

Early detection of subclinical ketosis and clinical diseases in dairy cows using peripartum motion activity

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ABSTRACT

Early detection of metabolic diseases like subclinical ketosis (SCK) and other disorders is crucial for animal welfare and economic efficiency. Automated monitoring systems become increasingly important here. This study examined motion activity of cows around calving and its suitability for early detection of SCK and clinical disorders. Activity data of 136 cows were collected over two weeks before to three weeks after calving and analyzed alongside blood test results for beta-hydroxybutyric acid (BHBA). Cows were categorized as clinically sick (S) or healthy (H), with group H further subdivided into group SCK (at least one BHBA result ≥ 1.2 mmol/L) and group non-SCK (BHBA consistently < 1.2 mmol/L). Antepartum, activity levels hardly differed between groups. Postpartum, groups H and non-SCK were significantly more active than groups S and SCK, respectively (mixed effects regression; estimated mean difference of 11.37, 95 % CI (Confidence Interval) [5.847, 16.9], $P = 0.0001$ for H vs. S, and estimated mean difference of 7.90, 95 % CI [0.544, 15.25], $P = 0.0353$ for non-SCK vs. SCK). Comparing antepartum and postpartum activity, a significant increase was observed only in groups H and non-SCK (mixed effects regression; estimated mean difference of -8.46 , 95 % CI $[-10.60, -6.327]$, $P < 0.0001$ and estimated mean difference of -12.35 , 95 % CI $[-15.18, -9.52]$, $P < 0.0001$, respectively). A lack of postpartum activity increase could serve as early warning indicator for cows at risk of disease and, with the development of animal-specific thresholds, may be integrated into automated monitoring systems.

1. Introduction

Subclinical ketosis (SCK) is a very common disease in early-lactating dairy cows. Incidences between 40 % and 60 % and prevalences between 8.9 % and 43 % are reported for the first two months of lactation (Simensen et al., 1990; Duffield et al., 1998; McArt et al., 2012; Berge and Vertenten, 2014). Rapidly increasing milk yield with insufficient dry matter intake leads to a negative energy balance (NEB) in the transition period which in turn can result in disturbances in carbohydrate and lipid metabolism with negative impact on the cow's health and productivity (Baird, 1982; Dohoo and Martin, 1984; Duffield, 2000; Rutherford et al., 2016). Increased susceptibility to secondary disorders, such as retained placenta, metritis, abomasal displacement, mastitis and lameness, reduced milk yield and fertility and an increased risk of culling in early lactation lead to poorer herd health and financial losses for dairy farms (McLaren et al., 2006; Ospina et al., 2010a, b; Chapinal et al., 2012; Dubuc et al., 2012; Berge and Vertenten, 2014). Therefore, an early detection of cows affected with and at risk of SCK is of great

importance in context of animal welfare and economic efficiency. It enables immediate intervention through therapeutic options, such as the supplementation of energy-rich feedstuffs like propylene glycol or glycerin, which are effective in treating the condition rapidly and efficiently, thereby reducing the risk of subsequent complications (Grummer, 2008). Constantly growing herd sizes with an unchanged number of farm personnel and the demand for working comfort require simple and automated solutions for animal monitoring (Zimmermann et al., 2016). The concept of precision livestock farming (PLF) is currently on everyone's lips. By using a variety of sensors and technologies, the behavior, productivity and well-being of dairy cattle can be constantly monitored and changes in these parameters can be detected immediately (Stygar et al., 2021; Dzermeikaite et al., 2023). In this way, management problems, as well as various diseases, can be discovered at an early stage. Early detection of diseases, ideally before more severe clinical symptoms appear, allows for earlier intervention, improves treatment response and mitigates the long-term consequences in terms of animal health and performance (Cascone et al., 2022). It is therefore

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an important tool for improving animal health and welfare in dairy cattle herds and has become indispensable in modern livestock medicine and management. However, a precondition for detecting changes in behavioral parameters is a profound knowledge of the behavioral patterns of healthy animals and animals with various diseases. A now frequently researched area for monitoring the health status of dairy cows is the recording of their motion activity. Originally developed and used for estrus detection, it has recently also been investigated regarding its suitability for early detection of metabolic (and other) disorders.

Several studies evaluated the relationship between changes in motion activity and the occurrence of fresh-cow disorders, such as SCK. [Edwards and Tozer \(2004\)](#) found cows with metabolic or digestive disorders to be less active than healthy cows for the first 14 days in milk (DIM) and ketotic cows could be detected 8–9 days before diagnosis based on a decline in activity. [Stangaferro et al. \(2016a\)](#) developed and evaluated an automated health monitoring system to identify cows with metabolic and digestive disorders based on a health index score (HIS) that combines motion activity and rumination time. Using this alert system, cows with ketosis were identified at least 5 days before clinical diagnosis. [King et al. \(2017\)](#) and [Steensels et al. \(2017\)](#) also detected lower activity levels for ketotic cows 7 and 5 days before clinical diagnosis, respectively. Compared to healthy cows, those affected with SCK showed lower activity from day 7 to day 11 and on days 13, 15, 16 and 17 postpartum, but the occurrence of SCK was not associated with the antepartum activity in the study of [Liboreiro et al. \(2015\)](#). Finally, [Najm et al. \(2020\)](#) found the activity level of healthy cows to be generally higher than that of ketotic cows, exceeding the latter by an average of 52.6 % for the period of 4–70 DIM. Compared to the herd mean daily motion activity, ketotic cows showed lower activity levels on 6–12 DIM ([Najm et al., 2020](#)). In a recent study conducted by [Antanaitis et al. \(2024\)](#), cows with SCK experienced a reduction of 27.36 % in the overall activity levels between 5 and 30 days postpartum. All these results show correlations between the occurrence of SCK and changes in motion activity. However, the focus has been mainly on the postpartum period and only few studies included antepartum activity levels, so far. Since previous studies showed that the activity of ketotic cows declines before changes in their production variables (e.g. milk yield) are noticeable ([Edwards and Tozer, 2004](#); [King et al., 2017](#)) and since many dairy farms already use activity monitoring systems nowadays, this study focused on the periparturient motion activity for an early detection of SCK.

The main objective of this study was to analyze the daily motion activity of cows around calving, comparing cows affected with SCK and healthy cows. Additionally, the study examined the periparturient daily motion activity of cows with signs of a clinical disease (deviations in normal behavior or feed intake, presence of fetal membranes outside the vulva, occurrence of vaginal discharge, lameness, udder health problems or increase of rectal temperature) to compare these activity patterns to those of clinically healthy cows. Finally, it was assessed how these findings gained at group levels could be applied to the individual cow for early detection of cows at risk of SCK or clinical disease.

2. Material and methods

2.1. Animals and herd management

The study was conducted at a commercial dairy farm in Saxony-Anhalt, Germany, with a total of 800 Holstein cows and an average milk production of around 11,000 kg per cow and year. The cows were kept in groups according to their production stage. Late-stage pregnant cows were grouped by parity (heifers vs. cows) two to three weeks before their expected calving date and placed in loose housing pens with straw bedding. Immediately after calving, primiparous and multiparous cows were brought together in another loose housing pen with straw bedding and kept there for approximately five to ten days postpartum depending on the course of puerperium. During the early postpartum period cows were routinely evaluated for deviations in normal behavior

or feed intake, presence of fetal membranes outside the vulva, occurrence of vaginal discharge, lameness, udder health problems or an increase of rectal temperature by trained farm personnel every day. If considered healthy, they were moved to a free stall barn. Cows with health disorders were treated by the farm staff. Examination results and treatments were documented and provided at the end of the data collection phase. Cows were milked three times per day in a rotary. Individual daily milk yield was not recorded.

2.2. Study design

All procedures were approved by the state administration office Saxony-Anhalt, department 203 (reference number 203.m-42502–3–907 LMU). Data were collected from June 2021 to September 2021. The study followed a longitudinal, observational, prospective design. To obtain statistically significant results with high probability, a two-sample *t*-test was used to determine the sample size of the number of cows needed. Since the cows were kept on a commercial dairy farm and were not specifically purchased for this study, a reserve of 10 % was planned to replace cows that left the farm unexpectedly due to farm decisions or had to be excluded from the study for other reasons. A power analysis for sample size determination was conducted by the G*Power statistical software and revealed that with a medium effect size (Cohen's $d = 0.5$), a power of 80 %, and an α -level of $P \leq 0.05$, a sample size of 128 animals was required. The addition of 10 % reserve animals resulted into 141 animals. According to the calculated sample size, 141 cows, regardless of their lactation number (median = second lactation, ranging from first to ninth lactation), were selected based on their estimated parturition date (calvings occurring between June and September) and originally enrolled in the study.

2.3. Blood sampling and laboratory analysis

Blood samples were taken three times a week (Monday, Wednesday, Friday) over a period of 14 days before the expected parturition date to 21 days after calving (– 14–21 days in milk, DIM). Blood was collected by puncturing the coccygeal vein using blood serum tubes with an inert, stable gel that separates the serum and the blood clot during centrifugation (BD Vacutainer SST II Advance Tubes, 8.5 mL, Becton, Dickinson and Company, Plymouth, UK). Approximately 30 – 60 min after collection the samples were centrifuged at 3000 x *g* for 10 min (Sigma 3K18, Sigma Laborzentrifugen GmbH, Osterode, Germany) and stored at –18 °C until assayed. Blood samples were analyzed for beta-hydroxybutyric acid (BHBA) and total calcium using the Cobas c311-Analyzer for clinical chemistry (Roche Diagnostics, Rotkreuz, Switzerland). Serum concentrations of BHBA were analyzed for detecting SCK, serum total calcium concentrations were assayed to consider decreased blood calcium levels as another possible cause of changes in motion activity.

2.4. Motion activity

Motion activity was recorded by the CattleData Classic system for animal identification, tracking and activity monitoring (CattleData GmbH, Friedberg, Germany) which was already used for estrus detection on the farm. The system recorded activity by using RFID (Radio Frequency Identification) technology. Cows were fitted with battery-free transponder ear tags that received signals from RFID-antennas installed above the walking areas of the barn and then sent these signals back when they were in signal range of the corresponding antenna. In this way, the barn was divided into adjacent zones, whereby an 'antenna zone' was defined as the area in which the respective antenna transmitted and received. The returned signals were registered via a reader connected to the antennas and sent to a computer. Time data, information on the antenna and the cow's identification number were processed by the associated CattleData software and daily motion

activity of a cow was output as the unit free sum of its movement underneath the antennas or more precisely, of its changes of zones over a 24-h period (00:00–23:59 h). The data were transferred to an excel file and saved for evaluation.

2.5. Group formation and data handling

Cows were grouped into various categories according to their health status on clinical and subclinical level (Fig. 1). Therefore, the farm's records on diseases and treatments and the results of the BHBA analyses were considered. The blood BHBA threshold used to define SCK in this study was 1.2 mmol/L, similar to other studies on SCK (Duffield et al., 1997; King et al., 2018; Sturm et al., 2020). On clinical level cows were divided into two groups, regardless of their BHBA results: a healthy and a sick group. The healthy group (group H) included all cows which showed no clinical signs of disease during the entire observation period. All cows with clinical signs of disease were assigned to group sick (group S). Clinical signs of disease were defined as a deviation in normal behavior or feed intake, the presence of fetal membranes outside the vulva, the occurrence of vaginal discharge, lameness or udder health problems or an increase of rectal temperature. To investigate the effect of subclinical ketosis in particular on motion activity, the healthy group was further subdivided into two groups according to their BHBA results. Cows who showed no clinical signs of disease and whose BHBA results were consistently < 1.2 mmol/L formed the non-SCK group. The SCK group consisted of all cows that were clinically healthy but had at least one BHBA result ≥ 1.2 mmol/L.

The observation period was divided into an antepartum and a postpartum section. Antepartum summarized the study period from 10 days up to and including one day before calving (–10 to –1 DIM). Postpartum covered the period from day one to day 21 after calving (1–21 DIM). Due to small sample sizes at –14 to –11 DIM, only the observation period from –10–21 DIM was considered for analyses. The day of calving itself (0 DIM) was excluded because regrouping within the barn distorted the activity values on that day. First, motion activity was compared

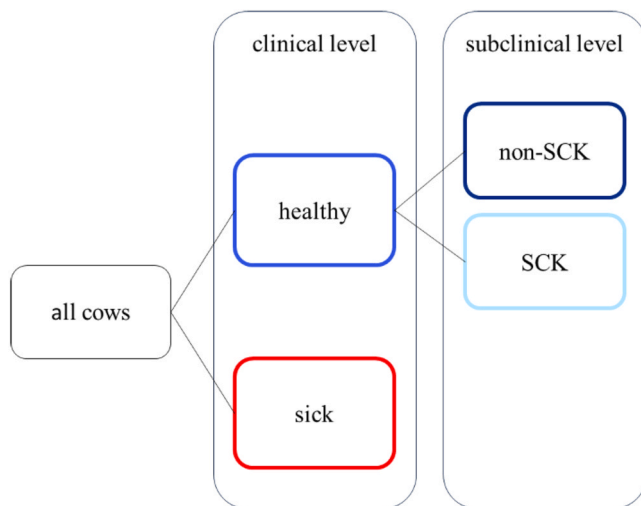


Fig. 1. Group formation according to the cows' health status on clinical level (based on the farm personnel's records on diseases and treatments) and on subclinical level (based on the beta-hydroxybutyric acid (BHBA) results). healthy = cows which showed no clinical signs of disease¹; sick = cows with clinical signs of disease; non-SCK = cows that were clinically healthy and whose BHBA results were consistently < 1.2 mmol/L; SCK = cows that were clinically healthy but had at least one BHBA result ≥ 1.2 mmol/L. ¹ Clinical signs of disease were defined as a deviation in normal behavior or feed intake, the presence of fetal membranes outside the vulva, the occurrence of vaginal discharge, lameness or udder health problems or an increase of rectal temperature.

antepartum and postpartum between groups (healthy vs. sick; non-SCK vs. SCK) and within each group (antepartum vs. postpartum) to find out if there were any differences in activity levels. In addition, motion activity was then compared between groups at each DIM (–10–21) and within each group around calving (–2–1 DIM). Finally, the applicability of the obtained results was tested at the individual animal level. For this purpose, it was investigated how many animals from the respective groups actually showed corresponding changes in their movement behavior and whether a threshold for these changes in motion activity could be determined to predict a cow's health status.

2.6. Statistical analyses

Of 141 cows originally included in the study, five had to be excluded due to severe lameness ($n = 1$) or lack of activity data ($n = 4$). Therefore, statistical analyses were performed with data of 136 cows using R statistical software, version 4.3.1 (2023–06–16) and, for descriptive statistics, IBM SPSS Statistic for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA). Due to the presence of repeated measures generalized linear mixed effects models, with group (Healthy, Sick, non-SCK, SCK), time of observation (antepartum / postpartum, individual DIM) and interaction between them as fixed effects (predictors) and the individual cow as a random effect, were chosen for analysis of the mean daily motion activity (response variable). The following model assumptions were always checked: (1) the normality of residuals was checked both visually and by the Shapiro–Wilk normality test, (2) the homogeneity of variances between groups was checked both visually and with Bartlett test, and (3) the heteroscedasticity (constancy of error variance) was checked with both visually and Breusch–Pagan test. In case assumptions were satisfied, generalized linear mixed effects models were used (R package – lme4). In case assumptions were violated, robust linear mixed effects models were applied (R package – robustlmm) (Bates et al., 2015; Koller, 2016). All contrasts (differences) between particular groups were assessed after model-fitting by the estimated marginal means (R package – emmeans) with Tukey p -value correction for multiple comparisons. Chi-square-test was used to determine a suitable threshold for changes in motion activity in the different groups (R package – ggstatsplot). Results with a P -value ≤ 0.05 were considered statistically significant. Additionally, results with $0.05 < P \leq 0.10$ were considered as tending to be statistically significant in order to identify subtle yet potentially interesting differences or trends between groups. These P -values suggest that the results do not provide sufficient evidence to reject the null hypothesis (no differences), but still exhibit a certain direction or tendency that should be considered within the context of the research question.

3. Results

3.1. Animals, health status and groups

Of 136 cows included in statistical analyses, 53.7 % ($n = 73$) showed no clinical signs of disease during the observation period (group H), whereas 46.3 % ($n = 63$) did (group S). The median day of occurrence of a clinical disease was 2 DIM (IQR = 1.5). The allocation of cows from

Table 1
Allocation of cows from group S (Sick) to different types of disorders.

Type of disorder	Number of cows
Systemic disorder ^a	45 (71.4 %)
Hoof disorder	1 (1.6 %)
Systemic disorder plus hoof disorder	6 (9.5 %)
Mastitis	7 (11.1 %)
Systemic disorder plus mastitis	4 (6.4 %)
Total	63 (100 %)

^a e.g. retained placenta, metritis, peritonitis, pneumonia, indigestion, displaced abomasum, clinical ketosis.

group S to different types of disorders is shown in Table 1. Of the 73 clinically healthy cows, 60.3 % ($n = 44$) consistently had BHBA concentrations < 1.2 mmol/L and therefore formed group non-SCK, whereas 39.7 % ($n = 29$) had at least one BHBA result ≥ 1.2 mmol/L and were consequently assigned to group SCK. The median day of diagnosis of SCK was 10 DIM (IQR = 7). There was no statistically significant association between the occurrence of a clinical disease or SCK and the number of lactation ($P = 0.2179$ and $P = 0.8776$, respectively).

3.2. Total calcium

Using the laboratory's threshold of 2.00 mmol/L for total calcium in blood serum samples, all animals, regardless of their group affiliation, were subclinical hypocalcemic in this study. The median blood serum calcium concentration measured was 1.69 mmol/L (IQR = 0.34). Therefore, no further subdivision into groups based on calcium values was made.

3.3. Motion activity of clinically healthy and sick cows

Comparing the mean daily motion activity during the antepartum period, group H tended to be more active than group S with an estimated mean difference of 5.15 (95 % CI (Confidence Interval) $[-0.836, 11.1]$, $P = 0.0917$). Postpartum, the activity level of group H was statistically significantly higher than that of group S with an estimated mean difference of 11.37 (95 % CI $[5.847, 16.9]$, $P = 0.0001$) (Fig. 2a). Mean daily motion activity of healthy cows exceeded that of sick cows by 12.9 % and 27.0 % on average, calculated over the last two weeks before calving and the first three weeks of lactation, respectively. Comparing antepartum and postpartum mean activity levels within each group,

there was a statistically significant increase of 18.8 % from antepartum to postpartum activity in group H (estimated mean difference of -8.46 , 95 % CI $[-10.60, -6.327]$, $P < 0.0001$). Mean daily motion activity in group S tended to increase from antepartum to postpartum by 5.7 % (estimated mean difference of -2.25 , 95 % CI $[-4.69, 0.181]$, $P = 0.0704$) (Fig. 2a).

Looking at the mean motion activity of both groups on each individual day (-10 – 21 DIM), consistently higher activity values were observed within the first 3 weeks after calving in healthy cows with statistically significant differences ($P < 0.05$) from 1 to 11 DIM (Fig. 2b, Table 2). On day 12 of lactation healthy cows still tended to be more active than sick cows (Table 2). Already one day before calving, cows of group H were significantly more active than those who developed a clinical disease postpartum and tended to be more active on DIM -4 and -7 (Table 2). Having nearly the same mean activity level two days before calving, the activity of healthy cows increased significantly by approximately 45.9 % from -2 – 1 DIM (estimated mean difference of -19.393 , 95 % CI $[-26.088, -12.698]$, $P < 0.0001$) whereas that of group S did not (approximately 13.6 % increase of activity, estimated mean difference of -5.745 , 95 % CI $[-12.974, 1.485]$, $P = 0.1194$). The mean activity of clinically healthy cows slowly decreases within the first two weeks after calving whereas that of group S increases steadily until both groups align in their activity levels approximately 14 days postpartum (Fig. 2b). Looking at the results at the level of the individual animal, 66.7 % ($n = 46$) of the clinically healthy cows showed an increase in activity from antepartum to postpartum, whereas 33.3 % ($n = 23$) did not. In group S, 50.9 % ($n = 29$) increased their motion activity from antepartum to postpartum and 49.1 % ($n = 28$) did not. There was no statistically significant association between group affiliation and the simple division of activity levels into “increase” and “no increase” (χ^2

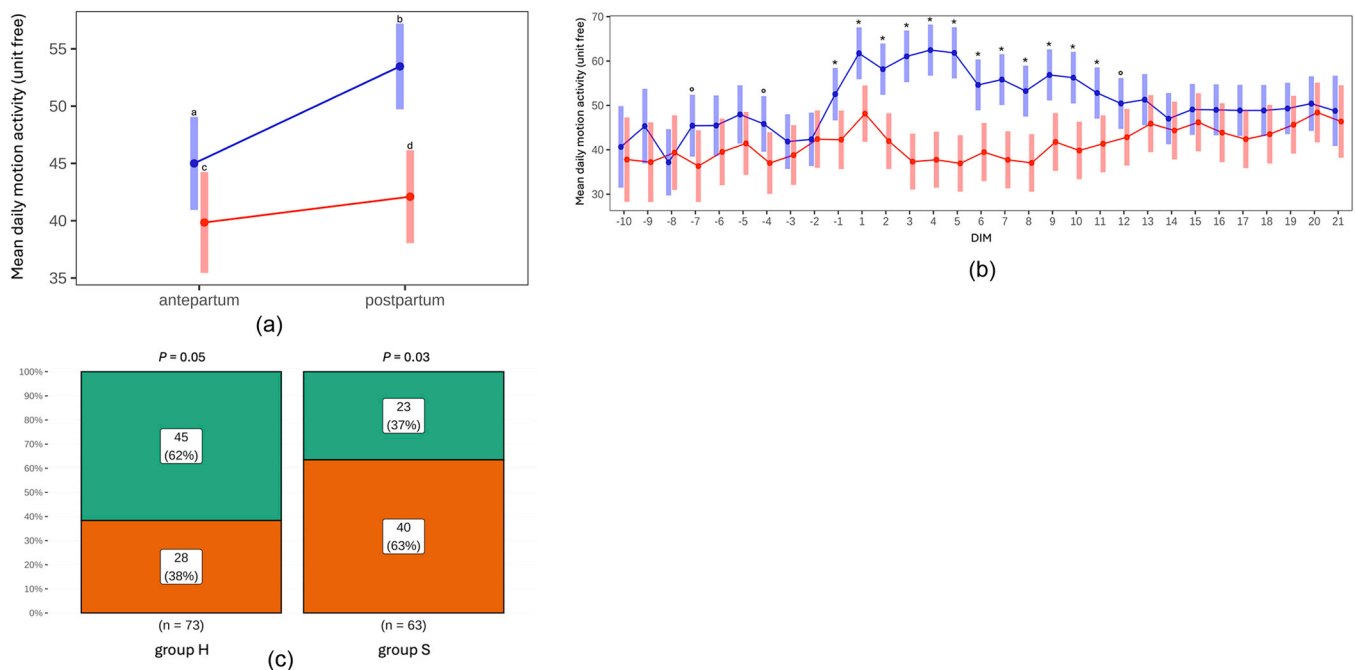


Fig. 2. a. Mean daily motion activity of group H (Healthy, $n = 73$; blue) and group S (Sick, $n = 63$; red) during the antepartum period (-10 to -1 days in milk) and the postpartum period (1 – 21 days in milk) predicted by robust linear mixed effects regression. The error bars show the 95 % confidence intervals of the means estimated by the model. Antepartum, differences between group H and group S tended to be statistically significant ($P = 0.0917$; a and c). Postpartum, differences between group H and group S were statistically significant ($P = 0.0001$; b and d). Activity increases significantly from antepartum to postpartum in group S ($P = 0.0704$; c and d). b. Mean daily motion activity of group H (Healthy, $n = 73$; blue) and group S (Sick, $n = 63$; red) on days in milk (DIM) -10 – 21 , excluding the day of calving (DIM 0), predicted by robust linear mixed effects regression. The error bars show the 95 % confidence intervals of the means estimated by the model. Days on which there were statistically significant differences ($P < 0.05$) between group H and group S are marked with *. Days on which differences between group H and group S tended to be statistically significant ($0.05 < P \leq 0.10$) are marked with °. c. Distribution of cows from group H (Healthy) and group S (Sick) to activity groups “activity increase $> 15\%$ from antepartum to postpartum” (green) and “activity increase $< 15\%$ from antepartum to postpartum” (orange) calculated by Chi-square-test. There was a statistically significant association between the threshold of 15 % activity increase from antepartum to postpartum and the affiliation to group H and group S ($P = 0.00346$).

Table 2

Results of robust linear mixed effects regression for the comparison of mean daily motion activity between group H and group S.

			95 % CI		
		Estimated mean difference	Asymptotic LCL	Asymptotic UCL	p-value
group H (n = 73) vs. group S (n = 63)	DIM -10	2.845	-10.401	16.09	0.6738
	DIM -9	8.115	-4.198	20.43	0.1964
	DIM -8	-2.197	-13.451	9.06	0.7020
	DIM -7	9.104	-1.560	19.77	0.0943
	DIM -6	5.943	-4.194	16.08	0.2505
	DIM -5	6.526	-3.164	16.22	0.1868
	DIM -4	8.804	-0.553	18.16	0.0652
	DIM -3	3.034	-6.097	12.17	0.5148
	DIM -2	-0.053	-8.902	8.80	0.9906
	DIM -1	10.272	1.414	19.13	0.0230
	DIM 1	13.595	4.951	22.24	0.0021
	DIM 2	16.210	7.654	24.77	0.0002
	DIM 3	23.728	15.154	32.30	< 0.0001
	DIM 4	24.719	16.154	33.28	< 0.0001
	DIM 5	24.905	16.313	33.50	< 0.0001
	DIM 6	15.126	6.408	23.84	0.0007
	DIM 7	18.082	9.452	26.71	< 0.0001
	DIM 8	16.189	7.529	24.85	0.0002
	DIM 9	15.112	6.406	23.82	0.0007
	DIM 10	16.401	7.689	25.11	0.0002
	DIM 11	11.449	2.801	20.10	0.0095
	DIM 12	7.612	-0.991	16.22	0.0829
	DIM 13	5.402	-3.246	14.05	0.2209
	DIM 14	2.663	-6.043	11.37	0.5488
	DIM 15	2.893	-5.828	11.61	0.5156
	DIM 16	5.136	-3.649	13.92	0.2518
	DIM 17	6.481	-2.225	15.19	0.1446
	DIM 18	5.334	-3.419	14.09	0.2323
	DIM 19	3.650	-5.074	12.37	0.4122
	DIM 20	2.009	-7.121	11.14	0.6662
	DIM 21	2.400	-8.997	13.80	0.6798

group H: healthy cows; group S: sick cows; DIM: days in milk; SE: Standard Error; CI: Confidence Interval; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit.

(Chi-squared) = 2.61, $P = 0.1063$). Applying a threshold of 15 % for the increase in activity from antepartum to postpartum, a statistically significant ($P = 0.00346$) association between this threshold and group affiliation and the medium effect size of this association (Cramér's $V = 0.24$, 95 % CI [0.00, 0.41]) were detected. In group H, 62 % ($n = 45$) of the cows increased their activity by more than 15 %, whereas 38 % ($n = 28$) showed an increase in activity of less than 15 %. This difference was statistically significant ($P = 0.05$) (Fig. 2c). Of the cows with signs of clinical disease, 37 % ($n = 23$) increased their activity level by more than 15 %. In 63 % ($n = 40$) of the cows in this group, an increase of less than 15 % was observed. This difference was also statistically significant ($P = 0.03$) (Fig. 2c).

3.4. Motion activity of cows with SCK and cows not affected with SCK

During the antepartum period no statistically significant differences in mean activity levels between group non-SCK and group SCK were observed (estimated mean difference of -2.23, 95 % CI: [-10.293, 5.84], $P = 0.5880$) (Fig. 3a). Postpartum, non-SCK cows had a significantly higher mean daily motion activity than SCK cows with an estimated mean difference of 7.90 (95 % CI [0.544, 15.25], $P = 0.0353$). Mean daily motion activity of group non-SCK exceeded that of group SCK by 16.1 % on average, calculated over the first three weeks of lactation. Comparing antepartum and postpartum mean activity levels within each group there was a statistically significant increase of 27.8 % from antepartum to postpartum activity only in group non-SCK (estimated mean difference of -12.35, 95 % CI [-15.18, -9.52], $P < 0.0001$). The activity of group SCK did not increase significantly from antepartum to postpartum (4.8 % increase of activity, estimated mean difference of -2.23, 95 % CI [-5.73, 1.27], $P = 0.2124$) (Fig. 3a).

Comparing mean motion activity on the individual DIM (-10–21) between the two groups, cows not affected with SCK showed consistently higher activity from -1–21 DIM with one exception on day 4 of lactation. On day 6, 7, 10, 11 and 13 postpartum the differences were statistically significant ($P < 0.05$) and tended to be statistically significant on day 1 and 16 postpartum (Fig. 3b, Table 3). One day before calving non-SCK cows tended to be more active than SCK cows (Table 3).

Statistically significant differences in activity levels were also found for day 9 and 4 before calving (Table 3). On these two days group SCK showed higher activity levels than group non-SCK. Having nearly the same activity level two days before calving, the activity of cows not affected with SCK increased by approximately 55.7 % from -2–1 DIM (estimated mean difference of -23.776, 95 % CI [-32.676, -14.877], $P < 0.0001$) whereas the activity of cows diagnosed with SCK increased by approximately 27.1 % during this time (estimated mean difference of -11.684, 95 % CI [-22.552, -0.816], $P = 0.0351$) (Fig. 3b). Translating the results to the level of the individual, 73.2 % ($n = 30$) of the cows not affected with SCK showed an increase in activity from antepartum to postpartum, whereas 26.8 % ($n = 11$) showed no increase. In group SCK, 57.1 % ($n = 16$) increased their activity level from antepartum to postpartum, whereas 42.9 % ($n = 12$) did not. There was no statistically significant association between group affiliation and the simple division of activity levels into “increase” and “no increase” ($\chi^2 = 1.27$, $P = 0.2598$). When testing different thresholds for the increase in activity from antepartum to postpartum, no threshold could be determined for group non-SCK and SCK in which there was a statistically significant association between the respective threshold and the group affiliation.

4. Discussion

The automated activity monitoring is already being used as a tool in the health management of dairy herds. In this study, motion activity of cows around calving was investigated for an early detection of clinically diseased cows and cows affected with SCK based on changes in their activity levels.

By considering both, antepartum and postpartum mean daily motion

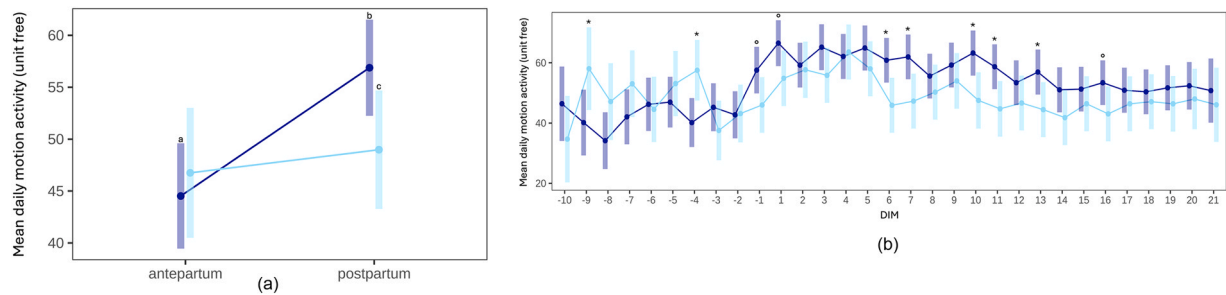


Fig. 3. a. Mean daily motion activity of group non-SCK (cows not affected with SCK, $n = 44$; dark blue) and group SCK (cows with SCK, $n = 29$; light blue) during the antepartum period (-10 to -1 days in milk) and the postpartum period (1 – 21 days in milk) predicted by robust linear mixed effects regression. The error bars show the 95 % confidence intervals of the means estimated by the model. Postpartum, differences between group non-SCK and group SCK were statistically significant ($P = 0.0353$; b and c). Activity increases significantly from antepartum to postpartum in group non-SCK ($P < 0.0001$, a and b). b. Mean daily motion activity of group non-SCK (cows not affected with SCK, $n = 44$; dark blue) and group SCK (cows with SCK, $n = 29$; light blue) on days in milk (DIM) -10 – 21 , excluding the day of calving (DIM 0), predicted by robust linear mixed effects regression. The error bars show the 95 % confidence intervals of the means estimated by the model. Days on which there were statistically significant differences ($P < 0.05$) between group non-SCK and group SCK are marked with *. Days on which differences between group non-SCK and group SCK tended to be statistically significant ($0.05 < P \leq 0.10$) are marked with °.

Table 3
Results of robust linear mixed effects regression for the comparison of mean daily motion activity between group non-SCK and group SCK.

		95 % CI			
		Estimated mean difference	Asymptotic LCL	Asymptotic UCL	p-value
group	DIM -10	11.730	-7.245	30.704	0.2257
non-SCK	DIM -9	-17.890	-35.458	0.321	0.0460
($n = 44$)	DIM -8	-12.966	-28.816	2.884	0.1089
vs.	DIM -7	-10.976	-25.334	3.382	0.1340
group SCK	DIM -6	1.675	-12.328	15.678	0.8146
($n = 29$)	DIM -5	-6.170	-19.894	7.555	0.3783
	DIM -4	-17.363	-30.304	4.422	0.0085
	DIM -3	7.691	-5.021	20.403	0.2357
	DIM -2	-0.419	-12.813	11.975	0.9472
	DIM -1	11.566	-0.499	23.630	0.0603
	DIM 1	11.673	-0.306	23.653	0.0561
	DIM 2	1.527	-10.43	13.483	0.8024
	DIM 3	9.355	-2.541	21.250	0.1232
	DIM 4	-1.481	-13.299	10.337	0.8060
	DIM 5	6.909	-4.909	18.727	0.2519
	DIM 6	14.941	3.159	26.723	0.0129
	DIM 7	14.682	2.900	26.464	0.0146
	DIM 8	5.305	-6.477	17.086	0.3775
	DIM 9	5.260	-6.606	17.125	0.3850
	DIM 10	15.735	3.744	27.727	0.0101
	DIM 11	13.971	2.105	25.836	0.0210
	DIM 12	6.743	-5.039	18.525	0.2620
	DIM 13	12.471	0.653	24.289	0.0386
	DIM 14	9.230	-2.589	21.048	0.1258
	DIM 15	4.867	-6.915	16.648	0.4182
	DIM 16	10.335	-1.447	22.117	0.0856
	DIM 17	4.507	-7.311	16.325	0.4548
	DIM 18	3.289	-8.493	15.071	0.5843
	DIM 19	5.322	-6.580	17.225	0.3808
	DIM 20	4.390	-8.395	17.175	0.5009
	DIM 21	4.711	-11.584	21.005	0.5710

group non-SCK: cows not affected with SCK; group SCK: cows with SCK; DIM: days in milk; SE: Standard Error; CI: Confidence Interval; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit

activity, it is interesting to note that, although the activity of clinically sick cows tended to increase, there is a statistically significant increase in activity from antepartum to postpartum only in cows not developing a clinical disease or SCK after calving (Figs. 2a and 3a). We are unaware of previous studies that have identified a similar markable increase in activity from antepartum to postpartum only in healthy cows. Clinically healthy cows (group H) had significantly higher activity levels during the postpartum period than those that showed signs of a clinical disease after calving (group S), which is consistent with results from previous studies (Edwards and Tozer, 2004; Liboreiro et al., 2015; Stangaferro et al., 2016a, b, c; Steensels et al., 2017). It is difficult to

determine whether the lower postpartum activity levels of group S (compared to group H) are a cause or a consequence of the occurring diseases, as we focused on the timing of calving rather than the timing of diagnosis. Since diseases occurred relatively early in this study (median day of occurrence = DIM 2), it can be assumed that the lower postpartum activity of sick cows is more likely a consequence of the disease. Other studies took a different approach and observed a decline in activity several days before clinical diagnosis (Edwards and Tozer, 2004; Stangaferro et al., 2016a; King et al., 2017; Steensels et al., 2017). In these cases, one might conclude that reduced activity is a cause of the subsequent development of disease. The truth likely lies somewhere in

between: an impairment of welfare during the early stages of disease development (even before clinical symptoms become clearly visible) certainly leads to reduced physical activity. Conversely, reduced activity is likely associated with decreased feed intake (as cows visit the feeding area less frequently), which in turn leads to a more severe negative energy balance and, subsequently, increased susceptibility to diseases. Ultimately, it may not be crucial whether lower postpartum motion activity is the cause or consequence of diseases; in any case, it can be used for the early detection of cows at risk of disease.

Comparing the antepartum activity levels between these two groups in the current study, healthy cows tended to be more active than cows that developed a clinical disease after calving (Fig. 2a). These observations match the study of Luo et al. (2022), that showed that physical exercise antepartum reduces metabolic stress in transition cows and suggested that thereby the overall health of postpartum cows is improved. Similarly, Goselink et al. (2020) found that physical exercise during the dry period influences the lipid metabolism of cows before calving. Antepartum lipid mobilization and utilization are thus induced, facilitating an early metabolic adaption to the onset of lactation. This may reduce the risk of postpartum hepatic lipidosis. Consequently, physical exercise before calving appears to have a beneficial effect on the health of fresh cows, a conclusion that is also supported by our observations.

Even if SCK cows were more active than non-SCK cows on days 4 and 9 before calving, no differences were found between the average daily activity levels of the two groups calculated over the last two weeks before calving. This fits the findings of Liboreiro et al. (2015), who found that the occurrence of SCK was not associated with the antepartum activity. The observed activity patterns of SCK cows and non-SCK cows during the postpartum period (Fig. 3a) are consistent with the results of several other studies: cows with SCK showed markedly reduced motion activity postpartum compared with non-SCK cows (Edwards and Tozer, 2004; Liboreiro et al., 2015; Najm et al., 2020). However, the difference in postpartum activity levels reported by Najm et al. (2020) was greater than it was in the current study (52.6 % and 16.1 %, respectively). These differing results could be based on the different observation periods, since our results refer to the first three weeks of lactation, whereas Najm et al. (2020) considered the period of 4–70 DIM. The longer observation period in the latter study may have allowed the long-term changes in the behavior of SCK cows, as described by King et al. (2018), to become more apparent. Moreover, health disorders other than ketosis, that may have had an impact on postpartum activity as well, were not taken into account in the study of Najm et al. (2020). Herein, the influence of clinical diseases on activity was investigated separately. Thus, only clinically healthy cows affected and not affected with SCK were compared in this part of the trial.

Similar to Liboreiro et al. (2015), all cows, regardless of their group affiliation, showed an increase in activity as they approached calving. Contrary to their results, those suffering postpartum from clinical disease or SCK showed markedly lower increase in motion activity between day 2 before and day 1 after calving than healthy cows did (Fig. 2b and 3b).

Applying the results observed at group levels to the level of the individual cow, we found that with the mere distinction between “increase” and “no increase” it was not possible to satisfactorily identify cows at risk of clinical disease or SCK. Setting a threshold for the increase in activity from antepartum to postpartum improved the results at least for cows developing a clinical disease after calving. In this study, 63 % of those that later became clinically sick would have been correctly identified as at risk of disease by falling below the threshold of 15 % activity increase (Fig. 2c). However, 37 % of those that later developed a clinical disease would still have remained undetected because their activity increased by more than 15 % (Fig. 2c). Therefore, they would have been wrongly classified as “not at risk of disease”. A possible explanation for the uncertainty of these results and for the difficulty of establishing a general threshold for the activity increase may be the wide range of

clinical diseases, which also differ in severity within a disease and affect the well-being of the cow to different extents. For example, Stangaferro et al. (2016a), (2016b), (2016c) reported different sensitivities and specificities in their HIS alert system depending on the type and severity of the investigated diseases. We already know that the changes in activity patterns of sick cows or cows at risk of disease are rather nonspecific and do not (yet) allow any inference about the underlying disease (Steensels et al., 2017). Furthermore, a cow's individually varying physical response to the same disease certainly also plays a role. All these factors lead to a large variation in changes in motion activity, as evidenced by the partly large confidence intervals. Unfortunately, it was not possible to identify a suitable threshold for the activity increase that showed a statistically significant association with group affiliation of clinically healthy cows affected and not affected with SCK. This could be due to the large physiological variation in individual movement behavior among healthy cows, with some cows being more active than others. As mentioned earlier, this study only included clinically healthy cows in this part of the trial, which were identified solely based on elevated BHBA results. Herein, the changes in motion activity from antepartum to postpartum in clinically healthy cows ranged from a decrease in activity of up to 76.4 % to an increase in activity of up to 482.1 %. These results, along with the partly large confidence intervals, show the huge range in the physiological variation of motion activity in healthy cows, thus also explaining the big difficulty of determining a general threshold for the desired increase in activity.

The development of an animal-specific threshold would therefore be desirable and should be an approach for future research to enable a better early detection of an individual cow at risk of disease or SCK.

Serum total calcium concentrations were additionally determined in this study to account for the potential influence of blood calcium levels on motion activity around calving. Surprisingly, all cows, regardless of their group affiliation, had blood calcium levels below the threshold in the current study. This fact led us to conclude that calcium was not decisive for the observed changes in motion activity in our study. Other authors also found no correlation between calcium concentrations and activity (Liboreiro et al., 2015). The occurrence of subclinical hypocalcemia was neither associated with antepartum nor with postpartum motion activity in their study. However, further research should be done to investigate the effect of subclinical hypocalcemia on periparturient motion activity.

We deliberately chose to use healthy cows as comparison groups instead of comparing with the herd average. King et al. (2018) reported that cows with health disorders deviate in their productivity and behavioral variables from healthy cows before they deviate from the herd average or their own baseline trajectory. They therefore propose to integrate a reference group of healthy cows with similar DIM into existing algorithms of health monitoring systems. Such a comparison with a healthy control group could, in their view, improve the ability of early disease detection models to discover subtle deviations in those variables (King and DeVries, 2018). And in fact, the differences in movement behavior between the healthy control group and the cows with SCK were rather subtle in this study. Comparing the SCK cows to the herd average, these differences would probably not have been noticeable.

Since Edwards and Tozer (2004) and King et al. (2017) showed that the movement behavior is the first parameter to change in ketotic cows, our study focused only on investigating motion activity. Other studies also looked for potential changes in further parameters, such as rumination time and milk yield, associated with various diseases and used them for early disease detection models (Edwards and Tozer, 2004; Stangaferro et al., 2016a; King et al., 2017, 2018). Combining our findings on the activity increase with these other parameters may enhance the development of a reliable early warning system for cows at risk of disease and should be further investigated in future studies.

As our study primarily addressed the diagnosis of subclinical ketosis and clinical diseases collectively, without differentiating between

specific disease types or severities, future research should investigate the changes in motion activity from antepartum to postpartum periods in relation to distinct diseases and their respective severity levels.

In this study, both conventional P -values ($P \leq 0.05$) and tendential P -values ($0.05 < P \leq 0.10$) were used. While P -values of $P \leq 0.05$ were considered sufficient to reject the null hypothesis (no differences in activity levels between groups) and were regarded as statistically significant, tendential P -values were interpreted as indications of potential trends and subtle associations. By considering statistical tendencies, subtle trends in activity levels which could be associated with the development of SCK or other diseases, were better detected. It is important to emphasize that these tendential P -values should not be interpreted as definitive evidence of a significant effect, but rather as indications of possible associations. A less stringent significance level ($\alpha = 0.10$) was incorporated here, as it can be useful to identify small, yet potentially interesting effects that should be further investigated in subsequent studies (Sterne and Davey Smith, 2001). This combination of methods allows for the identification of both, robust significant results and interesting tendencies that require further confirmation. Since subclinical ketosis is challenging to define, diagnose, and systemically investigate, relying solely on the conventional significance threshold ($P \leq 0.05$) may excessively increase the risk of Type II errors (failing to detect an existing effect). Therefore, in our study, we also considered tendencies ($0.05 < P \leq 0.10$) as potentially informative results, acknowledging that strict categorization based exclusively on the traditional P -value threshold of 0.05 may be overly restrictive for evaluating subtle and complex conditions such as subclinical ketosis.

5. Conclusion

At group level, the movement behavior of cows around calving showed similar patterns in SCK-affected cows and cows with clinical diseases: activity levels before calving were similar to those of the healthy control groups, but after calving, healthy cows were significantly more active than those that developed SCK or a clinical disease. The significant increase in activity from antepartum to postpartum observed in healthy cows was absent in affected cows, though the difference was less pronounced in SCK cows than in clinically diseased cows. At the individual animal level, setting a general threshold for activity increase as an early warning to flag up cows at risk of disease or SCK is challenging due to large individual variation in movement behavior. A more useful approach would be to develop animal-specific thresholds based on individual movement changes. Nonetheless, this study identified the lack of activity increase from antepartum to postpartum as a potential indicator of fresh-cow disorders, which could be used as part of an early warning system. Further research should explore combining productivity and behavioral variables to develop a reliable early detection system for cows at risk of disease, especially for subtle health issues like SCK.

CRedit authorship contribution statement

Rainer Martin: Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Charlotte Ahrens:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Holm Zerbe:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Yury Zablotzki:** Writing – review & editing, Software, Formal analysis.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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