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Original article

Prepartum behaviors as early indicators for postpartum energy associated biomarkers status in Holstein dairy cows

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Abstract

Our main aim was to investigate the predictive value of prepartum behaviors such as total daily rumination (TDR), total daily activity (TDA) and dry matter intake (DMI) as early indicators to detect cows at risk for hyperketonemia (HYK), hypoglycemia (HYG) or high non-esterified fatty acid (NEFA) status in the first (wk1) and second week (wk2) postpartum. In a case control study, 64 Holstein cows were enrolled 3 weeks before the expected time of calving and monitored until 15 days in milk (DIM). Postpartum blood samples were taken at D3 and D6 for wk1 and at D12 and D15 for wk2 to measure beta-hydroxybutyrate, NEFA and glucose concentration. Ear-mounted accelerometers were used to measure TDR and TDA. DMI and milk yield were obtained from farm records. Relationships between the average daily rate of change in prepartum TDR (ΔTDR), TDA (ΔTDA), and DMI (ΔDMI) with postpartum HYK, HYG and NEFA status in wk1 and wk2 post-partum were evaluated using linear regression models. Models were adjusted for potential confounding variables, and covariates retained in the final models were determined by backward selection. No evidence was found to support the premise that prepartum ΔTDR, ΔTDA or ΔDMI predicted postpartum HYK, HYG or NEFA status in wk1 or in wk2. Overall, prepartum ΔTDR, ΔTDA and ΔDMI were not effective predictors of HYK, HYG or NEFA status in the first 2 weeks postpartum.

Keywords: dairy cows, hypo-glycemia, ketosis, precision technology, transition cow

Introduction

Dairy cows face critical physiological and metabolic alterations during the period from late pregnancy to early lactation, which is traditionally defined as the transition period (Bauman and Bruce 1980, LeBlanc et al. 2005). Dairy cows during this vulnerable period experience nutrient deficit in early lactation due to a reduction in dry matter intake (DMI) in the late pregnancy and around the calving, followed by rapidly increased energy requirements postpartum to support colostrum and milk production (Caixeta et al. 2015, Schirmann et al. 2016). In the 3 weeks prior to calving, DMI declines by 30% (Hayirli et al. 2002). Following calving, requirements for amino acids, glucose, and fatty acids are 2 to 5 times higher than prepartum demands due to milk production (Bell 1995). As a part of adaptations to this new physiological state, dairy cows mobilize body reserves, especially fatty acids, to produce ketone bodies, which are used as an alternative source of energy (Herdt 2000). The normal adaptation to this new physiological state produces non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) in limited concentrations. Elevated concentrations of NEFA or BHB can indicate an unsuccessful adaptation as these increases have been associated with health disorders, decreased production and a reduction in the immune status (Hammon et al. 2006, Contreras et al. 2010). Many dairy cows with high BHB postpartum also suffer from low plasma glucose and insulin levels, as the rapid increase in milk yield can result in a demand for glucose that exceeds the cow's gluconeogenesis capacity (Herdt 2000, Ruoff et al. 2017), resulting in a hypoglycemic state.

The poor adaptation response to this new physiological state due to a prolonged or severe reduction in DMI around the calving can lead to subclinical ketosis (Grummer 1995), also defined as hyperketonemia (HYK) (McArt et al. 2012). Hyperketonemia is characterized by an increase in plasma BHB above the normal reference concentration ($\geq$ 1.2 mmol/L) without detectable clinical signs of disease. Moreover, HYK can be accompanied by a reduction in milk yield and poor reproduction performance (McArt et al. 2012, Ospina et al. 2013). Hyperketonemia also increases the odds of early removal from the herd due to increased economic losses (Duffield et al. 2009, Ospina et al. 2010). While NEFA functions to provide energy to tissues throughout the body, in excess mobilization they may be toxic (Overton 2001).

The gluconeogenic activity of liver tissue is found to be impaired by the development of fatty liver, which lowers blood glucose and reduces insulin secretion (Adewuyi et al. 2005). Gluconeogenesis is strongly liked to lactogenesis because the amount of available glucose determines the amount of milk produced (Mepham 1993). Lipolysis increases in response to a decrease in available glucose (hypoglycemia), releasing NEFA which circulates in the blood (Herdt 2000, McArt et al. 2012).

Measurements of prepartum changes in cow behaviors, such as rumination, animal activity, and dry matter intake (DMI), may be potential indicators to aid in the early detection of cows at risk of developing HYK and other transition health disorders (Weary et al. 2009), such as hypoglycemia (HYG) and high NEFA. Use of prepartum behaviors can allow for the development of management strategies and preventive measures to improve cow health during the transition period (von Keyserlingk et al. 2009, Paudyal 2021).

Precision dairy farming technologies aim to improve farm productivity and profitability by improving cow management (Wathes et al. 2008, Berckmans 2015). Moreover, these technologies provide scientists and farmers with research tools that can be used as early warning systems (Bikker et al. 2014). Monitoring rumination and animal activity behavioral patterns by automated technologies is used widely on dairy farms to predict postpartum diseases (Soriani et al. 2012, Liboreiro et al. 2015). However, to our knowledge, there is limited data about the association between energy related biomarkers status postpartum and the changes in prepartum rumination, activity and DMI behavioral patterns. The aim of this study was to investigate associations between the rate of change in daily rumination time (ΔTDR) and total daily activity (ΔTDA) across the last 3 days prior to calving, and dry matter intake (ΔDMI) across the last 2 days prior to calving, and HYK, NEFA, and HYG status during the first 2 weeks post-partum. This study was exploratory in nature. We hypothesized that cows that have an occurrence of HYK, high NEFA, or HYG in wk1 and or wk2 postpartum would have different prepartum behaviors when compared to cows that do not have an occurrence of metabolic disorders postpartum.

Materials and Methods

Animals and enrolment

All animal procedures were evaluated and approved by the Institutional Animal Care and Use Committee at the University of Minnesota (2002-37896A). An exploratory case control study included 64 Holstein dairy cows (27 primiparous and 37 multiparous), 16 of which were from unselected Holsteins (cows bred to maintain 1960's genetics; (Young 1977, Hansen et al.1979, Weber et al. 2007)) and 48 were high-yielding Holsteins. Cows were enrolled three weeks before the calving date and followed until 15 days in milk (DIM). The enrolled cows were apparently healthy and not suffering from any clinical disorders. Prepartum or postpartum TMR were formulated according to NRC recommendation and animals were fed a non-limiting amount of TMR once daily (Emam et al. 2023). Cows were housed in individual tie-stalls and were milked twice daily.

Data collection

Blood samples were taken from coccygeal vessels during the first 2 weeks postpartum with a 3-day interval (3, 6, 12, and 15 DIM). Vacuum tubes containing lithium-heparin (Vacutainer, Becton Dickinson, NJ, USA) with 20-gauge blood collection needles were used for blood collection. Following the morning milking, samples were collected and transported within 1 h of collection to the laboratory where samples were centrifuged at 1,500 × g for 15 min at 4°C for plasma separation. Samples were kept frozen at -20°C for later analysis. All plasma samples were thawed and run in duplicate using a small-scale chemistry analyzer (CataChemWell-T, Catachem Inc, Oxford, CT, USA) for the measurement of NEFA, BHB and glucose (Glu). Analyses used enzymatic colorimetric kits to determine NEFA (C514-0A, CataChem Inc., Oxford, CT) and glucose (C124-07, CataChem Inc., Oxford, CT) concentrations. An electronic cow-side device (Precision Xtra meter, Abbott Diabetes Care Inc, Alameda, USA) – previously validated for use in dairy cows (McArt et al. 2012), was used for measurement of blood BHB.

Cows were classified as hyperketonemic (HYK+) when plasma BHB concentrations were ≥ 1.2 mmol/L, with cows below that threshold considered HYK- (McArt et al. 2012). Cows were classified as having high NEFA (NEFA+) when plasma NEFA concentrations were ≥ 0.57 mmol/L and to be NEFA- below this threshold (Ospina et al. 2013). Cows were classified as hypoglycemic (HYG+) if plasma Glu concentrations were ≤ 40 mg/dL (Constable et al. 2016, Ruoff et al. 2017, Kabir et al. 2022), and HYG- otherwise.

Prepartum behaviors and milk yield

Ear-tag accelerometers (Cow Manager, Agis Automatisering BV, Harmelen, the Netherlands) was used for continuous recording of daily rumination and activity behaviors from 3 weeks prior to calving until the first 2 weeks postpartum. Total time spent ruminating or being active per day was based on a 24-h interval from 00h00 to 23h59. Dry matter intake from 3 weeks before calving until 14 DIM were obtained from the herd's feed management software (Feed Supervisors Systems, Dresser, WI, USA) and daily milk production for the first 15 days in milk were recorded from farm management software (DelPro, DeLaval, Tumba, Sweden).

Statistical analysis

The metabolic status of the cows during wk1 and wk2 was determined from their mean blood concentration of BHB, Glu and NEFA on D3 and D6 for wk1 and on D12 and D15 for wk2. The rate of change in prepartum behaviors (TDR, TDA and DMI) were the predictors of interest for this study. Daily rate of change was obtained from the slope of the linear regression of the daily behavioral measurements for each cow during the period of interest. Linear regression models were used to evaluate the association of prepartum ΔTDR, ΔTDA, and ΔDMI with HYK, HYG, and NEFA status of cows in wk1 and wk2 postpartum. Separate models (n=18) were created for each combination of predictor (ΔTDR, ΔTDA, and ΔDMI) and outcome (HYK, HYG, or NEFA status in wk1 and wk2). Two cows were missing D12 and D15 blood samples and were excluded from all wk2 analyses. Three cows were missing either prepartum dry matter intake data (2 cases) or baseline prepartum behaviors or postpartum milk yield. Another 11 cows had incomplete prepartum behavior data due to defects in their ear tag and were excluded from analysis.

The fixed effect of cow health status for HYK (HYK+ or HYK-), HYG (HYG+ or HYG-) and NEFA (NEFA+ or NEFA-) was enforced into their respective models. Parity (1, 2, or 3+), genotype (1960's or current Holstein genetics), baseline TDR (calculated as the average TDR from -14 to -7 days relative to calving), postpartum DMI and milk yield (calculated as the average across the first 6 DIM), and prepartum DMI (calculated as the average DMI across day -6 to -1 relative to calving) were offered to models in which ΔTDR was the predictor of interest. Parity, genotype, baseline TDA (calculated as the average TDA from -14 to -7 days relative to calving), postpartum DMI and milk yield, and prepartum DMI were offered to models in which ΔTDA was the predictor of interest. Parity, genotype, baseline DMI (calculated as the average DMI from -14 to -7 days relative to calving), and postpartum DMI and milk yield were offered to models in which ΔDMI was the predictor of interest. To determine which covariates were retained in the final models, backward selection was used. Collinearity diagnostics using the olsrr package in R (Hebbali 2020) were run on all models to confirm that variables were not multicollinear.

As this study was exploratory in nature, limitations exist in the number of animals that could be enrolled. To address sample size limitations, post hoc power calculations were performed and changes in prepartum

Table 1. Concentrations of blood beta-hydroxybutyrate (BHB), glucose (Glu) and non-esterified fatty acids (NEFA) during week1 (Wk1) and week 2 (Wk2) postpartum in dairy cows. Values are presented as least square means (95% Confidence Interval).

Variable	Wk1		Wk2	
	N[1]	Mean (95% CI)	N[1]	Mean (95% CI)
BHB concentrations, mmol/L[2]				
Overall		0.92 (0.78, 1.06)		0.96 (0.81, 1.12)
HYK+	24	1.52 (1.21, 1.83)	20	1.87 (1.47, 2.27)
HYK-	40	0.57 (0.52, 0.62)	43	0.55 (0.52, 0.59)
Glu concentrations, mg/dL[3]				
Overall		57.58 (53.64, 61.53)		57.45 (54.26, 60.65)
HYG+	23	41.10 (35.34, 46.86)	22	38.49 (34.02, 42.96)
HYG-	41	66.94 (62.92, 70.97)	40	67.88 (64.93, 70.83)
NEFA concentrations, mmol/L[4]				
Overall		0.45 (0.40, 0.50)		0.41 (0.37, 0.45)
NEFA+	23	0.70 (0.61, 0.79)	20	0.67 (0.57, 0.76)
NEFA-	41	0.31 (0.28, 0.34)	42	0.29 (0.27, 0.31)

[1] Total number of cows classified as each health disorder status in each week.
[2] Cows with BHB concentrations ≥ 1.2 mmol/L were classified as hyperketonemic (HYK+), otherwise as being non-hyperketonemic (HYK-).
[3] Cows with Glu concentrations ≤ 40 mg/dL were classified as hypoglycemic (HYG+), otherwise as being non-hypoglycemic (HYG-).
[4] Cows with NEFA concentrations ≥ 0.57 mmol/L were classified as having high NEFA (NEFA+), otherwise as not having high NEFA (NEFA-).

total daily rumination and activity power calculations were based on the ability to detect 20 min/d difference between groups and the power analysis for changes in prepartum DMI was based on the ability to detect 1 kg/d difference. Power for analyses of predictor ΔTDR ranged from 21.0 to 29.2 %, 44.0 to 53.5 % power for ΔTDA, and 52.3 to 66.4% power for ΔDMI.

Results

Descriptive Results

Twenty-seven, 13, and 24 of the cows enrolled in the study were parity 1, 2, or 3 or greater at the time of enrollment. Mean baseline TDR and TDA from -14 to -7 days relative to calving were 458.9±100.20 min/d and 343.8±74.99 min/d, respectively. Average daily DMI from -14 to -7 days relative to calving was 9.7±3.99 kg/d. The HYK- cows had 0.95 mmol/L and 1.32 mmol/L lower mean BHB in wk1 and wk2, respectively, than those of HYK+ (Table 1). Conversely, mean glucose concentrations were 25.84 mg/dL and 29.39 mg/dL greater in wk1 and wk2, respectively, in HYG- cows compared to HYG+. Mean NEFA concentrations in NEFA- cows was 0.39 mmol/L and 0.38 mmol/L lower in wk1 and wk2, respectively, than NEFA+ cows.

Rate of change in TDR (ΔTDR)

The rate of change in TDR for all cows with positive or negative disorders is documented in Table (2). No significant changes were observed in mean rate of change of total daily rumination (ΔTDR) between HYK- and HYK+ cows at wk1 (p=0.96) or wk2 (p=0.24; Table 2, Fig.1). Cows that were HYK- at wk2 had a numerically lower ΔTDR by comparison to HYK+ cows, with about 14 min difference (p=0.24). The NEFA+ cows had similar least-square means of ΔTDR to NEFA- in wk1 with only a 3 min difference (p=0.86). The opposite was true at wk2, with NEFA- cows having numerically lower least-square means of ΔTDR by 36 minutes compared to NEFA+ (p=0.13). No significant changes in the rate of change in TDR were found between HYG- and HYG+ cows at wk1 (p=0.65) and wk2 (p=0.39), The ΔTDR of HYG- cows was numerically higher than HYG+ by 7 min and 14 min for wk1 and wk2 respectively.

Rate of change in TDA (ΔTDA)

The values for ΔTDA presented by disorder status is presented in Table (2). The HYK- cows had numerically lower ΔTDA at both wk1 and wk2 at 10 and 13 min, respectively, compared to HYK+ cows, but we did not detect any significant changes at wk1 (p=0.30) or at wk2 (p=0.26; Fig. 2). There was no association between NEFA status and ΔTDA at wk1 (p=0.32) or at wk2 (p=0.15), with NEFA- cows in both models

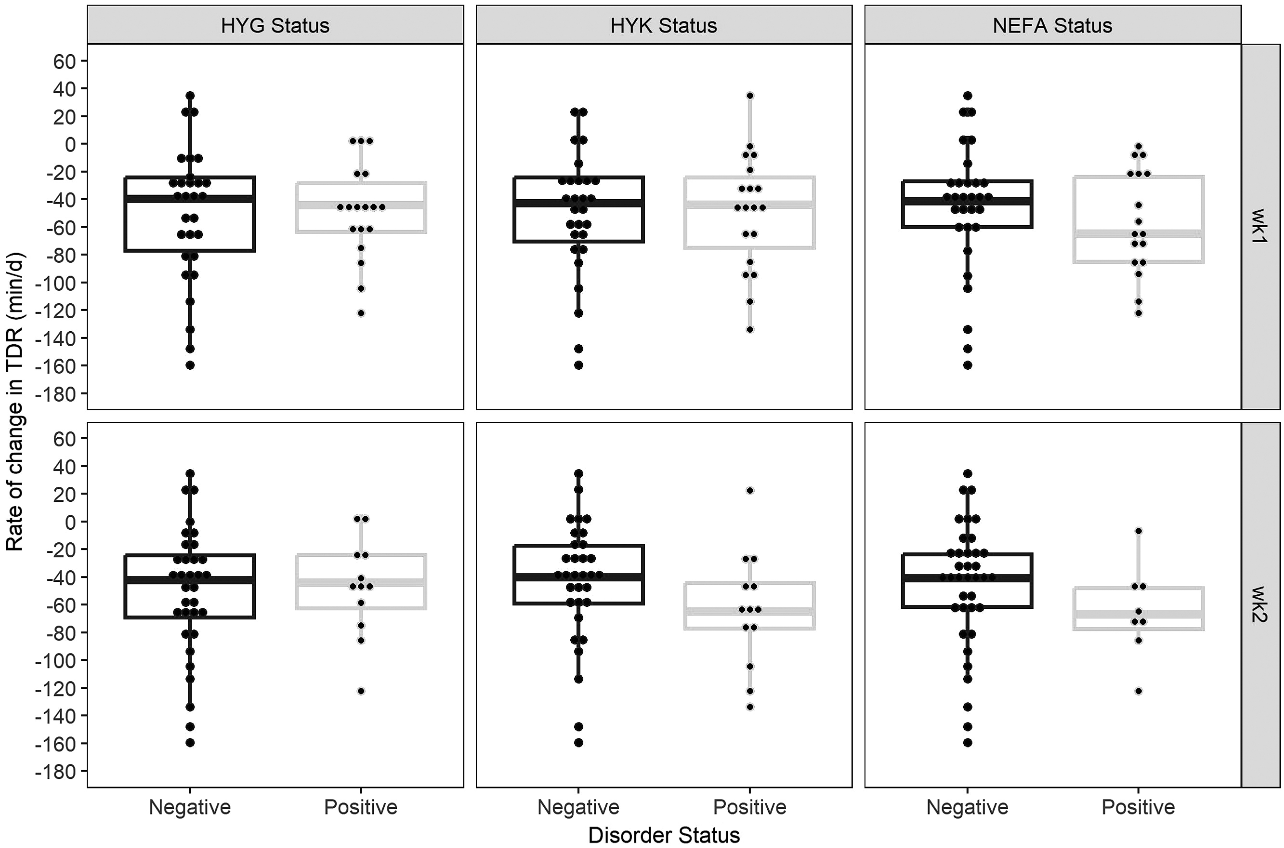

Fig. 1. Mean rate of change in TDR (ΔTDR) for cows that were hypoglycemic (HYG+; Glucose ≤ 40 mg/dL or were not hypoglycemic (HYG-) in wk1, (p=0.65) and in wk2, (p=0.39); for cows that were hyperketonemic (HYK+; blood beta-hydroxybutyrate ≥ 1.2 mmol/L) or were not hyperketonemic (HYK-) in wk1, (p=0.96) and in wk2, (p=0.24) and for cows that had high NEFA concentrations (NEFA+; blood NEFA ≥ 0.57 mmol/L or low NEFA (NEFA-) in wk1, (p=0.86) and in wk2, (p=0.13). The values for ΔTDR were detected from the linear regression of study cows from -3 days prepartum to calving. A disorder status of 'Negative' corresponds to HYK-, HYG-, or NEFA- and 'Positive' corresponds to HYK+, HYG+ or NEFA+.

having numerically lower daily activity in both weeks than NEFA+ cows. Similarly, no differences in the association between HYG status and ΔTDA were found at wk1 (p=0.16) or at wk2 (p=0.31), with HYG- cows in both models having numerically higher total daily activity in both weeks (14 min and 12 min, respectively) than HYG+ cows.

Rate of change in DMI (ΔDMI)

The least-square means for rate of change in DMI (ΔDMI) based on disorder status are presented in Table 2. Hyperketonemia status was not associated with ΔDMI at wk1 (p=0.62) or at wk2 (p=0.87), with similar decreases in DMI from -3 to -1 DIM regardless of HYK status (Fig. 3). Similarly, NEFA status at wk1 (p=0.76) and at wk2 (p=0.38) was not associated with ΔDMI. The least-square mean of ΔDMI was numerically lower in HYG+ cows compared to HYG- cows at wk1 with p=0.80 and at wk2 (p=0.08).

Discussion

The aim of this exploratory study was to investigate the relationships between the rate of change in prepartum behaviors across the 3 days prior to calving and HYK, NEFA, and HYG status in the first 2 weeks postpartum. The study looked at the rate of change in these behaviors, as opposed to mean prepartum behaviors seen in most equivalent studies, as certain deviations from normal behavioral patterns can then be used to alert caretakers of cows at increased risk of postpartum energy related disorders, also allowing for earlier intervention. We focused on rates of change from 3 days prior to calving as this is the period when behavioral changes are most pronounced. It was postulated that differences in behavioral patterns between disorder status groups would also be most evident at this time, while still being within a window of time in which intervention could successfully occur. However, we did not find any evidence of an association between the prepartum behaviors selected in cows with HYK, NEFA and HYG status at wk1 or at wk2 postpartum.

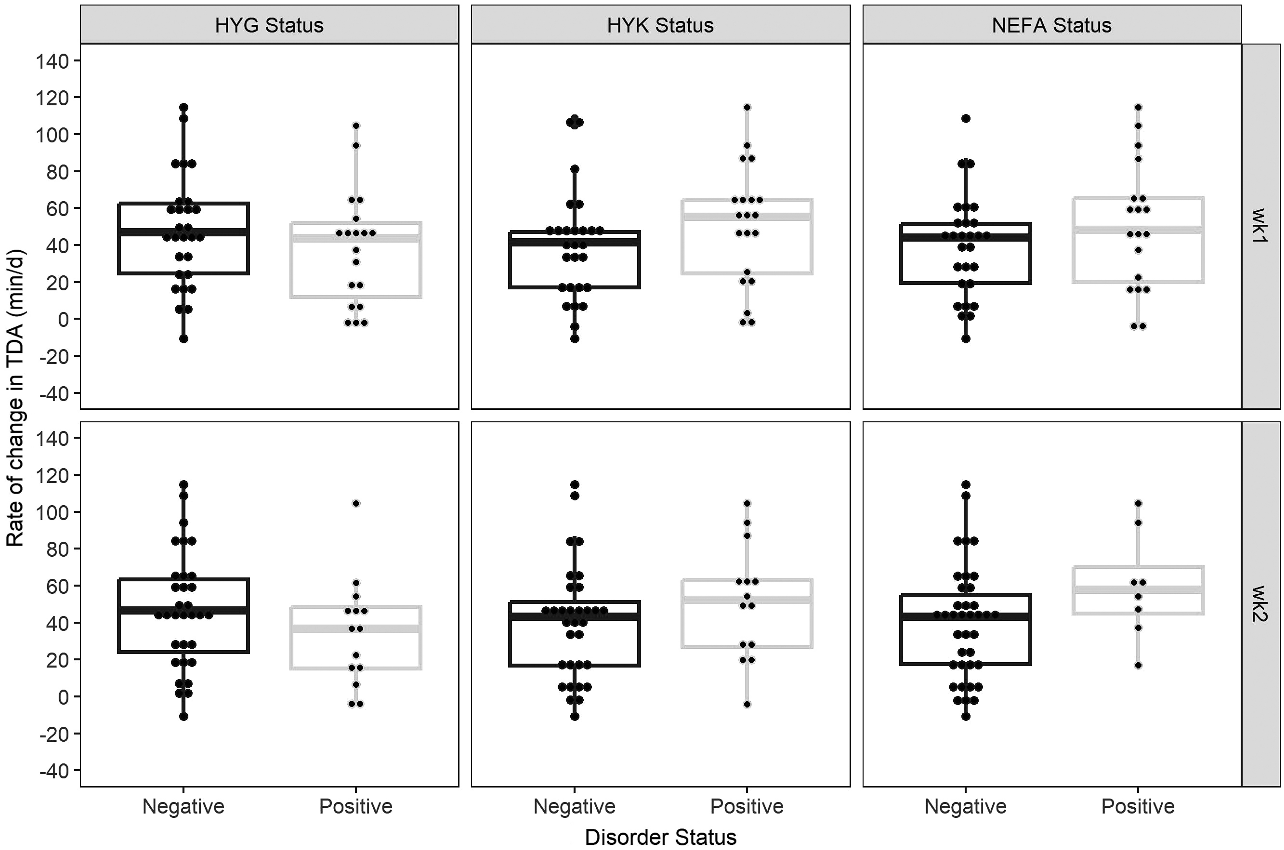

Fig. 2. Mean rate of change in TDA (ΔTDA) for cows that were hypoglycemic (HYG+; Glucose ≤ 40 mg/dL or were not hypoglycemic (HYG-) in wk1, (p=0.17) and in wk2, (p=0.31); cows that were hyper-ketotic (HYK+; blood beta-hydroxybutyrate ≥ 1.2 mmol/L) or were not hyper-ketotic (HYK-) in wk1, (p=0.30) and in wk2, (p=0.26) and cows that had high NEFA concentrations (NEFA+; blood NEFA ≥ 0.57 mmol/L or low NEFA (NEFA-) in wk1, (p=0.32) and in wk2, (p=0.15) . The values for ΔTDA were detected from the linear regression of the study cows from -3 days prepartum to calving. A disorder status of 'Negative' corresponds to HYK-, HYG-, or NEFA- and 'Positive' corresponds to HYK+, HYG+ or NEFA+.

The ability to identify energy related disorders early in their onset or, better yet, to predict the risk of their occurrence in a cow, represents a critical challenge for dairy producers (Kaufman et al. 2016). The evaluation of cow behaviors and activity during the transition period may aid in the detection of cows with higher risk of developing health disorders (Huzzey et al. 2007, Weary et al. 2009). In our study, we did not detect any statistical or biological differences in ΔTDR based on HYK status at wk1 or at wk2. These results agree with a previous study, in which no difference in rumination time – measured from two weeks before to 4 weeks after calving – was found between healthy cows and hyperketonemic cows in the first 4 weeks of lactation (Kaufman et al. 2016). In another observational study with more cows investigated (n=296), no association was detected between HYK status (using a cut-off for BHB of 1.0 mmol/L) and daily rumination time, with the authors concluding that HYK was not associated with prepartum or postpartum daily rumination time (Liboreiro et al. 2015).

Assessment of activity behaviors in dairy cattle plays vital role in the prediction of metabolic diseases (Edwards and Tozer 2004). However, the lack of evidence of any association between HYK status in wk1 or wk2 on ΔTDA in the current study suggests that this behavior is not as predictive of HYK as it is of clinical ketosis (González et al. 2008). In agreement with our results, another study reported that there was no association between the occurrence of HYK and prepartum total daily activity, with only reductions in postpartum activity found in HYK cows (Liboreiro et al. 2015). In contrast, a previous study reported that healthy cows spent more time per day feeding and engaging in social behaviors in the week prior to calving than cows diagnosed with HYK in the first week postpartum (Goldhawk et al. 2009). However, this study used a lower cut-off point for HYK diagnosis (BHB = 1 mmol/L) and investigated the mean of prepartum activity, whereas we used a 1.2 mmol BHB cut-off point for HYK detection which may lead to more HYK- cows in our investigation. Furthermore, we focused on the rate of change in the -3 days prior to calving where behavioral changes are most pronounced. Using a -7d interval as in previous studies might wash out meaningful differences that are important during the transition period.

Table 2. Mean rate of change in total daily rumination (ΔTDR), total daily activity (ΔTDA), and total daily dry matter intake (ΔDMI) based on disorder status. The rate of change for each predictor was calculated based on the slope generated from the linear regression of the behavior measurements and day of measurement from -3 days prepartum to calving.

Predictor/ Disorder	Disorder Status[1,2]				
	Week	Negative	Positive	Estimate	P-Value
ΔTDR					
HYK	1	-44.8 (-63.8, -25.8)	-44.1 (-66.0, -22.2)	-0.7	0.96
	2	-38.1 (-54.5, -21.8)	-56.2 (-82, -30.4)	18.0	0.24
HYG	1	-47.0 (-84.9, -29.2)	-40.5 (-62.8, -18.1)	-6.5	0.65
	2	-47.3 (-63.9, -30.7)	-33.7 (-60.0, -7.4)	-13.6	0.39
NEFA	1	-45.6 (-63.8, -27.3)	-42.8 (-65.9, -19.7)	-2.8	0.86
	2	-37.7 (-53.5, -21.9)	-73.9 (-116.8, -31.1)	36.2	0.13
ΔTDA					
HYK	1	41.1 (27.5, 54.7)	51.8 (36.3, 67.3)	-10.7	0.30
	2	41.7 (29.4, 54.1)	54.6 (35.3, 74)	-12.9	0.26
HYG	1	51.3 (38.5, 64.1)	37.0 (21.0, 53.1	14.3	0.17
	2	48.8 (36.6, 61.0)	37.2 (18.0, 56.5)	11.6	0.31
NEFA	1	41.6 (28.6, 54.7)	52.1 (35.7, 68.4)	-10.4	0.32
	2	42.2 (31.0, 53.4)	63.1 (36.8, 89.4)	-20.9	0.15
ΔDMI					
HYK	1	-0.81 (-1.29, -0.33)	-0.62 (-1.20, -0.04)	-0.19	0.62
	2	-0.76 (-1.18, -0.34)	-0.69 (-1.41, 0.03)	-0.07	0.87
HYG	1	-0.76 (-1.20, -0.32)	-0.67 (-1.27, -0.08)	-0.09	0.81
	2	-0.94 (-1.36, -0.52)	-0.25 (-0.90, 0.41)	-0.69	0.08
NEFA	1	-0.78 (-1.23, -0.32)	-0.66 (-1.26, -0.06)	-0.12	0.76
	2	-0.82 (-1.21, -0.42)	-0.35 (-1.29, 0.59)	-0.46	0.38

[1] Disorder status is positive if the cow was hyperketonemic (beta-hydroxybutyrate ≥ 1.2 mmol/L), hypoglycemic (Glucose ≤ 40 mg/dL) or having high non-esterified fatty acid (NEFA) concentrations (NEFA ≥ 0.57 mmol/L); otherwise, disorder status was negative

[2] Results for predictor values for each disorder type are presented as mean (95% Confidence Interval)

The reduction of DMI is a well-known indicator of clinical ketosis in dairy cattle, with cows showing reductions in DMI several days prior to diagnosis (Duffield et al. 2009). Measurement of feeding behavior and DMI are suitable for prepartum identification of animals developing postpartum subclinical ketosis (Goldhawk et al. 2009). In our study, we did not observe any evidence of an association between prepartum ΔDMI and HYK status in wk1 and in wk2, indicating that ΔDMI immediately before calving was not as good a predictor of HYK as it may be in predicting clinical ketosis cases (González et al. 2008). A previous report revealed that DMI of cows that went on to develop HYK after calving was 18% lower in the week prior to calving than DMI of healthy cows (Goldhawk et al. 2009). In the same study, the authors concluded that every 10-min reduction in average daily time spent at the feeder during the week prior to calving corresponded to a 1.9x increase in the risk of HYK development (Goldhawk et al. 2009). We focused on the rate of change in the last 2 days prior to calving and this time was intended to assist us in detecting more meaningful differences. Our results, however, revealed no differences. We recommend further investigations on HYK mechanisms with more animals as our study was underpowered and it is possible that a larger sample size may result in differences in prepartum behaviors that were not detected in the current study.

In our study we did not observe any evidence of an association between NEFA status postpartum and prepartum ΔTDR, ΔTDA or ΔDMI. Although the ΔTDR was approximately 20 min lower and ΔTDA was 20 min higher in NEFA+ cows than NEFA- cows at wk2, these differences were not significant. A previous study reported a weak correlation between NEFA and total daily rumination and a lack of association between animal activity and NEFA concentrations (Liboreiro et al. 2015). Our findings agree with a previous report that prepartum rumination time was not an effective estimator for post-partum health status

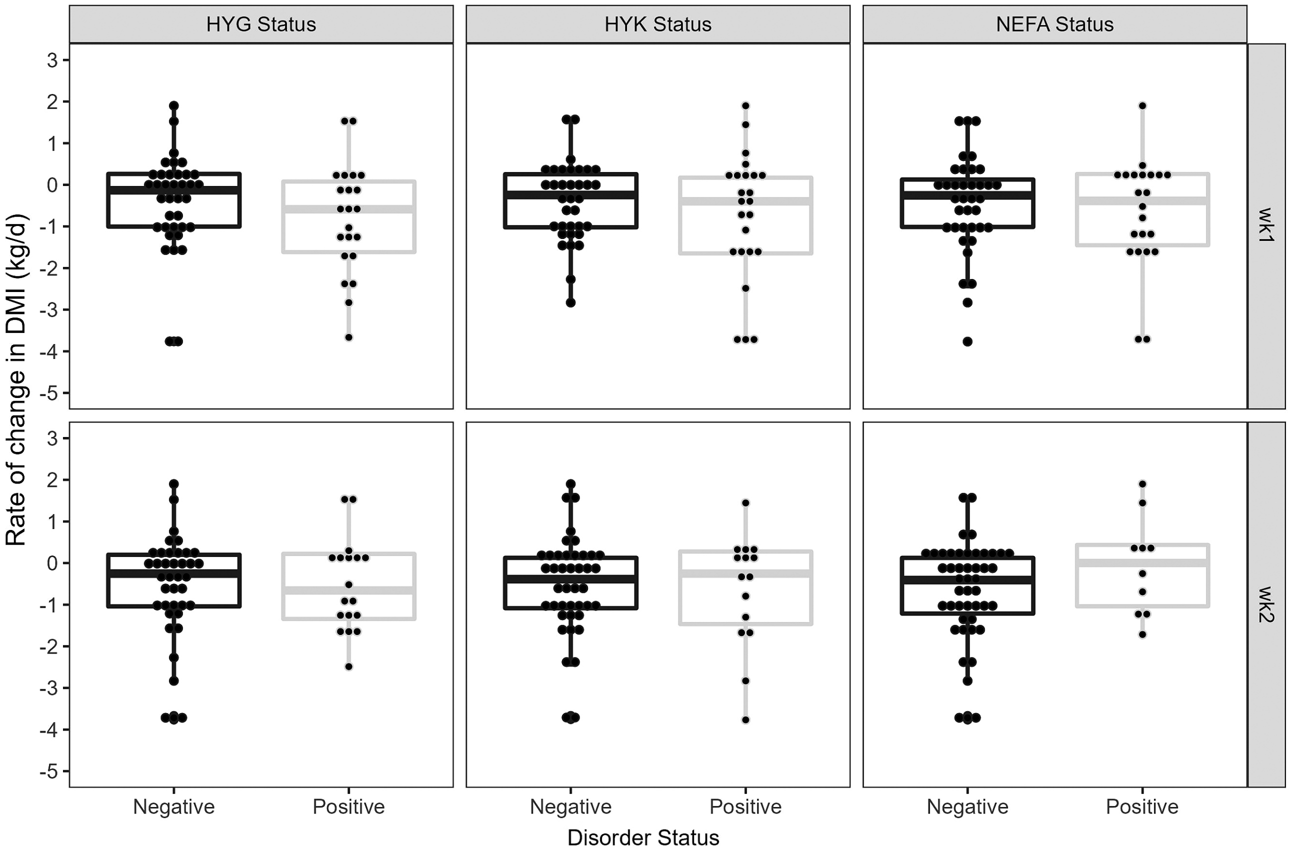

Fig. 3. Mean rate of change in DMI (ΔDMI) for cows that were hypoglycemic (HYG+; Glucose ≤ 40 mg/dL or were not hypoglycemic (HYG-) in wk1, (p=0.81) and in wk2, (p=0.08); cows that were hyper-ketotic (HYK+; blood beta-hydroxybutyrate ≥ 1.2 mmol/L) or were not hyper-ketotic (HYK-) in wk1, p=0.62) and in wk2, (p=0.87) and cows that had high NEFA concentrations (NEFA+; blood NEFA ≥ 0.57 mmol/L or low NEFA (NEFA-) in wk1, (p=0.76) and in wk2, (p=0.38). The value for ΔDMI was detected from the linear regression of study cows from -3 to -1 days relative to calving. A disorder status of 'Negative' corresponds to HYK-, HYG-, or NEFA- and 'Positive' corresponds to HYK+, HYG+ or NEFA+.

(Stevenson et al. 2020). Our results concerning the lack of associations between prepartum rumination, behavior and postpartum NEFA should be considered with caution as we did not have a sufficiently large sample size during the investigation.

Hypoglycemia status was not associated with prepartum ΔTDR. Cows that were HYG- had 14 min/d and 11 min/d higher ΔTDA than HYG+ cows at wk1 and w2, respectively. These differences are not significant and are in line with our findings regarding these predictors and HYK and NEFA status. Moreover, these results suggest that there is a correlation between glucose, NEFA and BHB, and many dairy cows with hypoglycemia suffer from high NEFA and BHB (Herdt 2000, McArt et al. 2012). A previous study reported that plasma glucose was positively correlated with feeding rate and the number of meals per day (van Hoeij et al. 2019). The ΔDMI was numerically greater in HYG- than HYG+ cows but nonetheless these differences were not significant. It is possible that glucose plays an important role in appetite and increases dry matter intake, which may warrant further investigation. To the authors' knowledge, this is the first study to investigate the association between HYG status postpartum and prepartum behaviors. Moreover, considering the small number of cows used in the study, we recommend further investigation with greater sample sizes. Interestingly, most ΔTDR, ΔTDA and ΔDMI models retained either baseline DMI (for DMI models) or prepartum DMI as well as postpartum DMI as covariates. Dry matter intake before calving is the primary source for most minerals, which are essential for the patterns of prepartum behaviors (Goff 2006, 2008). Future research should consider the importance of DMI either prepartum or postpartum when investigating the associations between postpartum metabolic disorders status and prepartum behaviors.

Limitations of our study include the use of only one farm and a relatively small number of animals. Furthermore, because the occurrence of clinical health disorders was not reported for cows in this study, we were not able to investigate the impacts of prepartum behaviors in the context of prepartum and postpartum health problems. Future work should look into these variations at different farms or consider other prepartum measurements as predictors of postpartum hyperketonemia, high NEFA, and hypo-glycemia disorders.

Conclusion

No evidence of associations was observed between prepartum rates of change in TDR or TDA or prepartum DMI and postpartum HYK, NEFA, or HYG status in wk1 or wk2. Our results indicate that ΔTDR, ΔTDA and ΔDMI in the 3 days leading up to calving might not be effective predictors of postpartum energy related biomarker status. Although our work was exploratory, our findings provide a basis for future research. Improving farm productivity and profitability by improving cow management is critical for dairy producers. Therefore, more investigations to understand the mechanisms of transition cow disorders are required.

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