Changes in behavior and fecal cortisol metabolites when dairy cattle are regrouped in pairs versus individually after calving

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ABSTRACT

Regrouping occurs when dairy cows are moved between pens, and this can negatively affect cow behavior and production. Pen changes occur frequently around calving, a time when cows are vulnerable to health problems. Regrouping cows along with a familiar conspecific after calving may make social integration easier, but little work has tested this hypothesis. The objective of this study was to compare lying behavior, feeding behavior, social behavior, and fecal cortisol metabolite concentrations (a physiological indicator of stress) between cows regrouped individually and cows regrouped with a familiar partner after calving. Holstein ($n = 8$) and Jersey ($n = 26$) cows were monitored from approximately 2 wk before until 7 d after calving. Cows were held in 1 of 2 identical maternity pens until they calved. After calving, cows were held in a transition pen for 3 d before being regrouped into the main lactation pen (regrouping of interest). On d 3 postpartum, cows were assigned to a treatment, balancing for parity and breed: regrouped into the lactation pen alone (individual; $n = 17$) or with a familiar partner (paired; $n = 17$). A familiar partner was defined as a cow that spent 3 d in the transition pen with the focal cow. Using live observation, we quantified the time cows spent feeding, standing or lying in the lying stalls, grooming, and standing in different locations in the pen during the 1 h immediately after regrouping. The frequency of competitive interactions at the feed bunk was also measured during this observation period. Lying behavior was monitored from 2 d before until 5 d after regrouping using a data logger. Fecal samples were collected for 5 d beginning on the day of regrouping to assess fecal cortisol metabolite concentrations (11,17-dioxoandrostane, 11,17-DOA). We found no effects of regrouping treatment on behaviors observed during the 1-h period immediately following regrouping. Cows that were regrouped with a partner had more lying bouts than

cows moved individually (9.2 ± 0.4 bouts/d vs. 7.5 ± 0.4 bouts/d) and shorter lying bout durations (66.6 ± 3.9 vs. 78.1 ± 3.5 min/d); however, we also observed these differences during the day before regrouping, suggesting that the differences in lying behavior could not be explained by the regrouping treatment alone. Individually regrouped primiparous cows had higher 11,17-DOA concentrations than individually regrouped multiparous cows but we found no differences in 11,17-DOA between parities when cows were moved with a familiar partner. In addition, 11,17-DOA was higher in primiparous cows moved alone compared with primiparous cows moved with a partner. More research is needed to understand the mechanisms by which social familiarity affects behavior and the physiological stress response following regrouping; this may provide new insight into how to better transition cows into new environments.

Key words: transition cow, behavior, cortisol, regrouping

INTRODUCTION

Regrouping is the practice of moving cows from one pen to another to accommodate factors such as a cow's stage of lactation, nutritional requirements, or health status. Within the 3-wk period before and after calving (the transition period), a cow may face up to 5 pen changes as she is moved to the dry-cow pen, the close-up pen, an individual maternity pen, a "transition" lactation pen (usually up to 21 d postpartum), and finally to the high-production lactation pen (Cook, 2019).

Regrouping alters behavior in dairy cattle. Competitive displacements at the feed bunk increase after regrouping (Raussi et al., 2005; von Keyserlingk et al., 2008; Lobeck-Luchterhand et al., 2014; Smid et al., 2019) as cows work to establish a new social hierarchy. Changes in feeding behavior, including lower DMI and decreased feeding rate and rumination time (von Keyserlingk et al., 2008; Schirmann et al., 2011) have also been reported. Regrouping has been shown to increase the time cows spend moving around the pen (Raussi et al., 2005; Gyax et al., 2009), possibly due

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to an increased motivation to explore their surroundings. Further work has reported shorter lying times (Hasegawa et al., 1997) and less time spent in the lying area (Gygax et al., 2009) after regrouping. Behavioral responses to regrouping vary based on a variety of factors, such as stocking density, cow comfort, and pen composition (Bøe and Færevik, 2003).

Studies investigating the effects of regrouping on production and physiology are limited. Milk yield has been reported to drop on the day a cow is regrouped (von Keyserlingk et al., 2008), and cows in pens that have frequent additions of new animals have higher cortisol concentrations than cows in pens with a stable social structure (Silva et al., 2013).

Most work investigating the effects of regrouping dairy cows has focused on either dry cows or heifers (Raussi et al., 2005; O'Connell et al., 2008; Schirmann et al., 2011), or on mid- to late-lactation dairy cows (von Keyserlingk et al., 2008; Silva et al., 2013). Very little work has described the behavioral and physiological changes that occur when cows are regrouped into the main lactation herd after calving. Changes in metabolism, immunity, behavior, and management during the time around calving are known to increase a cow's vulnerability to disease (Drackley 1999; Ingvarsen, 2006; Ingvarsen and Moyes, 2015). It is important to understand the behavioral and physiological effects of regrouping during this time to gain new insights on how to better manage the health and welfare of transition cows.

More work is also needed to determine whether management strategies can be implemented to mitigate the negative effects of regrouping. Limited research suggests that moving cows with a familiar partner may ease the stress of regrouping. Heifers regrouped with a partner spent more time in the feeding and lying areas (Gygax et al., 2009) and more time lying after regrouping than heifers regrouped individually (O'Connell et al., 2008). It has been suggested that regrouping cows with a familiar conspecific makes social integration easier, possibly by providing social support mechanisms that improve the likelihood of new cows gaining better access to resources such as the feed bunk or lying stalls (Bøe and Færevik, 2003); however, research on regrouping in pairs versus individually has focused on heifers only.

The objective of this study was to compare lying behavior, feeding behavior, social behavior, and fecal cortisol metabolite concentrations (a physiological indicator of stress) between cows regrouped individually or with a familiar partner after calving. We predicted that cows regrouped individually would experience more negative changes in behavior (e.g., reduced feeding and

lying time, greater likelihood of being displaced from the feed bunk) and physiology (e.g., higher cortisol) than cows regrouped with a partner.

MATERIALS AND METHODS

Animals, Housing, and Diet

This study was conducted from August 2017 to November 2018 at the California Polytechnic State University (San Luis Obispo, CA) dairy unit. A total of 34 cows, including 8 Holstein (5 primiparous and 3 multiparous; parity = 1.9 ± 1.4 , mean $\pm$ SD) and 26 Jersey (13 primiparous and 13 multiparous; parity = 1.9 ± 1.1) were used. Throughout the study, cows were housed in 1 of 3 experimental pens depending on their stage of lactation: a maternity pen, a transition pen, and a lactation pen. The maternity and transition pens consisted of an indoor and outdoor bedded-pack area (220 m² each) with straw and composted manure bedding. The maternity and transition pens also had 12 headlocks each. The lactation pen consisted of a free-stall barn that had 46 lying stalls bedded with composted manure and 48 headlocks. Cows in the lactation pen also had access to a large outdoor area of compact dirt and composted manure (Figure 1). A headlock feed barrier was used in all experimental pens, and feed was placed in front of the headlocks according to the number of animals in the group. Stocking density in the lactation pen was variable but never exceeded an 80% stocking rate (average stocking rate $\pm$ SD; 0.79 ± 0.16 headlocks/cow and 0.72 ± 0.17 lying stalls/cow in the lactation pen). The average stocking density in the transition pen was 0.34 ± 0.09 cows/headlock. No data were available to calculate the average stocking rate in the maternity pen; however, the farm protocol was to never exceed an 80% stocking rate. These stocking rates were consistent with industry recommendations for close-up and fresh cow pens (Cook, 2019). All cows had ad libitum access to water.

Cows in the maternity pen were fed twice daily at approximately 0500 and 1600 h. The prepartum TMR consisted of (mean $\pm$ SD) 19.9 $\pm$ 7.3% concentrate grain mix, 5.4 $\pm$ 3.8% wet brewers grain mash, 71.6 $\pm$ 9.7% forage mix (oat, barley, and rye hay and silage; corn silage) and 3.1 $\pm$ 2.1% mineral mix on a DM basis. Cows in the lactation and transition pens were milked and fed at approximately 0600 and 1700 h daily. Cows in the transition pen were milked with a manual milking machine while standing in a headlock in their pen; all cows in the lactation pen were milked in the milk parlor. The postpartum TMR consisted of (mean $\pm$ SD) 50.8 $\pm$ 0.8% concentrate grain mix, 7.8 $\pm$ 0.2%

Figure 1. Layout of the lactation pen with labels describing the areas that were observed for the behavioral time budget analysis. Circles with an attached line represent hinged gates allowing entry or exit to respective areas. The diagram is drawn to scale, and all relevant measurements have been denoted. WA = water alley.

wet brewers grain mash, $38.7 \pm 0.9\%$ forage mix (oat, barley, and rye hay and silage; corn silage) and $2.7 \pm 0.04\%$ mineral mix on a DM basis.

Experimental Design and Cow Participation

Before calving, cows were housed in 1 of 2 identical maternity pens located adjacent to each other and separated by parity (primiparous or multiparous). Cows entered the maternity pen 2 to 3 wk before their expected date of calving and remained in the pen until they calved. Within 12 h of calving, cows were moved to the neighboring transition pen. The physical layout of the transition pen was identical to that of the maternity pens, but it contained only fresh cows (1 to 3 DIM). On the third day after calving, all cows were regrouped from the transition pen into the lactation pen; this regrouping was the focus of our study. This timeline followed the dairy herd's standard operating procedure for transition cow management.

Two regrouping treatments were compared: (1) individual regrouping ($n = 17$), in which the focal cow was moved into the lactation pen alone; and (2) paired regrouping ($n = 17$), in which the focal cow was moved with a familiar partner into the lactation pen. We defined a "familiar partner" as a cow that had calved within 1 d of the focal cow and therefore spent the entire 3-d period with the focal cow in the transition pen. Because of the small herd size and number of cows calving each month, the partner cow was not required to be of the same breed or parity as the focal cow. Of the 17 paired cows, 4 pairs consisted of primiparous

cows only, 2 pairs of multiparous cows only, and 11 pairs of 1 primiparous and 1 multiparous cow. Further, 10 pairs consisted of Jersey cows only, 2 pairs of Holstein cows only, and 5 pairs consisted of 1 Jersey and 1 Holstein cow. Regrouping occurred between 1700 and 1800 h, except for 3 regroupings that occurred between 1100 and 1300 h because of temporary farm protocol changes that took place in October 2017. On the day of regrouping, the focal cow was added to the lactation pen (individually or in a pair) as the cows returned to the lactation pen after milking. Once regrouped into the lactation pen, the focal cow was followed for an additional 5 d to collect behavioral and physiological data.

Behavioral Data Collection

Lying behaviors were measured using a data logger (HOBO Pendant G Acceleration Data Logger; Onset Computer Corporation, Pocasset, MA) from the time of enrollment in the study (approximately 2 wk prepartum) to 5 d after regrouping. The logger was attached to the rear leg of the cow using vet wrap bandages (Co-Flex; Andover, Salisbury, MA). Each week, the logger was removed to download the data, after which it was reprogrammed and attached to an alternate leg for another week of recording. Data from the days a logger was changed were not included in any analyses of lying behavior. The logger was programmed to take measurements of leg orientation at intervals of 1 min, and this information was then submitted to an algorithm in SAS (version 9.4; SAS Institute Inc., Cary, NC) developed

by the UBC Animal Welfare Program (2013) to provide data on daily lying time (min), daily number of lying bouts, and daily lying bout duration (min).

The behavior of the focal cow was assessed using live observation during the 1-h period immediately following the focal cow's entrance into the lactation pen for the first time (i.e., regrouping). Multiple observers were trained to perform this assessment by a previously trained observer. Inter-observer reliability needed to exceed 0.95 for a new observer to be permitted to collect data alone. Observers were required to maintain visual contact with the entire body of the focal cow while also maintaining a minimum distance from the cow of approximately 11 m (the width of the inside pen; Figure 1).

Using instantaneous scan sampling, the cow's position within the pen was recorded every 30 s over the 1-h observation period. To facilitate this analysis, the lactation pen was divided into distinct areas for observation (Figure 1). Cows were recorded as standing in the back alley, feed alley, or 1 of the 2 water alleys when their front 2 feet were both visible in these respective zones. Cows were recorded as standing in the lying stall (i.e., standing with 2 or 4 feet in the lying stall) or lying in the stall. Cows were recorded as outside if their front 2 legs were positioned past the outer barrier of the indoor pen area, and they were recorded as standing or lying while outside. Cows were recorded as feeding if both ears were visible on the feed side of the headlock feed barrier. The time spent in these areas or engaged in these behaviors, was estimated by multiplying the number of times a cow was observed in each area of interest by the sampling frequency (e.g., 14 scans $\times$ 30 s = 7 min). One-zero sampling with 30-s sampling intervals was used to estimate the time a cow spent self-grooming (licking her own body or legs) or allogrooming (licking another cow anywhere on their body) during the 1-h period after regrouping. A cow was marked as grooming if she was observed to be grooming at any point in the 30-s sampling interval; the sum of these grooming events was multiplied by the interval duration (30 s) to provide an estimate of grooming time. One-zero sampling was chosen as the method for estimating grooming time because we expected grooming events to occur in short episodes that would likely not often coincide with an instantaneous scan. Finally, we used continuous observation to record instances of competitive behavior (as actor or reactor) at the feed bunk. A cow was noted as being the actor of a competitive displacement at the feed bunk when her head or body contacted the body of a feeding cow (the reactor), resulting in the feeding cow immediately withdrawing her head from the feed bunk.

Fecal Cortisol Metabolite Data Collection

Fresh fecal samples were manually collected, using a gloved hand, while cows were restrained in a headlock. The first fecal sample was collected on the day of regrouping just before the focal cow's movement into the lactation pen. Over the next 4 d, fresh fecal samples were collected after the focal cow returned to the lactation pen from the afternoon milking but not before any 1-h behavior observation period of a newly regrouped focal cow (to avoid disruption to behavior data collection). A total of 5 fecal samples were collected per cow. Fecal samples were stored at -20°C for later processing. Steroids from the fecal sample were extracted using the wet extraction method described by Palme and Möstl (1997); briefly, 0.5 g of raw fecal sample was combined with 5 mL of 80% methanol and vortexed for 30 min. Fecal cortisol metabolite concentration (11,17-dioxoandrosterone; **11,17-DOA**) was determined using a competitive enzyme immunoassay developed by Palme and Möstl (1997) and validated for use in cattle (Palme, 2019). The intra-assay and inter-assay CV for the 11,17-DOA assay were 7.5 and 4.7%, respectively. The correlation between 11,17-DOA and plasma cortisol has been validated in ruminants by ACTH and dexamethasone tests (Palme, 2019); it was determined that the concentration of 11,17-DOA paralleled that of cortisol in the blood with a delay time of approximately 10 to 12 h. Because the first fresh fecal sample in our study was collected just before the focal cow's introduction to the lactation pen, the concentration of 11,17-DOA in this sample would reflect the time period approximately 12 h before the regrouping event (i.e., d -1 relative to regrouping). Fecal samples were collected at the same time each day (~ 1830 h), so daily 11,17-DOA concentrations are expressed as d -1 , 0, 1, 2, and 3 relative to regrouping.

Statistical Analysis

All statistical analyses were performed in SAS (version 9.4; SAS Institute Inc.). The enrollment of cows in the pair treatment was more difficult than enrollment of cows in the individual treatment because of the need to wait for 2 cows to calve within 1 day of each other. For this reason, we measured more individual regroupings than paired regroupings over the duration of the study. To generate 2 balanced treatment comparison groups for the analysis and interpretation of results, we first selected all cows in the paired treatment that had a complete set of fecal samples for the analysis of fecal cortisol metabolites; this yielded 17 cows in the paired treatment. These cows were then match-paired with 17

cows enrolled in the individual treatment, balancing by breed and parity.

Differences in daily lying time, number of lying bouts and lying bout duration between treatments were analyzed using the MIXED procedure in SAS, which included the fixed effects of breed (Holstein vs. Jersey), parity (primiparous vs. multiparous), stocking density in the fresh pen (average number of cows per headlock over 3 d), stocking density in the lactation pen (number of cows per headlock on regrouping day), day (−2, −1, 0, 1, 2, 3, and 4 relative to regrouping), treatment (individual vs. paired), and treatment × parity and treatment × day interactions. Day 0 represents the day the cow was regrouped, and therefore would include data from before and after regrouping, because a day was defined as occurring from midnight to midnight. Day was considered a repeated measure using a spatial power covariance structure, and cow was considered a random effect. Model residuals were normally distributed. Standing behavior data were omitted from this analysis, because this variable could be calculated from the lying data and was therefore redundant.

Differences in behavior during the 1 h immediately following regrouping between treatments were tested using the MIXED procedure in SAS. For these analyses, some behaviors were consolidated because they occurred very infrequently or not at all. Time spent standing and lying in a stall were consolidated into a single behavior category (lying stall use); time spent standing or lying outside were also combined (outside), as was time spent at 1 of 2 water alleys (water alley). Behaviors analyzed using the MIXED procedure included the following: time at the feed bunk (feeding); time in the feed, water, or back alleys; time outside; time using a lying stall; time spent allogrooming or self-grooming; and frequency of being an actor or reactor during a displacement from the feed bunk. These models included the fixed effects of breed (Holstein vs. Jersey), parity (primiparous vs. multiparous), stocking density in the fresh pen (average number of cows per headlock over 3 d), stocking density in the lactation pen (number of cows per headlock on regrouping day), treatment (individual vs. paired) and the treatment × parity interaction. Model residuals were normally distributed.

Differences in 11,17-DOA between treatments were analyzed using the MIXED procedure in SAS, which included the fixed effects of breed (Holstein vs. Jersey), parity (primiparous vs. multiparous), stocking density in the fresh pen (average number of cows per headlock over 3 d), stocking density in the lactation pen (number of cows per headlock on regrouping day), day (−1, 0, 1, 2, and 3, relative to regrouping), treatment (individual

vs. paired), and the treatment × parity and treatment × day interactions. Time was considered a repeated measure using a spatial power covariance structure, and cow was considered a random effect. Model residuals were normally distributed.

RESULTS

Lying Behavior

Regrouping treatment had no effect on daily lying time (569.2 ± 28.5 vs. 578.9 ± 31.5 min/d for individual vs. pair treatments, respectively; $F_{1,25} = 0.06$, $P = 0.81$). Multiparous cows had longer daily lying times than primiparous cows (620.4 ± 27.9 vs. 527.7 ± 30.2 min/d; $F_{1,26} = 6.2$, $P = 0.02$). We also found an effect of regrouping day, with the longest daily lying times occurring before regrouping ($F_{6,107} = 2.2$, $P = 0.05$; Figure 2A). No other fixed effects influenced daily lying time, and we detected no interactions ($P \geq 0.20$).

Cows regrouped with a partner had approximately 2 additional lying bouts per day compared to cows regrouped individually across the observation period (9.2 ± 0.4 vs. 7.5 ± 0.4 bouts/d; $F_{1,32} = 10.2$, $P = 0.003$). We found an interaction between treatment and regrouping day ($F_{6,103} = 2.2$, $P = 0.05$; Figure 2B), whereby cows in the paired treatment had more lying bouts on d −1, d 1 and d 2 relative to regrouping. No other fixed effects influenced lying bout frequency ($P \geq 0.06$).

Cows moved with a partner had shorter lying bout durations than cows moved individually (66.6 ± 3.9 vs. 78.1 ± 3.5 min/d; $F_{1,41} = 5.4$, $P = 0.03$; Figure 2C). Holsteins had longer lying bouts than Jerseys (78.3 ± 5.1 vs. 66.4 ± 2.5 min/d; $F_{1,41} = 4.2$, $P = 0.05$), and multiparous cows had longer lying bouts than primiparous cows (80.9 ± 3.4 vs. 63.8 ± 3.8 min/d; $F_{1,40} = 13.7$, $P < 0.001$). No other fixed effects influenced lying bout duration, and we detected no interactions ($P \geq 0.45$).

Behavior 1 Hour After Regrouping

After accounting for the variation associated with breed, parity, stocking density in the transition and lactation pens, and a treatment × parity interaction, we detected no significant differences in any behaviors between cows moved individually and cows moved with a partner during the 1 h after regrouping ($P \geq 0.10$; Table 1).

Stocking density in the transition pen had an influence on self-grooming time during the 1 h immediately following regrouping. For every unit increase in stocking density (i.e., 100% increase), time spent self-grooming

was predicted to decrease by 17.8 min during the 1 h immediately following regrouping ($\beta = -17.8 \pm 7.4$; $F_{1,19} = 5.7$, $P = 0.03$).

We also observed an effect of both breed and stocking density in the lactation pen on the time cows spent in the water alleys. Holstein cows spent less time in the water alleys than Jersey cows (2.2 ± 0.8 vs. 4.2 ± 0.4 min per 1-h observation period; $F_{1,19} = 4.8$, $P = 0.04$).

Figure 2. Least squares means ($\pm$ SEM) for (A) daily lying time, (B) lying bouts and (C) lying bout duration for cows regrouped into the lactation pen individually (dashed line) or with a partner (solid line). Day 0 represents the day cows were regrouped. ** $P < 0.01$; * $P < 0.05$.

For every unit increase in stocking density (i.e., 100% increase), time spent in the water alley was predicted to increase by 6.9 min during the 1-h period immediately following regrouping ($\beta = 6.9 \pm 4.3$; $F_{1,19} = 8.9$, $P = 0.008$).

A 100-percentage-unit increase in stocking density in the transition or lactation pen (doubling the number of cows in the pen) would be very unlikely in a commercial dairy herd; this should be taken into account when interpreting the results above (i.e., predicted decrease in self-grooming time and predicted increase in time spent in the water alley) which, when using this reference unit, are also exaggerated. In this instance, the relative change in behavior (increase or decrease) to the increase in stocking density is likely of greater relevance.

Fecal Cortisol Metabolites

Overall, we found no differences in fecal cortisol metabolite concentrations between cows moved individually and cows moved with a partner (1.5 ± 0.2 vs. 1.2 ± 0.2 ng/mL, respectively; $F_{1,25} = 2.6$, $P = 0.12$; Figure 3). However, we detected a treatment $\times$ parity interaction ($F_{1,26} = 4.5$, $P = 0.04$) showing that individually regrouped primiparous cows had higher 11,17-DOA concentrations than individually regrouped multiparous cows across all sample days; when cows were moved with a partner, we detected no differences between parity (Figure 4). This interaction also revealed that primiparous cows had higher 11,17-DOA concentrations when moved individually than when moved with a partner (Figure 4). Overall, primiparous cows had greater 11,17-DOA concentrations than multiparous cows (1.7 ± 0.2 vs. 1.0 ± 0.1 ng/mL; $F_{1,26} = 14.8$, $P < 0.001$).

DISCUSSION

Our study predictions were that cows who were individually regrouped into the lactation pen 3 d after calving would experience more negative effects on behavior and physiology than cows regrouped with a partner. Results from the fecal cortisol metabolite analysis provided some support for this prediction, but additional work is needed to clarify the influence of these regrouping treatments on cow behavior.

Analysis of 11,17-DOA revealed a significant treatment $\times$ parity interaction. Primiparous cows moved individually had higher 11,17-DOA concentrations than multiparous cows moved individually, but this parity difference was not observed when cows were moved with a partner (Figure 4). Our results also showed that primiparous cows had higher 11,17-DOA concentra-

Table 1. General activity, grooming behavior (min; LSM $\pm$ SEM) and frequency of competitive interactions (n; LSM $\pm$ SEM) during the 60-min period immediately following regrouping (individually or with a partner) into the lactation pen

Variable	Individual	Paired	<i>P</i> -value ¹		
			Treatment	Parity	Treatment $\times$ parity
General activity, ² min/60 min					
Back alley	2.1 $\pm$ 0.9	2.6 $\pm$ 1.0	0.67	0.21	0.98
Feed alley	8.8 $\pm$ 3.8	3.8 $\pm$ 4.4	0.29	0.39	0.46
Feeding	23.0 $\pm$ 3.5	19.7 $\pm$ 4.0	0.51	0.09	0.86
Water alley	2.6 $\pm$ 0.5	3.9 $\pm$ 0.6	0.10	0.38	0.27
Outside	26.3 $\pm$ 3.4	28.7 $\pm$ 3.8	0.62	0.15	0.99
Using stall	1.9 $\pm$ 0.9	0.9 $\pm$ 1.1	0.46	0.85	0.90
Grooming behaviors, min/60 min					
Self-grooming	3.0 $\pm$ 1.0	3.7 $\pm$ 1.1	0.65	0.85	0.77
Allogrooming	1.6 $\pm$ 0.6	1.0 $\pm$ 0.7	0.45	0.19	0.11
Displacement from feed bunk, ³ no./60 min					
Actor	0.6 $\pm$ 0.2	0.5 $\pm$ 0.3	0.70	0.15	0.38
Reactor	1.4 $\pm$ 0.3	0.7 $\pm$ 0.4	0.13	0.09	0.62

¹*P*-values are provided for the effects of treatment, parity, and treatment $\times$ parity. The main effects of breed, stocking density in the transition pen, and stocking density in the lactation pen are not reported in this table but are reported in the text.

²Cows were always standing when positioned in the back, feed, and water alleys. While outside, cows may have been standing or lying. Stall use included cows lying or standing with 2 or 4 feet in the stall.

³The cow that initiated the displacement was the actor, and the cow that was displaced from the feed bunk was the reactor.

tions than multiparous cows. The combination of these findings suggests that primiparous cows may be more susceptible to stressors arising from regrouping than multiparous cows. Further, moving primiparous cows with a familiar partner may help mitigate their physiological stress response to regrouping. Conditions known to be present during regrouping, such as social instability and competitive feeding environments (Bøe and Færevik, 2003), have been associated with elevated cortisol concentrations in cows (Silva et al., 2013). Huzzey et al. (2012) also found that primiparous cows are less successful than multiparous cows in competing for access to the feed bunk, a trait (low competitive success) that is also associated with higher 11,17-DOA concen-

trations. In the present study, cows were regrouped into the lactation pen at the time of fresh feed delivery, a time when competitive interactions at the feed bunk are most likely to occur (DeVries et al., 2003), but we observed few displacements in either treatment group. Competition was likely low because the lactation pen was understocked (79% stocking rate at the feed bunk), and competitive displacements were observed for only 1 h immediately following regrouping. Consequently, due to the constraints of our study design, we can neither

Figure 3. Least squares means ($\pm$ SEM) fecal cortisol metabolite (11,17-dioxoandrostande, 11,17-DOA) concentrations before and after regrouping for cows regrouped into the lactation pen individually (dashed line) or with a partner (solid line). **Figure 4.** Least squares means ($\pm$ SEM) fecal cortisol metabolite (11,17-dioxoandrostande, 11,17-DOA) concentration across all sample days for primiparous and multiparous cows regrouped into the lactation pen individually (n = 8 and 9, respectively) or with a partner (n = 10 and 7, respectively).

confirm nor deny the influence of competition on the observed 11,17-DOA results in primiparous cows.

Differences in the physiological stress response of primiparous and multiparous cows after calving may be due in part to the stress of experiencing novel situations for the first time for primiparous cows. If familiarity with pen changes was the reason why multiparous cows had lower 11,17-DOA concentrations than primiparous cows in the individual treatment group, we might expect primiparous cows to have a decreased response to regrouping if they were regrouped often. Raussi et al. (2005) found this not to be the case; even after 16 regroupings in a period of 11 wk, primiparous cows still responded with increased competition and more frequent changes in activity compared to primiparous cows that were not regrouped.

Moving to the lactation pen with a familiar partner could bolster confidence and lower stress in primiparous cows if maintaining close physical proximity to a familiar partner had positive effects (e.g., protection from competitive interactions with unfamiliar cows). O'Connell et al. (2008) reported that when primiparous cows were housed with a partner for 7 d before regrouping, they spent more time than expected with their partner in various areas of the new pen and spent more time than expected in adjacent lying stalls. Gutmann et al. (2015) reported that when pairs of multiparous cows were housed together during their previous dry period, they were more likely to feed next to each other and engage in social interactions than cows without this long-term familiarity. To date, no work has explored the mechanisms by which social support (e.g., proximity of a familiar animal) may protect animals from agonistic interactions during regrouping and thus lower their physiological stress response, but we see this as a promising area for future research.

We did not observe differences in daily lying time between cows regrouped individually or in pairs, contrary to the findings of others who observed longer lying times among primiparous cows regrouped with a partner (O'Connell et al., 2008; Gygax et al., 2009). In the present study, daily lying time was affected by day relative to regrouping: lying times were longer before regrouping than after. Other researchers reported few to no changes in lying time in the days after regrouping (von Keyserlingk et al., 2008; Schirmann et al., 2011), theorizing that this may have been because obtaining adequate lying time is a behavior that is prioritized over feeding or social behavior (Munksgaard et al., 2005). In the current study, the observed decrease in daily lying time after regrouping was likely explained by the longer standing times required to accommodate movement to the milk parlor from the lactation pen twice a day, rather than the effect of regrouping per

se. This finding highlights the challenges associated with understanding the effects of regrouping during the transition period. This period is characterized by numerous environmental (e.g., pen changes, new social groups), management (e.g., milking in parlor, cow-calf separation, diet changes), and physiological changes (e.g., parturition, lactation onset, illness) over a relatively short period of time. Future research that aims to understand the effects of regrouping after calving will need to better account for these confounders.

In the present study, cows regrouped with a partner had more lying bouts (9 vs. 7 bouts/d) and shorter lying bouts (66 vs. 78 min/d) than cows regrouped individually. These results fell within the range of lying bouts (8 to 10 bouts) reported by others on the day of regrouping (von Keyserlingk et al., 2008; Schirmann et al., 2011). In the current study, lying bout frequency and duration were different between treatments both before and after regrouping. This finding suggests that factors beyond the regrouping treatment likely influenced lying behavior. We were able to control for parity, breed, and stocking density in the transition and lactation pens, and we did not enroll any cow that was deemed by the herdsmen to be sick (mastitis, ketosis, milk fever) at 3 DIM. As discussed earlier, the period around calving is marked by numerous factors that could have influenced these lying behavior results.

We did not observe differences in any behavior during the 1-h period immediately following regrouping between cows regrouped individually and cows regrouped with a partner. We predicted that because cows would be regrouped into the lactation pen at the same time as fresh feed delivery, the highly active social environment (DeVries et al., 2003) would offer a good opportunity to observe potential treatment differences in behavior despite the short time interval for observation; this proved to not be the case. During the 1-h observation period of this study, behaviors such as allogrooming and competitive interactions occurred infrequently and the high degree of variation between animals did not allow treatment effects to be seen. Using continuous video recordings over 3 d, von Keyserlingk et al. (2008) measured a decline in allogrooming and an increase in the frequency of displacements from the feed bunk among regrouped animals. These findings highlight the need to observe animals continuously for several days to assess acute behavioral changes associated with regrouping.

In the present study, the covariate stocking density also had an effect on some behaviors. Increased stocking density in the transition pen was associated with less self-grooming during the hour after regrouping and increased stocking density in the lactation pen was associated with longer standing times in the water alleys. In commercial dairies, cows within the 3-wk

period before and after calving are typically housed in pens with the lowest stocking densities (Cook, 2019). Industry stocking density guidelines for transition cow pens is 30 inches of linear feed bunk space per cow and 1 lying stall per cow (Cook, 2019). Many studies have shown that when stocking density exceeds industry recommendations, behavior is negatively affected, including reductions in DMI, feeding time, and increased competition (reviewed in DeVries, 2019). On the other hand, research has shown no change in feeding behavior when cows are provided more than the industry-recommended space (Huzzey et al., 2006). In the present study, cows were always stocked at $\leq 80\%$ of the space recommendation for transition cows, yet we still observed an effect of stocking density on some behaviors. The mechanisms behind these observations are unclear and warrant further investigation. Future research investigating the effects of regrouping on cow behavior and physiology must consider stocking density in the experimental pens, even if cows are understocked relative to industry guidelines.

CONCLUSIONS

This is the first study to report changes in fecal cortisol metabolite concentrations following regrouping after calving. When regrouped individually, primiparous cows had a greater cortisol response to regrouping than multiparous cows; however, when cows were regrouped with a familiar partner, we found no difference in fecal cortisol metabolite concentrations between parities. Regrouping treatment had no effect on the behavior observed during the 1-h period immediately following regrouping. Although we detected some differences in lying behavior between regrouping treatments, we also observed these differences during the day before regrouping, suggesting that factors other than the study treatment were at play. More research is needed to understand the mechanisms by which social familiarity affects behavior and the physiological stress response after regrouping; this may provide new insights on how to better transition cows into new environments.

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