Exposure to an unpredictable and competitive social environment affects behavior and health of transition dairy cows

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ABSTRACT

Social factors are important determinants of disease in humans and laboratory animals, but less research has been done using farm animals. The objective of this study was to determine if an unpredictable and competitive social environment affects behavior and health during the transition period when dairy cows are at high risk of disease. Five weeks before calving, 64 cows were assigned to a predictable and noncompetitive social environment (predictable) or an unpredictable and competitive social environment (unpredictable) using 8 groups of 4 animals per treatment. Each group consisted of 3 multiparous and 1 primiparous cow. At first enrollment (baseline; 5 wk before calving), all groups had access to 4 electronic feed bins. At 4 wk before calving, cows in the predictable groups were given access to 6 feed bins, and cows in the unpredictable groups were moved into a new pen with 4 resident cows each trained to consume feed from one bin. Each cow in the unpredictable group was then provided access to only 1 of the 4 feed bins which they shared with 1 resident cow (resulting in 2 cows/bin), creating a competitive feeding environment. To create an unpredictable environment, access to morning feed was delayed 0, 1, 2, or 3 h every other day. On alternate days, the cows in unpredictable groups were assigned to feed from a new feed bin (and thus had to compete with a new resident partner). Feeding and social behavior were collected electronically from the feed bins. Blood was sampled at baseline (wk -5), wk -2, wk -1, and wk +1 relative to calving to measure inflammatory (haptoglobin and tumor necrosis factor- α) and metabolic (nonesterified fatty acids, β -hydroxybutyrate, calcium, and glucose) biomarkers. Uterine cytology was performed 3 to 5 wk

after calving to diagnose cytological endometritis. Data were analyzed using mixed models including baseline data as a covariate, week as a repeated measure, treatment as a main effect, and a treatment by week interaction. The probability of cytological endometritis at the group level was analyzed using Mann-Whitney U tests. Parity was included in separate models to determine any parity $\times$ treatment interactions. Cows from both treatments consumed the same amount of feed, but cows in the unpredictable group spent less time feeding and had a higher rate of feed intake. Cows in the unpredictable groups also visited the feed bins less often, consumed more feed during each visit, and were involved in more social replacements at the feed bin compared with predictable groups. Cows in the unpredictable groups had higher serum concentrations of nonesterified fatty acids and tumor necrosis factor- α , but lower β -hydroxybutyrate compared with predictable groups. Multiparous cows in unpredictable groups were more likely to be diagnosed with cytological endometritis after calving compared with cows in the predictable groups, but primiparous cows in unpredictable groups showed a tendency for the opposite response. These results suggest that an unpredictable and competitive social environment before calving causes changes in feeding and social behavior, some physiological indicators of metabolism and inflammation, and increases the risk of uterine disease in multiparous cows after calving.

Key words: predictability, stress, competition, animal welfare

INTRODUCTION

Despite decades of research, periparturient diseases remain a major animal welfare and economic challenge of the dairy industry. A goal of many dairy producers is disease prevention, but this approach requires knowledge of disease determinants, including the animals' immune competence, nutrition, and social environment (LeBlanc et al., 2006; Sepúlveda-Varas et al., 2013). Al-

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though numerous advancements have been made in the understanding of transition cow immunity and nutrition (LeBlanc et al., 2006), far less is known about the role of the social environment as a mediator of disease in transition cows.

Dairy cows may experience several social challenges during the transition period, including group changes, overstocked feeding and lying areas, and occasionally unpredictable management practices such as delayed feeding times or other disturbances to their daily routines. Some research has identified the effects of a single factor, such as overstocking, on cow behavior and health (Proudfoot et al., 2009; Huzzey et al., 2012). For example, in some of our earlier work we found that when cows were overstocked at the feeding areas (2 cows/bin), they increased their rate of feed intake (Proudfoot et al., 2009). However, less research has attempted to measure the combined effects of multiple sources of social disturbance on transition dairy cows.

The social environment may also affect physiological biomarkers of metabolism and inflammation in dairy cows. For example, Huzzey et al. (2012) have found that overstocking both the feeding and lying areas during the dry period increased blood nonesterified fatty acids (**NEFA**), an indicator of adipose mobilization and metabolic health that has been linked to disease after calving (Ospina et al., 2010a). Other research has found little effect of moderate increases in stocking density on cow health and behavior, such as increasing stocking density from 80 to 100% at the feed bunk (Silva et al., 2014). In humans, another mediator of stress-induced illness is chronic, systemic inflammation measured using pro-inflammatory cytokines [e.g., tumor necrosis factor (**TNF**)- α , IL-1b, and IL-6; reviewed by Popa et al., 2007]; however, to our knowledge no studies have measured the effect of the social environment on pro-inflammatory cytokines in dairy cattle.

It is well known in the human and laboratory animal literature that unpredictable social environments may cause a physiological stress response, which in turn may lead to negative health outcomes if these stressors are additive, chronic, or severe in nature (Koolhaas et al., 2011). The research to date assessing the effect of social pressures on transition cows has focused on addressing a single factor, with little unpredictability in the cow's daily routine (e.g., Proudfoot et al., 2009; Huzzey et al., 2012; Silva et al., 2014). Thus, the objective of this study was to determine the effect of housing transition cows in an unpredictable and competitive social environment on feeding behavior, social behavior, indicators of metabolic health (**NEFA**, **BHB**, calcium, glucose), indicators of inflammation (**TNF**- α and haptoglobin), and uterine health (cytological endometritis)

after calving. We predicted that cows in the unpredictable and competitive environment would change their feeding behavior and social behavior, and show increases in metabolic and inflammatory markers, and show a higher probability of endometritis after calving compared with those housed in a predictable and noncompetitive environment.

MATERIALS AND METHODS

The experiment took place at the University of British Columbia's Dairy Education and Research Centre in Agassiz, BC, Canada, between September 2012 and April 2013. Cows were cared for under protocol #A14-0040 approved by the University of British Columbia's Animal Care Committee according to the guidelines provided by the Canadian Council on Animal Care (2009).

Animals and Housing

Sixteen groups of 4 Holstein dairy cows were used in this experiment ($n = 64$). Each group included 1 primiparous cow and 3 multiparous cows (average parity 2.4 ± 1 , mean $\pm$ SD; range 1 to 3) so the effects of parity could be assessed in the context of the treatment. All experimental cows were previously housed in 1 of 2 group freestall pens in the same building as the experimental pens. Cows were assigned to groups based on their expected calving dates to make the group assignments as random as possible. Groups were moved into 1 of 4 experimental pens approximately 5 wk before the average expected calving date of the cows in each group. To control for seasonal effects, groups from both treatments were moved into the pen at approximately the same time; when each group was finished with the experiment (i.e., when all of the cows had calved), a new group was added to the pen until all 16 groups completed the experiment.

Experimental pens (11.5×9.8 m) included 12 lying stalls fitted with mattresses (Pasture Mat, Promat Inc., Woodstock, Ontario, Canada) covered with 5 cm of sand bedding and vulcanized rubber floors in the alleys and crossovers (Red Barn Dairy Mat, North West Rubber Mats Ltd., Abbotsford, British Columbia, Canada). The pens also included 6 gated electronic feed bins (Insentec, Marknesse, Holland). To access the feed bins, cows were required to place their heads over a feed gate so that an antenna could read the unique radio frequency signal in their ear tag (Allflex, High-Performance ISO Half Duplex Electronic ID Tag, Allflex Canada, St-Hyacinthe, Quebec, Canada). Using a computer connected to the feeding system, experi-

menters could assign cows to access feed from one or all feed bins in the pen.

Cows were fed a close-up TMR twice daily at 0700 and 1600 h. The TMR samples were collected once weekly and were dried at 60°C to calculate the DM content of the feed. Before calving, cows were fed a TMR consisting of 26% corn silage, 31% ryegrass straw, 23% alfalfa hay, 7% grass silage, and 13% grain concentrate mash (DM = 49%, CP = 15.1% DM, ADF = 34.9% DM, NDF = 52.5% DM, and NE_L = 0.58 Mcal/kg). Diets were formulated according to the NRC (2001).

Cows were moved into individual maternity pens to give birth when signs of calving were imminent (e.g., restlessness, suddenly enlarged udder, relaxed pelvic ligaments, the observation of the amniotic sac, or a combination of these). After calving, cows were moved into 1 of 2 freestall pens.

Treatments and Experimental Design

Baseline. Each group was housed in an experimental pen for 7.7 ± 0.07 d starting at 5 wk before their predicted calving date (5 ± 0.5 wk before their actual calving dates, calculated retrospectively) to collect baseline data. During the baseline phase, all groups had access to 12 lying stalls (3 stalls: 1 cow) and free access to 4 of the 6 feed bins (1 bin: 1 cow).

Treatments. At 4 wk before their expected calving dates (27.3 ± 3.5 d before their actual calving dates, calculated retrospectively), 8 of the groups were assigned to a predictable and noncompetitive social environment (predictable treatment). Cows in the predictable groups remained in the same pen and were given free access to all 6 feed bins (1.5 bins: 1 cow) and 12 lying stalls (3 stalls: 1 cow) for the duration of the experiment. These groups were fed at approximately the same time each day (0700 and 1600 h).

The remaining 8 groups were assigned to an unpredictable and competitive social environment (unpredictable treatment). To create an unpredictable and competitive environment, we used a combination of overstocking at the feeding area, social instability (partner change), and unpredictable feeding times (delayed feed access). In addition, these unpredictable groups were moved into a new pen with 4 nonexperimental, multiparous resident cows (parity 4.5 ± 2). Each cow could access only 1 of 4 feed bins (1 bin: 2 cows). Cows had access to 8 lying stalls (1 stall: 1 cow). Resident cows were assigned to access one feed bin each, and this bin assignment remained the same for the duration of the trial. Cows in the unpredictable groups were randomly assigned to access 1 of the 4 feed bins on the first day they entered the pen, forcing them to compete for feed with one of the resident cows. On the following day,

the cows remained assigned to the same feed bin, but each group experienced a random delayed feed access at morning feeding of 0, 1, 2, or 3 h. To delay feeding, the bins were filled with fresh TMR at the normal hour (approximately 0700 h), but the gates were manually closed until the delayed feed access was complete; all cows in the same group experienced the same delayed feed access. On the third day, cows were reassigned to a new feed bin, which caused a partner change, and thus they were required to compete with a new resident partner for access to feed. These delayed feed access periods and partner changes were alternated daily until cows showed signs of calving. At 14.7 ± 0.07 d after entering the study, unpredictable groups were subjected to another regrouping event that involved a new pen with 4 new resident cows (pen change). To ensure that no cows habituated to the delayed feed access or bin change regimen, the order of bin changes (e.g., which bins cows were assigned to) and the order of delayed feed access times (e.g., whether it was 0, 1, 2, or 3 h) was randomized for the first group using the random function of Microsoft Excel (Microsoft Corp., Redmond, WA), and this same order was applied to all groups.

Behavioral Data Collection

Feeding Behavior. Feeding behavior data were automatically collected from the electronic feeding system (validated by Chapinal et al., 2007). The feed bins recorded the amount of feed consumed and amount of time spent feeding during each visit for individual cows. These data were used to calculate daily feed intake (kg/d), daily feeding time (min/d), average rate of feed intake (kg/min), the number of visits to the feed bins (no./d), the duration of each visit (min/visit), feed intake per visit (kg/visit), as well as the proportion of visits that were nonnutritive (e.g., the cow accessed the bin or attempted to access the bin but did not consume any feed).

Social Behavior. To measure social competition at the feed bins, physical replacements at the feed bins were calculated automatically from the feed bin data using methodology described by Huzzey et al. (2014). Briefly, a replacement was considered to occur if a cow was feeding at a bin (reactor), she left the bin, and another cow (actor) used the bin within 26 s of the reactor leaving. These data were summarized into the number of times a cow was involved in a replacement (either as an actor or a reactor) per day.

Serum Biomarkers

Blood samples were taken from the coccygeal vein (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) on

the last day of the baseline period and once weekly at wk -2, wk -1, and wk +1 after calving. Biomarkers of interest included indicators of inflammation (TNF- α and haptoglobin), and indicators of metabolic health (NEFA, BHB, glucose, and calcium). Blood sampling occurred at approximately 0900 h on each sampling day. To facilitate blood sampling, cows were moved from their home pens into a holding area where they were gently coerced into a head-lock using fresh hay. For 6 of the 8 treatment groups, the wk -1 blood sample was on the same day as a delayed feed access of 1 h ($n = 4$ groups) or 2 h ($n = 2$ groups); although the opportunity to consume feed before these measurements may affect the biomarkers, we chose to keep the 0900 h sampling time to ensure that our measurements were not affected by diurnal changes (see Nielsen et al., 2003). After collection, blood was centrifuged at $1,400 \times g$ for 10 min at 4°C, and serum was extracted using a pipette, divided into three 1-mL aliquots, and stored in a -20°C freezer until analysis. Serum concentration of TNF- α was determined with an ELISA (described by Farney et al., 2011), using bovine-specific polyclonal antibodies and bovine-specific recombinant protein (Thermo Fisher Scientific, Rockford, IL). The remaining biomarkers (NEFA, BHB, haptoglobin, glucose, and calcium) were analyzed using an automated analyzer (Hitachi 911 Analyzer, Randox, Laval, Quebec, Canada) with reagents supplied by Randox Laboratories Ltd. (Crumlin, UK). The NEFA and BHB were determined using Randox kits (Randox Laboratories Canada Ltd., Mississauga, ON, Canada; analytic sensitivity = 0.1 mmol/L for both, interassay control coefficients = 4.3 and 2.5% for NEFA and BHB, respectively). Glucose was measured using the Roche GLUC3 kit (Roche Diagnostics, Indianapolis, IN; analytical sensitivity = 0.1 mmol/L, interassay control CV = 1.13%). Calcium was measured using the Cobas Calcium Gen 2 kit (Roche Diagnostics; analytical sensitivity = 0.2 mmol/L, interassay control CV = 2.2%). Haptoglobin was measured using hemoglobin binding capacity (analytical sensitivity = 0.03 g/L, inter-assay control CV = 5.5%).

Cytological Endometritis Diagnosis

A trained veterinarian performed a uterine cytology exam 29 ± 6 d after calving as described by Dubuc et al. (2010). Prior to the exam, the vulva was cleaned with paper towels and an iodine solution, and the endometrial cytological sample was taken using a cytobrush adapted for use in cattle. The brush was rolled onto a clean glass microscope slide that was fixed with cytofixative (Cytoprep, Fisher Scientific, Nepean, Ontario, Canada). The slides were then stained with modified

Wright Giemsa stain (Protocol-Hema3, Biochemical Sciences, Swedesboro, NJ) and a differential cell count of 100 epithelial cells and neutrophils was conducted by one trained observer blinded to treatment; this process was replicated twice for each slide using 2 different locations on the slide. The average of the 2 counts was used as the final percentage for each cow; no cases were observed where the 2 counts were >10% different (mean = 1.75% different, SD = 2.25%). Cows were considered to have cytological endometritis if $\geq 6\%$ of the cells were identified as neutrophils.

Statistical Analysis

Analyses were performed with SAS software (version 9.4, SAS Institute Inc., Cary, NC). Treatment was applied at the group level, so group was considered the unit of analysis ($n = 8/\text{treatment}$). Nine animals were excluded from the analysis ($n = 5$ predictable, $n = 4$ unpredictable) because they calved early with twins ($n = 2$ predictable, $n = 2$ unpredictable), aborted ($n = 1$ predictable), were not pregnant ($n = 1$ unpredictable), or calved >2 wk early ($n = 2$ predictable, $n = 1$ unpredictable). An additional 8 animals were removed from the cytological endometritis analysis because we were not able to collect the data from the first group due to missing equipment (predictable, $n = 4$; unpredictable, $n = 4$). Data were screened for normality and extreme outliers. Extreme values were checked against 24-h video recording of the feed bins; values most likely due to a mechanical issues (e.g., a bin door malfunctioning) were removed before analysis (0.02% of the data). Some serum biomarkers (NEFA, BHB, TNF- α , and haptoglobin) were log-transformed to normalize the error distribution. No other variables required transformation.

The effect of treatment on daily feeding and social behavior was assessed using mixed models in SAS (PROC MIXED). Data collected during the baseline period (wk -5) were summed per cow and day, and then averaged across the baseline period and group to create one value per group. Data collected from the experimental period were summed per cow and day, and averaged across each week and group relative to calving (-4, -3, -2, and -1 wk). Models included baseline data for each variable as a covariate, treatment and week as main effects, and a treatment $\times$ week interaction. Week was considered a repeated measure, modeled with a heterogeneous autoregressive covariance structure chosen based on best fit using the Bayesian information criterion, and group ($n = 8/\text{treatment}$) was included as a random effect.

To determine effects of treatment on behavior during peak feeding times after fresh feed delivery, DMI data

were averaged for each hour during the experimental period for days when the bins were switched. Data from delayed feed access days were removed because the feeding pattern from the cows in the unpredictable groups was altered due to the experimental treatment. Data were summarized to create one value per treatment and hour of day (0 to 23 h). From these data, the 2-h periods after fresh feed delivery (0700 to 0859 h and 1600 to 1759 h) were summed to create one value per group. A mixed model in SAS (PROC MIXED) was used to determine the effect of treatment on feed intake during these peak feeding times; the model included treatment (predictable vs. unpredictable) as a fixed effect and group as a random effect.

The effect of treatment on serum biomarkers was assessed using mixed models in SAS (PROC MIXED). Each model included the baseline value (wk -5) as a covariate, treatment and week as main effects, and a treatment $\times$ week interaction. Week was considered a repeated measure modeled with a heterogeneous autoregressive covariance structure chosen based on best fit using the Bayesian information criterion, and group was included as a random effect.

To determine the effect of treatment on cytological endometritis, the data were summarized at the group level. The percentage of cytological endometritis cases (defined as neutrophils $\geq 5\%$) per group was calculated and compared between treatments using a Mann-Whitney U test in SAS (PROC NPAR1WAY).

We anticipated that first parity cows would respond to the treatment differently than older cows, particularly in the case of feeding and social behavior (see Neave et al., 2017). To test this prediction, we used mixed models in SAS (PROC MIXED) using the feeding, social, and serum data summarized at the level of parity (1 primiparous cow per group and the average of 3 multiparous cows per group). The models included treatment, week, and parity (first vs. second and greater [mean = 2.8 SD = 0.8]) as fixed effects, as well as parity $\times$ treatment interaction. Week was considered a repeated measure modeled with compound symmetry covariance structure chosen based on best fit, and group was included as a random effect. The percentage of cytological endometritis cases (defined as neutrophils $\geq 6\%$) per group was calculated and compared between treatments using a Mann-Whitney U test in SAS (PROC NPAR1WAY) separately for primiparous and multiparous cows. For any cases where there was a significant treatment $\times$ parity interaction ($P < 0.05$), pair-wise comparisons were made using a Bonferroni correction. Statistical significance was declared at $P < 0.05$ and statistical tendencies were declared at $P < 0.10$. Results are presented as least squares means $\pm$ standard errors.

RESULTS

Feeding Behavior

We found no difference between treatments in DMI throughout the study (predictable vs. unpredictable: 15.6 vs. 15.5 ± 0.3 kg/d; $F_{1,48} = 0.30$, $P = 0.59$; Table 1). However, overall differences were observed between treatments in the number of feeder visits; cows in unpredictable groups visited the feed bins 25% less often (69.2 vs. 93.9 ± 2.8 no./d; $F_{1,48} = 35.7$, $P < 0.0001$), consumed twice as much feed at each visit (0.4 vs. 0.2 ± 0.01 kg/visit; $F_{1,48} = 257.9$, $P < 0.0001$), and spent more time feeding during each visit compared with cows in the predictable groups (6.2 vs. 3.5 ± 0.2 min/visit; $F_{1,48} = 227.4$, $P < 0.0001$). Although unpredictable groups visited the feed bins less often than predictable groups, a higher proportion of those visits were non-nutritive (33 vs. $13 \pm 1\%$; $F_{1,48} = 396.8$, $P < 0.0001$). Despite spending more time feeding per visit, cows in the unpredictable groups spent less time feeding per day compared with cows in predictable groups throughout the study period (238.7 vs. 263.6 ± 4.5 min/d; $F_{1,48} = 15.6$, $P = 0.0003$). This lower daily feeding time may have been due to cows in the unpredictable groups consuming their feed at a faster rate than those in predictable groups (86 vs. 66 ± 2 g/min; $F_{1,48} = 67.9$, $P < 0.0001$; Figure 1).

The daily pattern of feed intake varied by treatment (Figure 2); cows in the unpredictable groups shifted their peak feeding time and consumed about 50% less feed during the first 2 h after morning and afternoon fresh feed delivery compared with predictable groups on days when feed access was not delayed (3.5 vs. 6.6 ± 0.4 kg/4 h; $F_{1,7} = 55.63$, $P < 0.0001$).

Social Behavior

We found differences in actor and reactor replacements at the feed bins; cows in unpredictable groups engaged in more actor (16 vs. 9 ± 1 no./d; $F_{1,48} = 39.6$, $P < 0.0001$) and reactor (15 vs. 10 ± 1 no./d; $F_{1,48} = 19.6$, $P < 0.0001$) replacements from the feed bins compared with those in the predictable groups (Figure 3).

Serum Biomarkers

We found differences between treatments in NEFA, BHB, and TNF- α throughout the study period, but no differences in serum glucose, calcium, or haptoglobin were detected. Cows in the unpredictable groups had higher NEFA [LSM (backtransformed means): -0.7 (0.20) vs. -0.8 (0.16) ± 0.05 ; $F_{1,34} = 4.2$, $P = 0.048$], lower BHB (2.6 (434.7) vs. 2.7 (548.0) ± 0.01 ; $F_{1,34} =$

22.2, $P < 0.0001$], and higher TNF- α [2.1 (117.3) vs. 1.9 (76.2) $\pm$ 0.1; $F_{1,34} = 4.3$, $P = 0.047$] compared with those in the predictable groups throughout the study period (Table 2).

Parity by Treatment Interactions

We detected some interactions between parity and treatment for feeding behaviors. A treatment by parity interaction was observed for the number of feeding visits ($P = 0.01$), whereby primiparous cows visited the feed bins less often in the unpredictable groups than in the predictable groups (72.0 vs. 117.8 $\pm$ 5.3 no./d; $P < 0.0001$), but multiparous cows did the opposite (91.6 vs. 64.4 $\pm$ 4.9 no./d; $P < 0.0001$). We also detected a treatment by parity interaction for the proportion of nonnutritive visits ($P < 0.0001$); primiparous cows had an equal number of nonnutritive visits in both treatments (predictable vs. unpredictable: 38 vs. 39 $\pm$ 2%; $P = 1.0$), but multiparous cows had higher nonnutritive visits in the unpredictable groups (43 vs. 31 $\pm$ 1%; $P < 0.0001$).

In addition, we detected treatment by parity interactions for both actor ($P = 0.009$) and reactor ($P = 0.03$) replacements. Multiparous cows in the unpredictable groups had higher actor (17.1 vs. 8.9 $\pm$ 1.1 no./d; $P < 0.0001$) and reactor (16.5 vs. 10.8 $\pm$ 1.0 no./d; $P < 0.0001$) replacements than those in the predictable groups; however, primiparous cows in both treatments only tended to differ in their actor replacements (predictable vs. unpredictable: 11.2 vs. 14.8 $\pm$ 1.8; $P = 0.06$) and did not differ in their reactor replacements (predictable vs. unpredictable: 12.8 vs. 14.3 $\pm$ 1.3, unpredictable vs. predictable; $P = 1.0$).

Overall, we detected no differences between treatments in the percentage of cases of cytological endo-

metritis (50 vs. 32%; unpredictable vs. predictable; $P = 0.11$); however, we detected a difference between in cases of cytological endometritis when the data were analyzed separately by parity. More cases of cytological endometritis were observed in the unpredictable groups for multiparous cows compared with the predictable groups (64 vs. 17%; $P = 0.02$). However, a tendency was observed for fewer cases of cytological endometritis in the unpredictable groups for primiparous cows compared with the predictable groups (17 vs. 57%; $P = 0.10$).

DISCUSSION

Cows exposed to an unpredictable and competitive social environment changed how and when they consumed their diet, and showed differences in some physiological biomarkers. These changes may have contributed to higher risk of cytological endometritis in multiparous cows after calving.

We did not detect differences in feed intake between cows in the predictable and unpredictable groups. This finding is similar to studies that have assessed the effect of competition at the feed bunk during the dry period (Collings et al., 2011; Proudfoot et al., 2009). For example, Collings et al. (2011) compared the feed intake of cows given access to one feed bin with those that had to compete for access to a feed bin with a partner, and found no effect of competition on feed intake. However, the lack of difference in DMI in our study is in contrast to previous research that has found that regrouping during the dry period resulted in reduced DMI on the day of regrouping, and this effect was greatest for cows that were also moved into a new pen compared with cows regrouped within their home pen (Schirmann et al., 2011). We did not measure the effect of regroup-

Table 1. Least squares means and SE for feeding behaviors during the transition period for groups ($n = 8$ /treatment) of dairy cows housed in either an unpredictable and competitive, or a predictable and noncompetitive social environment¹

Item	Week relative to calving				SEM	P_{trt}	P_{wk}	$P_{\text{wk} \times \text{trt}}$
	-4	-3	-2	-1				
Feed intake (kg of DM/d)								
Predictable	15.9	15.7	15.8	15.0	0.3	0.59	0.002	0.33
Unpredictable	16.1	16.0	15.4	14.2				
Feeding time (min/d)								
Predictable	269.8	262.9	280.5	241.2	4.4	0.0003	0.02	0.02
Unpredictable	229.9	253.9	239.7	231.2				
Duration of feeding visits (min/visit)								
Predictable	3.5	3.5	3.7	3.3	0.2	<0.0001	0.02	0.02
Unpredictable	5.4	7.0	6.2	6.5				
Nonnutritive visits (%)								
Predictable	14.8	12.7	13.7	11.5	1.5	<0.0001	0.01	0.23
Unpredictable	36.6	29.8	31.3	32.4				

¹Means are shown relative to week before calving. The table includes P -values for the test of treatment (trt), week (wk), and treatment by week interactions (trt $\times$ wk).

ing on DMI the days around regrouping in the current study, but it appears that a longer-term period of social instability had little effect on this measurement.

Cows in the unpredictable groups shifted their time budgets and consumed less of their diet during peak feeding time compared with those in the predictable groups. Cows in the unpredictable groups were forced to compete with resident cows for access to feed, and

unlike cows in the unpredictable groups, resident cows fed from the same feed bin for the duration of the study. Cows in the unpredictable groups may have learned to avoid social confrontation by giving up access to bins in the hours immediately after feed became available, instead electing to feed later in the day once their feeder partner was less motivated to defend the bin. A similar shift in peak feeding time was previously reported by Collings et al. (2011) when feed bins were overstocked (100 vs. 200%) and when feed availability was restricted in time (14 vs. 24 h per day).

Cows in the unpredictable groups increased how quickly they consumed their diet. An increase in feeding rate is a consistent response to a competitive, overstocked feeding environment, as cows attempt to access feed before being displaced by another cow (Olofsson, 1999; Hosseinkhani et al., 2008). The effect of a high feeding rate on cow health has not been well-studied; further work is encouraged to determine any negative consequences of consuming feed at a fast rate.

We found a reduction in the number of visits to the feed bins by cows in the unpredictable groups. By design, all cows in the predictable groups had access to all 6 bins, but each cow in the unpredictable groups had access to only 1 of 4 bins (shared with a resident cow), and this difference in the number of available bins likely affected the number of visits in 2 treatments. There is evidence that cattle will sample from different areas of a feed bunk, especially when the quality of feed is variable across the bunk (Huzzey et al., 2013). Thus, cows in the predictable groups may have been more likely to sample from all feed bins. Interestingly, studies that controlled for the number of available bins have failed to show an effect of overstocking on feeding visits (Collings et al., 2011), or showed the opposite effect of the current findings (Proudfoot et al., 2009). For example, cows fed competitively (2 cows per bin) had more visits (43 vs. 32 visits/d) compared with those fed noncompetitively (1 cow per bin; Proudfoot et al., 2009). Authors attribute this difference to an increase in social displacements at the feed bunk; however, in our case fewer feeding visits were not driven by lower social displacements for cows in the unpredictable groups.

Cows in the unpredictable groups consumed more feed during each visit and spent more time feeding during each visit compared with those in the predictable groups. These behaviors were not expected, as a previous study where cows were overstocked showed that a competitive environment decreased the amount of feed consumed during each visit (Proudfoot et al., 2009). The changes in the current study may have been driven by the shift in time budgets by the cows in the predictable group; these cows may have waited until their

Figure 1. Least squares means ($\pm$ SE) of the number of visits to the feed bins, intake per visit, and rate of feed intake per visit for groups of ($n = 8$ /treatment) dairy cows during a baseline period (5 wk before calving) and when cows were housed in either an unpredictable and competitive, or a predictable and noncompetitive social environment in the 4 wk before calving.

Figure 2. The diurnal feeding behavior of groups ($n = 8/\text{treatment}$) of dairy cows housed in either an unpredictable and competitive, or a predictable and noncompetitive social environment averaged across the 4 wk before calving. Data presented as LSM and SE.

resident partners had finished their meal, thus allowing the cows to spend more time during each visit without interruption from their partner.

Despite visiting the feed bins less often, cows in the unpredictable groups made more nonnutritive visits to the feed bins compared with those in the predictable groups. A nonnutritive visit could have been recorded if the cow had her head over a feed bin that she was not assigned to, or if she accessed her feed bin but did not consume any feed. This behavior may have been driven by a higher number of social replacements in the unpredictable groups, as cows may have attempted to access their feed bin but were replaced by their partner before consuming feed. This behavior may have also been driven by the unpredictability of their feed bin locations; cows in the unpredictable groups likely attempted to visit feed bins that they had been previously assigned to, despite not getting access to feed. Cows may have also attempted to access feed during periods of delayed feed access when the bins were not accessible. In any case, the high number of nonnutritive visits in the unpredictable groups may indicate frustration as a result of not receiving food when expected (LeDoux, 1995).

Social replacements at the feeder were higher for cows housed in the unpredictable groups. This difference was highest in the week following pen changes (wk -4 and -2), a finding consistent with earlier studies of regrouping (von Keyserlingk et al., 2008; Schirrmann et al., 2011). In previous studies, an increase in social replacements was associated with a higher number of

feeding visits, as cows would consume a small amount of feed before being replaced by another cow (Proudfoot et al., 2009). The higher number of both actor and reactor replacements were driven by multiparous cows, as primiparous cows in the unpredictable groups may have opted to avoid social interactions at peak feeding times.

Cows in the unpredictable groups had higher serum TNF- α concentrations compared with cows in the predictable groups. Tumor necrosis factor- α is a pro-inflammatory cytokine that is used as an indicator of inflammation in humans (Popa et al., 2007) and dairy cattle (Bradford et al., 2009), and has been associated with several clinical diseases in humans including heart disease, inflammatory bowel disease, fatty liver, and rheumatoid arthritis (Levine et al., 1990; Breese et al., 1994; Li et al., 2003; Davis et al., 2008). In human and laboratory animal research, an increase in inflammation is considered one of the mechanisms by which chronic stress contributes to poor clinical health outcomes; humans and laboratory animals exposed to chronic stressors enter a state of mild chronic inflammation characterized by high circulating concentrations of pro-inflammatory cytokines, including TNF- α (Rohleder, 2014). Less is known about the effect of high circulating TNF- α concentrations in transition dairy cows, and to our knowledge there are no known thresholds for biologically relevant concentrations of TNF- α for dairy cows as there are for metabolic biomarkers (e.g., NEFA and BHB; Ospina et al., 2010a). Evidence suggests that injections of TNF- α can contribute to fatty liver

(Bradford et al., 2009) and lower milk yield (Kushibiki et al., 2003). To our knowledge this is one of the first studies to assess the effect of a long-term social stressor (an unpredictable and competitive environment) on circulating TNF- α in dairy cows. More research is needed to better understand the relationship between social environment and inflammatory markers in transition dairy cows.

Despite higher TNF- α in unpredictable groups, we found no differences between groups in serum haptoglobin concentrations. Haptoglobin is an acute phase protein that is stimulated by pro-inflammatory cytokines such as TNF- α and IL-6 (Baumann and Gauldie, 1994), and is thus used as a nonspecific indicator of inflammation in dairy cattle. Other research has linked higher circulating haptoglobin concentration during the week after calving with the risk of puerperal metritis

and cytological endometritis after calving (Huzzey et al., 2009; Dubuc et al., 2010); cows with ≥ 0.8 g/L of haptoglobin in serum in the week after calving had greater odds of cytological endometritis after calving. In our case, the average haptoglobin concentrations in both treatments were low after calving (approximately 0.24 g/L).

Cows in the unpredictable groups had slightly higher NEFA but lower BHB throughout the transition period compared with those in the predictable groups. Elevated NEFA concentration before calving (e.g., ≥ 0.3 mEq/L during the 2-wk period before calving) has been associated with an increased risk of disease, reduced milk yield, and compromised reproductive performance (Ospina et al., 2010a,b). The average NEFA levels recorded in the current study were low (0.8 to 0.15 mEq/L in the 2 wk before calving), so it is unclear if these treatment differences were biologically relevant. An increase in NEFA has been thought to be a consequence of reduced feed intake, as cows begin to mobilize fat to compensate for inadequate dietary energy intake (Bauman and Currie, 1980). In our case, we found no effect of treatment on DMI, suggesting NEFA concentrations may be affected by more than just changes in DMI. In previous research, both acute stressors (routine handling and lock-up; Brickner et al., 2007; Leroy et al., 2011) and more long-term stressors (overstocking; Huzzey et al., 2012) have been shown to increase blood NEFA concentrations without associated changes in feeding behavior. Human and cattle studies have also found that TNF- α can promote the breakdown of fat stores, resulting in higher concentrations of blood NEFA (Kushibiki et al., 2003; de Heredia et al., 2012); thus, high blood TNF- α in our study may have contributed to higher NEFA levels in the unpredictable groups. More research is encouraged to determine the relationship between the social environment, inflammatory markers, and adipose mobilization in transition dairy cows.

Other studies that have measured the effect of the social environment on metabolic biomarkers have focused on one stressor. For example, Huzzey et al. (2012) found that cows housed in overstocked pens (200% stocking: 0.5 lying stalls/cow and 34.5 cm linear feed bunk space/cow) had higher circulating NEFA and glucose concentrations during the early dry period compared with cows housed at 100% stocking (1 lying stall/cow and 68.5 cm linear feed bunk space/cow). In contrast, Silva et al. (2013) found that regrouping cows weekly did not affect NEFA or BHB compared with stable groups before calving. Similarly, Coonen et al. (2011) found no effect of twice-weekly regroupings before calving on NEFA compared with stable groups.

Figure 3. Least squares means ($\pm$ SE) of the number of actor and reactor replacements for groups ($n = 8$ /treatment) during a baseline period (5 wk before calving) and when dairy cows were housed in either an unpredictable and competitive, or a predictable and noncompetitive social environment in the 4 wk before calving. A replacement was recorded when a cow left a feed bin within 26 s of another cow entering the bin (validated by Huzzey et al., 2014). The reactor was considered the cow that left the bin, and the actor was considered the cow that entered the bin.

Despite slightly higher NEFA levels throughout the transition period, unpredictable groups had lower BHB compared with predictable groups. β -Hydroxybutyrate is used as an indicator of ketosis during the few weeks after calving (McArt et al., 2012). Increased ketones in the blood after calving are thought to be a normal adaptive response to negative energy balance, but excess ketone accumulation is abnormal and predisposes cows to disease and poor reproductive performance (Walsh et al., 2007; Duffield et al., 2009). In our study, BHB concentrations were low in both treatments (ranging from 381 to 611 $\mu\text{mol/L}$), and well below the cut-off for subclinical ketosis ($>1,200 \mu\text{mol/L}$; McArt et al., 2012), suggesting that the higher BHB levels in the predictable group might not have been biologically relevant. Both NEFA and BHB have diurnal patterns (Nielsen et al., 2003); for this reason we chose to sample blood at the same time of day (0900 h) for all cows. However, for some cows this coincided with delayed feed access, which may have also affected these measures. Nielsen et al. (2003) found that plasma NEFA and BHB are both higher after delayed feed access; in our case, treatment cows had slightly higher NEFA and lower BHB compared with control cows, so the effects of these brief periods of feed deprivation are unknown.

We did not find an effect of treatment on cases of cytological endometritis when the data were summarized by group; however, when analyzed separately multiparous cows in the unpredictable groups had a

high prevalence of cytological endometritis after calving and primiparous cows had a tendency to show the opposite response. Endometritis has been defined as inflammation in the endometrium occurring beyond 21 d after calving, although there is evidence that most cows have some bacterial infection in the uterus during the first few weeks after calving (see LeBlanc, 2014 for a review). Many cows are able to clear this contamination by uterine involution and mobilization of the immune system; those without insufficient immune responses postpartum fail to clear this contamination, leading to uterine disease. To our knowledge, this is the first study to find a possible link between social stressors before calving and uterine disease after dairy cattle. These results are promising, but should be taken with caution due to our small sample size. We encourage further study on the association between social factors and an inflammatory uterine disease in the weeks after calving.

CONCLUSIONS

An unpredictable and competitive social environment changed the feeding and social behavior of dairy cows before calving. The treatment contributed to differences in some physiological biomarkers that indicate changes in inflammatory (higher $\text{TNF-}\alpha$) and metabolic processes (higher NEFA and lower BHB). For multiparous cows, an unpredictable and competitive social environment before calving increased the risk of uterine disease

Table 2. Least squares means and SE for serum biomarkers during the transition period for groups ($n = 8/\text{treatment}$) of dairy cows housed in either an unpredictable and competitive, or a predictable and noncompetitive social environment¹

Item	Week relative to calving			SE	P_{trt}	P_{wk}	$P_{\text{trt} \times \text{wk}}$
	−2	−1	+1				
NEFA [$\log(\text{mmol/L})$]							
Predictable	−1.1 [0.08]	−0.9 [0.13]	−0.4 [0.40]	0.06	0.048	<0.0001	0.23
Unpredictable	−0.9 [0.12]	−0.8 [0.14]	−0.4 [0.44]				
BHB [$\log(\mu\text{mol/L})$]							
Predictable	2.7 [524]	2.7 [517]	2.8 [607]	0.02	<0.0001	<0.0001	0.15
Unpredictable	2.6 [392]	2.6 [383]	2.7 [546]				
Glucose (mmol/L)							
Predictable	3.44	3.54	3.13	0.13	0.24	0.0007	0.59
Unpredictable	3.43	3.36	3.07				
Calcium (mmol/L)							
Predictable	2.10	2.26	2.10	0.10	0.90	0.79	0.21
Unpredictable	2.19	2.11	2.16				
$\text{TNF-}\alpha$ [$\log(\text{pg/mL})$]							
Predictable	1.9 [76.2]	1.9 [88.2]	1.8 [65.2]	0.06	0.047	0.91	0.71
Unpredictable	2.1 [122.8]	2.0 [108.5]	2.1 [121.2]				
Haptoglobin [$\log(\text{g/L})$]							
Predictable	−0.9 [0.13]	−0.9 [0.12]	−0.6 [0.25]	0.04	0.97	0.002	0.87
Unpredictable	−0.9 [0.14]	−0.9 [0.12]	−0.6 [0.23]				

¹Data that were log-transformed [nonesterified fatty acids (NEFA), BHB, tumor necrosis factor (TNF)- α , and haptoglobin] are back-transformed in brackets. Means are shown relative to week before calving. The table includes P -values for the test of treatment (trt), week (wk), and treatment by week interactions (trt $\times$ wk).

measured using cytological endometritis. Mitigating social stressors before calving may help improve the health of dairy cows during the transition period.

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