



## Metabolic-digestive clinical disorders of lactating dairy cows were associated with alterations of rumination, physical activity, and lying behavior monitored by an ear-attached sensor

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### ABSTRACT

The objective of this observational cohort study was to characterize the pattern of rumination time (RT), physical activity (PA), and lying time (LT) monitored by an automated health monitoring system, based on an ear-attached sensor, immediately before, during, and after clinical diagnosis (CD) of metabolic-digestive disorders. Sensor data were collected from 820 lactating Holstein cows monitored daily from calving up to 21 DIM for detection of health disorders (HD). Cows were grouped retrospectively in the no-clinical health disorder group (NCHD;  $n = 616$ ) if no HD were diagnosed, or the metabolic-digestive group (METB-DIG;  $n = 58$ ) if diagnosed with clinical ketosis or indigestion only. Cows with another clinical health disorder within  $-7$  to  $+7$  d of CD of displaced abomasum, clinical ketosis, or indigestion were included in the metabolic-digestive plus one group (METB-DIG+1;  $n = 25$ ). Daily RT, PA, and LT, and absolute and relative changes within  $-7$  to  $+7$  d of CD were analyzed with linear mixed models with or without repeated measures. Rumination time and PA were smaller, and LT was greater for the METB-DIG and METB-DIG+1 group than for cows in the NCHD group for most days from  $-7$  to  $+7$  d of CD of HD. In general, daily RT, PA, and LT differences were larger between the METB-DIG+1 and NCHD groups than between the METB-DIG and NCHD groups. In most cases, RT and PA decreased to a nadir and LT increased to a peak immediately before or after CD of HD, with a return to levels similar to the NCHD group within 7 d of CD. Absolute values and relative changes from 5 d before CD to the day of the nadir for RT and PA or peak for LT were different for cows in the METB-DIG and METB-DIG+1 group than for the NCHD group. For PA, the METB-DIG+1 group had greater changes than the METB-DIG group.

For cows affected by metabolic-digestive disorders, RT, PA, and LT on the day of CD and resolution of clinical signs were different than for cows in the NCHD group, but an increase in RT and PA or a decrease in LT was observed from the day of CD to the day of resolution of clinical signs. We conclude that dairy cows diagnosed with metabolic-digestive disorders including displaced abomasum, clinical ketosis, and indigestion presented substantial alterations in the pattern of RT, PA, and LT captured by an ear-attached sensor. Thus, automated health monitoring systems based on ear-attached sensors might be used as an aid for identifying cows with metabolic-digestive disorders. Moreover, RT, PA, and LT changes after CD might be positive indicators of recovery from metabolic-digestive disorders.

**Key words:** dairy cow, health monitoring, sensor, automation, ear-attached sensor

### INTRODUCTION

Poor adaption to the acute physiological, endocrine and metabolic changes experienced by lactating dairy cows during the transition from gestation to lactation predisposes cows to suffer from health disorders (HD; Bell, 1995; Goff and Horst, 1997). For example, clinical metabolic-digestive disorders, including hypocalcemia, clinical ketosis, indigestion, and displaced abomasum along with mastitis and metritis, are observed in early lactation (Ribeiro et al., 2013; Macmillan et al., 2021) with a reported overall incidence in commercial farms ranging from 30 to 37% (Ribeiro et al., 2013; Dubuc and Denis-Robichaud, 2017; Carvalho et al., 2019). As clinical HD impair cow well-being and reduce farm profitability through reduced milk production (Lucey et al., 1986; Bareille et al., 2003; Hostens et al., 2012) and increased treatment costs (Liang et al., 2017), greater likelihood of herd exit (Gröhn et al., 1998; Dubuc and Denis-Robichaud, 2017; Probo et al., 2018), and reduced reproductive performance (Carvalho et al., 2019; Pinedo et al., 2020), most dairy farms implement cow herd health monitoring programs (Espadamala

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et al., 2016; USDA, 2018). Typically, these programs include a combination of methods for identifying cows with clinical signs of disease, such as visual inspection, palpation, evaluation of body temperature, and clinical tests, while cows are restrained in headlocks, palpation lanes, chutes, or milking parlors (Espadamala et al., 2016). Systematic implementation of these cow health monitoring programs on tens or hundreds of cows per day can be time-consuming for farm personnel and disrupt cow routines (Mattachini et al., 2013). Moreover, methods for identifying cows with HD can be cumbersome and subjective because of variation in execution among farm personnel (Espadamala et al., 2016).

Automated health monitoring systems (AHMS), including wearable sensors that monitor behavioral, physiological, and performance parameters, might enhance, complement, or replace, partially or totally, other methods used for identifying cows with HD. These automated health screening technologies can only be effective if cows affected by HD manifest consistent alterations of the patterns of sensor-monitored parameters. Likewise, the deviations from expected trajectories and temporal shifts around clinical manifestation of HD must be of sufficient magnitude to be detected through visual inspection, statistical methods, or data analytical techniques (Rutten et al., 2013). In studies in which cows were fitted with accelerometer-based neck- (Soriani et al., 2012; Liboreiro et al., 2015) or ear-attached (Stevenson et al., 2020) sensors, cows affected by HD presented different trajectories of rumination, eating, physical activity (PA), and lying behaviors after calving when compared with cows not diagnosed with HD. Others documented temporal shifts in the patterns of behavioral parameters monitored by sensors immediately before, during, and after clinical diagnosis (CD) of HD in the early postpartum period. For example, using neck-attached rumination and PA monitoring tags, Stangaferro et al. (2016a,b,c) reported reduced rumination and activity in cows diagnosed with metabolic-digestive disorders, metritis, and mastitis. Likewise, Gusterer et al. (2020) reported differences in the patterns of behavioral parameters captured by an ear-attached sensor around CD of HD for cows with one or 2 HD. Although these previous studies provided valuable insights about the pattern of some behaviors monitored by AHMS, the current understanding of the effects of HD on sensor parameters with the potential value for identifying cows with HD is limited. Specifically, the effects of different types and severity of HD, the implications of more than one HD affecting a cow at the same time or within a short time frame, and the dynamics of sensor parameters from disorder diagnosis until the resolution of clinical signs (RCS) have been poorly characterized.

Therefore, the objective was to characterize the pattern of rumination time (RT), PA, and lying time (LT), as measured by an AHMS based on an ear-attached accelerometer-based sensor, immediately before, during, and after CD of HD for early lactation dairy cows. We hypothesized that HD would be associated with detectable alterations to the patterns of RT, PA, and LT captured by the ear-attached sensor compared with cows without HD. Our secondary hypotheses were that the occurrence of more than one HD at the same time or within a few days would be associated with a more notable alteration in the pattern of RT, PA, and LT than one HD, and that different types of HD would be associated with alterations of different duration and magnitude to the pattern of the parameters of interest. This manuscript presents data for cows grouped based on diagnosis of metabolic-digestive disorders as the primary disorder of interest, whereas a companion manuscript presents data for cows grouped with metritis or clinical mastitis as primary disorders of interest (Rial et al., 2023).

## MATERIALS AND METHODS

All procedures performed with cows were approved by the Animal Care and Use Committee of Cornell University (protocol #2017-0024) and University of Minnesota (protocol #1912-37705A).

### Cows and General Management

This study was conducted at a commercial dairy farm located in Long Prairie, Minnesota from January to September of 2020. This farm was selected for the study because the AHMS required for data collection was already installed and functional, DairyComp305 (ValleyAg Software, Tulare, CA) was used for dairy herd management, and the farm management team agreed to allow the research team to conduct the study procedures. The farm housed an average of 1,259 (range 1,231 to 1,287) milking and 166 (range 139 to 203) dry Holstein cows during the study period. Daily milk yield per cow during the study period for the herd was 37.2 kg/d (range 34.9 to 39.4 kg/d) and projected 305-d milk yield for cows that calved during the study period was 10,949 kg (range 3,629 to 18,048 kg). Cow numbers and milk yield data were retrieved from the dairy herd management software.

During the prepartum period, cows were housed in nulliparous and parous cow pens in the same freestall 3-row barn with deep, sand-bedded stalls, and fans and sprinklers for heat abatement. Every 1 h, farm personnel monitored cows visually during a walkthrough of each prepartum cow pen to identify cows with overt

signs of calving. Cows with signs of impending calving (i.e., observation of allantoic sac or calf, restlessness) were moved to a maternity pen bedded with straw. Calving was monitored and assistance was provided when needed, as determined by the attending calving manager. Immediately after calving, cows were milked once in a single side 3 milking unit parlor adjacent to the maternity pen. All multiparous cows received calcium and vitamin D3 supplementation through a ruminal bolus (Transition, MAI Animal Health, Elwood, WI) within 2 h of calving. Within approximately 3 to 12 h after calving, cows were transported to lactating cow 3-row barns where primiparous and multiparous cows were commingled in a single pen until 15 to 21 DIM.

Cows were milked 3 times daily at approximately 8 h intervals in a double-18 parallel parlor equipped with individual stall milk meters (Dematron 75, GEA, Düsseldorf, Germany) for capturing milk weights at every milking.

Cows had *ad libitum* access to water and a TMR delivered 2×/d and pushed 1×/h. The diets provided to cows during the prepartum period and in the fresh pen were the same for primiparous and multiparous cows. The prepartum TMR consisted of (DM basis) corn silage (38.1%), grass hay (18.9%), wheat straw (18.9%), corn gluten feed pellets (7.4%), canola meal (7.4%), soybean meal 47% (4.2%), and a dry cow mineral mix (5.1%). The TMR provided in the fresh pen consisted of (DM basis) corn silage (29.9%), alfalfa haylage (18.0%), dry corn (14.1%), high-moisture corn (14.1%), soybean meal 47% (5.0%), a lactating base mineral mix (5.0%), corn gluten feed pellets (4.9%), molasses mix (2.9%), canola meal (2.9%), wheat straw (1.1%), energy booster (1.1%), and a fresh cows vitamin and mineral pack (1.0%). A summary of a representative diet during the study period and the nutrient composition of the diet fed during the study period is presented in Supplemental Table S1 (<https://hdl.handle.net/1813/113489>).

### Automated Health Monitoring System

All cows were fitted with an ear-attached electronic accelerometer-based sensor tag as part of an AHMS (Smartbow, Zoetis, Parsippany, NJ) a minimum of 2 mo before expected calving. The tag (52 × 36 × 17 mm and weight of 34.1 g) was attached approximately to the upper third of the inner surface of the outer left ear cartilage. Research personnel monitored sensor placement and functionality for individual cows at enrollment and twice weekly until the end of the study.

The health monitoring system recorded RT (min/h), LT (min/h), and PA. For PA, the total min of every hour of the day were split into 3 mutually exclusive pos-

sible states of PA (i.e., inactive, active, and high active time). Thus, the system reported minutes of inactive, active, and high active time per hour. For this study, PA was generated by the summation of total minutes of active time and high active time per hour. Behavioral data (i.e., RT, PA, and LT) for each hour of the day was stored by the system software if at least 40 min of data per hour were available. Hourly periods that did not meet this criterium were included as missing data points for that hourly period. Failing to transfer data from the sensor to the system server was another reason for missing data. Altogether, the percentage of cow-days missing one or more hourly periods and removed from the data set was 9.4% (1,633/17,421).

Validation of RT data from the Smartbow system has been reported elsewhere (Borchers et al., 2016; Reiter et al., 2018). Validation results for LT and PA data from the Smartbow system have not been reported in peer reviewed publications and are not available from the manufacturer. Physical activity data created based on the combination of active and high active as used in this study has been validated for monitoring estrous behavior in lactating dairy cows (Schilkowsky et al., 2021).

### Study Design

The study followed a prospective cohort design. All cows that calved and initiated a lactation during the study period were eligible for enrollment. No exclusion criteria were implemented. Cows were enrolled in and remained in the study until 21 DIM or until exited the herd. Data from the AHMS was collected until cows were removed from the study. In total, 871 cows (314 primiparous and 557 multiparous) were enrolled at calving.

From 2 DIM until cows left the fresh pen (mean ± standard deviation: 18 ± 3 DIM), cow health was evaluated by 2 members of the research team in collaboration with a trained farm technician after the morning milking (~0600 to 0645 h). From 2 to 10 DIM, a screening procedure was implemented to identify cows for clinical examination while cows were restrained in self-locking headlocks and had access to a TMR.

The screening step conducted for all cows included visual observation (VO), recording of rectal temperature using an electronic thermometer (GLA M700, GLA Agricultural Electronics, San Luis Obispo, CA), evaluation of concentrations of acetoacetic acid in urine (Ketostix, Ascensia Diabetes Care US Inc., Parsippany, NJ), and milk deviation. Visual observation was conducted by a member of the team positioned in front of the cows to determine general attitude (normal = alert, head and ears held up, eyes bright and no recession

into the orbit; depressed = unresponsive, droopy ears, sunken eyes, lethargic), nasal discharge (0 = normal serous discharge; 1 = minimal cloudy discharge; 2 = cloudy and excessive discharge; 3 = copious, bilateral mucopurulent nasal discharge), and to identify cows with apparent poor appetite (i.e., not eating TMR or evidence that cow did not eat TMR). Simultaneously, another technician observed cows from behind to evaluate the presence of fetal membranes, udder appearance (i.e., shape, color, and consistency to the touch), feet appearance (i.e., shape, signs of pain), and manure consistency for cows that defecated while standing (1 = watery, 2 = loose, 3 = thick porridge forming a pile of 2 to 3 cm, 4 = well-formed and stacks in rings, 5 = dry). An exhaustive clinical examination was performed on cows that presented at least one of the following: depressed attitude, nasal discharge score  $>2$ , manure consistency scores of 1, 2 or 5, retained placenta, poor appetite, and signs of inflammation or pain in the udder, and milk deviation  $>15\%$  between 2 milking sessions.

Clinical examination conducted immediately after the screening procedure included abdominal auscultation to evaluate primary ruminal movements, abdominal finger percussion to detect resonant areas as evidence of abnormal gas accumulation, thoracic auscultation to evaluate the presence of abnormal lung sounds, and rectal palpation to evaluate rumen fill and obtain manure for determination of manure consistency, color, and odor. Palpation and milk forestripping (milk was stripped onto the floor and observed for flakes or clots) were used to evaluate conditions affecting the udder. Mastitis monitoring was also conducted during milking by milkers trained to identify signs of clinical mastitis. An aseptic milk sample was also collected for pathogen culture if mastitis was suspected. Cows identified with mastitis at milking underwent the complete clinical examination procedure after milking for confirmation of mastitis and determination of case severity. Cows with a urine ketone test outcome different from negative (trace = 5 mg/dL, small = 15 mg/dL, moderate = 40 mg/dL and large = 80 to 60 mg/dL) had a blood sample collected to determine blood circulating concentrations of BHB. Cows with pyrexia, defined as rectal temperature  $\geq 39.5^{\circ}\text{C}$  ( $103.1^{\circ}\text{F}$ ) during the cool season (January to April) and  $\geq 39.7^{\circ}\text{C}$  ( $103.5^{\circ}\text{F}$ ) during the warm season of the year (May to September), had their uterine discharge evaluated (i.e., consistency, color, and odor) after collection by transrectal uterine massage, had their udder evaluated as described above, and their lungs checked by thoracic auscultation. At 10 DIM, uterine discharge was evaluated in all cows, regardless of the outcome of the screening procedure.

After 10 DIM, all cows were evaluated daily through VO without restraint, at the same time as evaluating

cows 2 to 10 DIM. Additionally, a targeted evaluation through VO (i.e., all cows were identified and visually observed) was conducted in all cows at 14 and 21 DIM. Cows that met the criteria from the screening procedure or presented a decline in milk yield  $>15\%$  based on daily milk weight data underwent clinical examination, as described above.

All cows diagnosed with one or more HD were evaluated daily until RCS, defined as the first day at which no clinical signs of disorders were observed during the clinical examination procedure conducted, as described above.

Cows remained in the fresh pen up to 15 to 21 DIM depending on health status (i.e., removed only if had no evidence of clinical HD) and space constraints in the fresh pen. Cows that left the fresh pen were moved to high-lactating cow pens for primiparous or multiparous cows. Any cow that left the fresh pen before 21 DIM was under daily VO until 20 DIM and underwent a targeted VO evaluation at 21 DIM.

Cows that received antibiotic treatment or were diagnosed with clinical mastitis were moved immediately to a hospital pen where cows had *ad libitum* access to water and a TMR delivered  $2 \times /d$ . Cows returned to their respective pens after milk tested negative for antimicrobial residues or treatments were completed.

### Health Disorder Definitions

The following criteria were used to define HD of interest: clinical ketosis = blood BHB concentration  $\geq 1.2$  mmol/L and at least 2 of the following clinical signs: anorexia, depressed attitude, decreased primary ruminal movements,  $>15\%$  drop in milk yield and altered manure (i.e., too dry or too loose); displaced abomasum = abomasum in an abnormal position on the right or left side of the abdomen, detected by the presence of a metallic sound (i.e., “ping”), during simultaneous auscultation and finger percussion under an imaginary line traced from the tuber coxae to the elbow, and could extend from the 8th to 12th intercostal space; indigestion = either dry manure or diarrhea, lack of appetite, rumen and intestinal stasis (determined by presence of a dispersed metallic sound while simultaneously auscultating and percussion of either the right or left side of the abdomen) in absence of displaced abomasum and clinical ketosis. Other disorders of interest were defined as: metritis = watery, reddish, and fetid uterine discharge with or without pyrexia, as defined above; severe metritis = fetid, reddish uterine discharge with pyrexia and at least one of the following systemic signs of disease: depressed attitude, anorexia, dehydration, decreased primary ruminal movements, and a reduction in milk yield  $>15\%$  between 2 consecutive milking ses-



sions; mastitis = visibly abnormal milk secretion (i.e., off-color, presence of clots, or blood) with or without signs of udder inflammation, such as hardness, redness, and increased udder skin temperature to the touch; severe mastitis = visibly abnormal milk secretion with or without signs of udder inflammation, as described above, in addition to pyrexia and at least one of the systemic signs of disease described for severe cases of metritis; pneumonia = rapid and shallow breathing, moist cough, nasal discharge, wheezing, depressed attitude, with or without pyrexia. All HD definitions were adapted from Stangaferro et al. (2016a).

In addition, the following criteria were used to define health conditions that were considered potential confounders for the evaluation of outcomes of interest (RT, PA, and LT). Even though some of these are clinical HD, hereafter, these conditions will be referred to as risk factors (**RF**) and were: retained placenta = failure to expel fetal membranes for up to 24 h after calving; milk fever = inability to rise, or inability to rise and low temperature, muscle tremors and decreased ruminal motility observed up to 3 DIM; hyperketonemia = BHB concentration  $\geq 1.2$  mmol/L after a Ketostix outcome different from negative with no clinical signs of HD; and lameness, defined as a locomotion score  $\geq 3$  (1 = normal locomotion, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame).

For data analysis, cows were grouped retrospectively based on diagnosis of HD. Cows in the no-clinical health disorder (**NCHD**) group had no-clinical HD diagnosed during the study period. Cows in the metabolic-digestive (**METB-DIG**) group had only an event of clinical ketosis or indigestion and were not diagnosed with other HD during the study period. Cows in the metabolic-digestive plus one (**METB-DIG+1**) group had an event of displaced abomasum, clinical ketosis or indigestion and another metabolic-digestive or nonmetabolic-digestive disorder (metritis, mastitis, and pneumonia) within  $-7$  to  $+7$  d of CD of the metabolic-digestive disorder. Cows in all groups may or may not have had a RF recorded.

A similar approach was used for grouping cows for analysis of individual disorders of interest. Cows were retrospectively included in groups based on occurrence of clinical ketosis only (**CKET**) or indigestion only (**INDIG**) or groups in which cows were diagnosed with the disorder of interest and at least another clinical health disorder (metabolic-digestive or nonmetabolic-digestive) within  $-7$  to  $+7$  d of CD of the metabolic-digestive disorder (**DA+1** = displaced abomasum plus another HD, **CKET+1** = clinical ketosis plus another HD, and **INDIG+1** = indigestion plus another HD). All cows diagnosed with displaced abomasum had another disorder within the specified

window; therefore, there was not a displaced abomasum only group.

To test the hypothesis that cows affected by multiple disorders would have greater alterations in the parameters of interest, an additional category was created to include all cows that had multiple disorders (**MD**; cows diagnosed with 2 metabolic-digestive HD or one metabolic-digestive health disorder and a nonmetabolic-digestive health disorder during the period of interest) for use in certain analysis.

### **Blood Sample Collection and Estimation of Concentrations of Markers of Energy, Mineral, and Inflammation Status**

Blood samples were collected from a subgroup of cows in the NCHD and cows with any metabolic-digestive disorders for determination of plasma circulating concentrations of nonesterified fatty acids (**NEFA**), Ca, BHB, and haptoglobin (**Hp**). For cows with metabolic-digestive disorders ( $n = 30$ ), blood was collected on the day of CD of the disorder and the day of RCS. For cows in the NCHD group ( $n = 200$ ), blood was collected at 7, 14, 21 DIM but only the samples collected closest to the average DIM at which CD (7 DIM) and RCS (14 DIM) were observed for cows in the metabolic-digestive group were used. Blood samples were collected by puncture of the caudal vein or artery using evacuated tubes containing sodium heparin (Vacutainer BD, Franklin Lakes, NJ). After collection, samples were placed in a cooler with ice packs until processing within 1 h of collection. Samples were centrifuged at  $1,500 \times g$  for 15 min at  $4^{\circ}\text{C}$ . Plasma aliquots were harvested and transferred to Eppendorf vials for storage at  $-20^{\circ}\text{C}$  until assayed.

Plasma concentration of NEFA and Ca were analyzed at the University of Minnesota. Estimation of circulating concentrations of NEFA and Ca were conducted with an automated small-scale spectrophotometric chemistry analyzer (CataChemWell-T; Catachem Inc., Oxford, CT) using commercial kits provided by the manufacturer. Nonesterified fatty acids samples were analyzed in duplicate with quality control pooled samples ( $0.53 \pm 0.004$  mEq/L) run in triplicate. The range for the assay standard curve was  $0.1 \pm 0.007$  to  $1.5 \pm 0.01$  mEq/L. Samples with a coefficient of variation (**CV**)  $\geq 10\%$  were re-run in triplicate. The interassay CV was 16.9%. Circulating concentrations of Ca were estimated from samples run in duplicate with pooled samples ( $9.58 \pm 0.3$  mg/dL) included in triplicate. The range for the assay standard curve was  $2.6 \pm 0.2$  to  $20.4 \pm 0.3$  mg/dL. Samples with CV  $\geq 10\%$  were re-run in triplicate. The interassay CV was 13%.

Blood concentrations of BHB were analyzed immediately after sample collection using BHB strips measured

by the BHBCheck Plus (PortaCheck Inc., Moorestown, NJ).

Circulating Hp concentrations were evaluated in triplicate by a colorimetric assay that measures haptoglobin-hemoglobin complex by estimated differences in peroxidase activity as described by Eckersall et al. (1999), with further modifications as described by Brady et al. (2019). Estimations of Hp concentrations were conducted using a standard curve with reference concentrations ranging from 0 to 2.5 mg/mL (Tridelta Development Ltd., Maynooth, County Kildare, Ireland). Average concentration for a high concentration pooled sample was  $2.1 \pm 0.3$  mg/mL and for a low pooled sample was  $0.4 \pm 0.05$  mg/mL (both run once per assay). Samples with concentrations  $\geq 2.5$  mg/mL were re-analyzed after a 1:2 dilution in 2% bovine serum albumin. Measurements were done using a Spectramax 190 microplate reader. The interassay CV was 10% for the high pool and 19% for the low pool.

### Data Processing and Statistical Analyses

A sample size calculation was performed using the comparison between means option of the sample size menu of WinPepi v11.65 (Abramson, 2011). We estimated the sample size required to detect a difference in mean RT of 100 min/d with a standard deviation of 100 min (approximate standard deviation for RT from 2 to 21 DIM in data from Stangaferro et al., 2016a) for cows with no HD and 150 min (i.e., to compensate for larger variation) for cows with HD and when the ratio of sample size is 10:1 (no disorder to disorder) with a type I error rate of 5% and type II error rate of 20%. A total of 10 cows for the HD group of interest would have been required and 97 cows in the group with no HD. Thus, we enrolled sufficient cows to observe at least 10 cows with disorders (i.e., DA and INDIG) known to occur at a low incidence (1 to 2% range) and accounting for early lactation cow and data losses.

All data analyses were conducted using SAS software version 9.4 (SAS Institute Inc., Cary, NC).

Descriptive statistics and frequency distributions for daily values for the parameters of interest were generated with PROC MEANS and PROC UNIVARIATE. The normality of continuous data were evaluated before statistical analysis using graphical methods (histogram) and the Shapiro-Wilk test of normality generated by PROC UNIVARIATE. In addition, normality of residuals and homogeneity of variance for all models with continuous outcomes were evaluated using residual plots. We used univariable analysis with PROC MIXED to evaluate associations between covariates (season of calving and RF) and outcomes of interest. A covariate

was offered to multivariable statistical models if for the univariate analysis  $P < 0.10$ .

Daily patterns in minutes per day (min/d) for RT, PA, AT, HAT, LT, and daily milk yield (MY) were evaluated for the 7 d before to the 7 d after CD (d 0) for all the disorders of interest diagnosed during the study period. Two events of the same disorder were considered different events when diagnosed at least 7 d apart. For cows in the NCHD group, d 0 was considered to be the average DIM at CD for cows in the METB-DIG and METB-DIG+1 group or average DIM at CD for individual disorders. Data for the pattern of the parameters of interest were analyzed by ANOVA with repeated measurements using PROC MIXED. Four different models comparing different HD groups were developed for each outcome of interest: (1) comparing cows with NCHD, METB-DIG, and METB-DIG+1, (2) comparing cows with NCHD and DA+1, (3) comparing cows with NCHD, CKET, and CKET+1, and (4) comparing cows with NCHD, INDIG, and INDIG+1. Models for each outcome of interest included the fixed effects of HD groups of interest, time (i.e., days relative to CD), and the group by time interaction. Parity (primiparous vs. multiparous) was forced in all models as a covariate. Calving season (cold = January to April vs. warm = May to September) and records of the occurrence of at least one RF (i.e., yes or no) were offered as covariates if  $P < 0.10$  in the univariate analysis; however, covariates were removed by manual backward elimination if  $P > 0.10$ . Cow within group was included as a random effect in all models and cow was the subject of repeated measurements. Different covariance structures (i.e., compound symmetry, autoregressive order 1 with and without random effects, Toeplitz, and unstructured) were fitted to models for each response of interest. In all cases, Toeplitz minimized the Akaike's information criterion, and thus was selected as the covariance structure for all models. A similar analysis, but without repeated measures, was conducted to determine the interval in days between the nadir for RT, PA and MY and the peak for LT and the day of CD. Models were developed and selected as described above; however, only time was included as a fixed effect and models were only assessed for cows with the disorders of interest.

Additional analyses were conducted to evaluate the absolute values in units of measurement and the relative (%) differences for RT, PA, LT, and MY between 5 d before CD and the day of the nadir or peak for respective parameters. For the analysis of changes relative to CD, an interval of 5 d was used to avoid the effect of calving on the parameters of interest. The absolute change was calculated by subtracting the ab-

solute value for a parameter of interest at 5 before CD from the value observed on the day of the nadir or peak for the parameter. For relative changes, the absolute difference between the day of the nadir and 5 d before CD was divided by the value observed 5 d before CD and then multiplied by 100. Because cows in the NCHD group did not have a true nadir or peak, the value for d 0 was used as the equivalent to the day of the nadir or peak for cows with metritis and clinical mastitis. All analyses were conducted by ANOVA using PROC MIXED. Three models were run for each outcome of interest: (1) comparing cows with NCHD, METB-DIG, and METB-DIG+1, (2) comparing cows with NCHD, individual disorders (CKET, INDIG), and MD, and (3) comparing cows with NCHD and DA+1. Data for cows with DA were compared separately because all cows with this disorder had at least one other HD. Thus, all models included group of interest and parity, whereas calving season and RF were offered as covariates.

Analyses were conducted to compare sensor parameters, MY, and mean circulating concentrations of NEFA, Ca, Hp and BHB on the day of CD and RCS for cows diagnosed with metabolic-digestive disorders and the NCHD group on the day of CD and RCS. Data were analyzed by ANOVA with repeated measurements using PROC MIXED with models that included group (NCHD and metabolic-digestive disorders) and day relative to CD (i.e., day of CD or RCS) as fixed effects. Parity was forced in all models and season of calving and RF were offered as covariates. Cow was the subject of repeated measurements. The covariance structure used was heterogeneous Toeplitz because it does not assume equal variance among repeated measurements.

All explanatory variables and their interactions were considered significant if  $P \leq 0.05$  whereas  $0.05 < P \leq 0.10$  were considered difference of a marginal significance. When appropriate, the LSD post hoc mean separation test was used to determine differences between groups of means. Unless otherwise stated, all results are least squares means  $\pm$  standard error of the mean.

## RESULTS

### Proportion of Cows with Clinical HD and RF

From a total of 871 cows enrolled, 51 were excluded from data analysis: 10 cows were sold or died before 2 DIM and 41 cows had incomplete data due to sensor malfunction ( $n = 6$ ) or did not have a sensor tag from 2 to 21 DIM ( $n = 35$ ). Data from 34 cows that were sold or died from 2 to 21 DIM were included in analyses until the day of herd exit (mean days to herd exit and SD =  $11.1 \pm 8.5$  DIM). Out of the remaining 820 cows, 75.1% ( $n = 616/820$ ) did not present clinical HD, as

determined by the experimental procedures described, and thus were included in the NCHD group. Thirty-two percent ( $n = 199/616$ ) of the cows in the NCHD group presented at least one RF (hyperketonemia [ $n = 161$ ], lameness score  $\geq 3$  [ $n = 17$ ], milk fever = 3, retained placenta [ $n = 7$ ], and more than one of these disorders [ $n = 11$ ]). In contrast, 24.9% of the cows ( $n = 204/820$ ) had at least one clinical HD recorded during the study period, and out of these cows, 53.4% ( $n = 109/204$ ) had at least one RF. Out of all cows diagnosed with clinical HD, 40.7% ( $n = 83/204$ ) had at least one metabolic-digestive disorder, whereas the remaining 59.3% ( $n = 121/204$ ) had a nonmetabolic-digestive disorder and therefore, were excluded from data analysis. Data for cows with metritis and mastitis are presented in a companion manuscript (Rial et al., 2023). Out of the 83 cows diagnosed with metabolic-digestive disorders, 69.9% ( $n = 58$ ) were assigned to the METB-DIG group, whereas the remaining 30.1% ( $n = 25$ ) were assigned to the METB-DIG+1 group. The proportion of cows with metabolic-digestive disorders and DIM on the day of CD are presented in Table 1. All cows diagnosed with displaced abomasum had another concomitant metabolic-digestive disorder or nonmetabolic-digestive disorder, as defined for this study.

### RT, PA, and LT

#### Metabolic-Digestive Disorders Combined.

We observed an interaction between group and day ( $P < 0.001$ ) for RT, whereby the METB-DIG group ( $n = 58$ ) had less RT than the NCHD group ( $n = 616$ ) from  $-5$  to  $+5$  d relative to CD (Figure 1A). The METB-DIG+1 group ( $n = 25$ ) had less RT than NCHD group from  $-5$  to  $+3$  d relative to CD and had less RT than METB-DIG group from  $-5$  to  $+2$  d relative to CD. Primiparous cows had lower ( $P < 0.001$ ) RT than multiparous cows and cows that calved during the cool season had lower ( $P < 0.001$ ) RT than cows that calved during the warm season (Supplemental Table S2, <https://hdl.handle.net/1813/113489>).

An interaction between group and day ( $P < 0.001$ ) was observed for PA, whereby METB-DIG group had less PA than NCHD group from  $-5$  to  $+7$  d relative to CD (Figure 1B). Similarly, METB-DIG+1 group had less PA than NCHD and METB-DIG groups, from d  $-5$  to  $+6$  relative to CD and from  $-5$  to  $+3$  d relative to CD, respectively. Cows with RF had lower PA ( $P = 0.01$ ) than cows with no RF and cows that calved during cool season had lower PA ( $P < 0.001$ ) than cows that calved during warm (Supplemental Table S2).

For LT, there was an interaction between group and day ( $P < 0.001$ ), such that cows in the METB-DIG group had more LT than cows in the NCHD group

**Table 1.** Number and proportion of cows diagnosed with metabolic-digestive disorders and DIM at clinical diagnosis for cows evaluated from 2 to 21 DIM

| Health disorder group             | Cows (n/n) | Cows <sup>1</sup> (%) | DIM at CD <sup>2</sup> (mean $\pm$ SD) |
|-----------------------------------|------------|-----------------------|----------------------------------------|
| No-clinical HD <sup>3</sup> group | 616/820    | 75.1                  | —                                      |
| No risk factor                    | 417/616    | 67.7                  | —                                      |
| Risk factor                       | 199/616    | 32.3                  | —                                      |
| At least one HD group             | 204/820    | 24.9                  | 8.2 $\pm$ 5.2                          |
| No risk factor                    | 95/204     | 46.6                  | 9.2 $\pm$ 5.8                          |
| Risk factor                       | 109/204    | 53.4                  | 6.9 $\pm$ 4.6                          |
| All metabolic-digestive           | 83/820     | 10.1                  | 8.9 $\pm$ 5.1                          |
| METB-DIG <sup>4</sup>             | 58/83      | 69.8                  | 9.4 $\pm$ 5.4                          |
| METB-DIG+1 <sup>5</sup>           | 25/83      | 30.2                  | 7.8 $\pm$ 4.3                          |
| Displaced abomasum                | 19/820     | 2.3                   | 11.1 $\pm$ 4.5                         |
| DA+1 <sup>6</sup>                 | 19/19      | 100.0                 | 11.1 $\pm$ 4.5                         |
| Clinical ketosis                  | 48/820     | 5.8                   | 9.8 $\pm$ 5.8                          |
| CKET <sup>7</sup>                 | 27/48      | 56.3                  | 10.3 $\pm$ 5.7                         |
| CKET+1 <sup>8</sup>               | 21/48      | 43.7                  | 9.2 $\pm$ 6.0                          |
| Indigestion                       | 40/820     | 4.8                   | 9.9 $\pm$ 5.2                          |
| INDIG <sup>9</sup>                | 17/40      | 42.5                  | 9.5 $\pm$ 5.0                          |
| INDIG+1 <sup>10</sup>             | 23/40      | 57.5                  | 10.2 $\pm$ 5.5                         |

<sup>1</sup>Proportion of all disorders combined or individual disorders.<sup>2</sup>CD = clinical diagnosis.<sup>3</sup>HD = health disorder.<sup>4</sup>Cows diagnosed with one metabolic-digestive disorder (clinical ketosis or indigestion). All cows diagnosed with displaced abomasum had another clinical health disorder.<sup>5</sup>Cows diagnosed with a metabolic-digestive disorders and at least another metabolic-digestive or nonmetabolic-digestive health disorder (i.e., mastitis, metritis, and pneumonia) detected  $\pm 7$  d around clinical diagnosis.<sup>6</sup>Displaced abomasum and at least 1 other health disorder detected  $\pm 7$  d of clinical diagnosis of the disorder.<sup>7</sup>Cows diagnosed only with clinical ketosis during the period of interest.<sup>8</sup>Cows diagnosed with clinical ketosis at least 1 other health disorder detected  $\pm 7$  d of clinical diagnosis of the disorder.<sup>9</sup>Cows diagnosed only with indigestion during the period of interest.<sup>10</sup>Cows diagnosed only with indigestion during the period of interest at least 1 other health disorder detected  $\pm 7$  d of clinical diagnosis of the disorder.

from  $-4$  to  $+3$  d, and at d  $+6$  and  $+7$  relative to CD (Figure 1C). The METB-DIG+1 group had more LT than the NCHD group from  $-5$  to  $+7$  d relative to CD. Cows in the METB-DIG+1 group had more LT than METB-DIG group from  $-5$  to  $+4$  d relative to CD, except for d  $-3$ . Primiparous cows had lower LT ( $P < 0.001$ ) than multiparous cows, and cows with RF had marginally significantly greater LT ( $P = 0.09$ ) than cows with no RF, and cows that calved during the cool season had marginally significantly greater LT ( $P = 0.08$ ) than cows that calved during the warm season (Supplemental Table S2).

**Displaced Abomasum.** We observed an interaction between group and day ( $P < 0.001$ ) for RT, whereby cows in the DA+1 group ( $n = 19$ ) had less RT than cows in the NCHD group from  $-7$  to  $+5$  d relative to CD (Figure 2A). Primiparous cows had lower RT ( $P < 0.001$ ) than multiparous cows and cows that calved during cool season had lower RT ( $P < 0.001$ ) than cows that calved during warm (Supplemental Table S2).

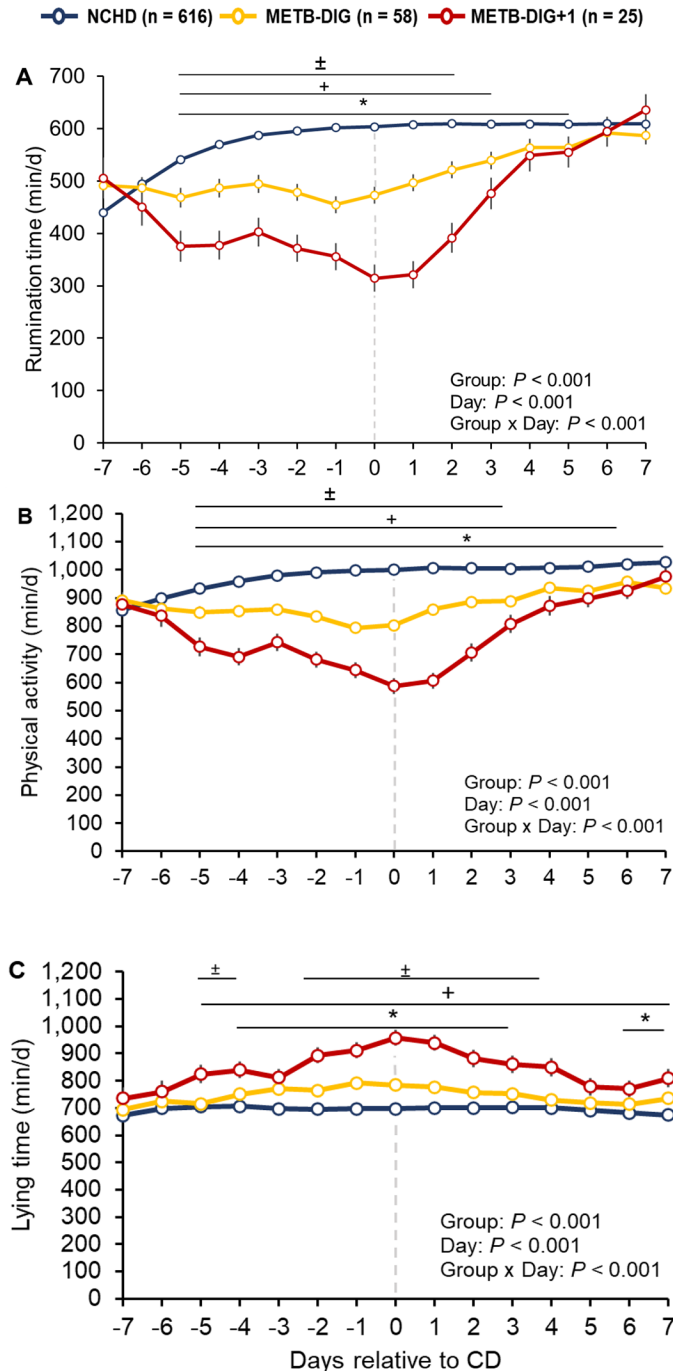
For PA, an interaction between group and day ( $P < 0.001$ ) was observed, such that cows in the DA+1 group had less PA than cows in the NCHD group from

$-7$  to  $+7$  d relative to CD, except for d  $+6$  (Figure 2B). Primiparous cows had lower PA ( $P < 0.001$ ) than multiparous cows and cows that calved during the cool season had lower PA ( $P < 0.001$ ) than cows that calved during the warm (Supplemental Table S2).

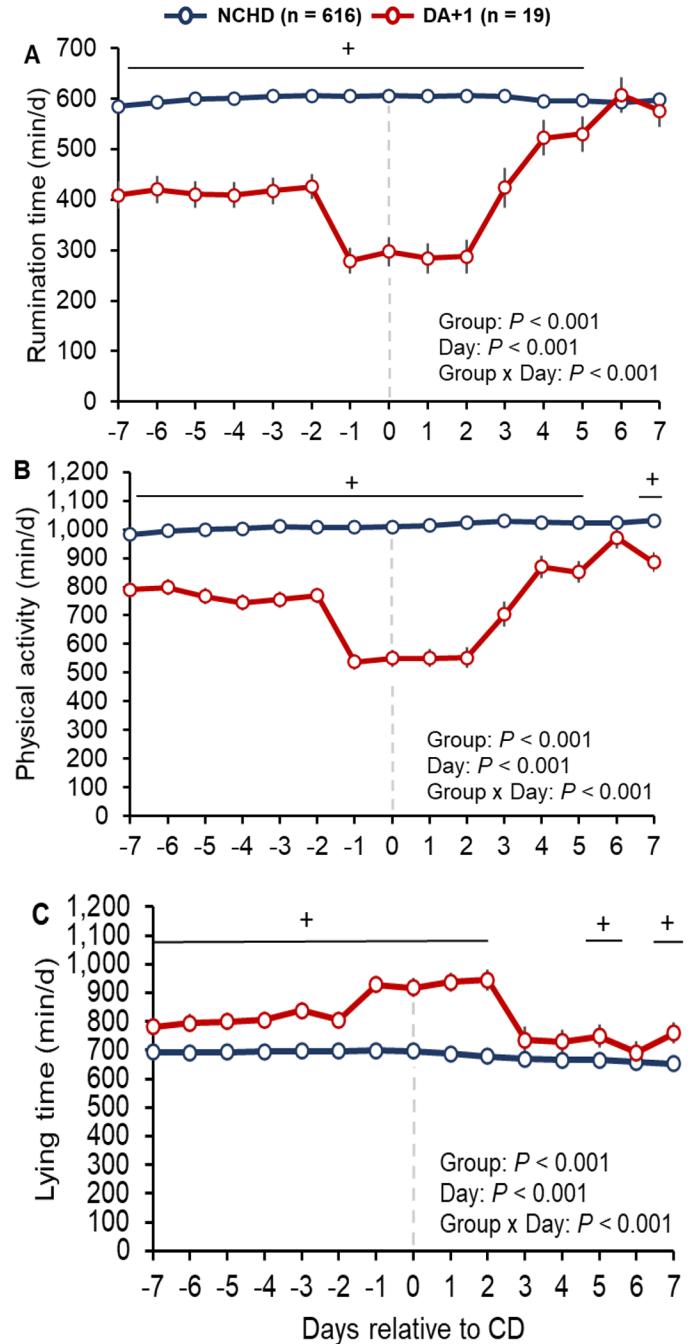
For LT, we observed an interaction between group and day ( $P < 0.001$ ), whereby cows in the DA+1 group had more LT than cows in the NCHD group from d  $-7$  to  $+2$ , and at d  $+5$  and  $+7$  relative to CD (Figure 2C). Primiparous cows had lower LT ( $P < 0.001$ ) than multiparous cows (Supplemental Table S2).

**Clinical Ketosis.** We observed an interaction between group and day ( $P < 0.001$ ) for RT, such that cows in the CKET group ( $n = 27$ ) had less RT than cows in the NCHD group from  $-4$  to  $+1$  d relative to CD (Figure 3A). Cows in the CKET+1 group ( $n = 21$ ) had less RT than cows in the NCHD group at d  $-7$ , from d  $-5$  to  $+3$ , and at  $+5$  and  $+6$  d relative to CD. Cows in the CKET+1 had less RT than cows in the CKET group at d  $-7$ , from d  $-3$  to  $+3$ , and at d  $+5$  and  $+6$  relative to CD. Primiparous cows had lower RT ( $P < 0.001$ ) than multiparous cows and cows that calved during the cool season had lower RT

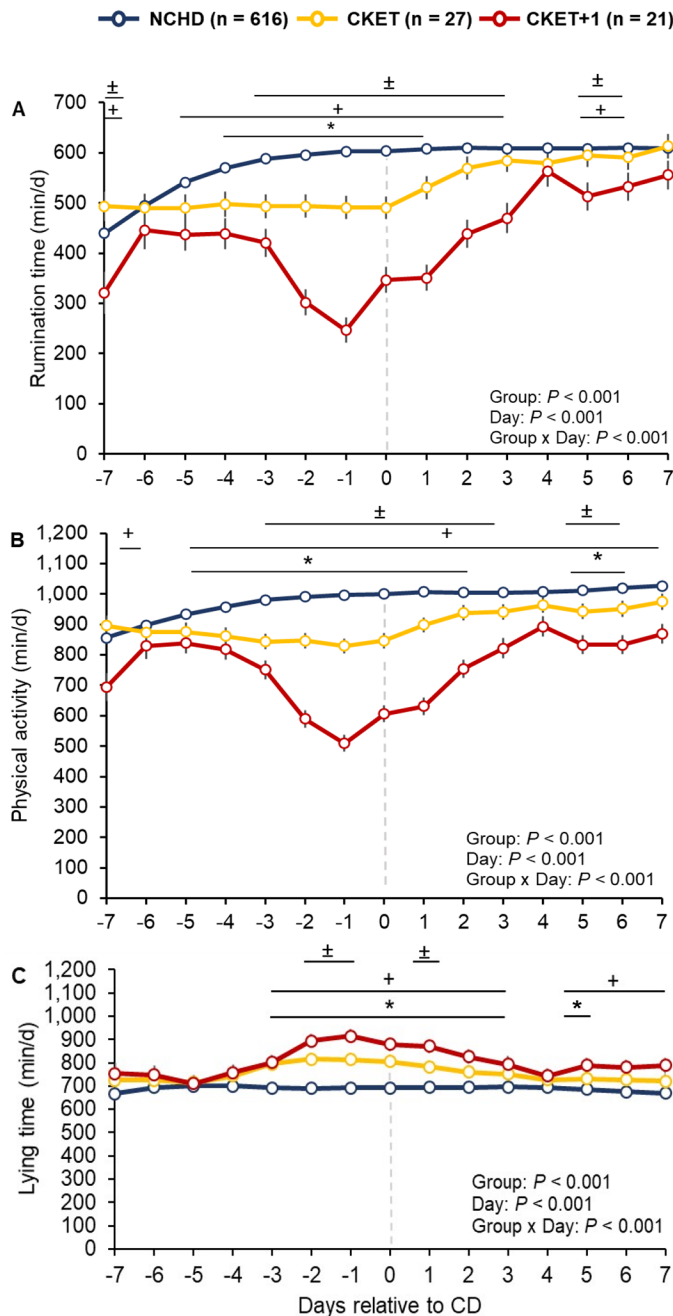




**Figure 1.** Pattern of daily rumination time (A), physical activity (B), and lying time (C) from 7 d before to 7 d after clinical diagnosis (CD) of metabolic-digestive disorders for cows diagnosed with metabolic-digestive disorders only (METB-DIG;  $n = 58$ ), a metabolic-digestive disorder and at least another health disorder (METB-DIG+1;  $n = 25$ ), and cows with no clinical health disorders diagnosed (NCHD;  $n = 616$ ) during the study period. For the NCHD group, the average DIM at CD for all cows with metabolic-digestive disorders (i.e., 9 DIM) was considered d 0. Values are LSM  $\pm$  SEM. Within a day, differences between groups based on the LSD test are represented as follows: \* METB-DIG different from NCHD; + METB-DIG+1 different from NCHD;  $\pm$  METB-DIG different from METB-DIG+1.



**Figure 2.** Pattern of daily rumination time (A), physical activity (B), and lying time (C) from 7 d before to 7 d after clinical diagnosis (CD) for cows diagnosed with displaced abomasum (DA+1,  $n = 19$ ) and cows with no clinical health disorders diagnosed (NCHD,  $n = 616$ ) during the study period. All cows with DA had at least another health disorder diagnosed. For the NCHD group, the average DIM at CD for all cows with DA+1 (i.e., 11 DIM) was considered d 0. Values are LSM  $\pm$  SEM. Within a day, differences between groups based on the LSD test are represented as follows: + DA+1 different from NCHD.



**Figure 3.** Pattern of daily rumination time (A), physical activity (B), and lying time (C) from 7 d before to 7 d after clinical diagnosis (CD) for cows diagnosed with clinical ketosis only (CKET,  $n = 27$ ), clinical ketosis and at least another health disorder (CKET+1,  $n = 21$ ), and cows with no clinical health disorders diagnosed (NCHD,  $n = 616$ ). For the NCHD group, the average DIM at CD for all cows with CKET (i.e., 11 DIM) was considered d 0. Values are LSM  $\pm$  SEM. Within a day, differences between groups based on the LSD test are represented as follows: \* CKET different from NCHD;  $\pm$  CKET+1 different from NCHD;  $\pm$  CKET different from CKET+1.

( $P < 0.001$ ) than cows that calved during the warm season (Supplemental Table S2).

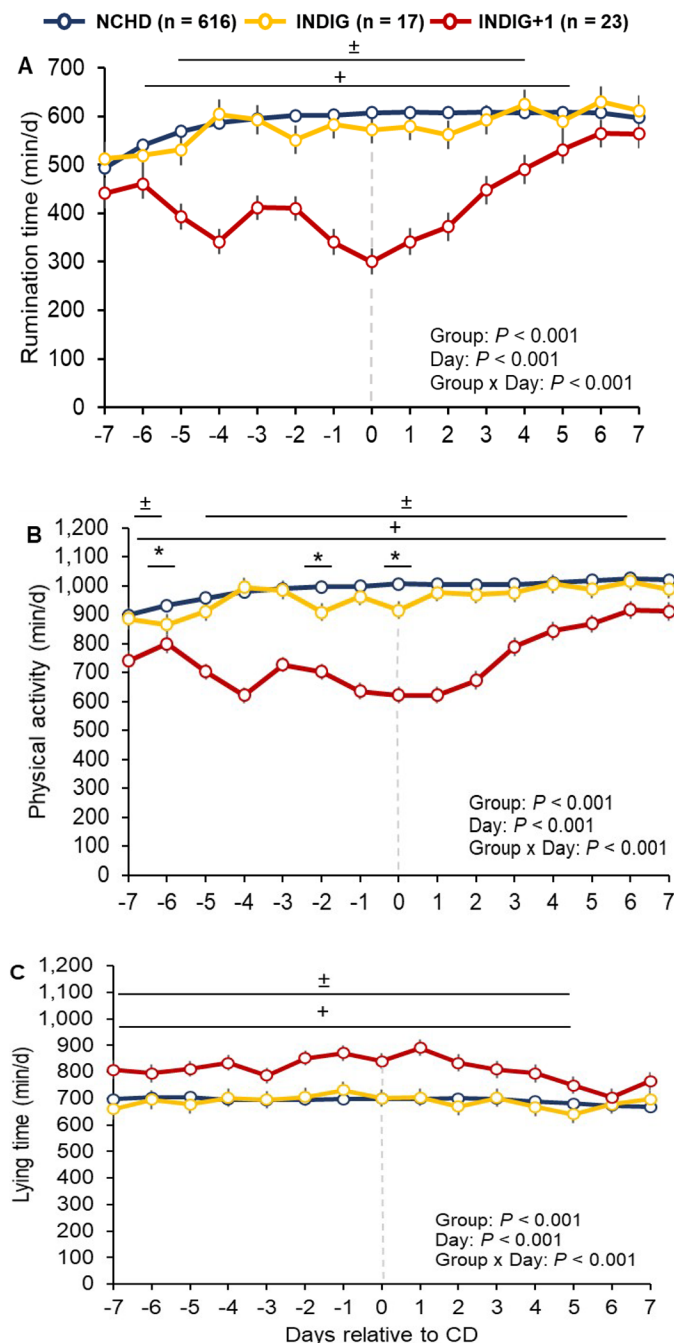
For PA, there was an interaction between group and day ( $P < 0.001$ ), such that cows in the CKET group had less PA than cows in the NCHD group from  $-5$  to  $+6$  d relative to CD, except on d  $+4$  (Figure 3B). Similarly, cows in the CKET+1 group had less PA than cows in the NCHD group from  $-7$  to  $+7$  d relative to diagnosis, except at d  $-6$ , and less PA than cows in the CKET group from  $-3$  to  $+3$ , and at d  $+5$  and  $+6$  relative to CD. Cows with RF had lower PA ( $P = 0.007$ ) than cows with no RF and cows that calved during the cool season had lower PA ( $P < 0.001$ ) than cows that calved during the warm season (Supplemental Table S2).

For LT, an interaction between group and day ( $P < 0.001$ ) was observed, whereby cows in the CKET group had more LT than cows in the NCHD group from d  $-3$  to  $+3$ , and d  $+5$  relative to CD (Figure 3C). Similarly, cows in the CKET+1 group had more LT than cows in the NCHD group from  $-3$  to  $+7$  d relative to CD, except for d  $+4$ . The CKET+1 had more LT than the CKET group at d  $-2$ ,  $-1$ , and  $+1$  relative to CD. Primiparous cows had lower LT ( $P < 0.001$ ) than multiparous cows (Supplemental Table S2).

**Indigestion.** We observed an interaction between group and day ( $P < 0.001$ ) for RT, whereby the cows in the INDIG+1 group ( $n = 23$ ) had less RT than the cows in the NCHD from  $-6$  to  $+5$  d relative to CD (Figure 4A), and less RT than the cows in the INDIG group from  $-5$  to  $+4$  d relative to CD. We did not observe differences between cows in the INDIG and NCHD groups ( $P = 0.54$ ). Primiparous cows had lower RT ( $P < 0.001$ ) than multiparous cows and cows that calved during the cool season had lower RT ( $P < 0.001$ ) than cows that calved during the warm season (Supplemental Table S2).

For PA, an interaction between group and day ( $P < 0.001$ ) was observed, whereby cows in the INDIG group had less PA than cows in the NCHD group at d  $-6$ ,  $-2$  and  $0$  relative to CD (Figure 4B). Cows in the INDIG+1 group had less PA than cows in the NCHD group from  $-7$  to  $+7$  d relative to CD, and less PA than cows in the INDIG group from  $-7$  to  $+6$  d relative to CD, except for day  $-6$ . Cows with RF had higher PA ( $P = 0.01$ ) than cows with no RF and cows that calved during the cool season had lower PA ( $P < 0.001$ ) than cows that calved during the warm season ( $P < 0.001$ ; Supplemental Table S2).

For LT, we observed an interaction between group and day ( $P = 0.002$ ), whereby cows in the INDIG+1 group had more LT than cows in the NCHD group



**Figure 4.** Pattern of daily rumination time (A), physical activity (B), and lying time (C) from 7 d before to 7 d after clinical diagnosis (CD) for cows diagnosed with indigestion (INDIG,  $n = 17$ ), indigestion and at least another health disorder (INDIG+1,  $n = 23$ ), and cows with no clinical health disorders diagnosed (NCHD,  $n = 616$ ). For cows in the NCHD group, the average DIM at CD for all cows with INDIG (i.e., 10 DIM) was considered Day 0. Values are LSM  $\pm$  SEM. Within a day, differences between groups based on the LSD test are represented as follows: \* INDIG different from NCHD; + INDIG+1 different from NCHD;  $\pm$  INDIG different from INDIG+1.

and cows in the INDIG group from d  $-7$  to  $+4$  relative to CD (Figure 4C). We did not observe differences for LT between the INDIG and NCHD groups ( $P = 0.56$ ). Primiparous cows had lower LT ( $P < 0.001$ ) than multiparous cows and cows that calved during the cool season had marginally significantly greater LT ( $P = 0.06$ ) than cows that calved during the warm season (Supplemental Table S2).

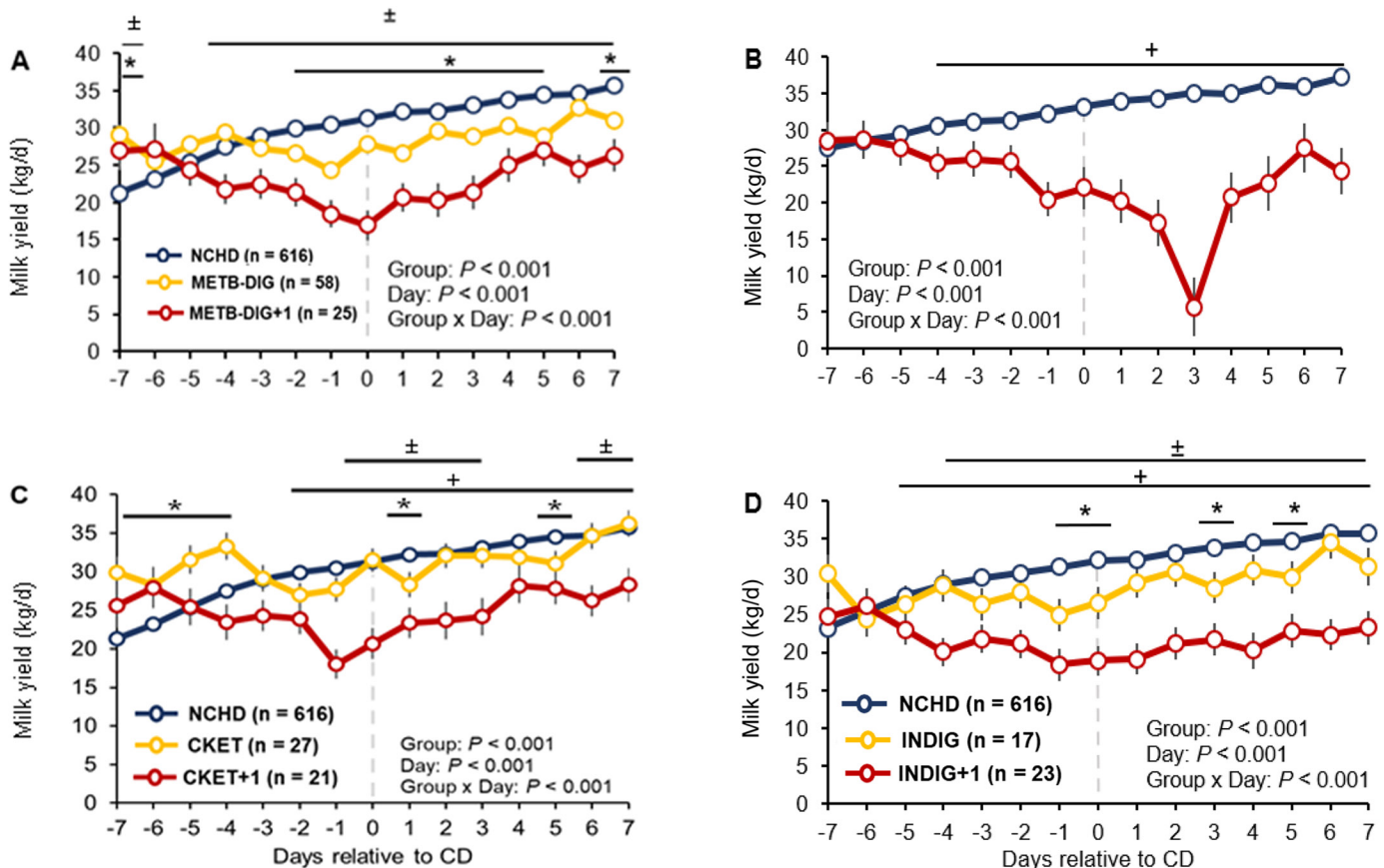
Data for AT and HAT for all analyses are presented in Supplemental Figures S1 and S2, respectively (<https://hdl.handle.net/1813/113489>).

### MY Relative to Clinical Diagnosis

**Metabolic-Digestive Disorders.** We observed an interaction between group and day ( $P < 0.001$ ), such that cows in the METB-DIG group produced more milk than cows in the NCHD group at  $-7$  d relative to CD, and then produced less milk than cows in the NCHD group from  $-2$  to  $+7$  d relative to CD, except for d  $+6$  (Figure 5A). Compared with cows in the METB-DIG+1 group, cows in the METB-DIG group produced more milk from  $-4$  to  $+6$  d relative to CD, except at d 5 relative to CD. Cows in the METB-DIG+1 group produced less milk than cows in the NCHD group from  $-4$  to 7 d relative to CD. Primiparous cows produced less milk ( $P < 0.001$ ) than multiparous cows, cows that calved during the cool season produced less milk ( $P = 0.005$ ) than cows that calved during the warm season, and cows with RF produced more milk than ( $P = 0.007$ ) cows with no RF (Supplemental Table S2).

**Displaced Abomasum.** We observed an interaction between group and day ( $P < 0.001$ ), such that cows in the DA+1 group produced less milk than cows in the NCHD group from  $-4$  to  $+7$  d relative to CD (Figure 5B). Primiparous cows produced less milk ( $P < 0.001$ ) than multiparous cows and cows with RF produced more milk ( $P = 0.005$ ) than cows with no RF (Supplemental Table S2).

**Clinical Ketosis.** We observed an interaction between group and day ( $P < 0.001$ ), whereby the CKET group produced more milk than the NCHD group from  $-7$  to  $-4$  d relative to CD (Figure 5C). Thereafter, we observed lesser MY for the CKET compared with the NCHD group on d  $+1$  and  $+5$  relative to CD. The CKET+1 group produced less milk than the NCHD group from  $-3$  to  $+7$  d relative to CD and produced less milk than the CKET group at d  $-1$ , 0, 2, 3, 6, and 7 relative to CD. Primiparous cows produced less milk ( $P < 0.001$ ) than multiparous cows, cows that calved during the cool season produced less milk ( $P = 0.02$ )



**Figure 5.** Daily milk yield for cows with metabolic-digestive disorders (A), displaced abomasum (B), clinical ketosis (C), and indigestion (D) from 7 d before to 7 d after clinical diagnosis (CD) for cows diagnosed with the respective disorder of interest only, the disorder of interest plus another health disorder, and cows with clinical health disorders (NCHD) diagnosed. For cows in the NCHD group, the average DIM at CD for all cows with the disorder of interest was considered d 0. Values are LSM  $\pm$  SEM. Within a day, differences between groups based on the LSD test are represented as follows: \* Disorder (either: METB-DIG, DA, CKET, INDIG) different from NCHD; + Disorder+1 different from NCHD;  $\pm$  Disorder different from disorder+1.

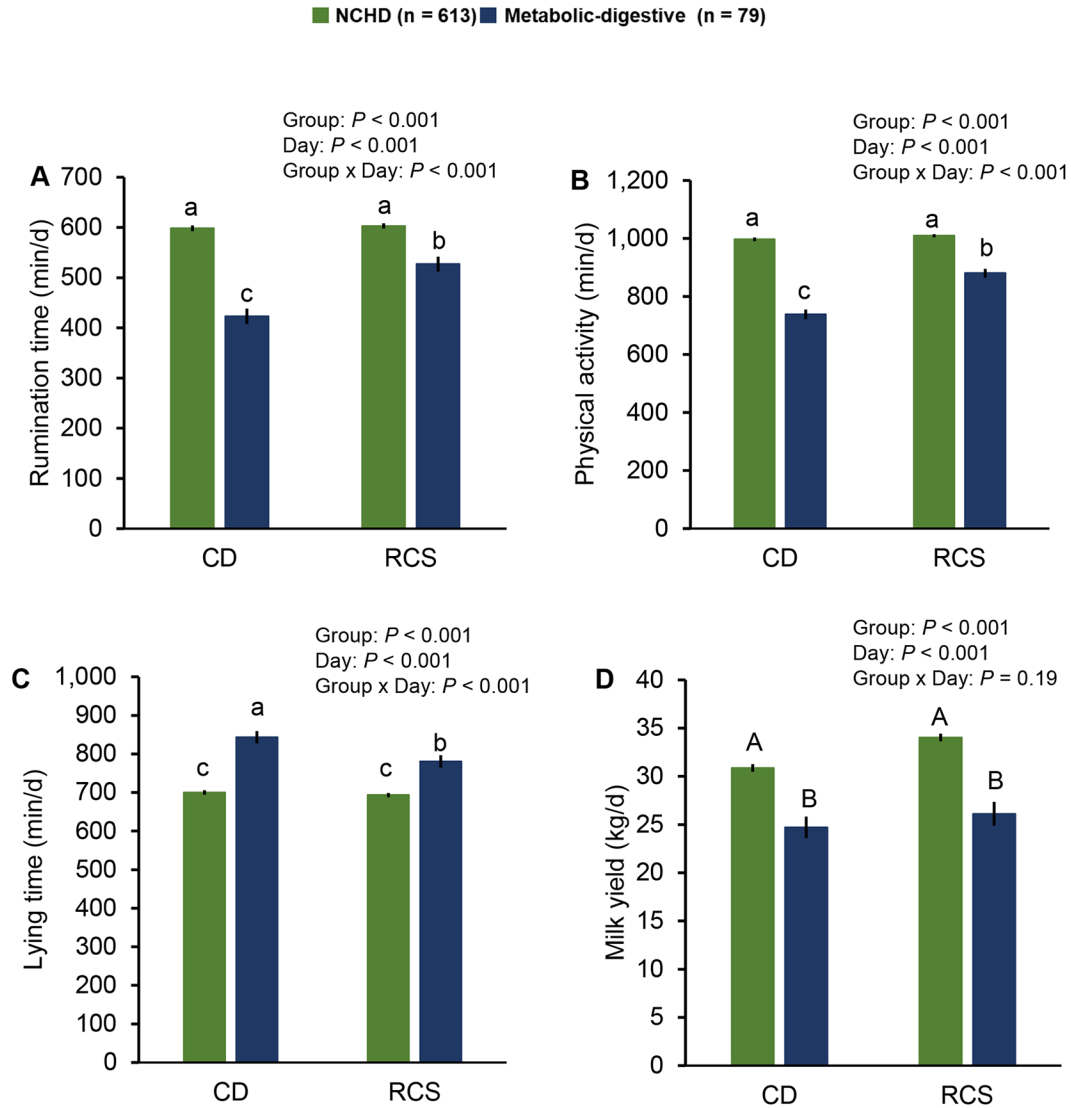
than cows that calved during the warm season, and cows with RF produced more milk ( $P = 0.002$ ) than cows with no RF (Supplemental Table S2).

**Indigestion.** Finally, we also observed an interaction between group and day ( $P < 0.001$ ) for MY for INDIG as yield for this group was less than for the NCHD group at d -1, 0, +3, and +5 d relative to CD (Figure 5D). Cows in the INDIG +1 group produced less milk than the NCHD group from -5 to +7 d relative to CD, and less milk than INDIG group from -4 to +7 d relative to CD, except for day -3. ( $P < 0.001$ ). Primiparous cows produced less milk ( $P < 0.001$ ) than multiparous cows, and cows that calved during the cool season ( $P < 0.001$ ) produced less milk than cows that calved during the warm season, and cows with RF produced more milk ( $P = 0.02$ ) than cows with no RF (Supplemental Table S2).

**Differences in Behavioral Parameters and MY Between the Day of CD and RCS.** For RT, we observed an interaction between group and day

( $P < 0.001$ ) as cows with metabolic-digestive disorders had less RT (Figure 6A) than the NCHD group on the day of CD ( $422.8 \pm 15.2$  vs.  $598.2 \pm 5.6$  min/d) and RCS ( $526.9 \pm 14.8$  vs.  $603.0 \pm 5.1$  min/d), and cows with metabolic-digestive disorders had more RT on the day of RCS than CD. Rumination time was greater ( $P < 0.001$ ) for multiparous cows than primiparous cows ( $564.2 \pm 7.7$  vs.  $511.3 \pm 9.3$  min/d), and greater ( $P < 0.001$ ) for cows that calved during the warm season than during the cool season ( $571.7 \pm 7.4$  vs.  $503.7 \pm 9.9$  min/d). For PA, there was an interaction between group and day ( $P < 0.001$ ), such that cows with metabolic-digestive disorders had reduced PA than cows in the NCHD group on the day of CD ( $738.4 \pm 16.5$  vs.  $997.1 \pm 6.3$  min/d) and on the day of RCS ( $880.4 \pm 16.5$  vs.  $1,009.4 \pm 5.5$  min/d), and both groups had reduced PA on the day of CD than RCS (Figure 6B). Physical activity was greater ( $P < 0.001$ ) for cows that calved during the warm season than during the cool season ( $930.4 \pm 7.1$  vs.  $882.2$

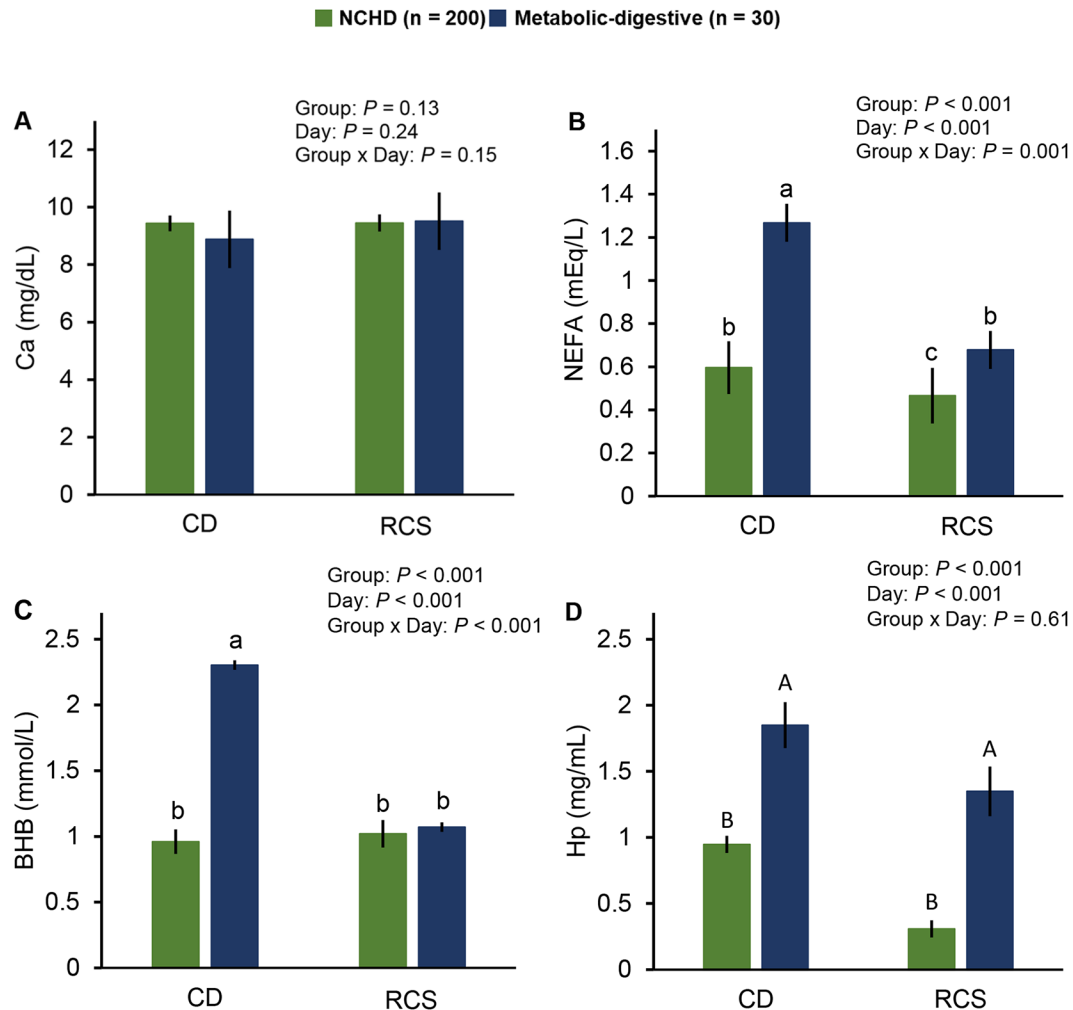




**Figure 6.** Rumination time (A), physical activity (B), lying time (C), and milk yield (D) average values on the day of clinical diagnosis (CD) and the day of resolution of clinical signs (RCS) in a subset ( $n = 79$ ) of cows diagnosed with metabolic-digestive disorders and cows with no clinical health disorders diagnosed (NCHD,  $n = 613$ ). For cows in the NCHD group, values for the day of CD and RCS were for those for the average DIM at CD (i.e., 7 DIM) and RCS (i.e., 14 DIM) for cows in the METB-DIG group. Values are LSM  $\pm$  SEM. Uppercase letter differences represent overall effects of group and lowercase letters indicate results of interactions based on the LSD test.

$\pm 10.4$  min/d) and were marginally significantly greater ( $P = 0.05$ ) for multiparous than primiparous cows ( $914.8 \pm 8.0$  vs.  $897.8 \pm 9.8$  min/d) and were marginally significantly greater ( $P = 0.07$ ) for cows with than without RF ( $898.3 \pm 9.7$  min/d vs.  $914.4 \pm 8.1$  min/d). For LT (Figure 6C), there was an interaction between group and day ( $P < 0.001$ ), such that LT was greater for cows with metabolic-digestive disorders than the NCHD group on the day of CD ( $843.3 \pm 15.8$  vs.  $700.0 \pm 5.8$  min/d) and RCS ( $780.6 \pm 16.1$  vs.  $693.3 \pm 5.6$  min/d). Additionally, cows with metabolic-digestive disorders had more LT on the day of CD than RCS. Lying time was greater ( $P < 0.001$ ) for multiparous

cows than primiparous cows ( $794.0 \pm 8.3$  vs.  $714.7 \pm 10.0$  min/d) cows, and greater ( $P = 0.03$ ) for cows that calved during the warm season than cool season ( $743.4 \pm 10.7$  vs.  $765.3 \pm 10.7$  min/d). Finally, MY (Figure 6D) was greater ( $P < 0.001$ ) for the NCHD cows than for cows with metabolic-digestive disorders ( $32.4 \pm 0.3$  vs.  $25.4 \pm 0.9$  kg/d), and overall, MY was greater on the day of RCS than CD ( $30.0 \pm 0.6$  vs.  $27.7 \pm 0.6$  kg). Milk yield was greater ( $P < 0.001$ ) for multiparous cows than primiparous cows ( $35.2 \pm 0.5$  vs.  $22.5 \pm 0.6$  kg/d), and greater ( $P = 0.01$ ) for cows that calved during the warm season than the cool season ( $29.7 \pm 0.5$  vs.  $28.0 \pm 0.7$  kg/d).



**Figure 7.** Plasma concentrations of calcium (A), nonesterified fatty acids (B), BHB (C), and haptoglobin (D) on the day of clinical diagnosis (CD) and the day of resolution of clinical signs (RCS) in a subset ( $n = 30$ ) of cows diagnosed with metabolic-digestive disorders and a subset of cows with no clinical health disorders diagnosed (NCHD,  $n = 200$ ). For cows in the NCHD group, values for the day of CD and RCS were values observed at the average DIM at CD (i.e., 7 DIM) and RCS (i.e., 14 DIM) for cows in the METB-DIG group. Values are LSM  $\pm$  SEM. Uppercase letter differences represent overall effects of group and lowercase letters indicate results of interactions based on the LSD test.

**Plasma Concentrations of Ca, NEFA, BHB, and Hp on the Day of CD and RCS.** We did not observe an association between HD group ( $P = 0.13$ ), day ( $P = 0.24$ ), or the interaction between HD group and day ( $P = 0.15$ ) for plasma Ca concentrations (Figure 7A). Conversely, we observed an interaction between group and day ( $P = 0.001$ ) for NEFA concentration (Figure 7B), such that plasma NEFA concentration was greater for cows with metabolic-digestive disorders than for cows in the NCHD group cows on the day of CD ( $1.2 \pm 0.1$  vs.  $0.6 \pm 0.08$  mEq/L) and RCS ( $0.6 \pm 0.1$  vs.  $0.5 \pm 0.08$  mEq/L), and NEFA concentrations were smaller on the day of RCS than CD for both groups. Season of calving ( $P = 0.07$ ) and RF ( $P = 0.05$ ) were marginally significantly associated with concentrations of NEFA, as NEFA concentrations were greater for cows that calved

during the warm season than the cool season ( $0.95 \pm 0.03$  vs.  $0.61 \pm 0.1$  mEq/L), and greater for cows with than without RF ( $0.83 \pm 0.09$  vs.  $0.73 \pm 0.09$  mEq/L). An interaction between group and day ( $P < 0.001$ ) was observed for plasma BHB concentrations (Figure 7C), such that BHB concentrations were greater for cows with metabolic-digestive disorders than for cows in the NCHD group on the day of CD ( $2.3 \pm 0.09$  mmol/L vs.  $0.9 \pm 0.03$  mmol/L) but not on the day of RCS ( $1.0 \pm 0.1$  vs.  $1.0 \pm 0.03$  mmol/L, respectively). Plasma BHB concentrations were greater ( $P = 0.01$ ) for cows with than without RF ( $1.4 \pm 0.04$  vs.  $1.2 \pm 0.04$  mmol/L) and were marginally significantly ( $P = 0.07$ ) greater for multiparous cows than primiparous cows ( $1.3 \pm 0.04$  vs.  $1.2 \pm 0.04$  mmol/L). Plasma Hp concentrations were greater in cows with metabolic-digestive disorders

than for cows in the NCHD group ( $1.6 \pm 0.1$  vs.  $0.6 \pm 0.04$  mg/mL) and greater ( $P < 0.001$ ) on the day of CD than RCS ( $1.4 \pm 0.09$  vs.  $0.8 \pm 0.09$  mg/mL; Figure 7D). Plasma Hp concentrations were also greater ( $P = 0.001$ ) for multiparous cows than primiparous cows ( $1.2 \pm 0.08$  vs.  $0.9 \pm 0.07$  mg/mL), and greater ( $P = 0.007$ ) for cows with than without RF ( $1.2 \pm 0.08$  vs.  $1.0 \pm 0.07$  mg/mL).

## DISCUSSION

Patterns of behavioral parameters recorded by the ear-attached accelerometer of an AHMS immediately before, during, and after clinical manifestation of metabolic-digestive disorders in lactating dairy cows were characterized in this study. In support of our primary hypothesis, cows diagnosed with the disorders of interest presented temporal shifts in the pattern of RT, PA, and LT. Rumination time and PA declined up to a nadir observed around the day of CD. Thereafter, both parameters increased to levels in the range observed before the decline around clinical disease, and similar to or slightly lower than for cows without clinical HD diagnosed at the same range of DIM. As expected, LT presented a similar behavior as RT and PA, except that levels peaked around the day of CD and declined thereafter. Our results using an ear-attached sensor agree with previous studies that reported similar patterns of RT and PA captured by a neck-attached sensor around CD (Stangaferro et al., 2016a) or the first day of health alerts (Silva et al., 2021) in dairy cows with metabolic-digestive disorders. The pattern of LT could not be directly compared with others because no previous study evaluated this parameter around CD of metabolic-digestive disorders only. Nevertheless, increases in LT before, during, and after health events (metabolic-digestive disorders included) were reported for the same ear-attached sensor used in our study (Gusterer et al., 2020). In addition, increased LT after calving has also been reported for multiparous cows with metabolic-digestive disorders (Piñeiro et al., 2019). Collectively, changes observed for RT, PA, and LT immediately before and during clinical manifestation of displaced abomasum, clinical ketosis, and indigestion demonstrates that alterations to the patterns of these sensor parameters are directly associated with these disorders. Additional evidence of a direct link between these HD and behavioral parameter changes was the dynamic of parameter values after CD. After reaching a nadir or peak, RT, PA, and LT returned to the same or approximately the same levels observed before CD by the time of RCS or within 1 wk after CD and treatment for the underlying health condition.

The magnitude of absolute and relative changes from 5 d before to the day of CD for RT, PA, and LT for cows diagnosed with metabolic-digestive disorders suggested potential for developing strategies to identify cows with metabolic-digestive disorders through monitoring of these sensor parameters. Likewise, the timing of the decline and the day of the nadir or peak for each parameter in relationship to the day of CD suggested that it might be possible to identify cows with these HD at a similar or earlier time than through traditional health monitoring programs. Although not directly comparable because of the different approach used to group cows, our results for relative changes and timing of the nadir for RT and PA agree with those reported by Stangaferro et al. (2016b). In the latter study, cows diagnosed with metabolic-digestive disorders and with health index score alerts (i.e., calculation based on RT and PA data) had significant negative relative changes in RT (30.9%) and PA (13.1%) from 5 d before to the day of CD compared with cows with no HD at the same range of DIM. Similarly, Stangaferro et al. (2016a) observed the nadir for RT and PA on the day of CD, and the day of the first health index score alert was observed either at the same time or slightly before CD of metabolic-digestive disorders. Collectively, evidence from the current and previous studies suggested that either visual inspection of sensor parameter patterns or alerts created with analytical methods, such as traditional statistics (Paudyal et al., 2018) or machine learning algorithms (Perez et al., 2020; Wagner et al., 2020; Zhou et al., 2022), could be developed and used to identify cows for examination and detection of HD. Although both visual inspection of sensor parameter data and alerts could be implemented, the latter is likely more feasible for practical application and might be more accurate by enabling a combination of multiple parameters (Perez et al., 2020; Wagner et al., 2020; Zhou et al., 2022). Visual inspection of AHMS data patterns for individual cows in real time presents major challenges including large variability within and among cows (J. O. Giordano and C. Rial, unpublished data), and the need to inspect individual cow patterns, which is labor-intensive and not practical for large herds.

Taken together, the observed temporal shifts, the magnitude of the absolute and relative changes, and the timing of the nadir and peak for RT, PA and LT around CD of metabolic-digestive disorders suggested that the AHMS used in this study including an ear-attached sensor might have practical value for the identification of dairy cows with metabolic-digestive disorders in early lactation. Nevertheless, the ability of this AHMS to identify cows with HD can only be validated through additional prospective observational

studies or randomized controlled trials under commercial farm conditions.

A better understanding of the relationship between the pattern of sensor parameters and the timing of RCS in cows diagnosed with HD adds value to data from AHMS. For the first time, our analysis for the evolution of sensor parameters from the day of CD to RCS of metabolic-digestive disorders demonstrated an association between clinical recovery and the patterns of RT, PA, and LT. Although in all cases clinical signs of the disorder were no longer observed and all behavioral parameters showed a significant increase (i.e., RT and PA) or decrease (i.e., LT) from the day of CD to the day of RCS, the sensor parameters levels were not the same as for cows not affected by HD. These observations suggested that relative gains or reductions (depending on the nature of the behavioral parameter) might be better indicators of clinical recovery than absolute sensor parameter values. Thus, relative increases and decreases in RT, PA, and LT could be used to monitor the clinical evolution and response to treatments of cows affected by metabolic-digestive disorders. The lack of a significant increase in MY from the day of CD to the day of RCS suggested that RT, PA, and LT monitored by wearable sensors, may be more sensitive indicators of RCS than MY monitoring.

In general, the concentration dynamics of blood markers of energy, inflammation, and mineral status were in line with patterns of sensor-monitored parameters. A reduction in energy intake in postpartum cows leads to fat mobilization reflected by an increase in circulating concentrations of NEFA and BHB (Ospina et al., 2010; Chapinal et al., 2012; van Hoeij et al., 2019). In our study, concentrations of NEFA and BHB for cows with metabolic-digestive disorders were not only greater than for cows without HD on the day of CD, but also declined significantly by the time of RCS. This dynamic was likely a reflection of the association between disorders and feeding behavior and are in line with the changes between the day of CD and RCS for RT, PA, and LT. The dynamic observed for Hp concentrations was most likely explained by a combination of factors. For example, 26% of the subset of cows with metabolic-digestive disorders included in the blood marker analysis had displaced abomasum. These cows typically present long-lasting inflammation (Stengärde et al., 2010) and surgical treatment (Ceciliani et al., 2012). Moreover, 30% of the cows with displaced abomasum also had systemic metritis or mastitis, which have been associated with elevated Hp concentrations (Eckersall et al., 2001; Huzzey et al., 2009; Stangaferro et al., 2016b,c). Monitoring blood concentrations of metabolic and inflammation markers and minerals can be costly. In addition, our data did not identify as

strong of an association between some markers, such as Ca and Hp, and RCS, as the behavioral parameters monitored in our study. Therefore, compared with the blood markers used in this study, sensor parameters such as RT, PA, LT may be more practical and reliable indicators of the clinical health status of cows diagnosed with metabolic-digestive disorders.

In support of our secondary hypothesis, cows in the METB-DIG+1 group presented greater shifts in the parameters of interest around CD than cows in the METB-DIG group; all parameters deviated (i.e., decreased or increased) at a faster rate and reached deeper nadirs or taller peaks, compared with cows in the NCHD group. For cows in the METB-DIG+1 group, PA also presented a greater relative change within 5 d of CD than for cows in the METB-DIG group. Similarly, cows diagnosed with MD had greater absolute and relative changes for sensor parameters than cows diagnosed with a single metabolic-digestive disorder. Our results agree with those of Stangaferro et al. (2016a) for cows diagnosed with metabolic-digestive disorders and another HD within 7 d of CD of the primary disorder. Gusterer et al. (2020) also reported that RT, measured with the same ear-attached sensor used in this study, was further reduced for cows diagnosed with more than one HD in the first 8 d after calving. This expected behavior for the sensor-monitored parameters in cows diagnosed with more than one HD was most likely due to the timing at which cows were affected by the secondary disorder (i.e., nonmetabolic-digestive or second metabolic-digestive condition) and the compounded effect of 2 different HD. Specifically, the timing at which cows were affected by the secondary condition altered the pattern of RT, PA, and LT either before or after the primary metabolic-digestive disorder was diagnosed (e.g., in one cow rumination started to decline 2 d before CD of displaced abomasum, which corresponded with the diagnosis of systemic mastitis). The compounding effect of multiple HD on the parameters monitored reflected the individual and combined effects of the primary and secondary disorder on the clinical status of cows (C. Rial and J. O. Giordano, unpublished data) and the behaviors monitored by the sensor (Gusterer et al., 2020). Indeed, it is well known that cows diagnosed concomitantly or within a few days by more than one HD manifest more intense clinical signs of disease and changes in behavior (Stangaferro et al., 2016b,c). Our MY results provide additional evidence of the compounding effect of multiple HD, as cows in the METB-DIG+1 group had a greater reduction in MY than cows in the METB-DIG group around CD of the primary metabolic-digestive disorder. Taken together, our current results support the notion that there is an association between the degree



of alteration of the pattern of behavioral parameters monitored by sensors and the clinical status of cows. Specifically, rumination, activity, and lying behaviors were altered considerably more in cows with multiple and potentially more severe clinical signs of disease. Therefore, AHMS that monitor and use behaviors such as RT, PA, and LT either directly or indirectly to generate health alerts might be more effective for identifying cows affected by HD that cause more severe alterations to cow behavior, or cows with multiple HD because these cows manifest multiple and more severe clinical signs of disease at the same time or within a timespan of a few days.

Analyzing patterns of sensor-monitored parameters for groups of cows diagnosed with more than one HD (Stangaferro et al., 2016a; Gusterer et al., 2020; Silva et al., 2021) also presents several limitations. Specifically, evaluating sensor data patterns for cows with different clinical HD is confounded by the effect of the temporal overlap of disorders, manifestation of the same clinical signs and behaviors in response to different disorders, and the effect of treatments applied at different time points. Conversely, analyzing sensor parameter patterns for cows diagnosed with specific disorders helps elucidate the precise effect of individual clinical disorders on sensor-monitored parameters (Liboreiro et al., 2015; Stangaferro et al., 2016a,b,c), serves to demonstrate that disorder severity is associated with the degree of sensor parameters changes (Stangaferro et al., 2016a,b,c), and could be used in the future to attempt predicting the type of disorder affecting individual cows (Perez et al., 2020). As expected, because of the well-known major disruption to cow health (Mokhber Dezfouli et al., 2013) and behavior caused by displaced abomasum (Radostits et al., 2006), cows in the DA+1 group presented stark differences with cows in the NCHD group for RT, PA, and LT. Data for RT and PA in our study agree with results from Stangaferro et al. (2016a) because cows with displaced abomasum had the greatest parameter changes and approximately 50% of cows with displaced abomasum had another disorder diagnosed within 7 d. Similarly, in our study, 52.4% of cows in the DA+1 group presented clinical ketosis before diagnosis of displaced abomasum, which is not surprising because hyperketonemia is a risk factor for displaced abomasum (Caixeta et al., 2018). Another reason for a more obvious alteration in sensor-monitored parameters for cows diagnosed with displaced abomasum was that on average, cases were diagnosed at slightly later DIM than cases of clinical ketosis and indigestion. Later DIM at diagnosis of HD results in more obvious shifts in RT, PA, and LT because these parameters reach a plateau and are more stable at later DIM (Gusterer et al., 2020; Stevenson et al., 2020; Banuelos and Stevenson, 2021).

Cows in the CKET or INDIG groups had less dramatic alterations to the patterns of RT, PA, and LT and, in general, the alterations were of lesser magnitude and duration than for cows in the DA+1 group. Conversely, greater alterations to sensor parameters were observed for cows with clinical ketosis and indigestion when a concomitant nonmetabolic-digestive or another metabolic-digestive disorder was diagnosed. This was in line with expectations based on the known effects and clinical manifestation of these conditions (Hart, 1988; Fogsgaard et al., 2012), and the case definitions used in our study. In addition, the mean duration for cases of indigestion was 2.2 d and 45% of the cases lasted only 1 d, which ultimately reduced the window of time to alter sensor parameter patterns. Further evidence of the milder effects of disease for cows in the single disorder groups (CKET and INDIG) was the transient and small reductions in MY, as compared with the greater losses for cows in the DA+1 group. Altogether, these observations suggested that the relatively mild effects of clinical ketosis and ingestion on cow health were the underlying reason for the less evident changes in RT, PA, and LT for cows in the CKET and INDIG groups. As such, using the evaluated AHMS to identify cows with only clinical ketosis and ingestion might be challenging.

### **Behavioral Parameters and MY Shifts up to the Nadir or Peak Around CD**

The absolute and relative changes between 5 d before CD to the day of the nadir for RT, PA, and MY, and the day of the peak for LT for cows in the NCHD, METB-DIG, and METB-DIG+1 groups are presented in Table 2. Unlike the NCHD group, which had an increase in RT, METB-DIG and METB-DIG+1 groups had a reduction in absolute and relative values for RT ( $P < 0.001$ ). Likewise, PA was reduced ( $P < 0.001$ ) for cows in the METB-DIG and METB-DIG+1 compared with the NCHD group, but the reduction was greater for the METB-DIG+1 than for the METB-DIG group. Absolute and relative values for LT were also different ( $P < 0.001$ ) among groups, as cows in the METB-DIG and METB-DIG+1 groups had more LT than cows in the NCHD group. Milk yield, which increased during the same range of DIM for the NCHD group, was reduced ( $P < 0.001$ ) in absolute and relative values for the METB-DIG and METB-DIG+1 groups. A greater reduction in the absolute values change of MY was observed for cows in the METB-DIG+1 than for cows in the METB-DIG group.

Data for absolute and relative changes for comparisons among cows in the NCHD group and cows grouped based on occurrence of individual or MD are presented

**Table 2.** Absolute values and relative changes from 5 d before clinical diagnosis to the day of the nadir for daily rumination time, physical activity, milk yield, or peak for daily lying time for cows not diagnosed with clinical health disorders, diagnosed with only one metabolic-digestive disorders, and diagnosed with a metabolic-digestive disorder and another clinical health disorder (metabolic-digestive or nonmetabolic-digestive disorder) from 2 to 21 DIM

| Item <sup>1</sup>     | Health disorder groups      |                                |                                  | P-value |
|-----------------------|-----------------------------|--------------------------------|----------------------------------|---------|
|                       | NCHD <sup>2</sup> (n = 616) | METB-DIG <sup>3</sup> (n = 58) | METB-DIG+1 <sup>4</sup> (n = 25) |         |
| Rumination time       |                             |                                |                                  |         |
| Absolute change (min) | 47.5 ± 7.3 <sup>a</sup>     | −141.1 ± 28.3 <sup>b</sup>     | −219.1 ± 43.5 <sup>b</sup>       | <0.001  |
| Relative change (%)   | 13.4 ± 1.7 <sup>a</sup>     | −26.8 ± 6.5 <sup>b</sup>       | −46.7 ± 10.0 <sup>b</sup>        | <0.001  |
| Physical activity     |                             |                                |                                  |         |
| Absolute change (min) | 64.4 ± 6.7 <sup>a</sup>     | −266.1 ± 29.6 <sup>b</sup>     | −381.9 ± 44.4 <sup>c</sup>       | <0.001  |
| Relative change (%)   | 9.0 ± 1.0 <sup>a</sup>      | −32.1 ± 4.4 <sup>b</sup>       | −49.4 ± 6.5 <sup>c</sup>         | <0.001  |
| Lying time            |                             |                                |                                  |         |
| Absolute change (min) | 0.04 ± 5.6 <sup>b</sup>     | 182.2 ± 21.8 <sup>a</sup>      | 205.4 ± 33.5 <sup>a</sup>        | <0.001  |
| Relative change (%)   | 1.3 ± 0.8 <sup>b</sup>      | 26.6 ± 3.5 <sup>a</sup>        | 29.6 ± 5.3 <sup>a</sup>          | <0.001  |
| Milk yield            |                             |                                |                                  |         |
| Absolute change (kg)  | 5.7 ± 0.3 <sup>a</sup>      | −4.6 ± 2.1 <sup>b</sup>        | −9.9 ± 1.4 <sup>c</sup>          | <0.001  |
| Relative change (%)   | 27.3 ± 1.5 <sup>a</sup>     | −21.5 ± 10.5 <sup>b</sup>      | −25.8 ± 6.7 <sup>b</sup>         | <0.001  |

<sup>a,b</sup>Different superscripts within a row indicate differences ( $P \leq 0.05$ ) between means based on the LSD post hoc test.

<sup>1</sup>Data presented include the absolute difference or the relative change between 5 d before clinical diagnosis and the day of the nadir for RT, AT, and MP, or the peak for LT. Values are LSM ± SEM.

<sup>2</sup>Cows not diagnosed with clinical health disorders during the period of interest.

<sup>3</sup>Cows diagnosed with one metabolic-digestive disorder (clinical ketosis and indigestion).

<sup>4</sup>Cows diagnosed with a metabolic-digestive disorders and at least another metabolic-digestive or nonmetabolic-digestive health disorder (i.e., mastitis, metritis, and pneumonia) detected ±7 d around clinical diagnosis.

in Table 3. Data for cows with displaced abomasum were compared separately because even though all cows with this disorder were included in the MD group we were interested on evaluating this groups separately. Compared with NCHD cows, cows in the CKET and INDIG groups had a reduction in absolute and relative values for RT and PA (all  $P < 0.001$ ), whereas cows with MD had the greatest changes among all groups. Similarly, for LT and MY all groups of cows with HD were different ( $P < 0.001$ ) from the NCHD group but no differences were observed among them. For cows with DA+1, the absolute and relative changes observed were different (all  $P < 0.001$ ) from the NCHD group for all parameters of interest. The effect of covariates for comparisons among cows in the NCHD group and cows grouped based on the occurrence of single or multiple metabolic-digestive disorders and other disorders of interest were consistent, regardless of the analysis. Parity ( $P < 0.05$ ) and season of calving ( $P < 0.05$ ) were significant, whereas the presence of RF was not significantly ( $P > 0.10$ ) associated with RT. Physical activity and LT were associated with parity ( $P < 0.05$ ) and presence of RF ( $P < 0.05$ ). Milk yield was associated with parity ( $P < 0.05$ ).

The interval in days between the observed nadir for RT, PA and MY and the peak for LT, and the day of the CD for cows with individual or MD are shown in Table 4. There were no differences ( $P > 0.10$ ) in the interval from nadir or peak to CD among cows

diagnosed with CKET, INDIG, or MD for RT, PA, or MY. Conversely, cows in the INDIG group had the peak for LT earlier ( $P = 0.04$ ) than cows in the CKET group. The interval between the nadir and CD was associated with season of calving for RT ( $P < 0.05$ ), PA ( $P < 0.05$ ), and MY ( $P < 0.05$ ), and the presence of RF for PA ( $P < 0.05$ ). Parity was associated with the interval between the peak for LT and CD ( $P < 0.05$ ).

### Study Limitations

Although data from this study would suggest that the AHMS evaluated might be useful for identifying cows with HD, results must be interpreted with caution because we did not evaluate the ability of the AHMS to detect individual cows with HD and our study design presented other limitations. Noteworthy weaknesses include limited generalizability, potential underdiagnosis of clinical HD, and confounding due to cow grouping strategies used. Generalizability was limited because the study was conducted at a single farm. Selecting cows for extensive clinical examination could have resulted in underdiagnosis of certain HD or clinical signs of interest. Including cows with RF, as defined in this study, in the NCHD could have confounded results by altering the magnitude of the differences between cows with and without metabolic-digestive disorders. Including RF group in statistical models (term retained only when  $P < 0.10$ ) likely reduced but did not fully

**Table 3.** Absolute values and relative changes from 5 d before clinical diagnosis of disorders of interest and the day of the nadir for daily rumination time, physical activity, milk yield, or peak for daily lying time for cows diagnosed with a single metabolic-digestive disorder or metabolic-digestive plus another health disorder diagnosed from 2 to 21 DIM

| Item <sup>1</sup>    | Health disorder groups      |                            |                             |                            | Health disorder groups |                         |                            |         |
|----------------------|-----------------------------|----------------------------|-----------------------------|----------------------------|------------------------|-------------------------|----------------------------|---------|
|                      | NCHD <sup>2</sup> (n = 616) | CKET <sup>3</sup> (n = 27) | INDIG <sup>4</sup> (n = 17) | MD <sup>5</sup> (n = 39)   | P-value                | NCHD (n = 616)          | DA+1 <sup>6</sup> (n = 19) | P-value |
| Rumination time      |                             |                            |                             |                            |                        |                         |                            |         |
| Absolute change, min | 48.0 ± 7.4 <sup>a</sup>     | -153.9 ± 36.8 <sup>b</sup> | -73.6 ± 47.7 <sup>b</sup>   | -255.8 ± 28.4 <sup>c</sup> | <0.001                 | 46.5 ± 7.3 <sup>a</sup> | -249.9 ± 39.8 <sup>b</sup> | <0.001  |
| Relative change, %   | 13.5 ± 1.7 <sup>a</sup>     | -25.1 ± 8.0 <sup>b</sup>   | -14.2 ± 10.9 <sup>b</sup>   | -54.8 ± 6.5 <sup>c</sup>   | <0.001                 | 13.3 ± 1.7 <sup>a</sup> | -54.2 ± 9.3 <sup>b</sup>   | <0.001  |
| Physical activity    |                             |                            |                             |                            |                        |                         |                            |         |
| Absolute change, min | 64.2 ± 6.9 <sup>a</sup>     | -243.0 ± 38.4 <sup>b</sup> | -128.5 ± 9.0 <sup>b</sup>   | -451.8 ± 29.7 <sup>c</sup> | <0.001                 | 63.1 ± 6.4 <sup>a</sup> | -499.0 ± 39.2 <sup>b</sup> | <0.001  |
| Relative change, %   | 9.0 ± 1.0 <sup>a</sup>      | -24.5 ± 5.5 <sup>b</sup>   | -13.9 ± 7.1 <sup>b</sup>    | -58.0 ± 4.1 <sup>c</sup>   | <0.001                 | 8.9 ± 0.9 <sup>a</sup>  | -64.6 ± 6.0 <sup>b</sup>   | <0.001  |
| Lying time           |                             |                            |                             |                            |                        |                         |                            |         |
| Absolute change, min | -0.003 ± 5.6 <sup>b</sup>   | 214.7 ± 28.6 <sup>a</sup>  | 126.7 ± 36.8 <sup>a</sup>   | 198.3 ± 22.8 <sup>a</sup>  | <0.001                 | -0.1 ± 5.5 <sup>b</sup> | 199.4 ± 30.5 <sup>a</sup>  | <0.001  |
| Relative change, %   | 1.4 ± 0.8 <sup>b</sup>      | 29.2 ± 4.3 <sup>a</sup>    | 20.6 ± 6.0 <sup>a</sup>     | 30.1 ± 3.5 <sup>a</sup>    | <0.001                 | 1.4 ± 0.8 <sup>b</sup>  | 27.3 ± 4.9 <sup>a</sup>    | <0.001  |
| Milk yield           |                             |                            |                             |                            |                        |                         |                            |         |
| Absolute change, kg  | 5.7 ± 0.3 <sup>a</sup>      | -10.4 ± 1.9 <sup>b</sup>   | -7.9 ± 2.5 <sup>b</sup>     | -9.4 ± 1.3 <sup>b</sup>    | <0.001                 | 5.7 ± 0.3 <sup>a</sup>  | -13.4 ± 1.9 <sup>b</sup>   | <0.001  |
| Relative change, %   | 26.5 ± 1.5 <sup>a</sup>     | -26.8 ± 8.2 <sup>b</sup>   | -23.7 ± 11.7 <sup>b</sup>   | -32.6 ± 6.4 <sup>b</sup>   | <0.001                 | 26.8 ± 1.5 <sup>a</sup> | -42.9 ± 9.5 <sup>b</sup>   | <0.001  |

<sup>a,b</sup>Different superscripts within a row indicate differences ( $P \leq 0.05$ ) between means based on the LSD post hoc test.<sup>1</sup>Data presented include the absolute difference or the relative change between 5 d before clinical diagnosis and the day of the nadir for rumination time, active time, and milk yield, or the peak for lying time. Values are LSM ± SEM.<sup>2</sup>Cows not diagnosed with clinical health disorders during the period of interest.<sup>3</sup>Cows diagnosed only with clinical ketosis during the period of interest.<sup>4</sup>Cows diagnosed only with indigestion during the period of interest.<sup>5</sup>Cows diagnosed with metabolic-digestive health disorder and either another metabolic-digestive disorder or a nonmetabolic-digestive disorder (i.e., mastitis, metritis, and pneumonia) during the period of interest.<sup>6</sup>Cows diagnosed with displaced abomasum and at least 1 other health disorder. All cows with displaced abomasum had at least one other health disorder detected ±7 d of clinical diagnosis of the disorder. Therefore, displaced abomasum cows are included in the MD group for general analysis and compared separately with cows in the NCHD group.

**Table 4.** Interval (in days) between nadir for daily rumination time, physical activity, and milk yield, and peak for daily lying time, and the day of clinical diagnosis of metabolic-digestive disorders diagnosed between 2 and 21 DIM<sup>1</sup>

| Parameter         | DA+I <sup>2</sup> (n = 19) | CKET <sup>3</sup> (n = 27) | INDIG <sup>4</sup> (n = 17) | MD <sup>5</sup> (n = 39) | P-value |
|-------------------|----------------------------|----------------------------|-----------------------------|--------------------------|---------|
| Rumination time   | -0.7 ± 3.3                 | -0.9 ± 0.4                 | -0.9 ± 0.4                  | -1.0 ± 0.3               | 0.97    |
| Physical activity | 0.6 ± 3.3                  | 0.02 ± 0.4                 | -0.5 ± 0.5                  | 0.1 ± 0.3                | 0.59    |
| Lying time        | -1.0 ± 2.8 <sup>b</sup>    | 0.25 ± 0.3 <sup>a</sup>    | -1.1 ± 0.4 <sup>b</sup>     | -0.5 ± 0.2 <sup>ab</sup> | 0.04    |
| Milk yield        | 0.4 ± 3.6                  | 0.2 ± 0.4                  | -0.8 ± 0.5                  | -0.4 ± 0.3               | 0.26    |

<sup>a,b</sup>Different superscripts within a row indicate differences ( $P \leq 0.05$ ) between means based on the LSD post hoc test.

<sup>1</sup>Days from the day of the nadir for daily rumination time, physical activity, milk yield, or peak daily lying time and the day of CD of the disorder of interest.

<sup>2</sup>Cows diagnosed with displaced abomasum and at least 1 other health disorder. All cows with displaced abomasum had at least one other health disorder detected  $\pm 7$  d of clinical diagnosis of the disorder. Therefore, DA cows are included in the MD group for general analysis and compared separately with cows in the NCHD group.

<sup>3</sup>Cows diagnosed only with clinical ketosis during the period of interest.

<sup>4</sup>Cows diagnosed only with indigestion during the period of interest.

<sup>5</sup>Cows diagnosed with metabolic-digestive health disorder and either another metabolic-digestive disorder or a nonmetabolic-digestive disorder (i.e., mastitis, metritis, and pneumonia) during the period of interest.

eliminate the confounding effect of including cows with RF in the NCHD group.

This study also had limitations from an on-farm AHMS use perspective. All comparisons were made retrospectively for groups of cows rather than individual cows. Evaluating data for groups with tens or hundreds of cows makes patterns of sensor parameters obvious and increases the likelihood of detecting statistically significant differences and large effect sizes. Under commercial farm conditions, individual cows must be identified, which may prove more challenging as sensor-monitored behavioral parameter patterns during healthy and diseased states vary widely, and the effects of disorders on these patterns for individual cows are of different magnitude. Although the retrospective nature of our analysis allowed us to identify associations between the sensor parameters and disorders of interest, this is not practical in a farm setting, as cows must be identified prospectively with a time granularity of days or hours. At a commercial farm, sensor data collected in real time can only be used for comparison with past data for individual cows and groups, reference values for the population of interest, or ingested by algorithms trained to identify anomalies in the pattern of interest. Thus, more research is needed to demonstrate that the AHMS used in this study has the ability to identify cows affected by or at risk of suffering metabolic-digestive disorders in real time under commercial farm conditions.

## CONCLUSIONS

Cows diagnosed with metabolic-digestive disorders and cows diagnosed with a metabolic-digestive disorder

plus another health disorder had detectable temporal shifts in the patterns of RT, PA, and LT captured by the ear-attached sensor of an AHMS within 7 d before and after disorder diagnosis. Moreover, based on the magnitude of the absolute and relative changes, the dynamic of the alterations, and the timing of the nadir and peak around diagnosis of metabolic-digestive disorders for each behavioral parameter monitored, the AHMS evaluated in this study might have potential value for identifying dairy cows with these disorders in early lactation.

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