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# Physiological and behavioural responses of grazing dairy cows to an acute metabolic challenge

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## Abstract

Due to seasonal changes in the quality and quantity of herbage, the nutrient supply to grazing dairy cows is not always sufficient, which may increase their metabolic load. To investigate the temporal pattern of behavioural changes in relation to concomitant metabolic alterations, we subjected 15 multiparous early lactating Holstein dairy cows (24 (*SD* 7.4) days in milk) to a short-term metabolic challenge, which we provoked by abruptly withdrawing concentrate for 1 week. Cows grazed full-time and were supplemented with concentrate in experimental week (EW) 1 and EW 3, whereas concentrate was withdrawn in EW 2. We analysed milk and blood samples to characterise the metabolic changes and found that the total yield of milk and protein decreased ($p < 0.05$) and fat yield, fat-to-protein ratio and acetone content increased ($p < 0.05$) from EW 1 to EW 2. Plasma glucose and insulin concentrations were lower ($p < 0.05$), and concentrations of nonesterified fatty acids and beta-hydroxybutyrate were higher ($p < 0.05$) in EW 2 compared with EW 1. Apart from ingestive and rumination behaviour and activity, we also monitored the use of an automated brush on pasture. While time spent eating and ruminating increased ($p < 0.05$) in EW 2 compared with EW 1, time spent idling decreased ($p < 0.05$). Concomitantly, while time standing and moving increased ($p < 0.05$) from EW 1 to EW 2, walking time decreased ($p < 0.05$). The daily proportion of cows using the automated brush decreased ($p < 0.05$) in EW 2 compared with EW 1, as did the duration of brushing per day. In conclusion, grazing cows experiencing a metabolic challenge try to compensate for the nutrient deficiency by increasing eating time, a behavioural element important for short-term survival. Due to the strong impact of weather conditions, we cannot currently recommend observation of outdoor brushing activity to address short-term alterations in the metabolic state of grazing cows.

KEYWORDS

automated brush, dairy cow, grazing, ingestive behaviour, metabolic load, well-being

# 1 | INTRODUCTION

Consumers are increasingly concerned about the husbandry conditions of livestock and desire farm systems in which dairy cows can display natural behaviours such as grazing (Von Keyserlingk, Cestari, Franks, Fregonesi, & Weary, 2017). Furthermore, herbage is the most economical nutrient source for dairy cows (Taweel et al., 2006), and feeding maximum herbage involves less competition with human food resources. However, the quality and availability of pasture herbage can vary considerably; thus, intermittent nutrient shortages are likely (Hopkins, 2000). In a zero-grazing study, herbage-fed cows not receiving a supplementary concentrate experienced a higher metabolic load than cows receiving concentrate supplementation (Zbinden et al., 2017). In addition, grazing cows expended around 20% more energy than herbage-fed cows kept indoors (Dohme-Meier et al., 2014), indicating that grazing cows receiving little or no supplementary concentrate are particularly susceptible to metabolic disorders and, hence, impaired well-being (Von Keyserlingk, Rushen, de Passillé, & Weary, 2009). In order to decrease the risk of reduced animal welfare in grazing dairy cows, a monitoring system based on noninvasive markers that immediately reflects changes in the animal's metabolic state is desirable. Changes in milk yield and composition that can be continuously and noninvasively monitored might be suitable biomarkers. However, milk composition alone is not sufficiently sensitive to estimate the energy status of an individual cow (Reist et al., 2002), and a high milk yield is no guarantee for good welfare because it is affected by various factors that are welfare neutral (Von Keyserlingk et al., 2009). Low plasma glucose concentrations and high plasma NEFA and BHB concentrations indicate a high metabolic load in early lactating cows as well as in production systems with compromised nutrient supplies (Gross & Bruckmaier, 2015; Gross, Van Dorland, Bruckmaier, & Schwarz, 2011) but may not necessarily be accompanied by inflammatory and stress markers such as cortisol and acute phase proteins that are commonly linked with animal well-being (Zbinden et al., 2017). Furthermore, frequent blood sampling is not practicable; therefore, identifying alternative noninvasive biomarkers is of major interest. González, Tolkamp, Coffey, Ferret, and Kyriazakis (2008) reported that automated monitoring of changes in feeding behaviour (e.g., eating time) could detect acute production diseases in dairy cows. However, core activities like eating and lying are essential for short-term survival and presumably not appropriate to assess an animal's welfare and longer-term fitness (Weary, Huzzey, & Von Keyserlingk, 2009). For this purpose, researchers suggest low-resilience behaviours (also referred to as "luxury activities") (Held & Špinka, 2011) that are characterised by reduced occurrence when time or energy resources are limited (Von Frisch, 1999). In dairy cows, self-grooming (Fogsgaard, Røntved, Sørensen, & Herskin, 2012) and using automated cow brushes (Mandel, Whay, Nicol, & Klement, 2013) were identified as low-resilience activities.

The objective of this study was to investigate the behavioural responses of grazing dairy cows in early lactation subjected to a transient metabolic challenge induced by an abrupt withdrawal of concentrate for 7 days. Apart from core activities like eating, ruminating, lying and locomotion, the use of an automated brush as a low-resilience activity was monitored on pasture. The hypothesis tested was that during the concentrate withdrawal, dairy cows undergo additional metabolic stress which is accompanied by shifts in their core activities. We expected an increase in eating activity to compensate for the shortage of nutrients. In addition, we assumed a decrease in using the automated brush.

# 2 | MATERIALS AND METHODS

## 2.1 | Animals and experimental outline

All experimental procedures were in accordance with the Swiss guidelines for animal welfare and approved (2016_07_FR) by the Committee of Animal Experiments of the Canton of Fribourg, Switzerland. Four weeks before the experiment started, fifteen multiparous Holstein dairy cows (parity: 3.2 ± 1.6, mean ± *SD*) were selected from the Agroscope dairy herd (Posieux, Switzerland) based on their expected calving date. Cows were clinically examined concerning vital parameters, as well as udder and claw health. At the onset of the experiment, cows were an average (mean ± *SD*) of 24.0 ± 7.4 DIM, produced 42.4 ± 4.7 kg of milk/day and had an initial BW of 639 ± 53 kg. The cows were managed as one group from 4 weeks before the experiment until its conclusion. The study comprised three experimental weeks (EW 1, EW 2, EW 3) with repeated measurements of individual cows and lasted from 16 May to 5 June 2016. Cows grazed from 08:00 to 14:30 and from 18:00 to 05:00. In EW 1 and EW 3, all cows were supplemented on a flat rate basis (mean ± *SD*; EW 1, 6.49 ± 1.25 kg/day; EW 3, 6.12 ± 1.92 kg/day (as-fed basis)) with a compound feed containing (g/kg): barley, 337; maize, 321; wheat, 297; maize gluten, 91; molasses, 30; $CaCO_3$, 18; NaCl, 4 and a trace elements-vitamin mix, 2. On the first day of EW 2, the concentrate was withdrawn. The reintroduction of concentrate in EW 3 was implemented gradually so that the full amount was eventually offered on day 3 of EW 3. The concentrate was fed in two equal meals in the free stall barn after returning from milking at 07:00 and 16:00 and controlled using automatic weighing troughs (Insentec B.V., Marknesse, the Netherlands). The cows had constant access to drinking water and mineral blocks in the barn and on pasture.

## 2.2 | Grazing management and climatic conditions

During the experiment, each of the thirteen paddocks used was split into two halves. When the cows completed grazing on one half, the second half was made accessible to them. The area of the paddocks was (mean ± *SD*) 0.357 ± 0.163 ha. The sward was composed of 89.6% grasses, 8.9% legumes and 1.6% herbs. Paddocks were rotationally grazed for 0.5–1.5 days and changed at a residual sward height of 4–5 cm. The sward surface height was measured with an electronic rising plate meter (FARMWORKS Plate Meter F200, Jenquip, Feilding, NZ). The average herbage mass offered was

**TABLE 1** Weather conditions during the experiment (mean ± *SD*)

| Item | EW 1 | | EW 2 | | EW 3 | |
|---|---|---|---|---|---|---|
| | Mean | *SD* | Mean | *SD* | Mean | *SD* |
| Temperature humidity index[a] | 61.2 | 5.8 | 62.2 | 7.1 | 61.6 | 2.0 |
| Temperature, daily maxima (°C) | 17.2 | 4.8 | 17.6 | 5.5 | 16.8 | 1.4 |
| Temperature, daily minima (°C) | 3.84 | 1.73 | 8.53 | 4.50 | 10.99 | 1.17 |
| Precipitation (mm/day) | 7.14 | 10.35 | 7.91 | 8.07 | 5.10 | 6.48 |
| Hours with rain occurrence (*n*/day) | 3.43 | 3.79 | 6.43 | 6.37 | 4.14 | 4.31 |
| Wind velocity (m/s) | 2.13 | 1.31 | 2.21 | 1.24 | 2.87 | 2.45 |
| Sunshine (min/day) | 455 | 294 | 276 | 248 | 54.1 | 49.6 |

*Notes*. EW: experimental week; *SD*: standard deviation.
[a]Calculated according to Mandel et al. (2013).

(mean ± *SD*) 698 ± 126 kg DM per ha over 4 cm and 24.4 ± 10.4 kg DM per animal and per day over 4 cm. This estimation was based on measurements from the electronic rising plate meter combined with mowing a defined area of herbage and consecutive weighing and DM analysis. The climatic conditions were recorded daily at the meteorological station in Grangeneuve (Meteo-Schweiz, Station Fribourg/Posieux, Switzerland), located about 1 km away from the experimental pastures (Table 1). The THI was calculated according to Mandel et al. (2013).

## 2.3 | Sample collection and laboratory analysis

Milk yield and body weight after milking were measured in the milking parlour twice daily throughout the experiment. Milk samples were taken from every cow at each milking on days 1–4 and 7 of each EW. Samples were pooled per day, preserved with Broad-Spectrum Microtabs II (Gerber Instruments AG, Effretikon, Switzerland) and stored at +5°C for later analysis of fat, protein and lactose (International Dairy Federation, 2000; method number 141C) using infrared spectrometry (Combifoss FT+, Foss, Hillerod, Denmark). A second milk sample per cow was taken and stored at −18°C for analysis of urea and acetone as described by Heublein et al. (2017). On days 1–4 and 7 of each EW, blood was collected in the morning after milking and before concentrate feeding by puncturing the jugular vein using EDTA-tubes (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria). The samples were immediately cooled on wet ice until they were centrifuged at 1,000 *g* for 20 min. The retrieved plasma was stored at −21°C for later analysis of glucose, NEFA and BHB as described earlier by Gross et al. (2011) and insulin using radioimmunoassay (RIA; Vicari, Van den Borne, Gerrits, Zbinden, & Blum, 2008). Herbage samples were hand-plucked once daily using electrical shears by following and mimicking the cows' grazing behaviour for half an hour. Samples were chopped and lyophilised; DM content was determined as the residue after lyophilisation. Subsequently, samples were pooled over two consecutive days for further analysis. Samples of the concentrates were collected twice a week during the experiment. DM content was determined by drying the samples at 105°C for 3 hr. The chemical composition of the herbage and concentrate samples was analysed as described by Heublein et al. (2017). Ethanol soluble carbohydrates were determined as described by Hall, Hoover, Jennings, and Webster (1999). The contents $NE_L$ and absorbable protein were calculated according to Agroscope (2016). Results of analysis and calculations are shown in Table 2.

## 2.4 | Data recording

To record and evaluate their ingestive and rumination behaviour and physical activity, cows were equipped with RumiWatch halters (version 6.0) and pedometers (Itin + Hoch GmbH, Fütterungstechnik, Liestal, Switzerland) as specified in Rombach, Münger, Niederhäuser, Südekum, and Schori (2018) and Alsaaod et al. (2015). Data were converted with the RumiWatch Converter (C31) (Itin + Hoch GmbH, Converter 0.7.3.31). Before the experiment, cows were accustomed to the halter and pedometer for 3 days. The halters were removed during days 6 and 7 of each EW to avoid skin alterations. Thus, ingestive and rumination behaviour was recorded on days 1–5 of each EW, and the following items were assessed according to Rombach et al. (2018): eating time, eating chews, prehension bites, rumination time, rumination chews, bolus count, idle time (jaw movements which cannot be assigned to eating, ruminating or drinking, or no detectable activity) and the number of changes between the different activities of eating, ruminating, drinking and idling. Data from the pedometers were recorded throughout the experiment. We focused on the behavioural components of lying, standing and walking time, and number of lie down events as defined by Alsaaod et al. (2015). As standing time also included movements in an upright position with less than three consecutive strides in the same direction with a period between two strides of 4 s or less (Alsaaod et al., 2015), we

**TABLE 2** Composition of the experimental feed (mean ± *SD*)

| Item | Herbage[a] | | | | | | Concentrate[b] | |
|---|---|---|---|---|---|---|---|---|
| | EW 1 | | EW 2 | | EW 3 | | | |
| | Mean | *SD* | Mean | *SD* | Mean | *SD* | Mean | *SD* |
| DM (g/kg of wet weight)[c] | 184 | 13 | 171 | 14 | 167 | 24 | 880 | 6 |
| Analysed nutrient and mineral composition (g/kg DM) | | | | | | | | |
| OM | 916 | 6 | 908 | 11 | 915 | 6 | 956 | 1 |
| Crude protein | 158 | 37 | 140 | 12 | 158 | 10 | 164 | 1 |
| Crude fat | –[d] | | – | | – | | 37.3 | 0.5 |
| NDF | 416 | 35 | 406 | 28 | 442 | 6 | 141 | 15 |
| ADF | 217 | 13 | 231 | 18 | 254 | 6 | 41.8 | 4.1 |
| WSC | 170 | 8 | 178 | 22 | 127 | 12 | 49.1 | 2.1 |
| ESC | 141 | 11 | 117 | 8 | 95.5 | 12.6 | 32.8 | 1.9 |
| Starch | – | | – | | – | | 589 | 4 |
| Calculated energy and protein supply per kg of DM[e] | | | | | | | | |
| $NE_L$ (MJ) | 5.77 | 0.35 | 5.60 | 0.11 | 5.75 | 0.14 | 8.20 | 0.00 |
| APDE (g) | 98.3 | 9.5 | 94 | 3.4 | 98.5 | 3.2 | 120 | 0.56 |

*Notes*. ADF, acid detergent fibre; APDE, absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen; DM, dry matter; ESC, ethanol soluble carbohydrate; EW, experimental week; NDF, neutral detergent fibre; $NE_L$, net energy for lactation; OM, organic matter; *SD*, standard deviation; WSC, water-soluble carbohydrate.
[a]Means of daily hand-plucked herbage samples, which were pooled for analysis over 2 days. [b]Means of six samples. [c]Means of 5 (EW 1) and 6 (EW 2 and 3) determinations per experimental week. [d]Not analysed. [e]According to Agroscope (2016).

called this item "standing and moving". At least 2 months prior to the experiment, all cows were accustomed to the automated brush (VPB2, Buri AG, Hasle-Rüegsau, Switzerland) installed in the exercise yard of the free stall barn, which was later used on pasture. Lifting the brush automatically initiates its rotating function for 25 s if no further activation occurs. The brush was installed on a trailer equipped with solar panels (to power the device). Two cameras (DH61E, ANNKE, City of Industry, USA) were installed outside the paddock at a 90° angle relative to each other and the brush. A recorder (four-channel compact digital recorder, ABUS Security Tech Germany, Wetter, Germany) was placed on the trailer. This setup (Figure 1) was installed 1 week before the start of the experiment so that the cows grew accustomed to the presence of the brush on pasture. Brushing behaviour was defined as follows: contact between cow and brush, excluding the brush support. The occurrence (daily proportion of cows using the brush at least once) and daily duration of brush usage were recorded. The videos were encoded with The Observer Version 11 software (Noldus Information Technology, Wageningen, the Netherlands). One person conducted the analysis; EW and day of EW were blinded. Intra-observer reliability was determined using the ICC, which was calculated with the following equation according to Zaiontz (2015):

$$ICC = var(\beta)/(var(\alpha) + var(\beta) + var(\varepsilon)),$$

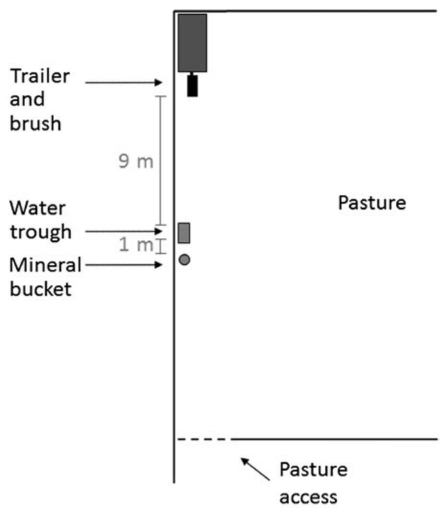


**FIGURE 1** Position of the automated brush on pasture

where $\beta$ represents the variability due to differences in the subjects, $\alpha$ represents the variability due to differences in the rating levels/scale used by the judges and $\varepsilon$ represents the variability due to differences in the evaluations of the subjects by the judges. The ICC's for occurrence of brushing per cow and overall sum of the duration of brushing per cow were 1 and 0.996 regarding short-term reliability (comparison between two consecutive days) and 1 and 0.986 regarding long-term reliability (bias over time during the 2 months of video analysis), respectively, which can be regarded as very good (Nunnally & Bernstein, 1994).

## 2.5 | Calculations and statistical analysis

Descriptive statistics, explorative graphics and parametric LMMs were performed using SYSTAT 13.0 software (Systat Software, Chicago, USA). The response variables were modelled by the categorical factor EW (1, 2, 3) and the covariates day within EW (1, 2, 3, … 7), temperature humidity index, precipitation (mm), rain (number of hr/day with occurrence of precipitation), sun (duration), wind (velocity), $NE_L$ content of herbage and DIM. Each cow represented its own control and was considered a random effect; the "within subject" error correlation was modelled as an autoregressive AR(1) structure. In some cases, log-transformed or square root-transformed responses were evaluated based on the residual diagnostics (i.e., normal probability plots and tests such as Lilliefors, Shapiro–Wilk and Anderson-Darling). Stepwise model reduction was performed on the basis of the *p*-values (type III tests). Outlying residuals were graphically identified, and the corresponding response observations were excluded if the *z*-scores supported this decision ($|z| > 3$). Tukey–Kramer tests were used for pairwise comparisons of the least squares means of the periods. Robust LMMs (R package robustlmm, Koller, 2016) were set up if the residuals of the parametric models showed obvious and significant deviation from normality. Stepwise model reduction was based on *t*-statistics (*p*-values were not available) by computing an approximate critical *t*-value for the two-sided significance level of 0.1. No least squares means or post hoc tests were available for these robust models (Koller, 2013). Binary and count data (occurrence and number of lie downs respectively) were modelled using GEE (R package geepack; binomial and Poisson model respectively). Then least squares means were computed (package lsmeans), and pairwise comparisons of the periods were corrected for multiple testing (package multcomp). Noninteger lie down counts as responses were analysed as Gaussian GEE models because Poisson regression is not applicable in this case. Results are presented as least squares means and *SEM* representing the highest

**TABLE 3** Body weight, milk yield and components and blood metabolites and hormones of cows during the experimental weeks[1]

| | Experimental week[2] | | | | | Effect (*p*) of day within EW | | |
|---|---|---|---|---|---|---|---|---|
| Item | EW 1 | EW 2 | EW 3 | *SEM* (≤) | Effect of EW (*p*) | EW 1 | EW 2 | EW 3 |
| Body weight (kg) | 636 | 628 | 630 | 12.6 | 0.797 | 0.001 | < 0.001 | < 0.001 |
| Milk yield and composition | | | | | | | | |
| Yield (kg/day per cow) | 43.3[a] | 37.3[c] | 40.2[b] | 1.01 | < 0.001 | 0.246 | < 0.001 | < 0.001 |
| Fat (%) [3] | 3.31[f] | 3.94[d] | 3.50[e] | 0.074 | –[4] | 2.393[5] | 0.581[5] | 2.068[5] |
| Fat yield (g/day) | 1,194[b] | 1,547[a] | 1,276[b] | 101.4 | 0.008 | 0.002 | 0.736 | 0.163 |
| Protein (%) | 3.11[a] | 3.01[b] | 2.95[b] | 0.043 | < 0.001 | 0.043 | 0.042 | 0.023 |
| Protein yield (g/day) | 1,337[a] | 1,139[b] | 1,161[b] | 30.7 | < 0.001 | 0.038 | < 0.001 | < 0.001 |
| Fat-to-protein ratio | 1.02[b] | 1.41[a] | 1.08[b] | 0.084 | < 0.001 | 0.023 | 0.983 | 0.004 |
| Lactose (%) | 4.87[a] | 4.86[a, b] | 4.82[b] | 0.032 | < 0.001 | 0.053 | < 0.001 | 0.142 |
| Lactose yield (g/day) | 2,090[a] | 1,815[c] | 1,919[b] | 48.6 | < 0.001 | 0.896 | < 0.001 | < 0.001 |
| Urea (mg/kg) | 179[a] | 101[b] | 171[a] | 13.1 | < 0.001 | < 0.001 | 0.635 | < 0.001 |
| Acetone (mg/kg)[3, 6] | 1.32[f] | 3.56[d] | 2.38[e] | 0.318 | – | 1.487[7] | 9.458[7] | −6.097[7] |
| Blood metabolites and hormones | | | | | | | | |
| Glucose (mmol/L) | 3.49[a] | 3.15[c] | 3.32[b] | 0.062 | 0.002 | 0.302 | < 0.001 | 0.518 |
| NEFA (mmol/L)[6] | 0.74[b] | 0.75[a] | 0.56[c] | 0.056 | < 0.001 | 0.448 | 0.040 | 0.641 |
| BHB (mmol/L)[6] | 0.48[c] | 0.86[a] | 0.63[b] | 0.049 | 0.023 | 0.427 | < 0.001 | 0.343 |
| Insulin (µU/ml)[6] | 6.91[a] | 4.15[b] | 7.00[a] | 0.500 | 0.003 | 0.480 | 0.218 | 0.223 |

*Notes.* Values in the same row with different superscripts (a,b,c) differ ($p < 0.05$). Values in the same row with different superscripts (d,e,f) differ ($\alpha/2 = 0.05$).
[1]SEM: standard error of the means, highest *SEM* is presented; BHB: beta-hydroxybutyrate; NEFA: nonesterified fatty acids. [2]Average days in milk of the cows (mean + *SD*): EW 1, 27 + 7.5; EW 2, 34 + 7.5; EW 3, 41 + 7.5; $N = 15$. [3]Statistical evaluation conducted with robust Linear mixed models. Trimmed means and corresponding standard error are presented. [4]No *p*-values and no overall *t*-value are calculated, see Koller (2013). [5]*T*-statistic, |*t*-value| > critical *t*-value is considered as significant ($\alpha/2 = 0.05$). Critical *t*-value: 2.132. [6]Log-transformed for statistical analysis. [7]*T*-statistic, |*t*-value| > critical *t*-value is considered as significant ($\alpha/2 = 0.05$). Critical *t*-value: 1.860.

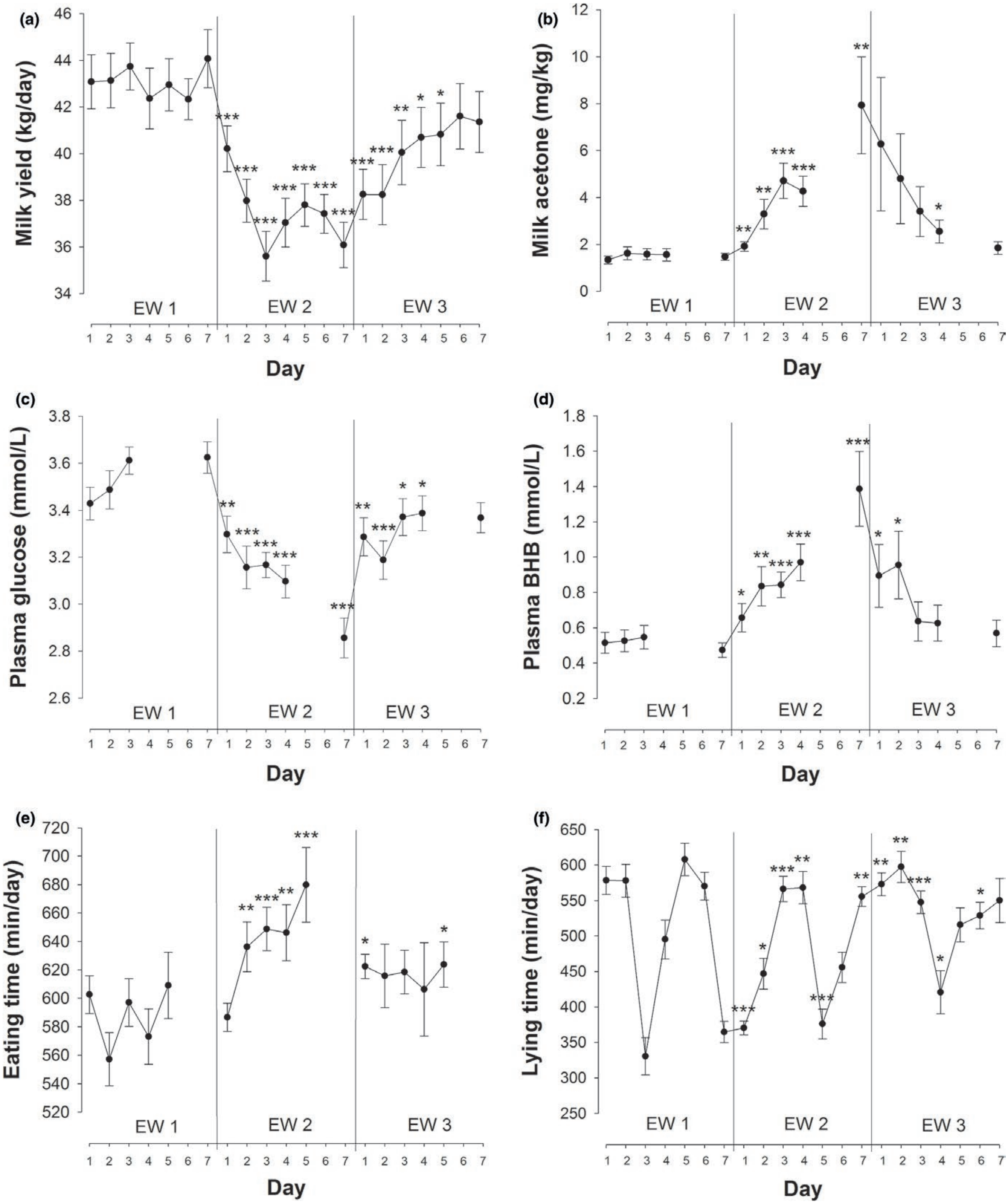


**FIGURE 2** Selected milk, metabolic and behavioural variables. Milk yield (a), milk acetone content (b), plasma glucose (c), plasma beta-hydroxybutyrate (BHB) (d), lying time (e) and eating time (f) of cows during experimental week (EW) 1 (pasture + concentrate), EW 2 (concentrate withdrawal) and EW 3 (concentrate reintroduction). Data are given as mean values ± standard error of the mean. Daily means of EW 2 and 3 were tested against overall mean of EW 1 using a paired *t* test: **p* < 0.05 ***p* < 0.01 ****p* < 0.001

*SEM* for LMMs and GEE models and as trimmed means (10.0%, two-sided) for robust models respectively. For brush duration, the arithmetic mean of the log-transformed data is presented because values of "0" occur often and are as important as other values. Significance was declared at $p < 0.05$. When variables are graphically displayed, daily means of EW 2 and EW 3 were tested against the overall mean of EW 1 using a paired *t* test. Due to technical problems in EW 1, data for milk fat analysis was lost; additional samples were collected on days 5–7 and analysed. Behavioural data for one cow that went lame during EW 1 was excluded completely from the statistical analysis.

## 3 | RESULTS

### 3.1 | Body weight, milk yield and milk components

Body weight did not differ ($p > 0.05$) among EW but changed ($p \leq 0.001$) across sampling days within each EW (Table 3). Total yields of milk, protein and lactose, as well as protein percentage and milk urea content, decreased ($p < 0.05$) in EW 2 compared with EW 1. Yield of milk and lactose rose again ($p < 0.05$) in EW 3 but remained lower than in EW 1. Protein yield and protein percentage did not change ($p > 0.05$) from EW 2 to EW 3, and urea content reached ($p < 0.05$) the level of EW 1. Fat percentage ($\alpha/2 = 0.05$), fat

**TABLE 4** Feeding and rumination behaviour, physical activity and brush usage of cows during the experimental weeks[1]

| | Experimental Week[2] | | | | | Effect ($p$) of day within EW | | |
|---|---|---|---|---|---|---|---|---|
| Item | EW 1 | EW 2 | EW 3 | *SEM* (≤) | Effect of EW ($p$) | EW 1 | EW 2 | EW 3 |
| Ingestive and rumination behaviour | | | | | | | | |
| Eating time (min/day) | 560[b] | 642[a] | 652[a] | 16.7 | 0.016 | 0.816 | 0.018 | 0.096 |
| Eating chews total (*n*/day) | 41,935[b] | 46,647[a] | 46,166[a] | 1,228.3 | <0.001 | 0.342 | 0.973 | 0.315 |
| Prehension bites (*n*/day) | 24,559[c] | 34,578[a] | 30,445[b] | 1,650.8 | <0.001 | <0.001 | 0.312 | 0.925 |
| Rumination time (min/day) | 448[b] | 526[a] | 461[b] | 14.8 | 0.004 | 0.271 | 0.030 | 0.048 |
| Rumination chews (*n*/day) | 28,788[b] | 33,991[a] | 29,684[b] | 1,181.2 | 0.083 | 0.336 | 0.292 | 0.008 |
| Rumination chews per minute (*n*/min) | 70.2 | 70.7 | 71.0 | 1.55 | 0.113 | 0.990 | 0.747 | 0.076 |
| Bolus count (*n*/day) | 494[b] | 596[a] | 511[b] | 18.8 | <0.001 | 0.062 | 0.714 | 0.149 |
| Time idle (min/day)[3] | 358[a] | 294[c] | 340[b] | 12.6 | <0.001 | <0.001 | 0.038 | 0.038 |
| Changes among different activities (*n*/day)[4] | 119 | 104 | 121 | 7.1 | 0.035 | 0.323 | 0.047 | 0.816 |
| Physical activity | | | | | | | | |
| Lying time (min/day) | 494[a, b] | 462[b] | 542[a] | 19.6 | <0.001 | 0.061 | <0.001 | 0.040 |
| Standing and moving time (min/day)[5] | 837[b] | 897[a] | 799[b] | 19.3 | <0.001 | 0.019 | <0.001 | <0.001 |
| Walking time (min/day)[6] | 101[a] | 90[b] | 96[a] | 3.3 | <0.001 | 0.006 | <0.001 | <0.001 |
| Lie down (*n*/day) | 8.3 | 8.5 | 7.5 | 0.46 | 0.23 | 0.392 | <0.001 | 0.693 |
| Brush usage | | | | | | | | |
| Occurrence[7] | 0.932[a] | 0.650[b] | 0.800[a,b] | 0.0780 | 0.024 | 0.004 | 0.045 | 0.049 |
| Duration (s/day)[8] | 1.59[d] | 1.34[f] | 1.36[e] | 0.103 | –[9] | −3.747[10] | −2.877[10] | −3.282[10] |

*Notes*. Values in the same row with different superscripts (a, b, c) differ ($p < 0.05$). Values in the same row with different superscripts (d, e, f) differ ($\alpha/2 = 0.05$).
[1]*SEM*, standard error of the mean, highest *SEM* is presented. [2]Average days in milk of the cows (mean + *SD*): EW 1, 27 + 7.5; EW 2, 34 + 7.5; EW 3, 41 + 7.5; $N = 15$. [3]Time spent with other activity than eating, rumination and lying. Log-transformed for statistical analysis. Statistical evaluation was conducted with robust linear mixed models. Trimmed means and corresponding standard error are presented. [4]Activities: eating, ruminating, drinking, idle. [5]Less than three consecutive strides in the same direction with a period between two strides of 4 s or less. [6]At least three consecutive strides in the same direction with a period between two strides of 4 s or less. [7]Proportion of cows using the brush at least once per day. [8]Log-transformed for statistical analysis. Statistical evaluation was conducted with robust linear mixed models. Arithmetic means and corresponding standard error of the log-domain are presented. [9]No $p$-values and no overall $t$-value are calculated, see Koller (2013). [10]$T$-statistic; |$t$-value| > critical $t$-value is considered as significant ($\alpha/2 = 0.05$). Critical $t$-value: 2.015.

yield ($p < 0.05$), fat-to-protein ratio ($p < 0.05$) and acetone content ($\alpha/2 = 0.05$) increased from EW 1 to EW 2. From EW 2 to EW 3, all these traits decreased ($p < 0.05$; $\alpha/2 = 0.05$), and fat yield and fat-to-protein ratios reached the level of EW 1. Lactose percentages did not differ ($p > 0.05$) between EW 1 and EW 2 but decreased ($p < 0.05$) in EW 3 compared with EW 1. The sampling day within an EW influenced almost all traits ($p \leq 0.05$; fat percentage, significant ($\alpha/2 = 0.05$) with $t > 2.13$; acetone content, significant ($\alpha/2 = 0.05$) with $t > 1.86$) except for total milk yield and lactose and acetone content in EW 1, fat percentage, fat yield, fat-to-protein ratio and urea content in EW 2 and fat percentage, fat yield and lactose percentage in EW 3 (Figure 2).

### 3.2 | Blood metabolites and hormones

Plasma glucose and insulin concentrations decreased from EW 1 to EW 2 ($p < 0.05$), whereas NEFA and BHB concentrations increased ($p < 0.05$) (Table 3). From EW 2 to EW 3, glucose and insulin concentrations rose ($p < 0.05$); insulin concentration reached the level of EW 1. The concentrations of NEFA and BHB decreased again ($p < 0.05$) in EW 3, and the NEFA concentration dropped below the level of EW 1. All metabolic variables were similar across sampling days within EW 1 and EW 3 but varied ($p \leq 0.04$) within EW 2, apart from insulin concentration, where the sampling day showed no effect ($p = 0.22$).

### 3.3 | Ingestive and rumination behaviour, physical activity and brush usage

Eating and rumination time, prehension bites, eating and rumination chews and bolus counts increased ($p < 0.05$) from EW 1 to EW 2 (Table 4). Eating time and eating chews remained elevated in EW 3, whereas prehension bites decreased, rumination time and chews decreased ($p < 0.05$) and rumination time and rumination chews reached the level of EW 1. Time spent idle decreased ($p < 0.05$) from EW 1 to EW 2 and increased again ($p < 0.05$) in EW 3 without reaching the level of EW 1. Rumination chews per minute and changes among different activities did not differ ($p > 0.05$) among the EW. Almost all traits characterising ingestive and rumination behaviour were unaffected ($p > 0.05$) by sampling day within EW 1, apart from prehension bites and time spent idle ($p < 0.001$). The latter, as well as eating and rumination time and changes among different activities, varied ($p < 0.05$) across sampling days within EW 2. Besides rumination time and time spent idle, rumination chews were influenced ($p < 0.05$) by sampling day within EW 3.

Time spent lying did not differ between EW 1 and EW 2 but increased ($p < 0.05$) in EW 3 compared with EW 2. Standing and moving increased from EW 1 to EW 2 ($p < 0.05$), whereas time spent walking decreased ($p < 0.05$). In EW 3, both activities returned to ($p > 0.05$) EW 1 levels. The number of lie down events did not differ ($p > 0.05$) among EW. Apart from lie down events, which were unaffected by sampling day within EW 1 ($p = 0.39$) and EW 3 ($p = 0.69$), and lying time, which was unaffected by sampling day within EW 1 ($p = 0.06$), the sampling day within EW affected all physical activities ($p < 0.05$).

The average proportion of cows using the automated brush at least once a day decreased ($p < 0.05$) from EW 1 to EW 2 and increased numerically in EW 3 without reaching the level of EW 1. Daily duration of brushing was higher ($\alpha/2 = 0.05$) in EW 1 compared with EW 2. In EW 3, duration of brushing increased ($\alpha/2 = 0.05$) again but did not reach the level of EW 1. Both daily occurrence ($p < 0.05$) and daily duration of brushing (significant ($\alpha/2 = 0.05$) with $t > 2.06$) varied across sampling days within an EW.

Table 5 presents weather characteristics, which were significant covariates in the evaluation of the selected behavioural traits.

## 4 | DISCUSSION

Grazing cows are exposed to short- and long-term variations in quality and quantity of herbage (Hopkins, 2000); thus, abrupt nutrient shortages may occur. Undersupply of nutrients often results from

**TABLE 5** Weather characteristics with significant influence on selected behavioural traits

| Variable | Eating time $p$ | Lying time $p$ | Occurrence[a] of brush usage $p$ | Duration of brush usage (s/day) $t^b$ |
|---|---|---|---|---|
| Temperature humidity index[c] | 0.023 | –[d] | 0.0036 | 5.321 |
| Precipitation (mm/day) | <0.001 | <0.001 | 0.0205 | – |
| Hours with occurrence of precipitation (n/day) | <0.001 | – | 0.0122 | 2.770 |
| Wind velocity (m/s) | – | 0.009 | 0.0154 | −4.131 |
| Sunshine (min/day) | 0.022 | – | – | – |

*Notes.* [a]Proportion of cows using the brush at least once per day. [b]*T*-statistic; |*t*-value| > critical *t*-value is considered as significant. Critical *t*-value ($\alpha/2 = 0.05$): 2.015. [c]Calculated according to Mandel et al. (2013). [d]Excluded from the model during stepwise model reduction.

a combination of lack of feed coupled with an imbalanced diet, especially in production systems with little or no supplementation via concentrate. In order to investigate grazing cows' behavioural response to a lack of nutrients and the resulting metabolic load, we simulated a nutrient restriction by abruptly withdrawing concentrate. As expected, milk and blood traits responded as previously reported when feed and energy intake were restricted (Gross et al., 2011; Heublein et al., 2017; Reist et al., 2002), and the cows showed no clinical signs of metabolic disorders. During the reintroduction of concentrate, insulin concentration, milk fat yield and milk urea content returned to the baseline level of EW 1, whereas most of the other traits remained on a level between EW 1 and EW 2, indicating that the recovery from a metabolic challenge does not occur within 1 week. Interestingly, NEFA concentration decreased in EW 3 and was lower than in EW 1, confirming the data of Gross et al. (2011) showing a clear decrease in NEFA concentration from 3 weeks postpartum onwards. Furthermore, when comparing blood traits of grazing cows in early (Zbinden et al., 2017) and mid-lactation (Heublein et al., 2017), the effect of the concentrate supplementation was the same, but the level of NEFA concentration was much higher in early lactating cows.

As hypothesised, withdrawal of concentrate caused changes in ingestive behaviour, suggesting that the cows tried to compensate for energy deficiency by increasing eating time, chews and prehension bites and, consequently, herbage intake (Bargo, Muller, Kolver, & Delahoy, 2003). However, the low milk urea content of cows in this study compared with unsupplemented cows in other grazing studies (Heublein et al., 2017; Thanner et al., 2014) indicates that an absence of dietary protein might motivate cows to increase herbage intake as well. In line with our findings, unsupplemented grazing cows increased time spent eating, number of eating chews and time spent standing and moving, a physical activity associated with grazing, compared to cows supplemented daily with 6 kg of concentrate (Heublein et al., 2017). However, despite longer eating time and higher herbage intake, unsupplemented cows were not able to reach the total DM intake of supplemented cows, a result similar to recent observations in herbage-fed cows kept indoors (Zbinden et al., 2017). This outcome seemed to be the case in the present study as well, as milk and metabolic variables indicated a lack of nutrients throughout the whole of EW 2, although time spent eating gradually increased. Bargo et al. (2003) concluded that herbage-only diets lead to lower total DM intake compared with those supplemented with concentrate, probably because of a higher eating rate (g feed/min) of concentrate compared with herbage (Beauchemin, 1991). In addition, cows on pasture required more time to consume 1 kg of herbage DM and generally had a lower total DM intake than cows in a barn who had access to feed of the same quantity and quality (Dohme-Meier et al., 2014). The longer eating time of grazing cows might be explained by the smaller bite size due to more selective grazing compared with herbage-fed cows in the barn (Oshita, Sudo, Nonaka, Kume, & Ochiai, 2008). On the other hand, Rook (2000) stated that the increase in grazing time is limited by other activities. Cows have an inelastic need for lying and even prioritise lying over eating when the available time for these activities is restricted (Munksgaard, Jensen, Pedersen, Hansen, & Matthews, 2005). These findings indicate that, during a shortage of nutrients, grazing cows can increase eating time but not to the extent necessary to ingest sufficient feed to satisfy their needs. Similar to time spent eating, time spent ruminating increased at the expense of time spent idle when concentrate was withdrawn. Eating time and numbers of prehension bites remained high or slightly decreased to an intermediate level between EW 1 and EW 2, respectively, when concentrate was reintroduced, suggesting that cows tried to compensate for the lack of nutrients by increasing their total feed intake. In contrast, rumination time decreased to EW 1 levels. This decrease is predictable because time spent ruminating is strongly associated with dietary fibre content (Tafaj, Maulbetsch, Zebeli, Steingass, & Drochner, 2005) and decreases with decreasing fibre content, which is the case in herbage diets containing concentrate compared with diets without concentrate. However, in feeding studies where the rumination behaviour of unsupplemented grazing cows was compared with those of supplemented grazing cows (Heublein et al., 2017; McCarthy et al., 2007), no effect on time spent ruminating was observed. Based on these findings, changes in time spent ruminating might be a suitable indicator to detect metabolic load due to an acute deficiency in nutrients in grazing cows.

Furthermore, low-resilience activities decrease when time or energy resources are limited or when the cost involved in the activity increases (Von Frisch, 1999). According to Weary et al. (2009), ill animals divert resources to those functions of critical short-term value, whereas low-resilience behaviours that offer only longer-term fitness will be most likely to decline with illness. Therefore, these behaviours may provide an effective means of assessing animal welfare and long-term fitness (Held & Špinka, 2011). Automated brush use, which was identified as a low-resilience behaviour for dairy cows (Mandel et al., 2013), was still reduced at the brush installed away from the feed bunk in the second week after diagnosis of metritis (Mandel, Nicol, Whay, & Klement, 2017), whereas clinical signs persisted in only 10.7% of the cows (R. Mandel, personal communication, 24 June 2017). Similarly, as we hypothesised, cows in the present experiment not only spent less time using the brush during concentrate withdrawal (EW 2) but also during the concentrate reintroduction (EW 3). Concomitantly, cows reduced their time spent walking, which is necessary to reach the brush and, simultaneously, a high energy-expending activity (Kaufmann et al., 2011). In general, cows are highly motivated to engage in brushing (DeVries, Vankova, Veira, & Von Keyserlingk, 2007). Correspondingly, the proportion of cows using the automated brush on pasture and the time spent brushing were high before concentrate withdrawal and decreased during the withdrawal, whereas time spent eating increased. This outcome confirms the precedence for feeding over brushing reported by Mandel et al. (2013), who found that brushing activity decreased when the brush was located farther from the feed bunk. Similarly, time spent standing and moving increased due to the increased grazing activity, and time spent lying, an inelastic need of cows (Munksgaard et al., 2005), did not change during the concentrate withdrawal.

In contrast to time spent eating, which rapidly responded to the metabolic challenge, the response of lying time seemed to be inconsistent, with strong day-to-day variation indicating it is affected by factors other than nutrient supply. Ketelaar-de Lauwere et al. (2000) reported that the time grazing cows spent lying decreased as rainfall increased. Similarly, precipitation and wind velocity inversely affected time spent lying in the present experiment. However, time spent eating was also influenced by weather conditions. Similarly, Schütz, Clark, Cox, Matthews, and Tucker (2010) reported that feed intake decreased 62% when cows were exposed to rain and the combination of rain and wind. The duration of brush usage and proportion of cows using the brush were also influenced by several weather-related variables, confirming the findings of Mandel et al. (2013). Nevertheless, those authors suggested that brush usage had the potential to indicate a range of health and welfare problems in cows. In the present study, time spent eating seemed to reflect the short-term variation in grazing cows' nutrient supply better than other behavioural characteristics. While brushing activity may be linked to a cow's metabolic state, strong day-to-day variation disqualifies it as a marker to detect the onset of a metabolic challenge.

## 5 | CONCLUSIONS

Withdrawing concentrate increased the metabolic load in grazing dairy cows without them developing clinical signs of metabolic disorders. It seems that cows try to compensate for the nutrient deficiency by increasing the amount of time spent eating on pasture. Eating time remained high when concentrate was reintroduced, indicating that cows need time to recover from nutrient deficiency. Time spent brushing and the proportion of cows using the brush decreased when the concentrate was withdrawn. However, weather conditions markedly influenced brushing activity and other behavioural patterns, such as time spent lying. Therefore, we cannot recommend observations such as brushing activity as a marker for early detection of short-term variations in the metabolic state of grazing cows without considering weather conditions.

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