calves decreased activeness relative to diagnosis of a diarrhea bout, and that there is value in considering an individual calf’s sickness behavior before disease diagnosis. Future research should evaluate if an algorithm that uses deviations from individual calf activity patterns can positively indicate calves at-risk for diarrheic bouts in individual housing.

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