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Lameness changes the behavior of dairy cows: daily rank order of lying and feeding behavior decreases with increasing number of lameness indicators present in cow locomotion

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ABSTRACT

Lameness is a major animal welfare problem in modern dairy farms. Although association between cow behavior and lameness is proven, functioning and reliable on-farm applications for automatic lameness detection is still lacking because large within and between farm variation in cow behavior exists. There were two aims in this study: 1) to investigate if a simple ranking of cows in an order based on their time spent on a specific behavior or number of behavior bouts could be used for normalizing behavior data, and 2) to study how daily lying and feeding behavior, and their rank order variables are affected by lameness. We followed in total 84 cows divided in two half-year trials (N = 45 and N = 49, ten cows enrolled in both trials). Cows were locomotion scored fortnightly and their lying,roughage feeding behavior, and step count were measured continuously with automatic sensors. In addition to using absolute values of daily behavior, animals were ranked in ascending order within experimental cows in the group for each day based on their absolute value (hours/number of bouts/number of steps) of the behavior in question. Daily behavioral and cow level factors were merged with temporally closest locomotion score. Two sets of linear mixed models were fitted: 1) to investigate the effect of trial on absolute and rank order values of behavior, and 2) to investigate effect of locomotion score, parity, breed and lactation stage on absolute and rank order values of behavior. There was a significant difference between the trials in lying behavior of the cows, but not in feeding behavior; cows spent more time lying down in the first trial (P= 0.0027). This difference was not evident in rank order values. Increasing the number of lameness indicators was associated with increasing lying time, increase in number of lying bouts, decreasing roughage feeding time and decrease in feeding bouts (P<0.0001). Similar differences were observed in rank order values of behaviors (P<0.0001). No consistent change in step count was observed with respect to increasing number of lameness indicators. Based on these data, ranking the cows within the herd based on their behavior did normalize the data between two trials, and rank order values behaved similarly to absolute daily behavior with respect to a cow's lameness status. Thus, using rank order variables could address some issues in development of automated lameness detection systems arising from within and between herd variability in dairy cow behavior.

Introduction

Lameness is a common problem on modern dairy farms affecting adversely animal welfare and farm economics in several direct and indirect ways (Bruijnis et al. 2010, Huxley 2013, Alvergas et al. 2019). Considering the negative effect of lameness on animal welfare, its prevalence is relatively high worldwide (e.g., Brazil, 41.1%

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(Bran et al. 2019), United Kingdom, 30.1% (Randall et al. 2019), Finland, 23.0% (Sarjokari et al. 2013) and North America, 15.0% (Westin et al. 2016a)). Hoof disorders that cause lameness are painful conditions causing hyperalgesia and chronic pain (Whay et al. 1998; Herzberg et al., 2019) and consequently reduction in animal welfare in several ways (Whay and Shearer, 2017). Lameness leads to production losses, impaired fertility and to higher odds for involuntary culling (Bruijnis et al., 2010; Huxley, 2013). Additionally, treatment of lame animals causes direct expenditures to the farmer.

According to the economic model created by Bruijnis et al. (2010) even 32% of the costs caused by lameness occur because of the subclinical cases that remain undetected and untreated. Indeed, prevalence of lameness is often underestimated on farms, even by the farmers that recognize lameness as a problem (Cutler et al., 2017). Additionally, farmers perceive lameness as more difficult to control than other health issues (Leach et al., 2010); locomotion scoring and treating lame animals is time consuming and often other tasks are prioritized (Horseman et al. 2014). Early treatment of claw disorders would lead to better recovery and it prevents lameness becoming a chronic state (Thomas et al., 2015). Thus, aid for detection of lameness is desperately needed. This applies especially to early detection of mild lameness as severe lameness cases are easily identified by visual observations (Alsaaod et al., 2019).

There has been plenty of research showing association between lameness and behavior of the cow (Van Nuffel et al., 2015; Alsaaod et al., 2019), and these changes in behavior may emerge already before visible signs of lameness (Norring et al., 2014) or appearance of actual hoof lesions (Omontese et al., 2020). In general, lame cows spend more time lying down (e.g., Ito et al., 2010, Solano et al., 2016; King et al., 2017) and less time feeding (e.g., Norring et al., 2014; Thorup et al., 2016; Barker et al., 2018) compared to their healthy conspecifics. Indeed, day-to-day variation in behavior has been suggested to be a useful tool for lameness detection (de Mol et al., 2013). However, functioning and reliable on-farm applications for automatic lameness detection still lack (Alsaaod et al., 2019; O'Leary et al., 2020). One possible reason for this could be a high variation in behavior and locomotion characteristics between individual cows and between herds (Alsaaod et al., 2012; Westin et al., 2016b; Piette et al., 2020) as, for example, lying behavior is affected by both cow and barn environment factors such as parity, lactation stage and stall surface (Ito et al., 2014; Solano et al., 2016; O'Leary et al., 2020). Thus, it would be advantageous to develop means to normalize behavior data within and between dairy cattle herds.

We had two aims in the present study. First, we wanted to investigate if a simple ranking of cows in order based on their time spent on a specific behavior or number of behavior bouts could be used for normalizing behavior data between experiments. If ranking is a suitable method for data normalization, then we need to study if a rank order behaves similarly to absolute values of daily behavior in association with lameness. Thus, the second aim was to study how daily lying and feeding behavior, and their rank order variables are affected by a locomotion score based on a sum of frequently used lameness indicators present in a cow locomotion.

Materials and Methods

Study was carried out in two trials: the first trial (1) between December 2016 and May 2017, and the second trial (2) between January and June 2019.

Housing and management

We conducted the study in the research barn of the Natural Resources Institute Finland (Luke) (Maaninka, 63° 10 'N, 27° 18'E), which is a free-stall curtain-wall barn with automatically scraped (Lely Discovery 90SW, Lely, The Netherlands) slatted passageways. The experimental cows were housed in two compartments of 24 cows (10 × 18 m), both compartments having fifteen 120 cm and nine 130 cm wide stalls with a total stall length of 250 cm and a body resting length of 180 cm from the brisket board. In the trial 1 stall mattresses were 7 cm thick with a rubber covering (Promat Inc, Canada) bedded with peat or recycled manure solids (Frondelius et al., 2020). In the trial 2 stall mattresses were 4 cm thick with a rubber covering (Soft Bed 4GS, Huber Technik GmbH and Co. KG, Germany) bedded with peat. Both compartments had their own concentrate feeder (Trial 1: Nedap, The Netherlands; Trial 2: DeLaval, Sweden) and twelve Roughage Intake Control (RIC) -feeders (Insentec BV, The Netherlands) with barrier structures described in Ruuska et al. (2014) to prevent stealing behavior. The cows had free access to total mixed ratio and water. The cows were milked twice daily in a 2 × 8 herringbone parlor.

Data collection

In trial 1, we followed a total of 45 lactating dairy cows on average for 166.6 days (min 7, max 141 days), and in trial 2, a total of 49 lactating dairy cows on average for 99.2 days (min 30, max 146 days). Ten animals were enrolled in both trials but considering a long period between the trials and the nature of our experimental setup, we decided to handle these individuals in the data as separate animals between the trials. The experimental cows were either primiparous (N = 22) or multiparous (N = 29 second parity, N = 43 parity 3-7) and were either Holstein-Friesian (N = 69) or Nordic Red (N = 25) breeds. During the experiment, the cows were between 1 and 299 days in milk (DIM) and their average milk yield was 32.7±8.0 kg/day (mean ± standard deviation (SD)).

We collected daily data on cow feeding and activity related behavior using automatic monitoring. Feeding behavior was collected using the RIC-system; the system measures individual cow's roughage intake, roughage feeding time and the number of visits to the feeder. For activity related measurements we used IceQube-sensors (IceRobotics Ltd, United Kingdom). The sensor was attached with a Velcro-strap to either of the hind legs of a cow just above the metatarsal joint. Based on the 3D-acceleration data the sensors measure individual cow's daily lying time, standing time, number of lying bouts, lying bout length and number of steps.

Additionally to the automated behavior measurements, we scored cow locomotion fortnightly (one of two trained observers authors L.F. or H.L.) using nine frequently used lameness indicators (Van Nuffel, 2009): 1) non-flexible joint movement, 2) tender placement of the hooves, 3) arching of the back, 4) reduced speed, 5) irregularity in the timing of the hoof placement, 6) irregularity in the location of the hoof placement, 7) reduced step overlap/tracking up, 8) increased abduction and 9) head movement. Observer collected the cows into the waiting area of the milking parlor and guided the cows back to their home compartment one at a time for the locomotion scoring. Locomotion was observed from the back and the side of the cow. After scoring, observed cow was moved to the separation pen to not to interfere the next cow's locomotion scoring. Indicators were marked as non-present (0) or present (1) in observed cow locomotion and the total number of the present lameness indicators (min 0, max 9) was used in the analysis. Locomotion was scored in total of 20 times during the two trials: nine times in trial 1 and eleven times in trial 2.

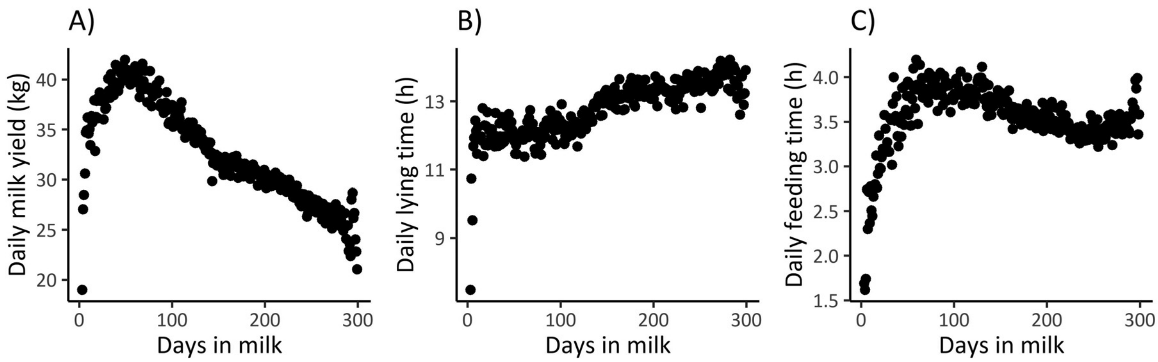

Figure 1. Average daily a) milk yield, b) lying time and c) feeding time across cows (N = 84) by lactation stage (days in milk).

Data processing and statistics

We had data in total from 84 lactating dairy cows and 10 111 observation days. Locomotion scoring affects the daily routine of the cows, and thus, scoring days were eliminated from the data. Observation days from single cows were also eliminated when it was known that the cow in question was in a sick pen. This left us with a dataset of 9,396 observation days.

Data contained some extreme values for measured behavioral variables, which were considered as artefacts in sensor data. For daily lying time there were only few outlier values that were not biologically plausible as total daily lying time and feeding time together accounted for more than 23 hours. These observations were eliminated leaving us with a dataset of 9,392 observation days for lying and activity behavior variables. In RIC data, there were both extremely low values in feeding time and high values in number of visits to the feeder. With this high number of observation days, it was inconvenient to check all these possible outlier values manually, and we decided to eliminate the lowest 0.5% of the values of the daily feeding time (<0.8 h) and the highest 0.5% of the values of the daily visit count (>120 visits/day). This left us with a dataset of 8,943 observation days for feeding behavior variables.

We used lactation stage as a categorical variable with three classes: 0-30, 31-150 and >150 days in milk. Uneven division between categories was based on the previous research showing diverging behavior during the first month after parturition compared to rest of the lactation (Maselyne et al. 2017). Based on a visual inspection, this was evident also in our data (Figure 1). Parity was also categorized into three classes as the number of cows with parity higher than 4 was markedly lower than number of cows with parity 1-2. Used classes were primiparous cows, second lactation cows and cows with parity ≥3. Same accounted for the number of lameness indicators: if there were 6-9 lameness indicators present, these observations were pooled in one category (≥6 lameness indicators present). Lower numbers (0-5) were used as independent categories.

Behavioral variables of interest were daily values for lying time (h), number of lying bouts, maximum length of a lying bout (h), roughage feeding time (h), number of visits to the roughage feeder and number of steps taken. Daily summaries of cow behavior have been reported most successful in terms of lameness detection performance (O'Leary et al. 2020). For all these behaviors daily rank order variables also were created: we ranked animals in ascending order within experimental cows in the group for each day based on their absolute value (hours/number of bouts/number of steps) of the behavior in question, and then divided this rank order with the number of cows in the data available on that day. Finally, daily behavioral variables and cow level factors were assigned with the number of present lameness indicators from the temporally closest fortnightly locomotion scoring.

Two sets of linear mixed models were fitted to test the associations between daily behavioral variables and predictor variables. Models were fitted for following continuous outcome variables leading to twelve individual models in both sets: daily lying time, number of lying bouts, maximum length of a lying bout, roughage feeding time, number of visits to the feeder, number of steps, and the rank order variables for all the above-mentioned absolute behavioral variables. The first set was univariate models testing the effect of a class variable trial on the behavioral variables. In the second set, multivariate models were created to test the effect of number of lameness indicators and cow factors on cow behavior. Fixed effects in these models were class variables as follows: sum of lameness indicators, DIM, parity, and breed. Milk yield was not included in the multivariate model as it correlated with DIM classes ($\rho = -0.44$, $P < 0.0001$). Degrees of freedom for fixed effects were estimated using Kenward-Roger 2 option. Cow was considered as a random effect. This accounted also for trial being a random effect as experimental animals were different or considered as separate individuals in two trials. We ascertained the appropriateness of the models from their residuals; approximately normal distribution of the residuals indicated that the data fulfilled the assumptions of the models. If the model residuals did not distribute normally, then the outcome variable was transformed by taking a natural logarithm. Outcome variables requiring transformation were number of lying bouts and number of steps. Residuals for maximum length of a lying bout also did not have a normal distribution and achieving normality by using a logarithmic transformation was not possible. Thus, we decided to use only the rank order values as an outcome for this behavioral variable, leaving us with eleven models in both sets. The least squares (LS) means with Tukey's adjustment were calculated for all class variables and transformed back to original scale when necessary. Ten cows enrolled in both trials were handled as separate individuals between the trials leading to N = 94 in statistical models.

Additionally, to estimate the possible variation in the data originating from individual cows we 1) investigated the solutions for random effects for the models of lying and feeding time (in second set) and extracted the minimum and maximum values from estimates of individual cows, and 2) modeled the variation caused by cow in lying and feeding time with variance component analysis. Then relative variance was calculated as a ratio of variance between cows and total variance of the behavior in question.

We set the statistical significance at $P<0.05$. All the analyzes were performed using SAS for Windows version 9.4 with the SAS Enterprise Guide version 7.1 (SAS Institute Inc., Cary, NC).

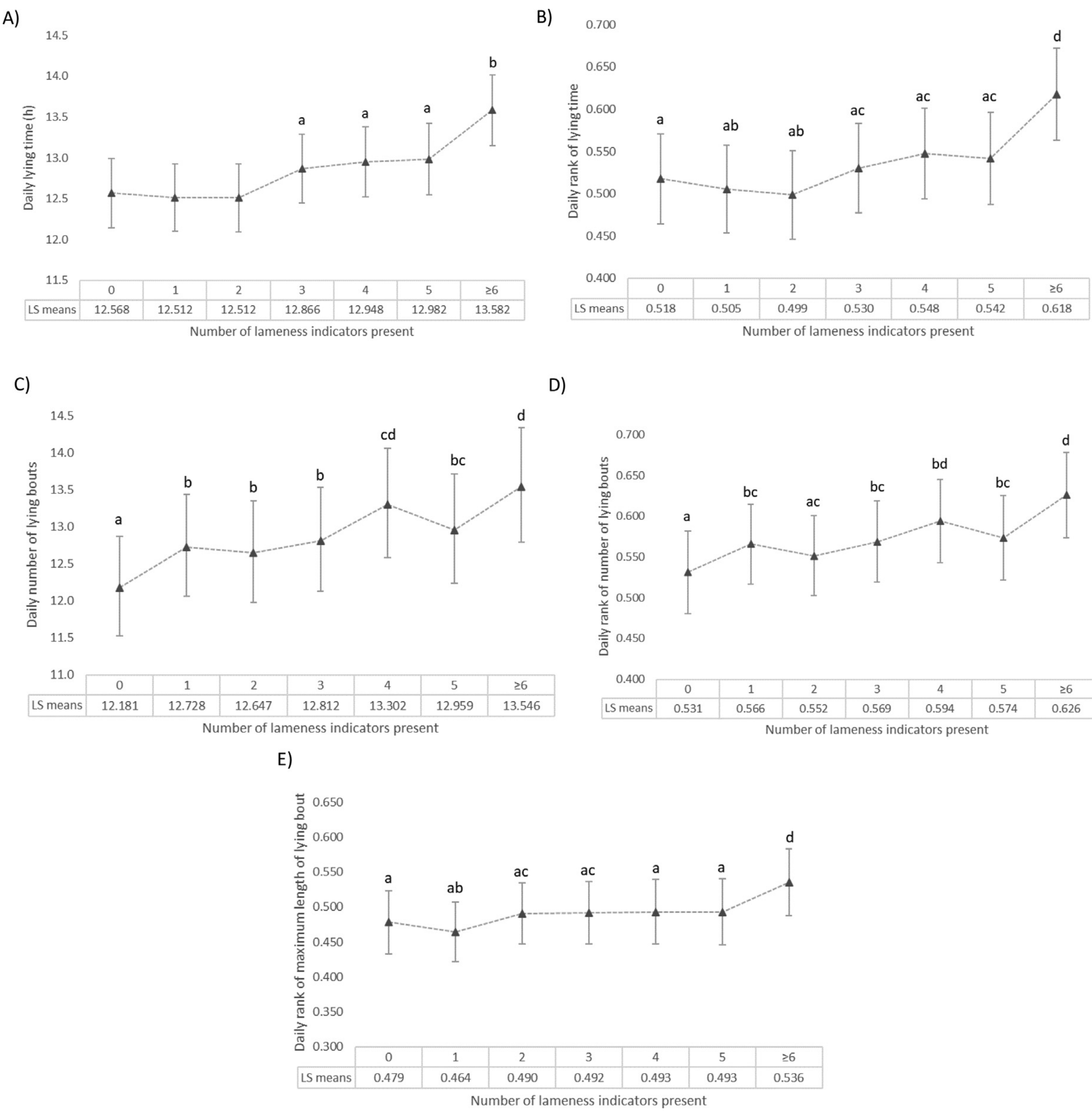

Figure 2. Least squares means and 95% confidence limits for daily a) lying time (h), b) rank of the lying time, c) number of lying bouts, d) rank of number of lying bouts, and e) rank of the maximum length of a lying bout by the number of lameness indicators present in cow locomotion for 9 392 observation days and 84 cows. Ten cows enrolled in both trials were handled as separate individuals leading to total N of 94 cows in statistical models. Values with different superscripts (a-d) are statistically different (Tukey's adjusted P <0.05).

Table 1
Mean ± standard deviation of daily behavior of dairy cows (N = 84) in two trials in the experiment.

Behavior	Trial 1	Trial 2
Lying time (h)	13.4±2.5	12.3±2.1
Number of lying bouts	11.5±3.5	13.6±4.0
Maximum lenght of the lying bout	2.5±0.6	2.1±0.7
Roughage feeding time (h)	3.5±0.8	3.7±1.0
Number of visits to the feeder	45.9±18.8	49.0±19.7
Number of steps	537.1±283.7	651.0±380.6

Results

Descriptive statistics for cow behavior by two trials are presented in Table 1. In 20 locomotion scorings during the two trials in total of 706 locomotion scores were given. The mean number of lameness indicators present in cow locomotion was 2.3±1.7. More detailed proportion of different locomotion scores is presented in Table 2.

As for variation originating from individual cows, in lying time 51.7% of the variation was explained by the differences between cows based on the variance component analysis and the minimum and maximum deviation from the intercept (12.4 h) in the multivariate model was -5.2 and +3.0 hours, respectively. In feeding time, the differences between cows explained 39.1% of the variation and the minimum and maximum deviation from the intercept (2.8 h) in the multivariate model was -1.2 and +1.6 hours, respectively.

The factors in multivariate models associated with lying behavior are summarized in Table 4. Increasing number of lameness indicators was associated with both higher daily lying time (Figure 2A) and higher lying time rank (Figure 2B) (P <0.0001). In pairwise comparisons of lying time, cows with 0-2 lameness

Table 2
Number and proportion (%) of scorings from all assessed locomotion scores (N = 706) according to the number of lameness indicators present in the locomotion of dairy cows. Locomotion was scored in total of 20 times during the two trials.

Number of present lameness indicators	Number of scores	Proportion (%) of scores
0	89	12.61
1	193	27.34
2	164	23.23
3	98	13.88
4	67	9.49
5	45	6.37
≥6	50	7.08

The associations between the behavioral variables and the trial are summarized in Table 3. Daily lying time was higher ($P = 0.0027$), and number of lying bouts ($P = 0.0006$) and number of steps ($P = 0.0146$) were lower in the first trial. None of the rank order variables nor feeding related variables were significantly different between the trials.

indicators had lower lying time than cows with higher number of lameness indicators (adjusted $P < 0.0001$ for lying time, and $P < 0.05$ for lying time rank), 3-5 indicators present were on a same level with each other, and cows with ≥6 indicators present had significantly higher lying time than all the other classes both in absolute and rank order values (adjusted $P < 0.0001$). Similar pattern was evident for the lying time rank, although statistically significant differences between pairs (Figure 2B) were not as coherent as in absolute lying time. Increasing DIM was associated with increasing lying time ($P < 0.0001$) but decreasing lying time rank ($P < 0.0001$). For the daily number of lying bouts similar increase with increasing number of lameness indicators was present in both absolute (Figure 2C) and rank order values (Figure 2D) ($P < 0.0001$). DIM was significantly associated only with number of lying bouts ($P = 0.0004$) but not with its rank order values; number of lying bouts decreased with increasing DIM. Increasing parity was also associated with decreasing number and rank of lying bouts ($P = 0.0026$ and $P = 0.0008$, respectively). The rank order value for maximum length of a lying bout was significantly affected by the number of lameness indicators ($P < 0.0001$), DIM ($P = 0.0207$) and breed ($P = 0.0272$). In pairwise comparison, cows with ≥6 lameness indicators present had significantly higher maximum length of a lying bout compared to lower number of lameness indicators (adjusted $P < 0.05$) (Figure 2E). Nordic red cows and cows in mid-lactation had shorter maximum lying bout length.

The factors in multivariate models associated with feeding behavior are summarized in Table 5. Increasing number of lameness indicators was associated with lower daily roughage feeding time (Figure 3A) and lower feeding time rank (Figure 3B) ($P < 0.0001$). In rank order values, it could be seen that cows with a high number (≥4) of lameness indicators present had a significantly lower rank in feeding time compared to cows with lower numbers (adjusted $P < 0.05$). Feeding time was lowest during the first 30 days after parturition both in absolute and rank order values ($P < 0.0001$). Parity was not associated with absolute values of feeding time, but it was significantly associated with feeding time rank ($P = 0.037$): cows in their second lactation had the highest feeding time rank. For roughage feeding bouts similar decrease with increasing number of lameness indicators was present in both absolute (Figure 3C) and rank order values (Figure 3D) ($P < 0.0001$). In pairwise comparison for absolute feeding bouts, only cows with 4 and ≥6 lameness indicators present differed statistically from cows with lower numbers (adjusted $P < 0.005$), but in rank order values significant decrease in feeding bouts followed more a pairing of lameness indicator numbers, 0-1, 2-3, 4-5, and ≥6.

Factors in multivariate models associated with daily number of steps are summarized in Table 6. Both the absolute number of steps taken (Figure 4A), and its rank value (Figure 4B) were affected by the number of lameness indicators present ($P < 0.0001$ and $P = 0.0341$, respectively). However, for rank order values no distinction between locomotion scores could be done in pairwise comparisons with adjusted P-values. In absolute number of steps taken cows with 1, 2, 4, and ≥6 lameness indicators present differed from cows with 0 indicators (adjusted $P < 0.05$) and 5 indicators present differed from 4 and ≥6 indicators present (adjusted $P < 0.05$). Cows with 3 indicators present did not differ significantly from any other classes in pairwise comparison. Cows with parity ≥3 took less steps both in absolute and rank order values ($P < 0.0001$). DIM was not associated with rank order values of step count, but it was significantly associated with the absolute number of steps taken ($P = 0.0026$): step count increased with progressive DIM. Breed was associated with neither absolute nor rank value of steps taken.

Discussion

In this study, we showed that a simple method of ranking the cows within the herd based on their daily behavior can be used for normalizing behavioral data between trials. Further these rank order values had a similar association to lameness as the absolute values of behavior. This association was systematic between increasing number of lameness indicators present in cow locomotion and changes in feeding and lying behavior.

Table 3
Univariate linear mixed models describing the associations between the trial and daily behavioral variables or their rank order variables with linear regression coefficient (β), standard error (SE) and P-value with N = 84. Ten cows enrolled in both trials were handled as separate individuals leading to total N of 94 cows in statistical model.

			Behavioral variable			Rank order variable		
Outcome variable	Fixed effect		β	SE	P-value	β	SE	P-value
Lying time (h)	Trial	1	Ref.			Ref.		
		2	-1.071	0.347	0.0027	0.008	0.045	0.8494
Number of lying bouts	Trial	1	Ref.			Ref.		
		2	0.1587[a]	0.045	0.0006	-0.002	0.044	0.9549
Maximum length of the lying bout	Trial	1	Ref.			Ref.		
		2				0.009	0.037	0.8160
Roughage feeding time (h)	Trial	1	Ref.			Ref.		
		2	0.140	0.122	0.2562	-0.037	0.044	0.4002
Number of visits to the feeder	Trial	1	Ref.			Ref.		
		2	1.547	3.022	0.6099	-0.020	0.047	0.6649
Number of steps	Trial	1	Ref.			Ref.		
		2	0.1834[a]	0.074	0.0146	0.005	0.048	0.9153

[a] Model fitted with outcome variable transformed by taking a natural logarithm.

Table 4
Linear mixed models describing the fixed effects associated with daily lying behavior variables and their rank order variables with linear regression coefficient (β), standard error (SE), P-value, least squares (LS) means and 95% confidence limits of the LS means (CL lower and upper). LS means and their standard errors are presented only for fixed effects with statistically significant (Tukey's adjusted P <0.05) association with the outcome variable. Data comprised 9,392 observation days and 84 cows. Ten cows enrolled in both trials were handled as separate individuals leading to total N of 94 cows in statistical models.

		Lying time (h)						Lying time -rank						Number of lying bouts[a]						Number of lying bouts -rank						Maximum length of a lying bout -rank					
Fixed effect		β	SE	*P*-value	LS means	CL lower	CL upper	β	SE	*P*-value	LS means	CL lower	CL upper	β	SE	*P*-value	LS means[b]	CL lower[b]	CL upper[b]	β	SE	*P*-value	LS means	CL lower	CL upper	β	SE	*P*-value	LS means	CL lower	CL upper
Number of present lameness indicators	0	Ref.		<0.0001	12.568	12.145	12.991	Ref.		<0.0001	0.518	0.464	0.571	Ref.		<0.0001	12.181	11.526	12.875	Ref.		<0.0001	0.531	0.481	0.582	Ref.		<0.0001	0.479	0.433	0.524
	1	-0.056	0.066		12.512	12.099	12.924	-0.012	0.008		0.505	0.454	0.557	0.044	0.009		12.728	12.060	13.434	0.035	0.008		0.566	0.517	0.615	-0.014	0.009		0.464	0.421	0.508
	2	-0.056	0.075		12.512	12.096	12.927	-0.019	0.009		0.499	0.447	0.551	0.037	0.010		12.647	11.978	13.352	0.021	0.009		0.552	0.502	0.601	0.012	0.011		0.490	0.447	0.534
	3	0.298	0.082		12.866	12.447	13.286	0.013	0.010		0.530	0.478	0.583	0.051	0.011		12.812	12.129	13.535	0.038	0.010		0.569	0.519	0.619	0.014	0.012		0.492	0.448	0.537
	4	0.380	0.094		12.948	12.520	13.376	0.030	0.011		0.548	0.494	0.602	0.088	0.012		13.302	12.579	14.067	0.063	0.012		0.594	0.543	0.645	0.015	0.014		0.493	0.447	0.539
	5	0.414	0.104		12.982	12.546	13.419	0.024	0.013		0.542	0.487	0.596	0.062	0.014		12.959	12.240	13.721	0.042	0.013		0.574	0.521	0.626	0.015	0.015		0.493	0.446	0.541
	≥6	1.014	0.106		13.582	13.146	14.018	0.100	0.013		0.618	0.563	0.673	0.106	0.014		13.546	12.796	14.342	0.095	0.013		0.626	0.574	0.678	0.057	0.015		0.536	0.488	0.583
Days in milk	≤30	Ref.		<0.0001	12.635	12.196	13.075	Ref.		<0.0001	0.580	0.525	0.635	Ref.		0.0004	13.214	12.476	13.996	Ref.		0.2899				Ref.		0.0207	0.508	0.459	0.556
	31-150	0.149	0.083		12.784	12.372	13.197	-0.063	0.010		0.516	0.464	0.568	-0.029	0.011		12.837	12.163	13.547	-0.002	0.010					-0.028	0.012		0.479	0.436	0.522
	>150	0.503	0.097		13.139	12.729	13.548	-0.064	0.012		0.515	0.464	0.567	-0.049	0.013		12.585	11.929	13.277	-0.012	0.012					-0.017	0.014		0.490	0.448	0.533
Parity	1	Ref.		0.7343				Ref.		0.3532				Ref.		0.0026	14.385	13.035	15.876	Ref.		0.0008	0.678	0.589	0.768	Ref.		0.0778			
	2	-0.312	0.473					-0.046	0.059					-0.127	0.062		12.672	11.636	13.800	-0.111	0.056		0.567	0.490	0.645	0.00	0.05				
	≥3	-0.029	0.443					0.028	0.056					-0.206	0.058		11.709	10.931	12.545	-0.205	0.052		0.473	0.411	0.536	0.08	0.05				
Breed	Ayrshire	Ref.		0.8917				Ref.		0.4599				Ref.		0.0924				Ref.		0.0546				Ref.		0.0272	0.538	0.496	0.581
	Holstein	0.054	0.394					0.037	0.050					-0.088	0.052					-0.091	0.047					0.09	0.04		0.447	0.375	0.518

[a] Model fitted with outcome variable transformed by taking natural logarithm.
[b] Back transformed least squares mean and its 95% confidence limits.

Table 5
Linear mixed models describing the fixed effects associated with daily roughage feeding behavior variables and their rank order variables with linear regression coefficient (β), standard error (SE), P-value, least squares (LS) means and 95% confidence limits of the LS means (CL lower and upper). LS means and their standard errors are presented only for fixed effects with statistically significant (Tukey's adjusted P <0.05) association with the outcome variable. Data comprised 8,943 observation days and 84 cows. Ten cows enrolled in both trials were handled as separate individuals leading to total N of 94 cows in statistical models.

		Roughage feeding time (h)						Roughage feeding time -rank						Number of visits to the feeder						Number of visits to the feeder -rank					
Fixed effect		β	SE	*P*-value	LS means	CL lower	CL upper	β	SE	*P*-value	LS means	CL lower	CL upper	B	SE	*P*-value	LS means	CL lower	CL upper	β	SE	*P*-value	LS means	CL lower	CL upper
Number of present lameness indicators	0	Ref.		<0.0001	3.482	3.340	3.625	Ref.		<0.0001	0.502	0.451	0.553	Ref.		<0.0001	46.654	43.299	50.009	Ref.		<0.0001	0.534	0.485	0.584
	1	-0.036	0.029		3.446	3.310	3.582	0.003	0.008		0.505	0.455	0.554	0.517	0.548		47.171	43.908	50.433	0.003	0.008		0.537	0.489	0.586
	2	-0.112	0.033		3.371	3.233	3.508	-0.002	0.010		0.500	0.450	0.550	-0.954	0.618		45.700	42.414	48.986	-0.020	0.009		0.514	0.465	0.563
	3	-0.162	0.036		3.320	3.180	3.461	-0.009	0.011		0.494	0.443	0.544	-0.871	0.685		45.783	42.458	49.109	-0.032	0.010		0.502	0.453	0.552
	4	-0.224	0.042		3.258	3.113	3.403	-0.045	0.012		0.457	0.405	0.508	-3.245	0.782		43.409	40.016	46.803	-0.067	0.011		0.467	0.417	0.518
	5	-0.307	0.046		3.175	3.025	3.326	-0.038	0.013		0.464	0.412	0.517	-1.446	0.864		45.208	41.736	48.679	-0.058	0.012		0.476	0.425	0.528
	≥6	-0.514	0.047		2.968	2.818	3.118	-0.126	0.014		0.376	0.324	0.429	-6.641	0.885		40.013	36.543	43.484	-0.139	0.013		0.395	0.344	0.447
Days in milk	≤30	Ref.		<0.0001	2.731	2.580	2.883	Ref.		<0.0001	0.407	0.354	0.460	Ref.		<0.0001	36.005	32.512	39.498	Ref.		<0.0001	0.424	0.372	0.475
	31-150	0.884	0.036		3.615	3.479	3.751	0.096	0.010		0.503	0.453	0.552	13.042	0.680		49.047	45.787	52.307	0.098	0.010		0.522	0.473	0.570
	>150	0.789	0.043		3.520	3.386	3.654	0.097	0.012		0.504	0.455	0.553	13.488	0.806		49.493	46.259	52.727	0.100	0.011		0.523	0.475	0.572
Parity	1	Ref.		0.1009				Ref.		0.0370	0.475	0.385	0.566	Ref.		0.0006	51.288	45.328	57.247	Ref.		0.0003	0.586	0.497	0.675
	2	0.252	0.154					0.057	0.057		0.532	0.454	0.610	-5.7751	3.7339		45.513	40.360	50.666	-0.077	0.056		0.508	0.431	0.585
	≥3	-0.018	0.145					-0.069	0.053		0.407	0.343	0.470	-13.5432	3.494		37.745	33.583	41.906	-0.211	0.052		0.375	0.313	0.437
Breed	Ayrshire	Ref.		0.3993				Ref.		0.6314				Ref.		0.5446				Ref.					
	Holstein	0.109	0.129					0.023	0.047					-1.892	3.111					-0.011	0.046	0.8096			

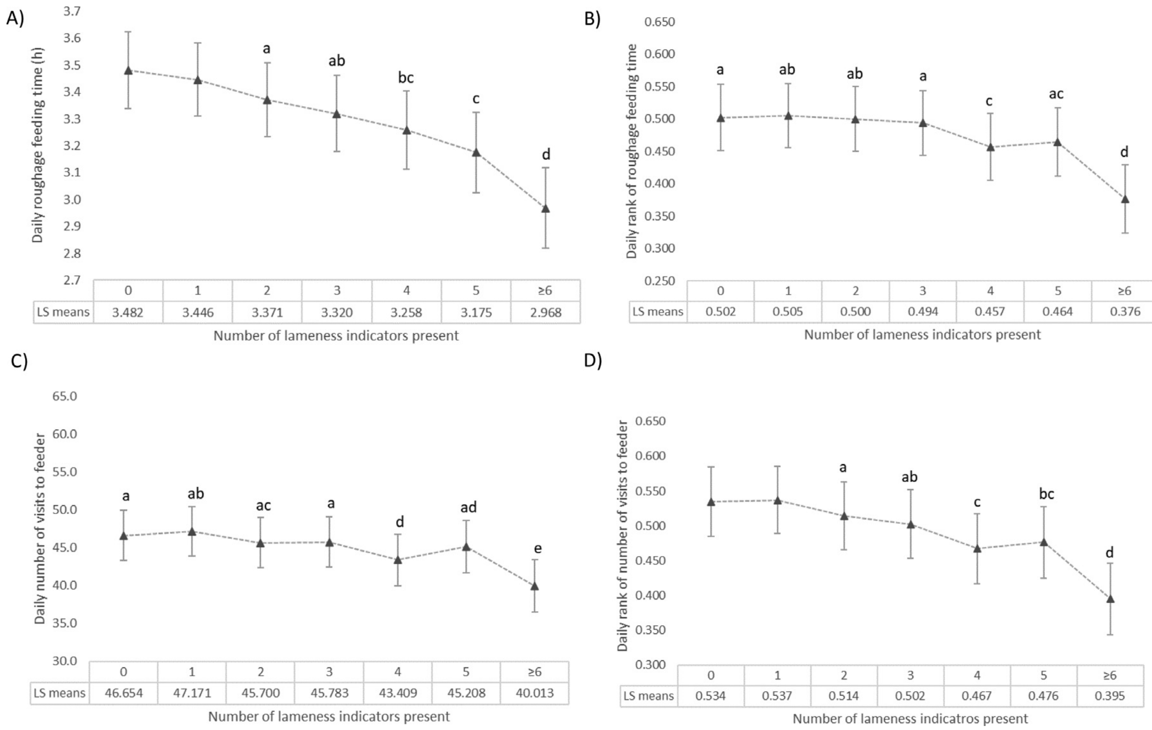

Figure 3. Least squares means and 95% confidence limits for daily a) roughage feeding time (h), b) rank of roughage feeding time, c) number of visits to the feeder, and d) rank of number of visits by the number of lameness indicators present in cow locomotion for 8 943 observation days and 84 cows. Ten cows enrolled in both trials were handled as separate individuals leading to total N of 94 cows in statistical models. Values with different superscripts (a-d) are statistically different (Tukey's adjusted *P* <0.05).

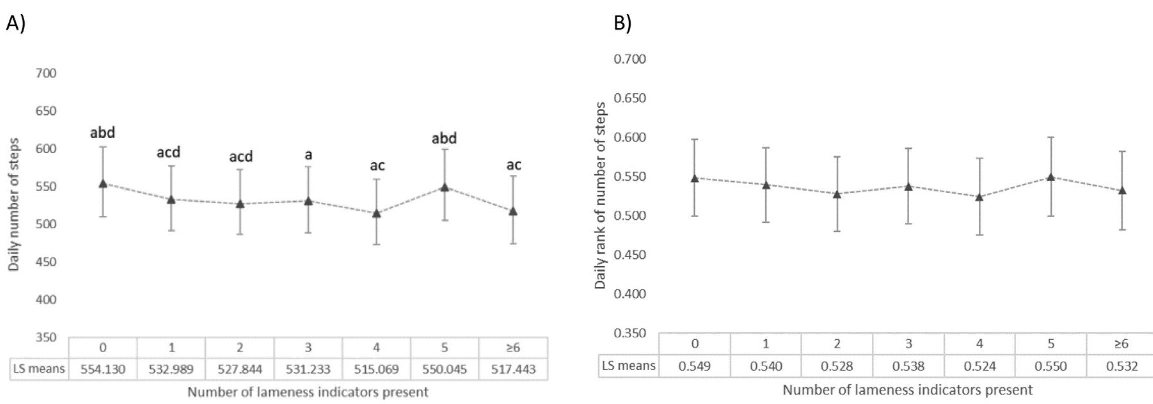

Figure 4. Least squares means and 95% confidence limits for daily a) number of steps and b) its rank order variable by the number of lameness indicators present in cow locomotion with 9 392 observation days and 84 cows. Ten cows enrolled in both trials were handled as separate individuals leading to total N of 94 cows in statistical models.

Normalization of behavior data using rank order

Between and within farm variability in animal behavior needs to be accounted for when, for instance, assessing animal welfare or developing automated methods for lameness detection. We used data in our study from two trials conducted in the same barn with approximately two years in between. Some environmental factors had changed during this time (e.g., stall mattresses and concentrate feeders), which could cause change also in animal behavior between the trials. Indeed, there was a significant difference in lying behavior between two trials, cows lying approximately one hour longer with fewer lying bouts and steps taken in the first trial. Ranking the animals in ascending order based on their absolute behavior values eliminated this difference between the trials. Thus, it seems that using the rank order of behavior is a simple and suitable method for diminishing variation in behavior data caused by environmental and management factors.

However, referencing to herd mates is most probably practical means for data normalization only for variables that are influenced by environment or management; if there is a significant within cow variation, then referring to individual's past behavior would be more appropriate (Thorup et al., 2015; O'Leary et al., 2020). Indeed, based on our examples of daily lying and feeding time, cow explained a large portion of the variation in the behavioral outcome variables; in lying time the difference between the lowest and highest deviation from group intercept was 8.2 hours. Taneja et al. (2020) noticed when using herd means of cattle activity in lameness detection, that some cows have, despite of their lameness status, consistently higher or lower activity levels than the herd mean. They used a clustering model to classify cows into active, normal, or dormant group based on their consistent deviation from the herd mean, and then built a different lameness detection model for each of these groups. This reduced the lameness classification error by 8%. Similar method could be used with the rank

Table 6

Linear mixed models describing the fixed effects associated with daily number of steps and its rank order variable with linear regression coefficient (β), standard error (SE), P-value, least squares (LS) means and 95% confidence limits of the LS means (CL lower and upper). LS means and their standard errors are presented only for fixed effects with statistically significant (Tukey's adjusted P<0.05) association with the outcome variable. Data comprised 9 392 observation days and 84 cows. Ten cows enrolled in both trials were handled as separate individuals leading to total N of 94 cows in statistical models.

		Number of steps[a]						Number of steps -rank					
Fixed effect		β	SE	P-value	LS means[b]	CL lower[b]	CL upper[b]	β	SE	P-value	LS means	CL lower	CL upper
Number of present lameness indicators	0	Ref.		<0.0001	554.130	509.995	602.026	Ref.		0.0341	0.549	0.500	0.597
	1	-0.039	0.013		532.989	491.617	577.842	-0.009	0.007		0.540	0.492	0.587
	2	-0.049	0.015		527.844	486.579	572.550	-0.021	0.008		0.528	0.480	0.576
	3	-0.042	0.016		531.233	489.312	576.745	-0.011	0.009		0.538	0.490	0.586
	4	-0.073	0.019		515.069	473.665	560.147	-0.024	0.011		0.524	0.475	0.574
	5	-0.007	0.021		550.045	504.920	599.143	0.001	0.012		0.550	0.500	0.600
	≥6	-0.068	0.021		517.443	474.993	563.687	-0.016	0.012		0.532	0.482	0.583
Days in milk	≤30	Ref.		0.0026	515.223	472.671	561.606	Ref.		0.0526			
	31-150	0.036	0.016		533.895	492.503	578.825	-0.021	0.009				
	>150	0.063	0.019		548.891	506.589	594.666	-0.015	0.011				
Parity	1	Ref.		<0.0001	633.462	546.427	734.287	Ref.		<0.0001	0.675	0.588	0.762
	2	-0.145	0.093		547.849	482.123	622.535	-0.125	0.055		0.550	0.475	0.626
	≥3	-0.376	0.087		435.110	392.446	482.413	-0.289	0.051		0.386	0.325	0.447
Breed	Ayrshire	Ref.						Ref.					
	Holstein	0.112	0.077	0.1493				0.073	0.046	0.114			

[a] Model fitted with outcome variable transformed by taking a natural logarithm.
[b] Back transformed least squares mean and its standard error.

order values for identifying cows with consistently high or low values in behavior in question to improve the differentiation between normal and lameness induced variation in cow behavior.

Limitation in our data is that it was collected only in one barn, although some environmental and management changes had been performed between two trials. There was a statistical difference only in lying behavior between two trials. However, it is known that there is large variation also in feeding behavior parameters depending on feeding management of the farm (von Keyserlingk and Weary, 2010; Grant and Ferraretto, 2018), and thus, using rank order for normalization of feeding behavior is probably appropriate. Regardless, validation of these results requires more data collected from several farms with different management systems.

Effect of lameness on cow behavior

In this study, we used number of frequently used lameness indicators as a measure of lameness instead of strict scoring system classifying cows lame or non-lame: there is no clear cut-off value when animal turns into clinical lameness, we only assume that when the number of lameness indicators increase, also the probability of being lame increases. There was a relatively consistent change in lying and feeding behavior of the cows with the increasing number of lameness indicators present in cow locomotion indicating that the scoring system was effective in assessing severity of lameness. These changes in behavior are likely a response to pain caused by hoof and other leg disorders (O'Callaghan et al., 2003; Gonzáles et al., 2008). These changes were evident both using the absolute daily values of cow behavior and in rank order of the cows. These results suggest that ranking cows based on their daily behavior indeed is a functional method for normalization of behavior data related to lameness induced changes.

Used locomotion scoring method makes the estimation of lameness prevalence in this study and comparing it to literature challenging. On the other hand, it is possible that this scoring method is more sensitive distinguishing lame animals, especially mildly lame cows, as it is shown that there is individual variation also in cow gait and lameness expression (Schlageter-Tello et al., 2014; Piette et al., 2020); use of the sum of the independent lameness indicators instead of using strict scaling of locomotion addresses better this individuality in lameness expression of cows. Grimm et al. (2019) also noticed that no explicit distinction between lame and sound cows can be made, and using binary classification always embodies elements of uncertainty. Pooling locomotion scores (e.g., low scores as healthy) may also underestimate the differences in behavior between different stages of lameness (Hut et al. 2021), and additionally pooling of the scores is often done variously in different studies (e.g., Grimm et al., 2019; Hut et al., 2021). However, we can assume in our data that at least cows having ≥4 lameness indicators present in their locomotion represent locomotion scores 2-3 in AHDB Dairy Mobility Score (AHDB 2020). This leads to lameness prevalence of 22.9% in this data, which is in accordance with earlier study conducted in Finland (Sarjokari et al., 2013).

Based on the literature, daily lying time is on average between 10.2-13.2 hours, and number of lying bouts between 8.0-13.3 depending on the study (Ito et al., 2010; Yunta et al., 2012; Solano et al., 2016; Westin et al, 2016b, King et al., 2017;, Blackie and MacLaurin, 2019; Grimm et al., 2019), and lying time and number of bouts in our data also laid within this range. Our results show a significant increase in lying time and lying bouts when the number of lameness indicators present increased, both when absolute values and rank order variables of behaviors were used as dependent variables in the models. Increased lying time in relation to lameness is supported by several other studies (e.g., Solano et al., 2016; Westin et al., 2016b; King et al., 2017; Hut et al., 2021), but for the number of lying bouts, more common result associated with lameness is decrease in the number of bouts (Solano et al., 2016; Westin et al., 2016; Hut et al., 2021). This is often reasoned with lame cows having more challenges in swapping position from standing to lying and vice versa, and that is why they prefer to stay lying after getting there. It is unclear why in our study there was a slight but significant increase in lying bouts when locomotion score increased (increase of 1.4 bouts from 0 to ≥6 lameness indicators present).

Difference in lying time between lame and non-lame cows is in many studies less than one hour (e.g., Thorup et al., 2015; Westin et al., 2016b; Hut et al. 2021). In our data, lying time and its rank order increased gradually with increasing number of lameness indicators, finally having more than one hour longer daily lying time for animals with severely impaired locomotion (≥6 lameness indicators present) compared to animals with 0-2 lameness indicators present. This result our decision to use more elaborate locomotion scoring instead of binary classification.

There are also some contradictory results regarding the association of lying time and lameness. For example, Yunta et al. (2012) and Blackie and MacLaurin (2019) reported no difference in daily lying time between lame and sound cows. Several factors – such as lactation stage – unrelated to lameness are known to affect lying behavior, and this is probable reason to varying results found in the literature about the association of lying time and lameness. Indeed, O'Leary et al. (2020) stated in their review that because of these reasons, lameness detection based solely on lying time measurements is unlikely to be successful. Part of our data supported the observation that lactation stage affected the daily lying time, so that the lying time is at the shortest in the early lactation (e.g., Blackie et al., 2011; Steensels et al., 2012; Maselyne et al., 2017).

Based on the literature, daily roughage feeding time on average is between 1.6-5.7 hours and number of visits to the feeder 21.5-46.5 (Miguel-Pacheco et al., 2014; Norring et al., 2014; Beer et al., 2016; Thorup et al., 2016; Grimm et al., 2019). Feeding time in our data also laid within this range and number of visits slightly exceeded the values found in the literature. Previous research shows consistently decrease in feeding time and number of feeding bouts in association with lameness (Miguel-Pacheco et al., 2014; Norring et al., 2014; Thorup et al., 2016; Barker et al., 2018; Grimm et al., 2019; Hut et al., 2021), and our results support this both in absolute values of behavior and in rank order variables. Especially in rank orders it could be seen that high numbers (≥4) of lameness indicators had significantly lower rank in feeding time compared to lower numbers of lameness indicators present in cow locomotion. Besides the number of lameness indicators also DIM significantly affected the feeding time and number of visits: animals in early lactation had shorter feeding time with fewer visits, which is in accordance with the results of Norring et al. (2014).

Difference between lame and non-lame cows in a daily feeding time has been reported to be as much as one hour or more (Miguel-Pacheco et al., 2014; Beer et al., 2016; Thorup et, al2016). In our data, decrease in feeding time was gradual with increasing number of lameness indicators, finally having a half an hour shorter feeding time for cows with severely impaired locomotion (≥6 lameness indicators present) compared to cows with zero lameness indicators present. In this study, we did not analyze the association between locomotion score and feed intake as it has been shown that dry matter intake is not affected by lameness, but rather lame cows compensate their reduced feeding time with higher feeding rate (Gonzáles et al., 2008; Thorup et al., 2016).

In this study, we concentrated only on how the amount of lameness indicators affects the individual behavioral variables. However, Grimm et al. (2019) discovered that there actually is a strong interaction between feeding and lying behavior when cow classified as lame or non-lame is the dependent variable; cow is likely to be lame only when there is a simultaneous decrease in feeding time and increase in lying time. This could partly be explained by the finding of Yunta et al. (2012) that lame cows stand up later when fresh feed is delivered and go back lie down earlier than their sound herd mates. This interaction between lying and feeding behavior should be considered also when rank order values of behaviors are used in development of predicting model.

Exact numbers of daily steps taken are rarely reported in the literature, but in the studies of Blackie and MacLaurin (2019) and Hut et al. (2021) step count was more than 2,500 steps per day. Our average daily step count was significantly lower, only 537 steps per day in trial 1 and 651 in trial 2. Reason for this could be that the experimental cows were housed in relatively small groups, in a small area and in an immediate vicinity of milking parlor. Blackie and MacLaurin (2019) used similar sensor to measure cow behavior, but animals were housed in larger groups and in a straw yard. Hut et al. (2021) used different acceleration-based sensor, and in their study, cows were also housed in much larger group than in ours. These differences in experimental setups may be part of the reason for such a high variability on daily step count and highlights again how different management practices can significantly affect animal behavior.

O'Leary et al. (2020) included in their review daily step count as a potential variable regarding automated lameness detection. Our results do not support this observation; the number of lameness indicators present had a significant effect on both daily step count and its rank order variable, but these associations were either inconsistent between different numbers of lameness indicators and absolute step count or not perceptible in the pairwise comparison in rank order values. Literature also reports contradictory results regarding the association between lameness and daily step count. In the study of Hut et al. (2021), daily number of steps taken decreased in association with lameness. On the other hand, several studies have reported increase in step activity in association with lameness (Alsaaod et al., 2012; Chapinal and Tucker, 2012; Thorup et al., 2015). This increase may relate to increasing discomfort while standing related to hoof disorders. The potential difference between lame and non-lame cows could also be obscured because the IceQube-sensor is attached only to one of the hind legs; Pastell (2007) observed a higher stepping activity in a milking robot for the leg that was affected by a hoof disorder. Thus, the measured step activity in lame cow is dependent on whether sensor is attached to the sick or the healthy leg.

There are several limitations in data reliability when using subjective locomotion scoring (Schlageter-Tello et al., 2014). Many environmental and cow factors affect locomotion independently of lameness (Van Nuffel et al., 2015). For example, in our experimental setup, locomotion scoring was conducted on slatted floors, which can alter normal gait. This can lead to cases where a healthy animal expresses lameness indicators in its gait. Cow's motivation to walk also affects the expression of lameness indicators both in lame and non-lame animals; cows with good motivation have a brisker pace, longer strides and less prominent head bob (Mokhtarnazif et al., 2020). Another problematic issue is merging the biweekly locomotion scoring with daily behavior data. In many studies, behavior data only from the days close to the locomotion scoring are used in models (e.g., Solano et al., 2016, Blackie and MacLaurin, 2019: Hut et al., 2021). We instead decided to use all the available behavior data and assign the timely closest locomotion score to each observation day. It is possible that this causes bias in our data as we do not know if there were alterations in cow's lameness status between the fortnightly scoring. However, many of the hoof disorders, especially claw horn disruption lesions, develop gradually over a long period of time (Hoblet and Weiss, 2001), and thus, also rapid changes in locomotion score are improbable. It is even possible that this approach acknowledges better the gradual development of hoof disorders and lameness.

Conclusions

Increasing number of lameness indicators present in cow locomotion was associated with increasing lying time, increase in number of lying bouts, decreasing roughage feeding time and decrease in feeding bouts. This was statistically evident both in the absolute values of daily behavior of cows as in the rank order of the cows based on their daily behavior. There was a significant difference between two trials in lying behavior of the cows but when using rank order values this difference was not evident. Thus, ranking the cows within the herd based on their behavior did normalize the data between the trials. Additionally, rank order values behaved similarly to absolute daily behavior in regard to cow's lameness status. Ranking could address some issues in development of automated lameness detection systems arising from within and between herd variability in dairy cow behavior. However, larger between-farm dataset would be needed to verify the generalizability of the results presented in this paper.

Ethical statement

This study design was reviewed and approved by Animal Welfare body (Government decree 564/2013 22§) of Natural Resources Institute Finland. Project authorization was not needed as the experiment did not cause the animals a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle (2010/63/EU).

Authorship statement

The idea for the paper was conceived by Matti Pastell and Lilli Frondelius. The experiments were designed by Lilli Frondelius, Matti Pastell and Heli Lindeberg. The experiments were performed by Lilli Frondelius and Heli Lindeberg. The data were analyzed by Lilli Frondelius. The paper was written by Lilli Frondelius, Heli Lindeberg and Matti Pastell.

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Conflict of interest

The authors declare no conflict of interest.

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