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Association of rumination time with subclinical ketosis in transition dairy cows

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ABSTRACT

The objective of this study was to characterize the relationship between rumination and subclinical ketosis (SCK) in transition dairy cows. A study was conducted on 4 commercial dairy farms in eastern Ontario, Canada. A total of 339 Holstein dairy cows (107 primiparous and 232 multiparous) were monitored for rumination activity and SCK from 14 d before calving until 28 d after calving. Rumination was recorded daily using an automated monitoring system. A blood sample was taken from the coccygeal vein of each cow for measurement of β -hydroxybutyrate (BHB) once weekly throughout the 6-wk observation period. Cows with BHB ≥ 1.2 mmol/L in any of the 4 postpartum samples were considered to have SCK. Cases of retained placenta, metritis, milk fever, or mastitis during the study period were also recorded. Cows were categorized into 1 of 4 groups: healthy cows (HLT) that had no SCK or any other recorded health problem ($n = 139$); cows treated for at least one health issue other than SCK (HLT+; $n = 50$); cows with SCK (hyperketonemia; HYK) with no other health problems during transition ($n = 97$); or cows (HYK+) that had SCK and one or more other health problems ($n = 53$). Daily rumination time was summarized by week and comparisons were made between HLT and HYK and HYK+. From 2 wk before calving (wk -2) to 4 wk after calving (wk +4), there was no difference in rumination time (409 ± 9.8 min/d) among HLT, HYK, and HYK+ cows in their first lactation. Multiparous cows in HLT spent an average of 459 ± 11.3 min/d ruminating from wk -2 to wk +4. Multiparous HYK cows ruminated 25 ± 12.8 min/d less than HLT cows, whereas HYK+ cows ruminated 44 ± 15.6 min/d less than HLT cows. The largest differences in rumination time between HLT and HYK+ cows were seen during wk -1, +1, and +2,

when HYK+ cows ruminated 48 ± 17.2 , 73 ± 16.0 , and 65 ± 19.4 min/d less than HLT cows, respectively. In multiparous cows, increased odds of HYK were associated with greater milk yield in the previous lactation, greater loss of body condition over the transition period, greater stall stocking density in wk -1, and reduced rumination time in wk -1. Increased odds of HYK+ were associated with higher parity, longer dry period, greater stall stocking density in wk -1, and reduced rumination time in wk +1. These results suggest that rumination monitoring across the transition period might contribute to identification of SCK and other health problems in multiparous cows.

Key words: transition, rumination, hyperketonemia, automated monitoring

INTRODUCTION

The transition period commences 3 wk before calving and lasts until 3 wk after calving (Drackley, 1999). It is both a critical and vulnerable period for the dairy cow. Essentially all dairy cows experience a negative energy balance (NEB) in early lactation (Sovani et al., 2000), due to decreased DMI around calving and slower acceleration of DMI than of milk production (Grant and Albright, 1995; Schirmann et al., 2013). An excessive or prolonged decrease in DMI around calving may result in nonadaptive NEB, which may lead to subclinical ketosis (SCK; Grummer, 1995), also referred to as hyperketonemia (McArt et al., 2012).

McArt et al. (2012) reported an average cumulative SCK incidence of 43% among cows tested thrice weekly from 3 to 16 DIM, with the peak incidence at 5 DIM. This condition can result in lower milk production (McArt et al., 2012), reduced reproductive performance (Walsh et al., 2007), and increased risk of other illnesses, including fatty liver, displaced abomasum, and metritis (Suthar et al., 2013). Technological advancements have improved detection of SCK. Cows in NEB begin to mobilize fat stores in an attempt to meet the high energy demand during early lactation, which results in partial oxidation of fatty acids and

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releases ketone bodies (i.e., BHB) into the blood (Bauman and Currie, 1980; Goldhawk et al., 2009; LeBlanc, 2010). An electronic cow-side test for the quantification of blood BHB concentration (Precision Xtra, Abbott Diabetes Care, Saint Laurent, QC, Canada), has been validated in dairy cows (Iwersen et al., 2009; Voyvoda and Erdogan, 2010), making on-farm diagnosis of SCK accurate and reliable.

The current challenge for producers is identifying SCK at an early stage. There is growing evidence that measurements of cow behavior can be used to identify cows at risk for illness (Weary et al., 2009). Huzzey et al. (2007) found that transition cows with decreased feed intake spent less time feeding before calving and were at an increased risk of developing metritis. It has been estimated that for every 1-kg decrease in DMI and 10-min decrease in feeding time during the week before calving, the odds of developing SCK increase by 2.2 and 1.9 times, respectively (Goldhawk et al., 2009). Cows diagnosed with SCK have been observed to have lower rumination times than healthy cows in the first week after calving (Soriani et al., 2012; Liboreiro et al., 2015). There is conflicting evidence in the literature regarding the association between rumination time and blood BHB concentration. Liboreiro et al. (2015) did not find any association between these factors over the transition period; however, Soriani et al. (2013) did find a negative association of rumination time with blood BHB concentration in early lactation cows. Rumination

behavior may be a promising indicator of metabolic conditions (Soriani et al., 2012), particularly during the postpartum period because it is likely affected by changes in feeding behavior and DMI (Okine and Mathison, 1991).

The objective of this study was to characterize changes in rumination behavior across the transition period and determine if rumination behavior might be used to identify cows at risk for SCK postcalving. We hypothesized that dairy cows with reduced rumination activity, both pre- and postcalving, would be at higher risk of experiencing SCK during early lactation.

MATERIALS AND METHODS

Herd Selection

This prospective observational study was conducted on 4 commercial dairy farms located in eastern Ontario, Canada, between March and October 2014. Herds were selected as a convenience sample according to proximity to the University of Guelph, Kemptville Campus (Kemptville, ON, Canada). Participating dairies milked between 125 and 400 Holstein cows (Table 1). All cows were housed in a freestall facility, fed a TMR 1×/d, and milked in a parlor 3×/d. Animal use, data collection, and study design were approved by the University of Guelph's Animal Care Committee (AUP#2518) and Research Ethics Board (REB#14JA015).

Table 1. Descriptive summary of farm-level variables for lactating cows in an observational study of the associations of rumination time from 2 wk before to 4 wk after calving and subclinical ketosis

Variable	Herd 1	Herd 2	Herd 3	Herd 4
No. of milking cows	400	145	250	125
Fresh cows				
Fresh period (DIM)	1 to 11–14	1 to 14–21	1 to 28	1 to 21–28
Stall base	Mats and waterbeds	Bedded pack	Rubber mats	Deep bedding
Bedding type ¹	Shavings	Straw	Compost	Sand
Stocking density ² (% ± SD)	62 ± 12.5	134 ± 7.4	73 ± 4.5	99 ± 3.2
Stall length (cm)	165	—	178	178
Stall width (cm)	118	—	116	132
Feed bunk design	Post/rail	Headgates	Post/rail	Headgates
Feed bunk space ³ (cm/cow ± SD)	84 ± 27.2	45 ± 3.4	99 ± 5.6	44 ± 1.4
Lactating cows				
Lactating period (DIM)	11–14 to 28	14–21 to 28	— ⁴	21 to 28
Stall base	Mats and waterbeds	Deep bedding	—	Deep bedding
Bedding type ¹	Shavings	Sand	—	Sand
Stocking density ² (% ± SD)	97 ± 4.6	94 ± 7.1	—	100 ± 9.0
Stall length (cm)	161	160	—	174
Stall width (cm)	121	127	—	117
Feed bunk design	Post/rail	Headgates	—	Headgates
Feed bunk space ³ (cm/cow ± SD)	43 ± 2.1	25 ± 1.7	—	39 ± 3.4

¹Surface of stall base in freestall pens.

²Stocking density (ST) for freestall pens was calculated as $ST = [\text{no. of cows in pen}] / [\text{no. of stalls}]$. Bedded packs were calculated as $ST = [\text{no. of cows in bedded pack}] / \{(\text{dimensions, m}^2) / [\text{recommended space allowance, 11 m}^2; \text{Nordlund (2009)}]\}$.

³Feed bunk space = $[\text{length of feed bunk (cm)}] / [\text{no. of cows in the pen}]$.

⁴Sample cows did not occupy this pen during the sample period; they remained in the fresh pen up to 4 wk postcalving.

Table 3. Feed analysis¹ summary for close-up dry cow and fresh cow feed rations at each participating dairy farm in an observational study of the associations of rumination time and subclinical ketosis over the transition period

Ration component	Herd 1	Herd 2	Herd 3	Herd 4
Close-up dry cow ration				
DM (%)	46.6	46.6	43.1	45.7
NDF (% of DM)	38.4	37.5	33.3	41.1
ADF (% of DM)	26.2	23.9	23.0	25.9
NFC (% of DM)	30.0	32.1	34.5	31.8
CP (% of DM)	15.4	14.6	15.8	12.5
NE _L (Mcal/kg)	1.5	1.4	1.5	1.4
Fresh cow ration				
DM (%)	47.8	45.9	48.6	44.7
NDF (% of DM)	32.2	27.8	27.6	29.2
ADF (% of DM)	21.8	19.2	18.6	19.7
NFC (% of DM)	36.7	38.1	39.4	40.3
CP (% of DM)	15.0	17.9	16.6	14.7
NE _L (Mcal/kg)	1.6	1.6	1.6	1.6

¹Values were obtained from chemical analysis of feed samples. NFC = 100 - (% CP + % NDF + % fat + % ash); NE_L was calculated based on NRC (2001) equations.

together. Pooled samples were sent to Cumberland Valley Analytical Services Inc. (Maugansville, MD) for analysis of DM (135°C; AOAC International, 2000: method 930.15), ash (535°C; AOAC International, 2000: method 942.05), ADF (AOAC International, 2000: method 973.18), NDF with heat-stable α -amylase and sodium sulfate (Van Soest et al., 1991), and CP ($N \times 6.25$; AOAC International, 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI). Nonfiber carbohydrate content was also calculated, as $100 - (\% \text{ CP} + \% \text{ NDF} + \% \text{ fat} + \% \text{ ash})$ (NRC, 2001). Nutrient composition of the feed rations for each participating farm are summarized in Table 3.

Statistical Analyses

Cows that had aborted ($n = 2$), were sold ($n = 2$), or were diagnosed with SCK before calving ($n = 3$) were not included in the statistical analysis. Cows that were sold ($n = 22$) or died ($n = 1$) during the postcalving period with behavioral and health measurements recorded until the day they left the herd were included in the analysis. The final data set included 339 cows (107 primiparous and 232 multiparous) categorized as HLT ($n = 139$), HLT+ ($n = 50$), HYK ($n = 97$), and HYK+ ($n = 53$).

For all further analyses described, comparisons were made between HLT and HYK cows and HLT and HYK+ cows, respectively; HLT+ cows were not considered in the analysis. Statistical analyses were performed with SAS (version 9.4, 2013; SAS Institute Inc., Cary, NC) using cow within farm ($n = 289$) as the experimental unit. Daily rumination times (min/d) were summarized by cow and week (14 to 8 d before calving, 7 to 1 d before calving, 1 to 7 DIM, 8 to 14 DIM, 15 to 21

DIM, and 22 to 28 DIM), such that these data aligned with the once-weekly testing of SCK. These data were analyzed in a general linear mixed model (MIXED procedure in SAS), treating week as a repeated measure. The model for rumination time included the random effects of farm and cow within farm (accounting for repeated measures) and the fixed effects of health status, parity, and week, the interactions of health status with parity and health status with week, as well as the 3-way interaction of health status, parity, and week. The covariance structure was heterogeneous compound symmetry, selected by best fit according to Schwarz's Bayesian information criterion. A 3-way interaction was found between health status, parity, and week ($P < 0.01$); thus, data from first-lactation (primiparous) and multiparous cows were analyzed separately. These separate models included the fixed effects of health status, week and the interaction between health status and week, with farm and cow within farm included as random effects. Differences in rumination time between health categories and weeks were compared using the least squares means procedure with the PDIF option. Significance was declared at $P \leq 0.05$, and tendencies were reported if $0.05 < P \leq 0.10$.

In the analysis of the effect of health status on rumination time, as described above, differences were only found between health categories for multiparous cows. Thus, multivariable logistic regression was only performed on data from multiparous cows and not on primiparous cows. This analysis was performed using the GLIMMIX procedure (distribution = binomial and link = logit) in SAS to model to effects of rumination time and other cow-level factors on the presence or absence of SCK. This was done using 2 models: one model compared HLT and HYK cows, whereas the other compared

HLT and HYK+ cows. Parity and precalving BCS were both treated as categorical variables. Multiparous cows (HLT, HYK, and HYK+) were characterized as second lactation (2; $n = 99$) or third lactation and greater (3+; $n = 103$). Body condition score precalving was categorized into 3 groups: underconditioned, BCS <3; normal, BCS = 3 to 3.5; overconditioned, BCS >3.5. Parity, precalving BCS category, change in BCS over the transition period (BCS at enrollment – BCS at time of removal from study), length of dry period, milk yield from the previous lactation, as well as rumination time and stall stocking density during the weeks before the mean day of diagnosis (wk –2, –1, and +1 relative to calving), were all assessed for an association with presence or absence of HYK and HYK+ using univariable logistic regression models. Variables with $P \leq 0.25$ were then used to construct a multivariable logistic regression model. The CORR procedure in SAS was used to check for correlations between the explanatory variables (parity, BCS precalving, change in BCS, dry period length, 305-d milk yield, stall stocking density, and rumination time) included in the multivariable model. If 2 variables were highly correlated ($r > 0.8$), the variable with the lowest P -value and most biological relevance was retained for the multivariable model. Manual backward elimination of variables with $P > 0.10$ was used to create the final models. Using the final resultant models, all biologically plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those variables retained in the final multivariable models are presented.

RESULTS

A descriptive summary of cow-level variables, characterized by herd, is given in Table 4. Of the 339 cows, 139 (41%) did not have SCK or any other health problems. Table 5 describes the prevalence of ketosis. In total, 150 cows had ketosis (44%) and of these, 53 were treated for at least one other health problem (16% of all cows). The incidence risks for diseases other than SCK are described in Table 5, with metritis being the most common treated illness, followed by retained placenta, mastitis, milk fever, foot problems, and displaced abomasum.

Figure 1 illustrates how rumination time evolved across the transition period for both the primiparous and multiparous cows. Among cows in their first lactation, from 2 wk before calving until 4 wk after calving, no differences in rumination time ($P = 0.5$) were observed among HLT, HYK, and HYK+ cows (Table 6). Rumination time in primiparous cows varied by week ($P < 0.001$). Primiparous cows ruminated less in wk –1 than in wk –2 ($P = 0.001$), and rumination

Table 4. Descriptive summary (\pm SD) of focal cows sampled in each herd during an observational study of the associations of rumination time and subclinical ketosis over the transition period

Herd	No. of cows	Mean parity	Mean 305-d milk production (kg)	Mean length of dry period (d)	Mean precalving BCS ¹	Mean postcalving BCS ¹	Change in BCS ²	% lame precalving ³	% lame postcalving ³
1	79	2.5 \pm 1.35	10,710 \pm 1,458.1	59 \pm 27.7	3.6 \pm 0.46	2.9 \pm 0.49	–0.6 \pm 0.33	7	14
2	98	2.2 \pm 1.45	11,205 \pm 2,229.5	61 \pm 4.9	3.4 \pm 0.39	2.9 \pm 0.40	–0.5 \pm 0.36	2	4
3	91	2.2 \pm 1.11	11,294 \pm 1,610.5	60 \pm 18.7	3.6 \pm 0.46	3.0 \pm 0.41	–0.6 \pm 0.39	6	9
4	71	2.1 \pm 1.01	11,016 \pm 1,704.5	58 \pm 16.2	3.4 \pm 0.36	2.9 \pm 0.41	–0.5 \pm 0.34	1	4
All	339	2.3 \pm 1.26	11,066 \pm 1,781.1	59 \pm 18.7	3.5 \pm 0.43	3.0 \pm 0.43	–0.5 \pm 0.37	4	8

¹Precalving BCS was recorded at the time of enrollment in the study, 2 to 3 wk before the expected calving date; postcalving BCS was recorded at the time of removal from the study, 4 wk after the calving date.

²Change in BCS = BCS at time of removal of study – BCS at enrollment.

³Precalving lameness score was recorded at the time of enrollment in the study, 2 to 3 wk before the expected calving date; % lame precalving = [(no. of cows with a lameness score ≥ 3 precalving)/(total number of cows scored precalving)] \times 100; postcalving lameness score was recorded at the end of the study, 4 wk after the calving date; % lame postcalving = [(no. of cows with a lameness score ≥ 3 postcalving)/(total number of cows scored postcalving)] \times 100.

Table 5. Health status summary of focal cows sampled in each herd during an observational study of the associations of rumination time and subclinical ketosis over the transition period

Herd	Mean \pm SD d diagnosed ketotic (DIM)	Subclinically ketotic ¹ (%)	Treated for RP ² (%)	Treated for metritis ³ (%)	Treated for DA ⁴ (%)	Treated for MF ⁵ (%)	Treated for foot problems (%)	Treated for mastitis (%)	HYK ⁶ (%)	HYK+ ⁷ (%)	HLT+ ⁸ (%)
1	6 \pm 6.3	56	9	11	0	4	0	5	39	17	6
2	6 \pm 7.1	27	11	32	1	2	0	5	12	14	24
3	5 \pm 6.4	51	9	13	3	0	4	4	35	15	10
4	11 \pm 7.6	48	3	27	0	3	3	0	31	17	10
All	7 \pm 7.1	44	8	21	1	2	2	4	29	16	15

¹Cumulative incidence over 4 tests, once weekly in the first 4 wk postpartum based on a blood BHB >1.2 mmol/L measured with Precision Xtra (Abbott Diabetes Care, Saint Laurent, QC, Canada).

²Percentage of cows with retained placenta.

³Percentage of cows treated for metritis.

⁴Percentage of cows treated for displaced abomasum.

⁵Percentage of cows treated for milk fever.

⁶Percentage of cows with subclinical ketosis and no other health issue (HYK).

⁷Percentage of cows with subclinical ketosis and at least one other health issue (HYK+).

⁸Percentage of cows that were not subclinically ketotic but had at least one other health issue (HLT+).

time increased from wk +1 to wk +2 ($P < 0.001$) and decreased from wk +3 to wk +4 ($P = 0.04$). An interaction for rumination time was found between parity and week ($P = 0.05$). There was no difference in rumination during wk -1 and +1; however, primiparous cows tended to ruminate less in wk +2 (26 ± 15.9 min/d less, $P = 0.06$). During wk +3 and +4, primiparous cows ruminated less (38 ± 15.9 min/d, $P = 0.02$; and 51 ± 17.8 min/d, $P < 0.01$; respectively) than multiparous cows.

For multiparous cows, an interaction was found between health status and week ($P = 0.01$; Table 6). There was an effect of time ($P < 0.001$) across all health statuses: rumination time decreased in wk -1 compared with wk -2 but increased each week from wk -1 to +2. Multiparous HYK cows tended to ruminate less than multiparous HLT cows during wk -1 and wk +1 (Table 6). The largest differences in rumination time between multiparous HLT and HYK+ cows were seen during wk -1, +1, and +2. Figure 2 illustrates how the daily rumination time evolved across the transition period for HLT, HYK, and HYK+ multiparous cows.

Table 7 shows the unconditional associations of the multivariable model. Increased odds of SCK with no other recorded health problems (HYK) were associated with higher parity (3+ compared with second-lactation cows), greater milk yield during the previous lactation, longer dry period, cows being in the overconditioned category precalving, greater stall stocking density during wk -2, -1, and +1, and greater loss in BCS over the transition period. Decreased odds of SCK with no other health problems, relative to HLT, were associated with a greater stall stocking density during wk +1 and greater rumination time during wk -1. Four of these variables were retained in the final multivariable model (Table 8). Greater rumination time during the week before calving was associated with decreased odds of HYK, whereas greater milk yield in the previous lactation, greater loss of BCS over the transition period, and greater stall stocking density in the week before calving were all associated with increased odds of HYK relative to HLT.

Unconditional associations of the independent variables for HLT versus HYK+ cows are shown in Table 9. There were increased odds of developing SCK combined with another health problem with higher parity (3+ compared with second-lactation cows), greater milk yield during the previous lactation, longer dry period, higher BCS precalving, cows being in the overconditioned category precalving, greater change in BCS over the transition period, and greater stall stocking density during wk -2 and -1. The odds of SCK with another health problem (HYK+) with greater rumination time were decreased during wk -1 and +1. Four of

Table 6. Least squares means (\pm SE) for rumination time (min/d) for healthy cows without subclinical ketosis or other recorded illnesses (HLT), subclinically ketotic cows with no other health problems (HYK), and subclinically ketotic cows with other health problems (HYK+) during each week of the study period

Health status	No.	Period (relative to calving)					
		wk -2	wk -1	wk +1	wk +2	wk +3	wk +4
Primiparous							
HLT	52	407 \pm 13.7	376 \pm 11.8	375 \pm 10.6	439 \pm 12.5	439 \pm 14.2	421 \pm 17.0
HYK	21	421 \pm 25.2	374 \pm 21.3	393 \pm 16.7	465 \pm 19.7	460 \pm 22.3	434 \pm 26.7
HYK+	14	380 \pm 25.8	342 \pm 22.4	366 \pm 20.5	451 \pm 24.1	428 \pm 27.3	395 \pm 32.9
Multiparous							
HLT	87	420 \pm 12.1	402 \pm 12.0	430 \pm 11.5	509 \pm 13.0	504 \pm 14.1	489 \pm 14.8
HYK	76	406 \pm 12.7	374 \pm 12.9 \dagger	408 \pm 12.1 \dagger	484 \pm 13.7	477 \pm 15.1	453 \pm 15.8 \dagger
HYK+	39	405 \pm 16.6	354 \pm 16.0**	356 \pm 15.2***	444 \pm 17.7**	463 \pm 20.0 \dagger	468 \pm 21.7

$\dagger P \leq 0.10$; ** $P \leq 0.01$; *** $P \leq 0.001$: significance level for difference between HYK and HLT cows and HYK+ and HLT cows within weeks.

these variables were retained in the final multivariable model (Table 10). Greater rumination time during the week after calving was associated with decreased risk of HYK+, whereas being in the third parity or higher,

having a longer dry period, and experiencing greater stall stocking density in the week before calving were all associated with increased risk of HYK+ relative to HLT.

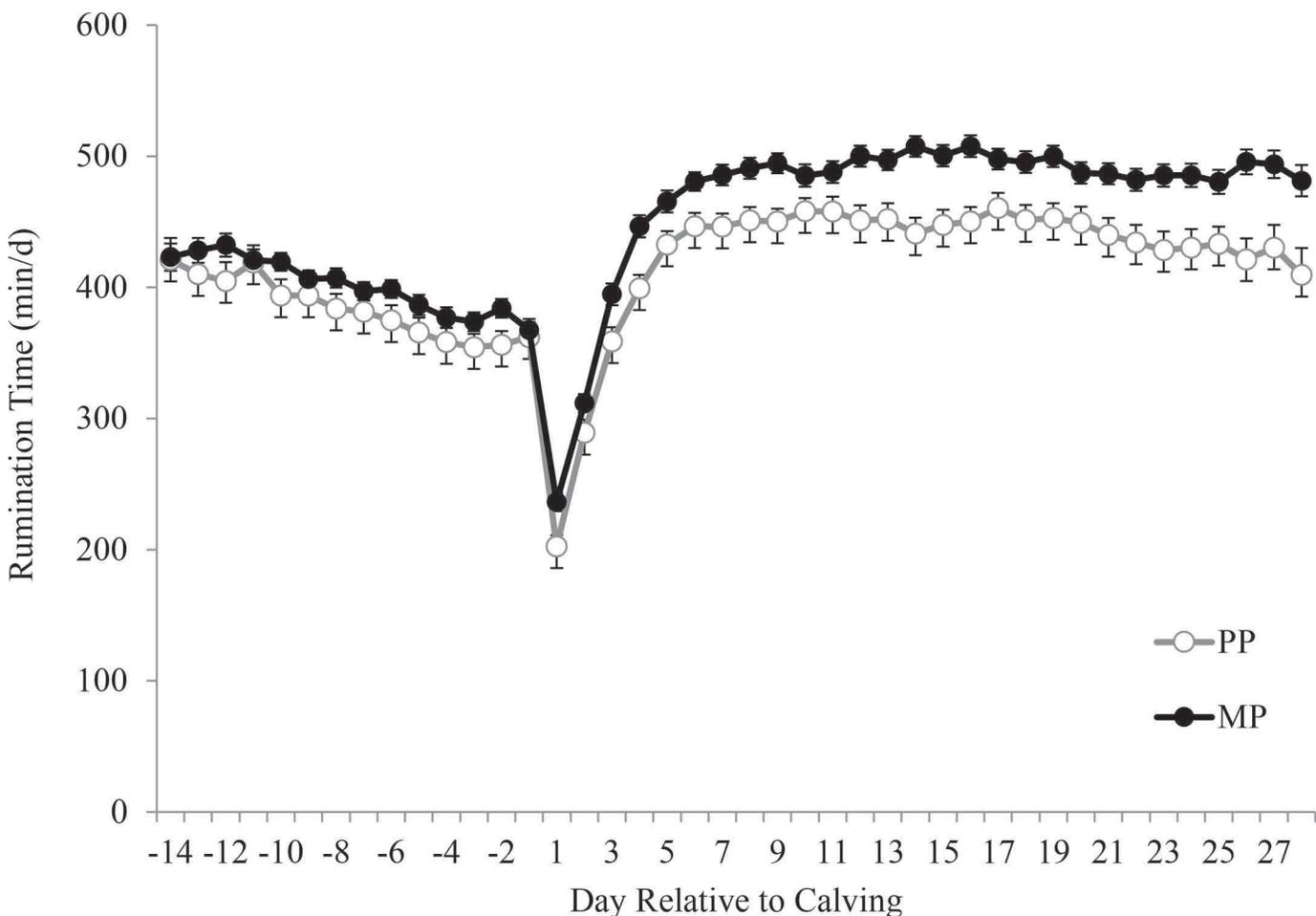


Figure 1. Daily rumination time (mean \pm SE; min/d) over the transition period (-14 to 28 d) for primiparous (PP, n = 87) and multiparous (MP, n = 202) cows.

DISCUSSION

In this study, we characterized the changes in rumination behavior across the transition period. Both primiparous and multiparous cows experienced a reduction in rumination time from wk -2 to -1 prepartum, which, as suggested by Liboreiro et al. (2015), may be associated with the common reduction in DMI leading up to calving. Similarly, the rumination time of primiparous and multiparous cows began to increase from wk +1 to +2, again potentially reflective of changes in DMI. Dry matter intake typically decreases as the cow approaches calving and begins to increase rapidly after calving (Grant and Albright, 1995). Although an association of rumination time and DMI is not consistently

reported in the literature, there are examples of these being positively associated. Cows have been found to spend more time ruminating about 4 h after periods of high feed intake (Schirmann et al., 2012); however, there was no correlation between periods of DMI and rumination time in that study, possibly due to large variations of these variables both between and within cows. Clément et al. (2014) recently found that rumination time was a significant, but small, contributor in a DMI prediction model. These researchers suggested that the variability of rumination time within weeks and cows makes it difficult to predict DMI from rumination time.

Rumination time is more consistently associated with dietary NDF intake (Welch and Smith, 1970; Beauche-

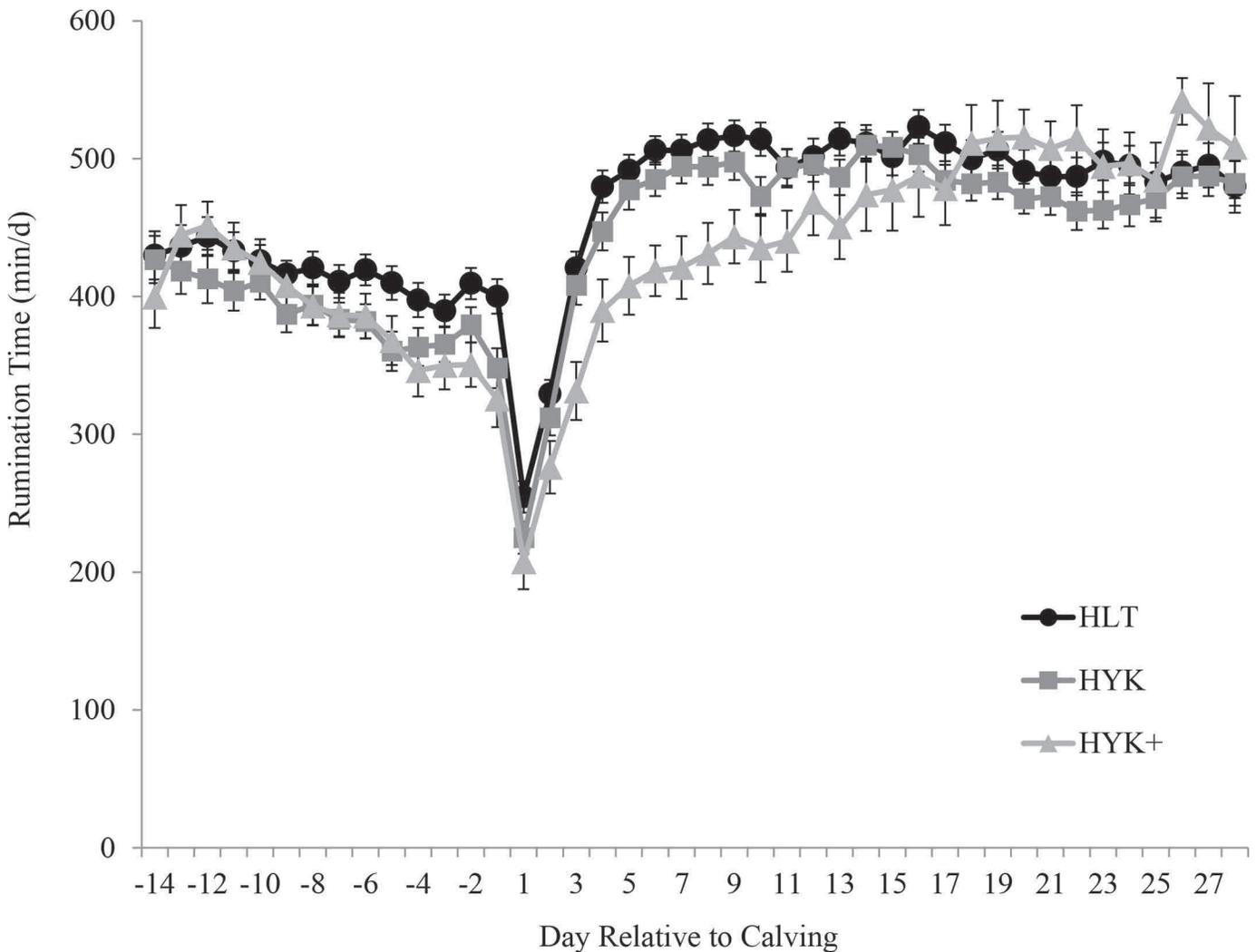


Figure 2. Daily rumination time (mean \pm SE; min/d) over the transition period (-14 to 28 d) for healthy multiparous cows with no other recorded illnesses (HLT; n = 87), subclinically ketotic multiparous cows with no other health problems (HYK; n = 76) and subclinically ketotic multiparous cows with other health problems (HYK+; n = 39).

Table 7. Unconditional estimates for factors associated with the incidence of subclinical ketosis with no recorded clinical disease (HYK; n = 76) relative to healthy animals (HLT; n = 87), in multiparous cows

Variable	Percentage or mean (\pm SD) ¹	Odds ratio (95% CI) ²	P-value
Parity (%)			0.002
2	51	Referent	—
3+	49	2.9 (1.49 to 5.65)	—
Previous 305-d milk yield (kg)	11,060 (1,785.0)	1.2 (0.88 to 1.73)	0.21
Length of dry period (d)	59 (19.0)	1.7 (1.00 to 2.86)	0.049
BCS precalving	3.4 (0.44)	1.3 (0.96 to 1.88)	0.083
BCS category precalving ³ (%)			0.17
Normal	70	0.9 (0.20 to 4.32)	—
Underconditioned	5	Referent	—
Overconditioned	25	2.1 (0.95 to 4.54)	—
Change in BCS ⁴	0.5 (0.37)	1.6 (1.13 to 2.29)	0.0081
Stall stocking density ⁵ (%)			
wk -2	84 (23.6)	1.6 (1.15 to 2.29)	0.0063
wk -1	79 (24.5)	1.6 (1.15 to 2.16)	0.0053
wk +1	81 (14.8)	0.6 (0.36 to 0.87)	0.011
Rumination time (min/d)			
wk -1	382 (85.6)	0.7 (0.46 to 0.97)	0.036

¹Proportion of animals for categorical variables or mean and standard deviation for continuous variables.

²Odds ratio and 95% CI for 1 SD increase in the variable presented.

³Cows were placed into 1 of 3 categories based on their body condition score precalving: normal (BCS 3–3.5), underconditioned (BCS <3.0), overconditioned (BCS >3.5).

⁴Change in BCS = BCS at enrollment – BCS at time of removal of study.

⁵Stocking density (ST) for freestall pens was calculated as $ST = [\text{no. of cows in pen}]/[\text{no. of stalls}]$. Bedded packs were calculated as $ST = [\text{no. of cows in bedded pack}]/\{(\text{dimensions, m}^2)/[\text{recommended space allowance, 11 m}^2; \text{Nordlund (2009)}]\}$.

min et al., 1994) and particle size (Kononoff et al., 2003; Beauchemin and Yang, 2005). Rumination time increases as particle size increases (Beauchemin et al., 1994); unfortunately, we did not measure particle size in this study, which may have provided greater insight into differences in rumination times observed. The NDF content in the fresh cow diets ranged from 28 to 32% of DM across the 4 commercial dairy farms in this study. Dado and Allen (1995) showed that rumination time

in early lactation dairy cows, measured using a halter that recorded jaw movements, increased from 380 to 500 min/d when the NDF content of the ration was increased from 25 to 35%. Rumination time averaged 418 and 481 min/d for healthy; lactating primiparous and multiparous cows respectively, which is comparable to that reported by Dado and Allen (1995). These averages are also within the reported range of 340 to 540 min/d for lactating cows (using jaw movement re-

Table 8. Final logistic regression model for factors associated with the incidence of subclinical ketosis with no other health issues (HYK; n = 76) relative to healthy animals (HLT; n = 87), in multiparous cows

Variable	Coefficient	SE	Odds ratio (95% CI) ¹	P-value
Intercept	-3.27	2.042		0.21
Previous 305-d milk yield (kg)	0.00024	0.000133	1.5 (0.96 to 2.47)	0.073
Change in BCS ²	1.68	0.622	1.9 (1.18 to 2.94)	0.0083
Stall stocking density ³ (%)				
wk -1	0.02	0.009	1.7 (1.10 to 2.58)	0.018
Rumination time (min/d)				
wk -1	-0.01	0.003	0.6 (0.38 to 0.97)	0.037

¹Adjusted odds ratio and 95% CI for 1 SD increase in each variable in the model. The mean \pm SD for each variable are as follows: 11,060 \pm 1,785.0 kg, 305-d milk yield; 0.5 \pm 0.37, change in BCS; 80 \pm 24.8%, stall stocking density (wk -1); 382 \pm 85.6 min/d, rumination time (wk -1).

²Change in BCS = BCS at enrollment – BCS at time of removal of study.

³Stocking density (ST) for freestall pens was calculated as $ST = [\text{no. of cows in pen}]/[\text{no. of stalls}]$. Bedded packs were calculated as $ST = [\text{no. of cows in bedded pack}]/\{(\text{dimensions, m}^2)/[\text{recommended space allowance, 11 m}^2; \text{Nordlund (2009)}]\}$.

Table 9. Unconditional estimates for factors associated with the incidence of subclinical ketosis with other health problems (HYK+; n = 39) relative to healthy animals (HLT; n = 87), in multiparous cows

Variable	Percentage or mean (\pm SD) ¹	Odds ratio (95% CI) ²	P-value
Parity (%)			<0.001
2	51	Referent	—
3+	49	5.5 (2.35 to 12.92)	—
Previous 305-d milk yield (kg)	11,061 (1,785.0)	1.5 (1.02 to 2.18)	0.039
Length of dry period (d)	59 (19.0)	1.9 (1.05 to 3.27)	0.034
BCS precalving	3.4 (0.44)	1.3 (0.90 to 1.94)	0.15
BCS category precalving ³ (%)			0.14
Normal	70	Referent	—
Underconditioned	5	1.1 (0.20 to 6.25)	—
Overconditioned	25	2.4 (1.00 to 5.87)	—
Change in BCS ⁴	0.5 (0.37)	1.5 (0.97 to 2.24)	0.068
Stall stocking density ⁵ (%)			
wk -2	84 (23.6)	1.4 (0.97 to 2.13)	0.071
wk -1	79 (24.5)	1.9 (1.22 to 2.92)	0.0048
Rumination time (min/d)			
wk -1	382 (85.6)	0.6 (0.40 to 0.92)	0.019
wk +1	407 (87.4)	0.4 (0.25 to 0.63)	<0.001

¹Proportion of observations for categorical variables or mean and standard deviation for continuous variables.

²Odds ratio and 95% CI for 1 SD in variable presented.

³Cows were placed into 1 of 3 categories based on their BCS precalving: normal (BCS 3–3.5), underconditioned (BCS <3.0), or overconditioned (BCS >3.5).

⁴Change in BCS = BCS at enrollment – BCS at time of removal of study.

⁵Stocking density (ST) for freestall pens was calculated as $ST = [\text{no. of cows in pen}]/[\text{no. of stalls}]$. Bedded packs were calculated as $ST = [\text{no. of cows in bedded pack}]/\{(\text{dimensions, m}^2)/[\text{recommended space allowance, 11 m}^2; \text{Nordlund (2009)}]\}$.

corders) found in the literature (Kononoff et al., 2003; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006).

Even though the close-up dry cow diets were greater in NDF than the fresh cow diets in the study herds, the expected changes in DMI across this period would result in much greater intake of total NDF in the postpartum period. Thus, it is possible that the changes in rumination time reflected the changes in DMI across this period. More research on the association of DMI

and rumination during the transition period is needed, particularly accounting for changes in physical and chemical composition of diets from pre- to postcalving.

In this study, primiparous cows ruminated less than multiparous cows during wk +3 and +4 after calving. Maekawa et al. (2002) found that primiparous cows ruminated 52 min/d less than multiparous cows in mid lactation, using visual observation; this difference was attributed to the greater DMI of multiparous cows, which also had greater BW and higher milk yields than

Table 10. Final logistic regression model for factors associated with the incidence of subclinical ketosis with other health problems (HYK+; n = 39) relative to healthy animals (HLT; n = 87), in multiparous cows

Variable	Coefficient	SE	Odds ratio (95% CI) ¹	P-value
Intercept	-3.67	2.750	—	0.27
Parity				<0.001
2	Referent	—	—	—
3+	2.09	0.580	8.1 (2.55 to 25.43)	—
Length of dry period (d)	0.06	0.032	2.9 (0.87 to 9.56)	0.083
Stall stocking density ² (%)				
wk -1	0.02	0.012	1.8 (1.01 to 3.27)	0.046
Rumination time (min/d)				
wk +1	-0.01	0.003	0.5 (0.27 to 0.80)	0.0063

¹Adjusted odds ratio and 95% CI for 1 SD increase in each variable in the model. The mean \pm SD for each variable are as follows: 59 \pm 19.0 d, length of dry period; 80 \pm 24.8%, stall stocking density (wk -1); 407 \pm 87.4 min/d, rumination time (wk +1).

²Stocking density (ST) for freestall pens was calculated as $ST = [\text{no. of cows in pen}]/[\text{no. of stalls}]$. Bedded packs were calculated as $ST = [\text{no. of cows in bedded pack}]/\{(\text{dimensions, m}^2)/[\text{recommended space allowance, 11 m}^2; \text{Nordlund (2009)}]\}$.

primiparous cows. Beauchemin and Rode (1994) monitored rumination time with a strain gauge that assessed jaw movement; they observed lactating multiparous cows to have a longer daily rumination time; primiparous and multiparous cows regurgitated a similar number of boluses; however, multiparous cows spent more time chewing each bolus. Soriani et al. (2012) suggested that primiparous cows suffer more from the stress of environmental changes at the initiation of lactation, and thus show a slower increase in rumination time after calving compared with multiparous cows. Other researchers have measured rumination time over the transition period and reported no difference between primiparous and multiparous cows (Soriani et al., 2013; Calamari et al., 2014; Liboreiro et al., 2015); unfortunately, no discussion of this lack of difference was presented in those studies.

Rumination time for HLT multiparous cows during the dry period (408 min/d) is within the range of 400 to 450 min/d reported by Adin et al. (2009) for close-up cows fed a similar diet. Soriani et al. (2012) found that daily rumination time averaged 522 min/d during d -10 to -2 precalving, which was higher than what was observed in this study for HLT cows during the same period. This difference in rumination time is probably due to the greater amount of NDF in their dry cow diet, which was 56% of DM (Soriani et al., 2012), compared with an average of 37% of DM in this study.

No difference in rumination time was found between health categories for primiparous cows (Table 6). This may be due to a low sample size among the subgroups with SCK (HLT, $n = 52$; HYK, $n = 21$; HYK+, $n = 14$). The initial sample size calculation was based on comparing only primiparous cows with SCK versus without SCK. With a larger sample size and greater number of HYK and HYK+ cows, it may have been possible to detect differences between healthy primiparous cows and primiparous cows with SCK; we recommend further research to address this. In our study, multiparous cows with only SCK tended to ruminate less during the week before and the week after calving. Recently, Liboreiro et al. (2015) sampled both primiparous and multiparous cows and did not find an association between SCK and precalving or postcalving daily rumination time. However, in that study, cows diagnosed with SCK had reduced daily rumination time from d 0 to 8 postcalving, and tended to have reduced daily rumination time on d 11 postcalving compared with cows without SCK. It is unclear whether the subjects in Liboreiro et al. (2015) were only diagnosed with SCK or could have had multiple health disorders, which may have had a further effect on rumination time, as demonstrated in the present study.

Lower rumination times were observed in multiparous HYK+ cows during wk -1, +1, and +2 compared with HLT cows. Soriani et al. (2012) categorized cows into 3 groups based on rumination time before calving: longer rumination time, middle rumination time, and shorter rumination time. Cows in the shorter rumination group showed a higher incidence of clinical disease (including mastitis, lameness, ketosis, and DA) and these cows had a decreased rumination time after calving, similar to what was seen in HYK+ cows in the current study.

This also agrees with the observations made by Calamari et al. (2014), who found that 90% of cows in the low rumination group postcalving had a clinical health problem, compared with 45% of cows categorized in the high rumination group. Liboreiro et al. (2015) recently reported similar effects of postpartum illness on rumination time; those researchers found that cows with milk fever on the day of calving and cows diagnosed with metritis between d 2 and 9 postpartum had reduced rumination time.

In the present study, the odds of developing SCK and another clinical disease were 1.2 times greater for every 20 min/d decrease in rumination time during the week after calving. Although there was also a difference in rumination during the week before calving, the depression in rumination time was much greater in the week after calving, possibly because of the combined effect of multiple transition disorders occurring postcalving, some of which may have preceded the diagnosis of SCK. In this study, SCK was only diagnosed once weekly, which was a limitation of the study. If cows were ketotic on the day of diagnosis, we did not know whether that was the first day of SCK or if the cow had been ketotic for multiple days. McArt et al. (2012) found the median time to resolution of SCK without treatment was 5 d; by testing only once per week in the current study, the onset and recovery of SCK may have occurred between sample days, causing the misclassification of subclinically ketotic cows as healthy cows. This type of misclassification may have created a bias toward the null during the analysis, meaning the differences in rumination time between health categories may have been underestimated. This also limited our ability to fully understand how rumination changes directly before and after the onset of SCK. Future studies monitoring this association should monitor SCK more frequently to understand the detailed changes in rumination around the onset of illness.

When comparing HYK with HLT cows, we found that for every 20 min/d decrease in rumination time during the week before calving, the odds of becoming HYK postcalving increased 1.1-fold. Low DMI and reduced feeding time are considered important risk fac-

tors for SCK. Studies by González et al. (2008) and Goldhawk et al. (2009) observed a 10-kg reduction in fresh feed intake per day and a 3-kg reduction in daily DMI, respectively, during the week before being diagnosed ketotic. Shorter rumination times in the current study may be indicative of low DMI in the prepartum period (Clément et al., 2014); however, many cow-level and management-related factors vary between farms and have a great effect on rumination time.

The cumulative incidence of SCK across 25 Ontario farms ranged from 8 to 80% during the first 9 wk postpartum, with a mean of 43% of cows that experienced SCK (Duffield, 2000). McArt et al. (2012) also found a 43% cumulative incidence of ketosis with thrice weekly testing between 3 and 16 DIM, with the peak incidence of ketosis occurring at 5 DIM. These estimates are in line with the 44% cumulative incidence of SCK within the first 4 wk postpartum observed in the present study.

There is much evidence in the literature supporting the notion that severe NEB in the transition period increases the risk for postpartum diseases such as retained placenta, milk fever, metritis, mastitis, displaced abomasum, and SCK (Dohoo et al., 1983; Duffield et al., 2009; LeBlanc, 2010). LeBlanc (2010) estimated that 30 to 50% of cows experience some form of health problem around the time of calving. Similar to that, in the current study, 35% of cows diagnosed with SCK had at least one other recorded health problem during the first 4 wk postpartum.

It is apparent that SCK and other postpartum diseases are common in commercial dairy herds, but the causes are not always apparent as there are numerous factors, including parity, breed, BCS, milk yield, dry cow nutrition, and management factors that have been associated with risk of both SCK and clinical ketosis (Andersson, 1988; Duffield, 2000). Increasing parity is a known risk factor for SCK (Suthar et al., 2013; Berge and Vertenten, 2014; Vanholder et al., 2015), which was also found in the present study; for example, the odds of SCK in HYK+ cows were 8 times higher in 3+ lactation cows compared with HLT cows in their second lactation. Cows with higher milk production have higher nutrient demands, putting them at a higher risk of developing SCK (Bauman and Currie, 1980; Gröhn et al., 1995; Fleischer et al., 2001), which is why cows in the present study with greater 305-d milk yield in the previous lactation were at increased odds for having SCK with no other health issues. Vanholder et al. (2015) found that cows in both the moderate ($3.5 \leq \text{BCS} \leq 3.75$) and fat BCS categories ($\text{BCS} \geq 4$) had an increased risk of SCK compared with cows in the thin category ($\text{BCS} \leq 3$). Cows with greater BCS precalving have a greater decrease in DMI before calving (Hayirli et al., 2002), which is a major contributing factor for develop-

ing SCK postpartum (Goldhawk et al., 2009). Cows in a state of NEB will deplete fat stores to compensate for the high energy demands of lactation (Goldhawk et al., 2009). Therefore, it is not surprising that in our study, a greater loss of BCS over the transition period was associated with increased risk of experiencing SCK in cows with no other health problems.

In the present study, each extra 5 d dry above the mean (59 d) increased the odds of developing SCK combined with another postpartum health disorder 1.3-fold. Vanholder et al. (2015) similarly observed this positive association between the length of the dry period and SCK. It is possible that cows with a longer dry period become overconditioned. Cows consuming the close-up ration longer than the recommended 3 wk have been shown to have increased BCS and risk of metritis postpartum (Mashek and Beede, 2001). We could also hypothesize that these cows with long dry periods became pregnant later in lactation and were already overconditioned before dry off.

Increasing stall stocking density by 5% during the week before calving was found to increase the risk of ketosis by 10% in both HYK and HYK+ cows. Overcrowding can limit the ability of cows to access their desired resources, whether that be lying areas, feed, or water, at the times they would prefer. This has the potential to decrease lying time (Munksgaard et al., 2005) and may impel cows to lie down sooner after milking (Fregonesi et al., 2007), rather than consume feed at the feed bunk, potentially limiting DMI. Proudfoot et al. (2009) demonstrated that when subjected to a competitive feeding environment, multiparous cows showed a decrease in DMI 1 wk before calving. It should be noted that both stall stocking density and feed bunk stocking density were highly variable among the study farms. However, in general, more space was provided on these farms than typically seen on commercial dairy farms for transition cows (von Keyserlingk et al., 2012). In any case, these results suggest that dry cow management should aim to reduce competition for resources by reducing stocking density in close-up dry cow pens.

CONCLUSIONS

Primiparous cows showed no difference in rumination time between health statuses; however, multiparous cows with ketosis, with or without other health problems, were found to ruminate less than healthy multiparous cows. Higher rumination times during the week before calving and the week after calving were associated with decreased odds of HYK and HYK+, respectively, in multiparous cows. Other factors that were found to decrease the odds of SCK in multiparous cows included lower stall stocking density during the

