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Associations between peripartum lying and activity behaviour and blood non-esterified fatty acids and β-hydroxybutyrate in grazing dairy cows

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ABSTRACT

During early lactation, most dairy cows experience negative energy balance (**NEB**). Failure to cope with this NEB, however, can place cows at greater risk of developing metabolic disease. Our objective was to characterise, retrospectively, lying behaviour and activity of grazing dairy cows grouped according to blood non-esterified fatty acids (**NEFAs**) and β-hydroxybutyrate (**BHB**) as indicators of postpartum metabolic state. Blood was sampled weekly for up to 4 weeks precalving, on the day of calving (day 0), daily between 1 and 4 days postcalving, and then at least weekly between week 1 and week 5 postcalving for analysis of plasma NEFAs and BHB concentrations. Two hundred and forty-four multiparous Holstein-Friesian and Holstein-Friesian × Jersey cows were classified into one of three metabolic status groups based on maximum blood NEFAs and BHB concentrations during week 1 and 2 postcalving. A cow was classified as having either: (1) low NEFAs and low BHB (**Lo–Lo**; n = 78), when all blood samples were <1.0 mmol/L for NEFAs and ≤1.0 mmol/L for BHB during the first 2 weeks postcalving; (2) high NEFAs and low BHB (**Hi–Lo**; n = 134), when blood NEFAs were ≥1.0 mmol/L and blood BHB was ≤1.0 mmol/L at the same sampling time point during the first 2 weeks postcalving; or (3) high NEFAs and high BHB (**Hi–Hi**; n = 32), when blood NEFAs were ≥1.0 mmol/L and blood BHB was ≥1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving. Accelerometers (IceTag or IceQube devices; IceRobotics Ltd.) were used to monitor lying and activity behaviours peripartum (−21 to +35 days relative to calving). Changes in lying behaviour and activity occurred before the mean day that cows were classified Hi–Hi and Hi–Lo (2.2 and 3.5 d postcalving, respectively). Up to 3 weeks preceding calving, Hi–Hi cows were more active, had fewer daily lying bouts (**LBs**), and spent less time lying than Lo–Lo cows. In addition, Hi–Hi cows had fewer daily LBs and were less active up to 4 weeks postcalving than Lo–Lo cows, but these differences were biologically small. Groups of grazing cows classified as experiencing a more severe metabolic challenge behave differently up to 3 weeks precalving than their herdmates with lower blood NEFAs and BHB postcalving. These altered behaviours may allow identification of individual cows at risk of a metabolic challenge, but further research is required.

Implications

Dairy cows experience some degree of negative energy balance during early lactation due to the mobilisation of body tissue to support lactation. Maladaption to negative energy balance can have detrimental impacts on cow production, health, and welfare. Therefore, early detection of cows experiencing a greater degree of metabolic maladaptation is of interest to both farmers and researchers. This study demonstrated that the behaviour of grazing dairy cows differed up to 3 weeks before calving between cows classified into divergent postcalving metabolic status groups. Altered behaviours before calving could identify individual cows at risk of experiencing a metabolic challenge postcalving.

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Introduction

Following parturition, all cows experience some degree of negative energy balance (**NEB**), whereby they mobilise body tissue to support the demands of lactation (Nielsen and Ingvartsen, 2004). Negative energy balance is characterised by elevated non-esterified fatty acids (**NEFAs**) in circulation; however, if the liver is unable to process all the available NEFAs, this can lead to hepatic lipidosis (i.e., fatty liver disease) and incomplete oxidation of fat. Subsequently, increased production of ketone bodies as an alternative energy source may occur, which can be identified as elevated blood β-hydroxybutyrate (**BHB**) concentrations (hyperketonemia) and may result in the development of metabolic disorders. Hence, excessive NEB can have detrimental impacts on cow production and health (Ospina et al., 2010a and 2010b).

Elevated blood NEFAs (≥0.6–1.0 mmol/L) (LeBlanc et al., 2005; Ospina et al., 2010a, 2010b, and 2013) and elevated blood BHB concentrations (≥1.0–1.4 mmol/L) within 1–2 weeks postcalving (LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010a and 2013; McArt et al., 2012) have been independently associated with increased risk of developing clinical ketosis, and adverse milk production, reproduction, and health outcomes (e.g., displaced abomasum, metritis) in housed cows. Unfortunately, there are presently no cow-side diagnostic tests for measurement of blood NEFAs, meaning that monitoring is limited by the cost and delay of laboratory analyses. Further, although routine, validated blood or milk testing programmes to monitor BHB cow-side are available (Iwersen et al., 2009), they are still impractical at the individual level in pasture-grazed systems, where large numbers of cows calve within a seasonally concentrated timeframe.

Other detection methods, such as those using electronic devices to remotely and continuously monitor individual cow behaviour are, therefore, of increasing interest to researchers and producers, and are practical options for use in commercial pasture-grazing systems (Borchers et al., 2016). They may also offer the advantage of early prediction of metabolic maladaptation based upon behavioural changes. However, successful on-farm implementation of such technologies requires quantitative research to improve our understanding of behaviour changes that are associated with altered metabolic status.

Further, due to the inherently greater blood BHB in cows fed predominantly grazed pasture compared with those fed high amounts of concentrates (Roche et al., 2010), it is inconclusive whether elevated blood BHB (e.g. ≥1.2 mmol/L) is suitable as a sole indicator for diagnosing metabolic maladaptation in grazing cows and whether this indicates a grazing cow is sick or at risk of transition cow disease, such as subclinical ketosis (Compton et al., 2015; Phyn et al., 2017). Based on our understanding of the mechanisms relating to NEB, a combination of elevated blood NEFAs and BHB concentrations may provide a more robust indicator of maladaptation to early lactation NEB (Tremblay et al., 2018). Circulating NEFAs and BHB concentrations can be used as markers of metabolic status by reflecting the degree of mobilisation of stored fat and the incompleteness of oxidation of this fat in the liver resulting in ketone production, respectively (Ospina et al., 2010b).

Several studies have been undertaken in housed cows to describe the associations between rumination, lying, and activity behaviours and elevated blood NEFAs (Adewuyi et al., 2006; Liboreiro et al., 2015; van Hoeij et al., 2019) or BHB concentrations (Soriani et al., 2012; Kaufman et al., 2016; Piñeiro et al., 2019; van Hoeij et al., 2019). To our knowledge, there have not been any studies that have investigated these associations in grazing dairy cows, using either biomarker as a sole indicator or in combination; this is a logical initial step when investigating whether behaviour variables are suitable for predicting or detecting metabolic maladaptation. Grazing dairy cows engage in a high degree of physical activity to meet their nutrient needs and to walk to and from the milking parlour (Kaufmann et al., 2011; Beggs et al., 2018); the latter also means they can spend long times away from the paddock (Neave et al., 2021). This system places constraints on the time budgets of grazing cows and is generally associated with lower daily lying times (Hendriks et al., 2019), longer daily eating times (Huzzey et al., 2005; Matthews et al., 2012), and a different competitive environment for feeding relative to housed cows. Therefore, it is possible that grazing cows may be more likely to sacrifice energy-expensive 'maintenance' behaviours and engage in energy-conserving behaviours when experiencing metabolic stress compared with housed cows. We hypothesised that notable increases in lying time and reductions in activity would occur postcalving in grazing cows with elevated blood NEFAs and BHB concentrations compared with better metabolically adapted cows. Our objective was to investigate whether grazing cows retrospectively classified according to elevated blood NEFAs and BHB postcalving displayed behavioural differences before, at the time of, and after classification, when compared with cows classified with lower NEFAs and BHB postcalving.

Material and methods

Animal handling, experimental design, and management

Data for the present study were selected from a dataset of 310 cows described by Hendriks et al. (2019) and included pooled data of multiparous Holstein-Friesian and Holstein-Friesian × Jersey cows from three previous experiments [body condition score (**BCS**), feed, and zeolite studies described in Roche et al. (2015, 2017, and 2018), respectively, with additional information provided in Crookenden et al. (2020) for the zeolite study]. The BCS, feed, and zeolite studies were undertaken in each of 3 years (2013, 2014, and 2016, respectively) and two locations [Scott Farm; BCS study, and Lye Farm; feed and zeolite studies (both Hamilton, New Zealand, 37°46′S 175°18′E)]. All cows were eligible for inclusion in the study, and a subset of 244 cows was selected for further analyses.

Briefly, cows from Roche et al. (2015) (BCS study) were managed to be BCS 4.0 or 5.0 at 1 month before calving (10-point scale, where 1 is emaciated and 10 obese; Roche et al., 2004), and then, within each BCS category, cows were allocated one of three levels of metabolisable energy (**ME**) intake during the 3 weeks preceding calving (75, 100, or 125% of estimated ME requirements; Roche et al., 2015). Cows from Roche et al. (2017) (feed study) were managed to be in one of two BCS categories at dry-off (approximately 4.25 and 5.0 on a 10-point scale). Following dry-off, cows in both BCS categories were managed to achieve a BCS 5.0 at 1 month before calving. Cows within each 'far-off' feeding level treatment were then allocated to one of three levels of ME intake during the 3 weeks preceding calving (65, 90, or 120% of estimated ME requirements) (Roche et al., 2017). Cows from Roche et al. (2018) (zeolite study) were allocated to one of two treatment groups (Control and Zeolite) during the precalving period. Cows were BCS 5.0 at the start of the experimental period (~3 weeks precalving). Treatment cows received 500 g/cow per day Zeolite A (80% sodium aluminosilicate, synthetic embedded in starch; X-Zelit/Optimate MF+, Blue Pacific Minerals, New Zealand) mixed into 2 kg DM/cow per day maize silage and supplementation ceased at the first signs of calving (Crookenden et al., 2020). Control cows received the same allocation of maize silage without Zeolite A.

During the experimental period, non-lactating and lactating cows were offered pasture once daily between ~0745 h and 0930 h, and ~0800 h and 0830 h, respectively. Pasture offered

was a mixture of fresh perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Different-sized grazing areas (range: 23–60 m^2/cow) were allocated to cows depending on their treatment ME allocation. Cows were rotationally grazed as described by Roche et al. (2005) and managed as a typical commercial herd of grazing cows under a spring-calving system. During the experimental period, non-lactating cows received pasture silage in the feed study at the same time as fresh pasture was allocated. In the zeolite study, all non-lactating cows were brought to a covered feeding facility at ~0800 h to be individually fed maize silage before a fresh allocation of pasture. Lactating cows received pasture silage in all studies and maize silage in the BCS study as supplementary feeds at the same time as fresh pasture was allocated following the morning milking. During the postcalving period, cows were milked twice daily in a rotary parlour. Total time spent standing while being milked and walking to and from the milking parlour ranged from ~40 to 90 min/day.

Blood sampling and analyses

The blood sampling protocols and analyses are described in detail in the studies mentioned above. Briefly, blood was sampled on the day of calving (day 0), and day 1, 2, 3, and 4 postcalving in all studies. Blood was also sampled weekly, for 4 weeks precalving until 5 weeks postcalving in the BCS and feed studies. In the zeolite study, blood was sampled weekly for 3 weeks precalving until 4 weeks postcalving, with a subset of 20 cows sampled daily from 2 weeks before expected calving date until day 10 postcalving and on day 18 and 24 postcalving.

Blood was sampled by coccygeal venipuncture into evacuated blood tubes containing lithium heparin anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Heparinised samples were placed immediately into iced water and centrifuged within 30 min of collection at 1 500*g* for 12 min at 4 °C. Following centrifugation, aspirated plasma was stored at −20 °C until assayed for metabolite analyses. Full details of the metabolite analyses are described in Roche et al. (2015), Crookenden et al. (2020), and otherwise in detail below.

Plasma samples were analysed for metabolites by Gribbles Veterinary Pathology Ltd. (Hamilton, New Zealand). Blood metabolites were assayed using colorimetric techniques at 37 °C with a Hitachi Modular P800 analyzer (Roche Diagnostics, Indianapolis, IN). Roche reagent kits were used in the BCS and feed studies and the Randox kit was used in the zeolite study to measure plasma concentrations of BHB (mmol/L; reduction of NAD+ to NADH during oxidation of D-3-hydroxybutyrate to acetoacetate). Plasma NEFAs concentrations (mmol/L) were measured using Wako Chemicals (Osaka, Japan) kit NEFAs HR2 measuring oxidative condensation of 3-methyl-N-ethyl-N-β hydroxyethyl aniline with 4-aminoantipyrine in the BCS and feed studies and using the acyl-CoA synthetase, acyl-CoA oxidase (**ACS-ACOD**) colorimetric method using the Wako NEFAs C kit in the zeolite study. The inter- and intra-assay coefficients of variation for all assays were <5.5% and ≤15%, respectively, as reported in Roche et al. (2015 and 2017) and Crookenden et al. (2020).

Classification of metabolic status

Cows were classified according to commonly reported blood NEFAs and BHB cut-points during the first 2 weeks postcalving, and retrospectively allocated to one of three metabolic status groups. Due to slight differences in sampling frequency during the first 2 weeks postcalving between the three studies, the maximal blood NEFAs and BHB values were extracted by week using PROC MEAN. These maximum blood NEFAs and BHB values within week were used to classify cows. Further, due to the retrospective classification of cows according to both blood NEFAs and BHB, we investigated a range of classification strategies to determine an appropriate approach based on: (1) critical thresholds reported in literature (blood NEFAs ≥0.6, ≥0.7, ≥0.9, ≥1.0 mmol/L and BHB ≥1.0, ≥1.2, ≥1.4 mmol/L) (LeBlanc et al., 2005; Duffield et al., 2009; McArt et al., 2012; Ospina et al., 2010a, 2010b, and 2013), (2) maximum NEFAs and BHB exceeding the critical thresholds during weeks 1 and 2 independently and during both weeks 1 and 2 postcalving, and (3) sufficient numbers in each category (n > 20). We chose the upper critical threshold for NEFAs (≥1.0 mmol/L) and the middle threshold for BHB (≥1.2 mmol/L) and categorised cows according to maximal NEFAs and BHB during either week 1 or 2 postcalving, or during both weeks 1 and 2 postcalving, as this classification strategy allowed for sufficient numbers in each group (n > 20).

Of 310 cows available from the dataset, 17 cows were removed prior to classification due to missing blood metabolite records during week 1 or 2 postcalving. A total of 293 cows were available for selection and none of the cows were recorded as exhibiting any symptoms of clinical disease or lameness during the experimental periods of the three parent experiments. A cow was classified as having low NEFAs and low BHB (**Lo–Lo**; n = 78) when all samples were <1.0 mmol/L for NEFAs and ≤1.0 mmol/L for BHB during the first 2 weeks postcalving. A cow was classified as having high NEFAs and low BHB (**Hi–Lo**; n = 134) when blood NEFAs was ≥1.0 mmol/L and blood BHB was ≤1.0 mmol/L at the same sampling time point during the first 2 weeks postcalving (i.e., during either week 1 or 2 postcalving, or during both weeks 1 and 2 postcalving). A cow was classified as having high NEFAs and high BHB (**Hi–Hi**; n = 32) when blood NEFAs was ≥1.0 mmol/L and blood BHB was ≥1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving. To complete the factorial, cows were classified as having low NEFAs and high BHB (**Lo–Hi**) when blood NEFAs was <1.0 mmol/L and blood BHB was ≥1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving, as others have reported cases where hyperketonemia occurs without a concurrent increase in blood NEFAs (Luke et al., 2019); however, due to a low number of subjects (n = 15), this group was deemed insufficient for meaningful statistical analysis and was excluded.

Due to some ambiguity in literature surrounding the appropriate cut-points for BHB (Gordon et al., 2013; Compton et al., 2015), we chose two divergent cut-points to categorise high (≥1.2 mmol/L) and low (<1.0 mmol/L) blood BHB to increase the likelihood of detecting differences in behaviour. As a result, 34 cows were not allocated to a group due to their blood BHB concentrations falling in between the threshold set for classification (e.g., blood BHB was >1.0 and <1.2 mmol/L). Therefore, 244 cows were included in the final dataset for further statistical analysis.

All cows included in the study were multiparous (i.e., approaching their second or greater parity at the time of calving), and parity was grouped as follows: parity 2–3 (n = 158), and parity 4–9 (parity 4+; n = 86) (Table 1). The mean parity ± SD across the three metabolic status groups was 3.21 ± 0.41, 3.40 ± 0.49, and 3.50 ± 0.51 for the Lo–Lo, Hi–Lo, and Hi–Hi groups, respectively.

Milk, body condition score, BW, and breed

Cows were milked twice daily, and milk yield was measured daily from 1 to 35 days in milk (DIM). Milk was sampled weekly on consecutive afternoon and morning milkings, and a composite sample was analysed for milk composition by infrared analysis (FT120, Foss Electric, Hillerød, Denmark). Energy-corrected milk (**ECM**) yield was calculated as Nielsen et al. (2009):

Table 1
Number of dairy cows (n) by parity, breed, and study for three metabolic status groups.

	Metabolic status group		
n (cows)	Lo–Lo (n = 78)	Hi–Lo (n = 134)	Hi–Hi (n = 32)
Parity 2–3[1]	62	80	16
Parity 4+[1]	16	54	16
Breed (HF)	53	105	25
Breed (HF × J)	25	29	7
BCS study[2]	40	59	9
Feed study[2]	37	47	12
Zeolite study[2]	1	28	11

Abbreviations: Lo–Lo = (blood non-esterified fatty acids (NEFAs) < 1.0 mmol/L and blood β-hydroxybutyrate (BHB) ≤ 1.0 mmol/L at all samplings during the first 2 weeks postcalving; Hi–Lo = blood NEFAs ≥ 1.0 mmol/L and blood BHB ≤ 1.0 mmol/L at the same sampling time point during the first 2 weeks postcalving; Hi–Hi (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≥ 1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving; BCS = body condition score; HF = Holstein-Friesian; HF × J = Holstein-Friesian × Jersey.

[1] Parity 2–3 = cows approaching their second or third parity at the time of calving; parity 4+ = cows approaching their fourth to ninth parity at the time of calving.

[2] Cows were selected from the BCS study as described by Roche et al. (2015), the feed study as described by Roche et al. (2017), or the zeolite study as described by Roche et al. (2018) and Crookenden et al. (2020).

$$\text{kg of ECM} = [\text{kg of milk} \times (383 \times \text{fat}\% + 242 \times \text{protein}\% + 780.8)]/3\ 140$$

Body weight was recorded and BCS was determined weekly following the morning milking or at approximately 0800 h during the non-lactating period. All BCS assessors were trained and recalibrated at the start of the experiment following the procedures set out in Macdonald and Roche (2011). Animal evaluation data for cow breed were provided by Livestock Improvement Corporation Ltd. (Hamilton, New Zealand).

Behavioural data and editing

A full description of the behavioural data collection and editing methods are described in Hendriks et al. (2019). Behavioural data were available for analysis for the period –21 days precalving to +35 days postcalving, relative to the day of calving (day 0). In brief, a triaxial accelerometer (IceTag or IceQube; IceRobotics Ltd., Edinburgh, Scotland) was fitted to each cow on the lateral side of a hind leg and behavioural data were recorded. Data were downloaded using the IceManager 2010 software (IceRobotics Ltd.) from the on-board memory of the device. Two summary files were generated for each individual cow; one file consisted of lying time (s) and number of steps recorded at 1- and 15-min sampling intervals, for the IceTag and IceQube, respectively, and the other file contained lying bouts (**LBs**) recorded by date, timestamp (hh:mm:ss), and duration (s). These summary files were used to calculate hourly and daily lying time (h/day), LBs (no./day), mean LB duration (min/day), and number of steps taken (steps/day) for each individual cow. A LB was defined as the period between the accelerometer changing from vertical to horizontal and back to vertical. Data excluded from the analysis included data recorded on the day that accelerometers were removed from, or fitted to the cows, and incorrect recordings due to technical errors.

From the output data sets, the sampling dates for each individual cow were assigned an experimental day relative to day 0 based on the recorded calving date. Farm staff collected newborn calves and dams once daily, and dams were milked for the first time at the afternoon milking. Consequently, there can be a discrepancy of up to 24 h for recording the date of calving. Therefore, lying behaviour and activity were adjusted, where appropriate, using activity data to re-assign calving day as previously described by Hendriks et al. (2020a). Briefly, we assumed that for individual cows where LBs were <14 on day 0, but were ≥14 LBs on day –1, the latter was likely associated with a calving event. Otherwise, it was assumed that the recorded calving date was correct. The methodology used to adjust for the discrepancy in the assignment of calving day has not been validated in a grazing system, which is an acknowledged limitation. All data including blood BHB and NEFAs were adjusted, where appropriate, according to re-assigned calving day, and these transformed datasets were the basis of subsequent analyses.

Weather

Daily rainfall (mm; 24-h period) and daily air temperature (°C; recorded at 0900 h) data were retrieved from The National Climate Database (National Institute of Water and Atmospheric Research, 2018) for the duration of the three experiments. Data were retrieved from station agent number 26 117 (37.8°S, 175.3°E) for all three studies (National Institute of Water and Atmospheric Research, 2018). The distance from the climate station to the study site for the three studies is ∼3 km. Mean daily rainfall for the study duration was 2.75 mm (min = 0 mm; max = 52.6 mm), and mean daily air temperature was 8.38 °C (min = –0.90 °C; max = 16.5 °C).

Statistical analyses

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Results are presented as least squares means ± SEM in the text and mean SED in the tables and figures. Where we have presented least squares means for the three metabolic status groups, we have presented group mean effects. The covariance structures selected were compound symmetry or autoregressive based on the lowest Akaike information criterion. Study and treatment from the three previous experiments were concatenated to create a categorical variable study group.

Study group (categorical) and calving season day within the herd (difference in days between adjusted calving date and the first day in June) were included to adjust for possible confounding due to different treatments and different calving dates within the three previous experiments in all models described below. Due to the greater risk of subclinical ketosis in older pasture-grazed cows, all models were adjusted for parity (categorical; parity 2–3 or parity 4+) (Compton et al., 2015). All repeated-measures ANOVA models were pairwise comparison-adjusted using Tukey-Kramer.

Blood metabolite markers

The sampling dates for blood data were assigned an experimental day relative to day 0 based on the re-assigned calving day. Blood data for energy metabolites (NEFAs and BHB) were summarised into six periods (i.e., day –14 to –1 precalving and day 0–2, 3–7, 8–14, 15–21, and 22–28 postcalving). Mean sampling times within these six periods were as follows: day 7 precalving (n = 597) and day 1 (n = 599), 4 (n = 528), 11 (n = 286), 18 (n = 263), and 25 postcalving (n = 261) with a mean ± SD of ± 2.1 days. To examine the differences in metabolites profiles among metabolic status groups and across periods, a repeated-measures ANOVA was undertaken using PROC MIXED, with cow as a random effect, period as a repeated measure, and the fixed effect of metabolic status, period, and metabolic status × period interactions. Variables were checked for skewness and to meet the assumption of normal distribution. Log-transformation was used to normalise blood BHB for the analysis, and untransformed least squares means, SEM, and SED are presented.

Behavioural parameters

In our study, based on previously determined thresholds for IceRobotics sensors, LBs <33 s and ≤2 min were discarded from the raw data recorded by the IceQube and IceTag devices, respectively, as described by Hendriks et al. (2020b). Behaviour data were summarised for 15 periods: day –21 to –15,–14 to –8, –7 to –4 precalving, daily precalving (day –3 to –1), day 0, daily postcalving (day 1–3), day 4–7, 8–14, 15–21, 22–28, and 29–35 postcalving due to known variation in behaviour across the peripartum period (Hendriks et al., 2019). Differences in daily lying time, daily number of LBs, mean LB duration, and number of steps taken among the three metabolic status groups were analysed using a repeated-measures ANOVA (PROC MIXED) with cow as a random effect, period as a repeated measure, and the fixed effect of metabolic status, period, and metabolic status × period interactions. Behavioural analyses included fixed effect of daily rainfall (continuous) and mean air temperature at 0900 h (continuous), and their interactions as potential explanatory variables. Variables were checked for skewness and to meet the assumption of normal distribution. Log-transformation was used to normalise LBs and LB duration, whereas step counts were transformed using square root for statistical analysis and *P*-values presented. Untransformed least squares means, SEM, and SED are presented for ease of interpretation.

Metabolic status, parity, and metabolic status × parity interactions were also investigated for all models described above; however, no interactive metabolic status by parity effects were detected for daily lying time ($P = 0.99$), daily LBs ($P = 0.96$), mean LB duration ($P = 0.90$), or number of steps taken ($P = 0.46$).

Within-day behavioural parameters

The behaviour data within a 24-h period were summarised by hour, where 0000 h was equivalent to the period from midnight until 0059 h (0000 h = 0000–0059 h, 0100 h = 0100–0159 h, and so on). Behaviour data were analysed using a repeated-measures ANOVA (PROC MIXED) to investigate the association between metabolic status and time of day and lying time and number of steps taken over a 24-h period during day –21 to –8 precalving due to significant differences in daily behaviour measures among metabolic status groups identified during this time. Included in the models were cow as a random effect, hour as a repeated measure, and the fixed effect of metabolic status, hour, and metabolic status × hour interactions.

Milk, body condition score, and BW

Weighted means for weekly milk yield were calculated using daily yields on a per-cow basis for week 1–7 postcalving using PROC MEAN. Weighted means for milk yield were used to calculate weekly milk component yields and ECM yield for week 2–6 postcalving. Due to a lack of records for milk composition from 141 cows during week 1 postcalving (i.e. the weekly herd test date coincided with their colostrum period), these data were excluded from ECM yield and milk protein and fat composition analysis. To examine the differences in milk and ECM yield, and milk protein and fat composition, among metabolic status groups within 6 weeks postcalving, a repeated-measures ANOVA was performed using PROC MIXED. Cow was included as a random effect, week as a repeated measure, and metabolic status as a fixed effect.

Body condition score and BW were summarised into two periods pre- (–4 to –1 weeks) and postcalving (1–6 weeks). A repeated-measures ANOVA was undertaken using PROC MIXED, with cow as a random effect, week as a repeated measure, and the fixed effect of metabolic status to investigate the differences in BCS and BW (4 weeks pre- and 6 weeks postcalving) among the three metabolic status groups pre- and postcalving. Additional analyses were undertaken to investigate the associations between BCS and BW, and metabolic status and week. A repeated-measures ANOVA was performed using PROC MIXED, with cow as a random effect, week as a repeated measure, and the fixed effect of metabolic status, week, and metabolic status by week interactions. Mean week –5 to –6 precalving BCS and BW were included as covariates in the models investigating BCS and BW, respectively.

Results

Metabolic status indicators, body condition score, and BW

The mean ± SD day that cows were classified Hi–Hi and Hi–Lo was 2.2 ± 4.16 days and 3.5 ± 4.80 days postcalving, respectively. Mean blood NEFAs and BHB concentrations differed ($P < 0.001$) among the three metabolic status groups, with metabolic status × period interactions ($P < 0.001$) detected for both metabolites (Fig. 1a and b, respectively).

During day –14 to –1 precalving, the Hi–Lo group had lower ($P < 0.05$) blood NEFAs concentrations (0.46 ± 0.02 mmol/L) than the Lo–Lo group (0.56 ± 0.03 mmol/L), but neither group differed ($P \geq 0.58$) from the Hi–Hi group (0.51 ± 0.05 mmol/L). During day 0–2 and day 3–7 postcalving, blood NEFAs concentrations were greatest ($P < 0.01$) in the Hi–Hi group (0.85 ± 0.04 and 1.14 ± 0.05 mmol/L, respectively) followed by the Hi–Lo group (0.68 ± 0.02 and 0.91 ± 0.02 mmol/L, respectively), which, in turn, had greater ($P < 0.001$) blood NEFAs than the Lo–Lo group (0.46 ± 0.03 and 0.56 ± 0.03 mmol/L, respectively). During day 8–14 postcalving, blood NEFAs did not differ between the Hi–Hi and Hi–Lo groups ($P = 0.36$; 1.06 ± 0.06 and 0.97 ± 0.03 mmol/L, respectively), but both groups had greater ($P < 0.001$) concentrations than the Lo–Lo group (0.69 ± 0.04 mmol/L). There were no differences ($P \geq 0.15$) in blood NEFAs among metabolic status groups during day 15–21 postcalving. During day 22–28 postcalving, Hi–Lo cows had greater ($P < 0.05$) blood NEFAs than Lo–Lo cows (0.68 ± 0.03 and 0.54 ± 0.04 mmol/L, respectively). The Hi–Hi cows were intermediate (0.58 ± 0.06 mmol/L), but not different ($P \geq 0.32$) from either group.

During day –14 to –1 precalving and day 0–2 postcalving, blood BHB concentrations were greatest ($P < 0.001$) in the Hi–Hi group (0.59 ± 0.02 mmol/L and 0.85 ± 0.02 mmol/L, respectively) compared with the Hi–Lo (0.45 ± 0.01 and 0.66 ± 0.01 mmol/L, respectively) and Lo–Lo groups (0.43 ± 0.02 and 0.63 ± 0.02 mmol/L, respectively), which were not different from each other during either period ($P = 0.10$ and 0.28, respectively). Between day 3–7 and day 8–14 postcalving, blood BHB concentrations were greatest ($P < 0.001$) in the Hi–Hi group (0.97 ± 0.02 and 0.80 ± 0.03 mmol/L, respectively), and the Hi–Lo group (0.68 ± 0.01 and 0.65 ± 0.02 mmol/L, respectively) had greater ($P < 0.05$) blood BHB concentrations than the Lo–Lo group (0.62 ± 0.02 and 0.58 ± 0.02 mmol/L, respectively). These differences among metabolic status groups were reduced by day 15–21 and day 22–28 postcalving (Fig. 1b).

Precalving mean BCS and BW were not different among the metabolic status groups; however, postcalving mean BCS and BW were lowest, on average, in the Hi–Hi group compared with the Hi–Lo and Lo–Lo groups (Table 2). There were metabolic status by week interactions ($P < 0.001$) for BCS and BW during the 4 weeks precalving and 6 weeks postcalving (Fig. 2a and b, respectively). During week 1–6 postcalving, cows in the Hi–Hi group consistently lost more BCS and BW than cows in the Hi–Lo ($P < 0.05$) and Lo–Lo groups ($P < 0.01$). Cows in the Hi–Lo group lost more BCS ($P < 0.01$) and BW ($P < 0.001$) than Lo–Lo cows, but these differences were not apparent until week 4–6 and week 3–6 postcalving, respectively.

Mean milk and ECM yield and fat and protein composition during the first 2 months of lactation for the three metabolic status groups are presented in Table 2. Briefly, ECM yields during the first

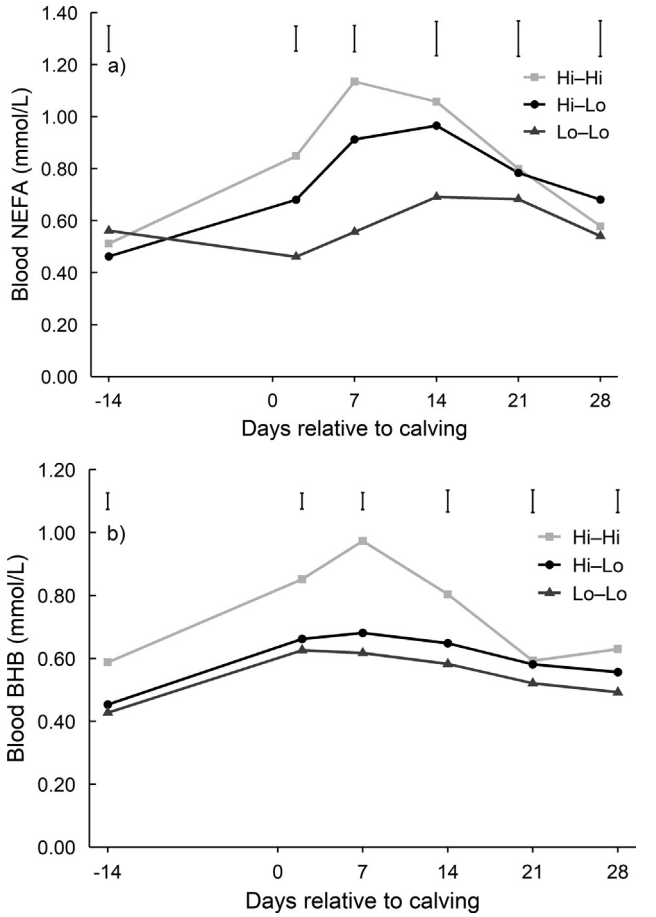

Fig. 1. Blood non-esterified fatty acids [NEFAs; (a)] and blood β-hydroxybutyrate [BHB; (b)] concentrations (mmol/L) during day –14 and –1 precalving, day 0–2, 3–7, 8–14, 15–21, and 22–28 postcalving for dairy cows classified into three metabolic status groups [Lo–Lo (blood NEFAs < 1.0 mmol/L and blood BHB ≤ 1.0 mmol/L at all samplings during the first 2 weeks postcalving); Hi–Lo (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≤ 1.0 mmol/L at the same sampling time point during the first 2 weeks postcalving); Hi–Hi (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≥ 1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving)]. Error bars represent 2 × mean SED.

2–6 weeks in milk were, on average, 1.3 kg/day greater in the Hi–Lo group relative to the Lo–Lo group; the Hi–Hi group were intermediate, but not different from the Hi–Lo and Lo–Lo groups. Milk protein % was lower, on average, in Hi–Hi and Hi–Lo cows compared with Lo–Lo cows. Milk fat % only differed between groups at week 2 and 5 in milk; the Hi–Hi group tended to have a lower milk fat % (4.95 ± 0.11 and 4.09 ± 0.11%, respectively) than the Lo–Lo group [4.65 ± 0.08 (P = 0.07) and 4.41 ± 0.08% (P = 0.06), respectively]. The Hi–Lo group (4.80 ± 0.05 and 4.34 ± 0.05%) were intermediate, but not different (all $P \geq 0.12$) from either Lo–Lo or Hi–Hi groups.

Lying behaviour and activity pre- and postcalving

There were metabolic status × period interactions ($P < 0.001$; Table 2) for daily lying time, daily LB number, mean LB duration, and number of steps taken per day during day –21 pre- to 35 postcalving (Fig. 3a–d, respectively). Furthermore, the number of daily LBs differed among the groups during this period: the Hi–Hi group had fewer ($P < 0.05$) daily LBs, on average, than the Lo–Lo group, but neither group differed from the Hi–Lo group (Table 2).

Lying time, lying bouts, and activity precalving

Cows classified as Hi–Hi spent less time lying than Lo–Lo cows, and had fewer daily LBs and took more steps than Hi–Lo and Lo–Lo cows, during the weeks before calving (Fig. 3a, b, and d).

During day –21 to –15 and day –14 to –8 precalving, the Hi–Hi group spent less time lying [9.46 ± 0.28 ($P < 0.05$) and 9.93 ± 0.27 h/day (P = 0.07), respectively] than the Lo–Lo group (10.4 ± 0.20 and 10.7 ± 0.19 h/day, respectively). The Hi–Lo group spent a similar amount of time lying ($P \geq 0.13$) each day (10.0 ± 0.14 and 10.2 ± 0.13 h/day, respectively) compared with the Hi–Hi and Lo–Lo groups (Fig. 3a).

The greatest differences in lying time among metabolic status groups occurred on day –3 to –1 precalving (Fig. 3a). The Hi–Hi group spent less time ($P < 0.05$) lying down than the Lo–Lo group during day –3 (9.36 ± 0.39 vs. 10.6 ± 0.26 h/day; $P < 0.05$), –2 (8.25 ± 0.39 vs. 9.98 ± 0.26 h/day; $P < 0.001$), and –1 (7.83 ± 0.39 vs. 9.01 ± 0.26 h/day; $P < 0.05$). However, the Hi–Hi group was only different ($P < 0.01$) from the Hi–Lo group on day –2 (9.52 ± 0.19 h/day). Further, although the Hi–Lo group was not different (P = 0.32) from the Lo–Lo group on day –2, they spent less time ($P < 0.05$) lying down on day –1 (8.14 ± 0.19 h/day) and tended to spend less time (P = 0.09) lying down on day –3 (9.88 ± 0.19 h/day) than the Lo–Lo group.

During day –21 to –15 and day –14 to –8 precalving, the Hi–Hi group also had fewer LBs [6.39 ± 0.44 and 7.20 ± 0.42 no./day (both $P < 0.001$), respectively] than the Lo–Lo group (8.41 ± 0.31 and 9.13 ± 0.29 no./day, respectively) (Fig. 3b). During the same period, the Hi–Lo group were intermediate for number of LBs (7.53 ± 0.21 and 8.14 ± 0.20 no./day, respectively) between the Hi–Hi ($P \leq 0.01$) and Lo–Lo groups ($P \leq 0.06$).

Despite the large decrease in total daily lying time in cows in the Hi–Hi group in the 3 days precalving, daily LB during this time were not different ($P \geq 0.23$) among groups, however, the number of LB increased in all three groups as the cows approached calving. Further, LB duration (Fig. 3c) was only different on day –2 precalving, where the Hi–Hi group had shorter (both $P < 0.05$) bouts of lying (49.9 ± 4.09 min/bout) than the Hi–Lo and Lo–Lo groups [62.5 ± 2.00 and 65.7 ± 2.72 min/bout, respectively], which were not different from each other (P = 0.20).

During day –14 to –8 and day –7 to –4 precalving, and on day –2 precalving, cows in the Hi–Hi group were more active and took, on average, between 511–758 and 384–668 more steps/day (range = minimum and maximum difference in least squares means across all three periods) than cows in the Hi–Lo ($P < 0.05$) and Lo–Lo groups ($P < 0.01$), respectively. During day –14 to –8 precalving, cows in the Hi–Lo group tended (P = 0.07) to take, on average, 227 more steps/day than Lo–Lo cows, but step counts did not differ among groups during day –7 to –4 and on day –2 precalving ($P \geq 0.54$) (Fig. 3d). On day –1 precalving, activity levels for the cows in the Hi–Hi and Hi–Lo groups were not different (3 453 ± 210 vs. 3 114 ± 102 steps/day; P = 0.36); however, both groups were more active ($P < 0.01$ and $P < 0.05$, respectively) than the Lo–Lo group (2 658 ± 139 steps/day).

Lying time, lying bouts, and activity postcalving

Daily lying time from day 0 to 35 postcalving did not differ ($P \geq 0.12$) among groups of grazing cows varying in metabolic status (Fig. 3a); however, there were differences among groups in daily LB number, mean LB duration, and number of steps taken postcalving (Fig. 3b–d).

On the day of calving and day 2 postcalving, cows in the Hi–Hi group had ($P < 0.05$) fewer LBs (15.5 ± 0.60 and 8.50 ± 0.60 no./day, respectively) than the Lo–Lo group (18.8 ± 0.40 and 10.4 ± 0.40 no./day); although neither group differed ($P \geq 0.33$ and P = 0.11, respectively) from the Hi–Lo group (17.2 ± 0.30 and 9.17 ± 0.29 no./day). On the day of calving, cows in the Hi–Hi group took more

Table 2
Means for cow performance and behaviour parameters for three metabolic status groups.

	Metabolic status group				*P*-value	
Parameter	Lo–Lo	Hi–Lo	Hi–Hi	Mean SED	Metabolic status	Metabolic status × Week
Cow performance						
BCS[1] (10-point scale)						
Precalving	4.82	4.88	4.86	0.02	0.106	
Postcalving	4.38[a]	4.32[a]	4.17[b]	0.03	<0.001	
BW[1] (kg)						
Precalving	559	557	558	1.76	0.535	
Postcalving	495[a]	487[b]	473[c]	2.39	<0.001	
Milk yield[2] (kg/day)	24.7[b]	26.2[a]	25.6[a]	0.40	0.005	0.040
ECM yield[3] (kg/day)	26.9[b]	28.2[a]	27.2[ab]	0.40	0.008	0.141
Milk fat[3] (%)	4.48	4.48	4.38	0.06	0.471	0.010
Milk protein[3] (%)	3.60[a]	3.48[b]	3.45[b]	0.02	<0.001	0.199
Behaviour[4]						
Daily lying time (h/day)	8.85	8.59	8.45	0.18	0.348	<0.001
Daily LB (no./day)	9.93[a]	9.24[ab]	8.71[b]	0.28	0.053	<0.001
Mean LB duration (min/bout)	59.3	61.1	64.4	1.74	0.213	<0.001
Steps taken (steps/day)	3 603	3 586	3 740	87.6	0.494	<0.001

Abbreviations: Lo–Lo = (blood non-esterified fatty acids (NEFAs) < 1.0 mmol/L and blood β-hydroxybutyrate (BHB) ≤ 1.0 mmol/L at all samplings during the first 2 weeks postcalving; Hi–Lo = blood NEFAs ≥ 1.0 mmol/L and blood BHB ≤ 1.0 mmol/L at the same sampling time point during the first 2 weeks postcalving; Hi–Hi (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≥ 1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving; BCS = body condition score; ECM = energy-corrected milk; LB = lying bouts.
[a–c]Means with different superscripts are significantly different at the 5% confidence level.
[1] BCS (10-point scale, where 1 is emaciated and 10 is obese; Roche et al., 2004) and BW during the 4 week pre- and 6 week postcalving period. Metabolic status by week interactions for BCS and BW for the period encompassing 4 week pre- and 6 week postcalving are presented in the text and Fig. 2a and 2b, respectively.
[2] Milk yield during the first 7 weeks in milk.
[3] Energy-corrected milk yield, milk fat and protein % during weeks 2–6 in milk.
[4] Overall means for behaviour parameters presented for the period –21 d precalving to 35 d postcalving.

steps (P = 0.05) than Lo–Lo cows (4 175 ± 210 vs. 3 540 ± 139 steps/day, respectively); the Hi–Lo group (3 901 ± 102 steps/day) did not differ from the Hi–Hi and Lo–Lo groups (P = 0.46 and 0.15, respectively) (Fig. 3d). In the days immediately postcalving (days 1–7), we did not detect any further differences in daily lying time, LBs, or activity among the three metabolic status groups.

During day 8–28 postcalving, further differences among the three metabolic status groups were evident for LB number and duration. Cows in the Hi–Hi group engaged in fewer LBs (∼1/day) during day 8–14 and day 15–21 postcalving (P = 0.06 and P < 0.05, respectively) than their Lo–Lo peers. The Hi–Lo cows were intermediate and not different compared with either group ($P \geq 0.14$; Fig. 3b). During day 22–28 postcalving, cows in the Hi–Hi group had fewer but longer LBs (6.83 ± 0.42 no./day and 71.6 ± 2.65 min/bout, respectively) than cows in the Hi–Lo [8.04 ± 0.21 no./day; (P < 0.01) and 62.6 ± 1.31 min/bout, respectively; (P < 0.01)] and Lo–Lo groups [8.53 ± 0.30 no./day; (P < 0.001) and 58.8 ± 1.88 min/bout, respectively; (P < 0.001)], which were not different ($P \geq 0.17$) from each other.

Furthermore, Hi–Lo cows took fewer steps during day 15–21 postcalving (4 639 ± 64 steps/day; P = 0.07) and day 22–28 postcalving (4 562 ± 66 steps/day; P < 0.001) than Lo–Lo cows (4 930 ± 93 and 5 059 ± 94 steps/day, respectively). The Hi–Hi group were intermediate (4 607 ± 133 and 4 681 ± 134 steps/day), but not different ($P \geq 0.20$) from the Hi–Lo and Lo–Lo groups (Fig. 3d).

Within-day profiles for daily lying time and steps precalving

The within-day profiles of lying time and activity during 2–3 weeks precalving indicate that there were metabolic status by hour interactions for hourly lying time and number of steps taken (Fig. 4a and b, respectively). Differences in precalving behaviour among metabolic status groups were evident within distinct periods of the day and the range of mean values (range = minimum and maximum least squares means and SEM) within the hours specified is reported below.

During the early morning, Hi–Hi cows spent less time lying (P < 0.05) between 0300 and 0700 h (range = 28.7 ± 1.26–49.8 ± 1.32 min/h) than Lo–Lo cows (range = 33.8 ± 0.81–54.3 ± 0.85 min/h). They also spent less time lying (P < 0.05) between 0300 and 0500 h, and 0600 and 0700 h than Hi–Lo cows (range = 32.3 ± 0.58–53.5 ± 0.61 min/h). In turn, Hi–Lo cows spent less time lying (P < 0.01) between 0500 and 0600 h than Lo–Lo cows (49.9 ± 0.59 vs. 53.4 ± 0.82 min/h, respectively), but were not different from Hi–Hi cows at this time (48.6 ± 1.28 min/h). There were no further differences ($P \geq 0.29$) between 0300 and 0500 h and 0600 and 0700 h (Fig. 4a). The Hi–Hi cows also took more steps (range = 96 ± 9–368 ± 9 steps/h; P < 0.05) between 0600 and 0700 h, and 0900 and 1000 h than Hi–Lo cows (range = 78 ± 4 and 303 ± 4 steps/h), which in turn, took more steps (P < 0.01) than Lo–Lo cows (range = 60 ± 6 and 266 ± 6 steps/h) (Fig. 4b). The Hi–Hi and Hi–Lo cows took a similar number of steps between 0700 and 0800 h (P = 0.14).

Similarly, during late afternoon (between 1900 and 2000 h); while not different (P = 0.50) from each other, Hi–Hi and Hi–Lo cows spent less time (P < 0.05) lying down (37.6 ± 1.29 and 39.2 ± 0.60 min/h, respectively) than Lo–Lo cows (41.6 ± 0.83 min/h). The Hi–Hi cows also took more steps (190 ± 9 steps/h; P < 0.05) between 1500 and 1600 h than the cows in the Lo–Lo group (152 ± 6 steps/h), but neither group were different ($P \geq 0.21$) from Hi–Lo cows (174 ± 4 steps/h).

Discussion

Investigating the associations between behaviour and metabolic status in groups of grazing dairy cows will improve our understanding of whether these behaviours have potential as early indicators of disease. To our knowledge, this is the first study to characterise the lying behaviour and activity before, at the time of, and after calving in groups of grazing cows classified according to both blood NEFAs and BHB concentrations postcalving.

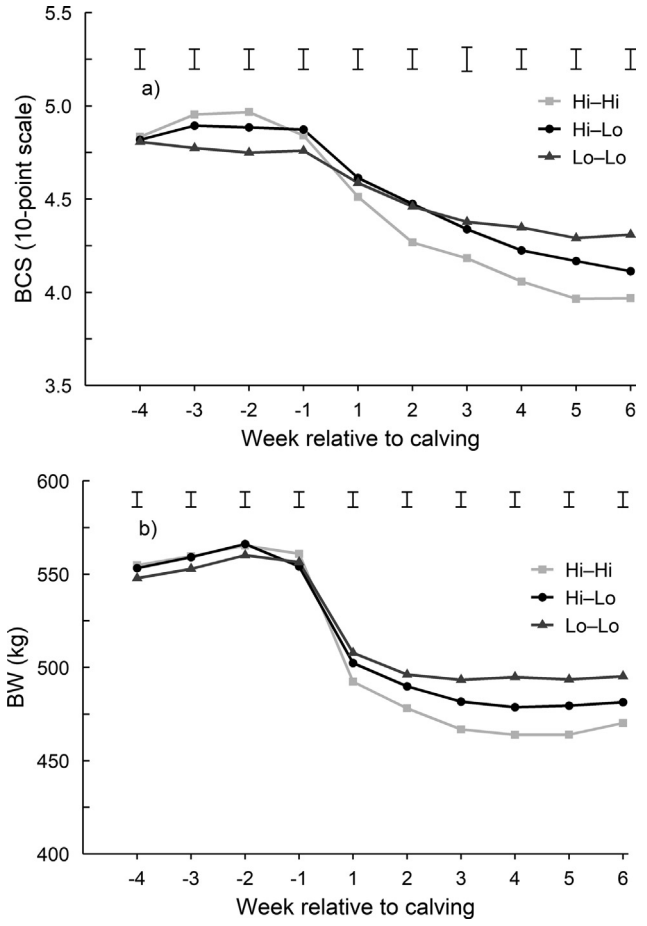

Fig. 2. Mean body condition score [BCS (a); 10-point scale, where 1 is emaciated and 10 is obese; Roche et al., 2004] and BW [(b); kg] during the 4 week pre- and 6 week postcalving for dairy cows classified into three metabolic status groups [Lo–Lo (blood non-esterified fatty acids (NEFAs) < 1.0 mmol/L and blood β-hydroxybutyrate (BHB) ≤ 1.0 mmol/L at all samplings during the first 2 week postcalving); Hi–Lo (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≤ 1.0 mmol/L at the same sampling time point during the first 2 weeks postcalving); Hi–Hi (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≥ 1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving)]. Error bars represent 2 × mean SED.

Classification of metabolic status

Studies undertaken in housed cows investigating independent critical thresholds for concentrations of postcalving blood NEFAs and BHB to predict risk of transition cow diseases (e.g., clinical ketosis, displaced abomasum, metritis), and associations with milk production and reproductive parameters indicate a range of cut-points (blood NEFAs ≥ 0.6–1.0 mmol/L and BHB ≥ 1.0–≥1.4 mmol/L) depending on the outcome of interest (LeBlanc et al., 2005; Duffield et al., 2009; McArt et al., 2012; Ospina et al., 2010a, 2010b, and 2013). Further, in grazing cows, it is inconclusive whether blood BHB (≥1.2 mmol/L) alone is able to distinguish animals that are ill from those that may be able to tolerate and process higher circulating concentrations of ketone bodies (Gordon et al., 2013; Compton et al., 2015; Phyn et al., 2017); however, the presence of both elevated blood NEFAs and BHB can be used as markers of metabolic status (Ospina et al., 2010b; Tremblay et al., 2018; Xu et al., 2019). Therefore, in our study, cows were classified according to elevated NEFAs and BHB (Hi–Hi) or elevated NEFAs without elevated BHB (Hi–Lo), relative to cows with lower concentrations of NEFAs and BHB (Lo–Lo) in blood postcalving.

Overall, our classification of cows using NEFAs and BHB concentrations during the first 2 weeks postcalving was successful as indicated by the differences in blood metabolite profiles for the Hi–Hi, Hi–Lo, and Lo–Lo groups. Cows in the Hi–Hi group were experiencing a greater degree of body fat mobilisation and more severe NEB early postpartum than cows in the Hi–Lo and Lo–Lo groups, as indicated by their greater NEFAs and BHB concentrations during day 0–7 postcalving and lower BCS and BW postcalving. These cows had greater concentrations of circulating NEFAs and production of ketone bodies, likely due to incomplete liver oxidation of NEFAs (LeBlanc, 2010). Cows in the Hi–Lo group were also experiencing a greater NEB and likely also increased body fat mobilisation relative to cows in the Lo–Lo group, as indicated by their increased blood NEFAs concentrations during the first 2 weeks postcalving. However, our data support that the Hi–Lo group were non-ketotic, less likely to be maladapted and were able to cope with and oxidise NEFAs reaching the liver compared with the Hi–Hi group, as indicated by their lower blood BHB postcalving and lesser rate of body fat mobilisation (LeBlanc, 2010). The lack of difference in BCS and BW during the first 2 weeks postcalving and relatively small increase in blood BHB in the Hi–Lo group compared with the Lo–Lo group also supports this premise.

Differences in lying and activity behaviour precalving among postpartum metabolic status groups

Lying time, lying bouts, and activity before calving

Consistent differences in lying and activity behaviours among postpartum metabolic status groups occurred in the weeks before calving. We determined that the Hi–Hi cows had greater step activity, fewer daily LBs, and spent less time lying up to 2–3 weeks precalving; however, the greatest variation and differences (∼1.4 h) in lying time between the two most divergent metabolic status groups (Hi–Hi vs. Lo–Lo) occurred during the 3-day lead-in to calving. Similarly, Itle et al. (2015) reported longer standing times (i.e., ∼2h less time spent lying) during the week before calving in housed cows subsequently diagnosed with clinical ketosis (blood BHB > 3.0 mmol/L at three consecutive samplings during 3 weeks postcalving). Nevertheless, despite this similarity, the rest of our results differ from others, who have reported longer lying times (−30 to −1 days precalving) in housed cows with elevated blood BHB (≥1.4 mmol/L within 15 DIM; Rodríguez-Jimenez et al., 2018), or no associations between blood BHB (>1 mmol/L within 21 DIM) or NEFAs and prior activity (−21 to −1 days precalving; Liboreiro et al., 2015). Further, in contrast to our findings, there were no associations between daily LB (2–4 weeks precalving) and blood BHB (≥1.4 mmol/L within 15 DIM and ≥1.2 mmol/L within 28 DIM, respectively; Kaufman et al., 2016; Rodríguez-Jimenez et al., 2018) in previous studies undertaken in housed cows. The lack of consistency between our study and previous studies could be due to differences in production management systems, behaviour monitoring devices, or in the classification, cut-points, and timing of postpartum metabolic status using blood NEFAs or BHB.

Within-day lying time and activity before calving

Differences in lying and activity behaviours among postpartum metabolic status groups were also evident within-day in the 2–3 weeks before calving. We determined that, during the early morning (prior to fresh feed allocation) and at certain times during mid- to late afternoon, Hi–Hi cows in more severe NEB after calving were more active and spent less time lying than their less metabolically challenged peers (Lo–Lo and Hi–Lo cows). The differences within-day in the Hi–Hi group were small (∼40–100 more steps/h and ∼4–5 more minutes/h lying) compared with the Lo–Lo group, but are supported by the findings of Itle et al. (2015). Similarly, housed cows diagnosed with clinical ketosis postcalving spent more time standing at certain times during late afternoon

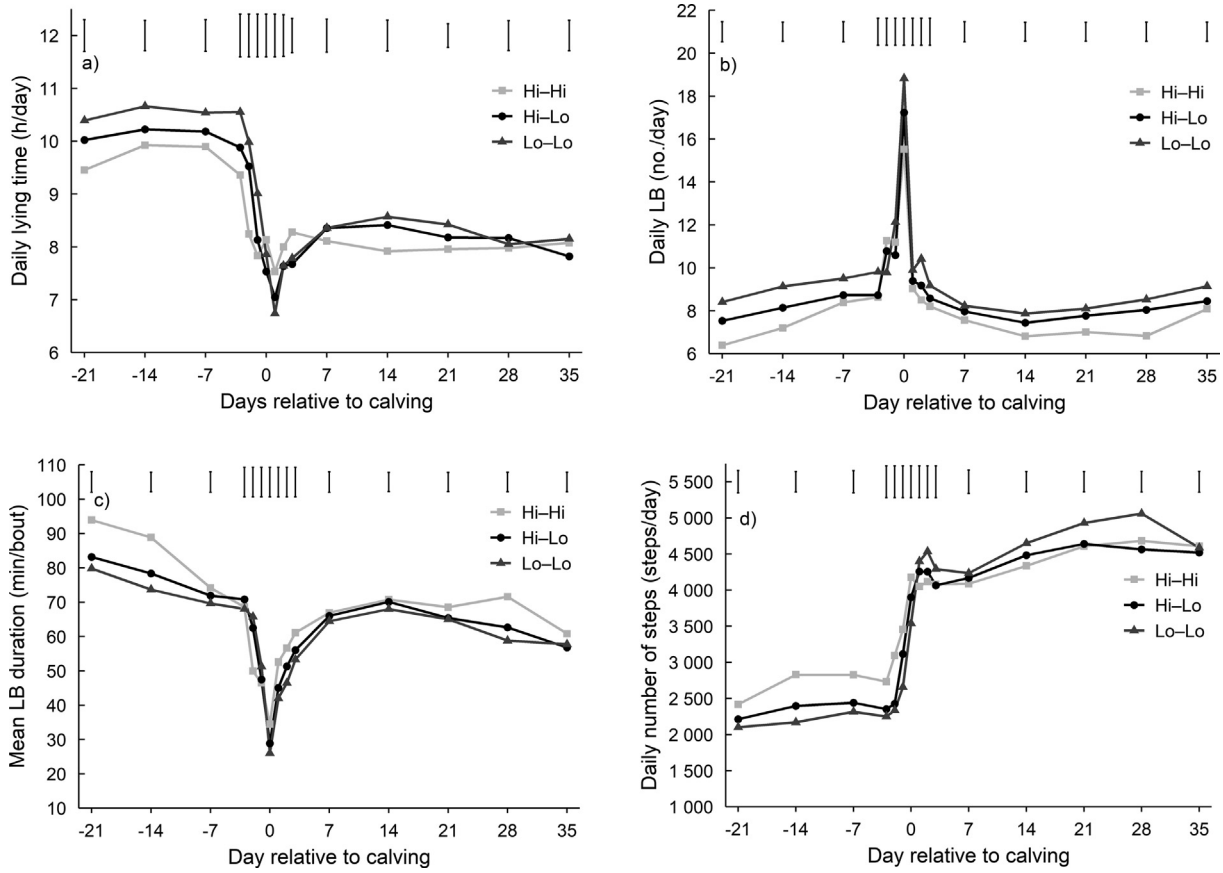

Fig. 3. Daily lying time (a; h/day), lying bouts [LBs (b); no./day], mean LB duration (c; min/bout) and number of steps (d; steps/day) during day −21 to −15, −14 to −8, −7 to −4 precalving, daily precalving (day −3 to −1), day 0 (the day of calving), daily postcalving (days 1–3), day 4–7, 8–14, 15–21, 22–28, and 29–35 postcalving in grazing dairy cows classified into three metabolic status groups [Lo–Lo (blood non-esterified fatty acids (NEFAs) < 1.0 mmol/L and β-hydroxybutyrate (BHB) ≤ 1.0 mmol/L at all samplings during the first 2 weeks postcalving); Hi–Lo (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≤ 1.0 mmol/L at the same sampling time point during the first 2 weeks postcalving); Hi–Hi (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≥ 1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving)]. Error bars represent 2 × mean SED.

and evening in the week before calving than non-ketotic cows, but authors reported more consistent differences across the 24-h day (Itle et al., 2015). Our results and those of Itle et al. (2015) may suggest that within-day behaviour patterns and postcalving metabolic status are dependent upon the severity of metabolic challenge; however, further research is needed to investigate this possibility.

Due to the retrospective nature of our study, we cannot determine the cause and effect of precalving behavioural differences within-day and across days among the three metabolic status groups. It is plausible that cows in our study were more active and spent less time lying both across the day and at specific times of the day precalving due to another interacting factor such as social rank. Ungerfeld et al. (2014) reported that lower-ranking cows spend more time walking and grazing, and continue to graze while higher-ranking cows are not grazing; hence, the time budgets of lower- and higher-ranking cows differ. If lower-ranking cows experience unequal access to resources (e.g., feed) and reduced grazing efficiency (Ungerfeld et al., 2014), it is plausible that social rank may influence how animals cope with metabolic changes postcalving. To support this hypothesis, Goldhawk et al. (2009) reported that cows with blood BHB ≥ 1.0 mmol/L within 1 week postcalving displayed more subordinate behaviours by initiating fewer aggressor behaviours 1 week before calving compared with cows that were healthy postcalving. Further research would need to investigate how hierarchial placement alongside other possible interacting factors (e.g., social behaviour, grazing behaviour, energy requirements, access to resources, management) might influence these associations.

Irrespective of the cause or effect of this altered behaviour, our findings indicate that grazing dairy cows classified as Hi–Hi according to elevated blood NEFAs and BHB postcalving perform different behavioural activities at specific times of the day and across days precalving than their less metabolically challenged herdmates (Hi–Lo and Lo–Lo groups). Therefore, divergent daily and within-day lying and activity behaviours before calving may have the potential to identify cows at risk of excessive NEB postpartum before they calve. Future research should consider reporting behavioural data for both daily and within-day associations due to the additional information these data can provide.

Differences in lying and activity behaviour postcalving among postpartum metabolic status groups

Lying time, lying bouts, and activity from the day of calving

Transient differences in LBs and activity occurred from the day of calving. On the day of calving and day 2 postcalving, the cows with high NEFAs and BHB (e.g., Hi–Hi group) engaged in fewer LBs than the cows with low NEFAs and BHB (e.g., Lo–Lo) and they were also more active on the day of calving. Others have reported findings consistent with our results; however, they typically determined much larger group differences on the day of calving. For example, Itle et al. (2015) reported that cows diagnosed with clinical ketosis had 6.3 fewer standing bouts (indicative of fewer LBs) on the day of calving compared with healthy cows. The reduction in LBs and increase in steps taken on the day of calving in the Hi–Hi cows may reflect an early manifestation of metabolic stress

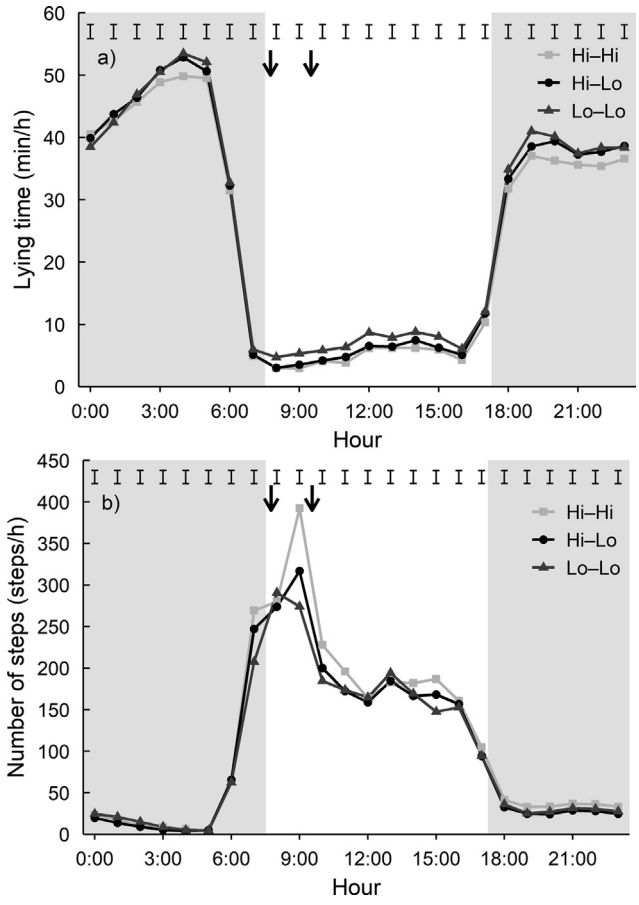

Fig. 4. Mean lying time (a; min/h) and number of steps (b; steps/h) across hourly time intervals during day −14 to −1 precalving for dairy cows classified into three metabolic status groups [Lo–Lo (non-esterified fatty acids (NEFAs) < 1.0 mmol/L and blood β-hydroxybutyrate (BHB) ≤ 1.0 mmol/L at all samplings during the first 2 weeks postcalving); Hi–Lo (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≤ 1.0 mmol/L at the same sampling time point during the first 2 weeks postcalving); Hi–Hi (blood NEFAs ≥ 1.0 mmol/L and blood BHB ≥ 1.2 mmol/L at the same sampling time point during the first 2 weeks postcalving)]. Error bars represent 2 × mean SED. Time intervals include data within each hour specified (e.g., 0100 h covers the period 0100–0159 h). Shaded areas represent the hours of darkness (sunrise = 0731 h, sunset = 1717 h). Cows were allocated fresh pasture once daily (between ∼0745 h and 0930 h; indicated with arrows).

in this group of cows. Care should be taken when interpreting the findings reported surrounding calving in our study due to the reassignment of calving day, the variation in the day cows were classified into metabolic status groups (range: 0–14 days postcalving), and an inability to clearly distinguish between behaviours that occurred prior to, and after individual cows were classified into metabolic status groups, which could mask some effects.

Activity in the grazing cow is comprised primarily of walking short distances to acquire feed and walking long distances to and from the milking parlour (Beggs et al., 2018), which are energy-expensive activities (Kaufmann et al., 2011). Further, changing from a standing to a lying position and vice versa is also an energetically expensive behaviour (Itle et al., 2015). Cows in a more severe state of NEB are more at risk of subclinical and clinical metabolic and infectious diseases (Ospina et al., 2010a and 2010b); therefore, they may spend more time lying or engage in less energetically expensive behaviours in an attempt to conserve energy or due to feeling unwell (Proudfoot and Huzzey, 2017).

In our original hypothesis, we speculated that notable increases in lying time and reductions in activity would be evident in grazing cows with elevated blood NEFAs and BHB postcalving. However, we found no differences in daily lying time from day 0 to 35 postcalving among metabolic status groups and no differences in activity after the day of calving between the two most divergent groups (Hi–Hi and Lo–Lo). In contrast, others have reported that housed cows with elevated blood BHB (≥1.2 mmol/L within 3 weeks postcalving) spent more time lying during the first 4 weeks postcalving than healthy cows (blood BHB < 1.2 mmol/L and no other health conditions within 3 weeks postcalving) (Tsai et al., 2020; Kaufman et al., 2016). Others (Liboreiro et al., 2015; Tsai et al., 2020) have also reported that housed cows with elevated blood BHB (>1.0 mmol/L and ≥1.2 mmol/L within 3 weeks postcalving, respectively) had reduced activity during the first 3 weeks in milk. Furthermore, in our study, cows in the Hi–Hi group tended to have fewer, yet longer, LBs for up to 4 weeks after they were classified as Hi–Hi. In contrast, studies in housed cows have reported no effect of blood BHB ≥ 1.2 mmol/L during the first 4 weeks postcalving on the number of LBs within 4 weeks postcalving (Kaufman et al., 2016) and an increase of ∼3 more LB/day within 3 weeks postcalving in cows with blood BHB ≥ 1.4 mmol/L within 15 DIM (Rodríguez-Jimenez et al., 2018). The differences in our study were relatively small in the Hi–Hi group (∼1–2 LB fewer/day and ∼13 min longer/bout) compared with the Lo–Lo group and may not reflect biologically meaningful behaviours as they did not result in altered daily lying time or activity to support our hypothesis that cows with both elevated NEFAs and BHB would engage in notably fewer energy-expensive behaviours postcalving. The lack of material differences between the two most divergent metabolic groups is an important finding. Cows that were intermediate for blood BHB (e.g., blood BHB was >1.0 and <1.2 mmol/L) were omitted from our study to create more distinct groups. Future studies should consider including these intermediate groups to determine whether it would be possible to distinguish between cows with a low, moderate, or high risk for compromised metabolic status postcalving within a herd. Nevertheless, it appears that there are conflicting associations when comparing our results and the wider literature, which may be influenced by cow- and system-level factors, the definition of metabolic state or disease (method of classification, timing, and cut-points used), the behaviour of interest, and the time relative to calving or relative to classification of metabolic status that the behaviour was reported.

Interestingly, the cows with high NEFAs and low BHB (Hi–Lo) had reduced activity up to 4 weeks after they were classified as Hi–Lo (∼290–500 steps fewer/day) compared with the Lo–Lo group. In agreement, others have reported lower activity 10–13 d postcalving in housed cows with blood NEFAs ≥ 0.7 mmol/L between 2 and 13 d postcalving (Adewuyi et al., 2006) and a negative correlation between step count and plasma NEFAs during week 4 postcalving (van Hoeij et al., 2019). But, Liboreiro et al. (2015) reported no association between blood NEFAs and activity postcalving. The Hi–Lo cows in our study reflect an intermediate metabolic group, where blood NEFAs are elevated but blood BHB is below 1.0 mmol/L and the reduced activity among these cows could reflect a multitude of cause and effect associations. In our study, these cows had 1.3 kg greater ECM yield compared with Lo–Lo cows, and therefore, a reduction in activity may reflect an attempt to conserve energy needed to support milk production or these cows may be higher producing because they are less active reflecting greater feed conversion and grazing efficiency (Gregorini et al., 2015). But, without understanding the interactions between other behaviours (e.g., rumination, grazing, and social) as well as individual traits (e.g., energy requirements, DMI, genetics, personality, other underlying conditions), it is difficult to determine a casual link between behaviour postcalving and metabolic status. Further research is needed to better understand interacting factors influencing behaviour and metabolic state in grazing cows such as those mentioned above.

In conclusion, groups of grazing cows classified as experiencing more severe metabolic challenge in the first 2 weeks postcalving were more active, spent less time lying, and had fewer LBs for up to 3 weeks before calving than cows with lower blood NEFAs and BHB postcalving. In the weeks before calving, these more metabolically challenged cows also displayed differences in their within-day behavioural profiles that indicated they were more active and lay less in the early morning. In contrast, following calving, they had fewer yet longer LBs, but these differences were biologically small. Further research is needed to better understand whether differences in lying and activity behaviours have potential in predicting or monitoring the metabolic status of individual cows, particularly in grazing systems.

Ethics approval

The data were provided by DairyNZ Ltd. from three previous experiments that were approved by The Ruakura Animal Ethics Committee (Hamilton, New Zealand; BCS study: RAEC#12799; Feed study: RAEC#13141; Zeolite study: RAEC#13871). All animal manipulations were in accordance with the New Zealand Animal Welfare Act (Ministry for Primary Industries, 1999).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study are available upon request.

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Declaration of interest

There is no conflict of interest to declare.

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