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Behavioural variability, physical activity, rumination time, and milk characteristics of dairy cattle in response to regrouping

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A B S T R A C T

In the commercial dairy industry worldwide, it is common practice to periodically regroup cows as part of their management strategy within housed systems. While this animal husbandry practice is intended to improve management efficiency, cows may experience social stress as a result of the social environment changes, which may have an impact on their behavioural patterns, performance, and welfare. We investigated whether regrouping altered dairy cows' behaviour and impacted their cortisol concentration (a physiological marker of stress), oxytocin, milk yield, and quality in a robotic milking system. Fifty-two lactating cows (17 primiparous; 35 multiparous) were moved in groups of 3–5 individuals into established pens of approximately 100 cows. Behaviour of the regrouped cows was directly observed continuously for 4 h/day across 4-time blocks (day-prior (**d-1**), day-of regrouping (**d0**), day-after (**d + 1**), and 6-days after (**d + 6**) regrouping). Cows were categorised as being with others, alone, or feeding every 2.5 min prior to the assessment of behavioural dynamics. Milk yield (**MY**) and composition, total daily activity, and rumination time (**RUM**) data were extracted from the Lely T4C management program (Lely Industries, Maassluis, the Netherlands), and milk samples were collected for cortisol and oxytocin concentration analyses; data were analysed using linear mixed-effect modelling. Primiparous cows were less likely to be interacting with others on d + 1 than d-1 compared with multiparous. However, average bout duration (minutes) between being alone and feeding activity states were similar on d-1, d + 1, and d + 6, for both primiparous and multiparous cows. A reduction in the average alone and feeding bout duration was observed on d0. Multiparous cows spent significantly more total time being alone on d0 compared to d-1. Neither regrouping nor parity statistically influenced milk DM content, energy, or cortisol concentration. Primiparous cows produced 3.80 ± 2.42 kg (12.2%) less MY on d + 1 compared to their d-1, whereas multiparous cows did not change MY. A significant decrease of 0.2% fat was found in both parity groups following regrouping and remained low up to d + 6. Daily activity in both parity groups increased significantly and RUM reduced after regrouping. A significant decrease in oxytocin concentration was observed in all cows on d + 1. The results, specifically for primiparous cows, indicated a negative impact of regrouping on social interactions, due to changes in the social environment which may lead to short-term social instability. Multiparous cows may benefit from previous regrouping experiences.

Implications

Dairy cattle are frequently regrouped with unfamiliar conspecifics in housed systems. We investigated whether regrouping altered behaviour and impacted on stress, oxytocin levels, and milk production. Regrouping had a significant impact on behavioural dynamics, activity budgets, and milk production depending on parity over 6 days. Regrouping increased activity and reduced rumination time but did not affect cortisol concentration (stress marker). Primiparous cows decreased milk yield and oxytocin levels for a day, while all cows had a drop in milk fat % over 6 days. Our findings show that the social impact of regrouping is greater in primiparous cows over the short-term.

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Introduction

Within housed systems across the world, the management practice of regrouping cows is widely employed in commercial dairy farming, including cows in automatic milking systems (**AMS**). Cows are typically regrouped based on various characteristics (e.g. lactation stage, age, production level, nutritional requirements or reproductive status) (Grant & Albright, 2001; Schirmann et al., 2011). However, evidence suggests that this practice can have a direct impact on productivity, behavioural patterns, and welfare due to changes in the social environment (von Keyserlingk et al., 2008; Zelena et al., 1999). It introduces animals to new individuals and a new environment and could contribute to increased social stress (Bøe and Færevik, 2003; Proudfoot and Habing, 2015; von Keyserlingk et al., 2008). Cows have to regularly re-establish social relationships with others using physical and non-physical interactions (Arave and Albright, 1976; Jensen and Proudfoot, 2017).

Several studies have demonstrated negative welfare and productivity consequences of regrouping, including reductions in milk yield, feed intake, rumination and lying times, and increased aggression (Hultgren and Svensson, 2009; Nogues et al., 2020; Raussi et al., 2005; von Keyserlingk et al., 2008). Increased aggressive interactions such as head butting are more pronounced immediately after regrouping compared to the day-prior (von Keyserlingk et al., 2008). Cows introduced into novel pens may decrease their feed consumption due to displacement (Boyland et al., 2016; Schirmann et al., 2011; von Keyserlingk et al., 2008).

In addition, the milk ejection and reflex process in lactating animals is mainly associated with nervous and endocrine regulation (Bobić et al., 2011), which under stressful situations can be hampered (Bruckmaier et al., 1993) through the activation of the sympathetic-adrenal system (Moberg, 2000). In dairy systems, previous work has shown an increased faecal cortisol metabolites concentration following regrouping in individually regrouped primiparous cows (Mazer et al., 2020).

Earlier studies indicated short-term effects of regrouping and/or relocation on the milk production traits in ewes (Sevi et al., 2001) and cows (Brakel and Leis, 1976). For instance, Hasegawa et al. (1997) reported a decline in milk yield in primiparous up to 2 weeks after social exchange while Broucek et al. (2017) revealed a reduction of 23% in milk yield following regrouping. An additional effect of regrouping included a decrease in milk fat and protein content in ewes (Sevi et al., 2001). The duration of the social instability in cows may differ depending on several factors, including breed, space availability, stocking density, and individual experience of the practice (regrouping) among others (Bøe and Færevik, 2003).

The transient effects of these recurrent social perturbations on milk production, and how to mitigate them, are not clear. As regrouping is a challenge, cows may change their behaviour in order to meet their daily physiological requirements. This can be achieved in different ways, for example, reduction in aggressive interactions. The individual demands and the environment in which the animals live in, dictate their activity budget. As regrouping is an internationally recognised practice, it is important to have a comprehensive understanding of whether regrouping has a behavioural effect that could have an impact on milk production and composition in cows housed in a robotic milking system under commercial conditions. We expected cows to change their daily behavioural time budgets as they adapt to their new environment during regrouping. Therefore, the aims of the study were to assess whether regrouping: (i) altered cows' behaviour, and (iii) had an impact on cortisol, oxytocin levels, and milk yield and composition. We hypothesised that the cow would either be alone or interact with other cows following regrouping. The preliminary results of the current study have been published in abstract forms (Marumo et al., 2020; Marumo et al., 2021b).

Material and methods

Study site and cows

Data collection was carried out on a commercial dairy farm located in Aberdeenshire, Scotland in the United Kingdom, between September and October 2018. All cows were housed in a free-stall housing with sand-bedded lying cubicles throughout their entire production life with no access to pasture. All the cows had free-traffic flow to the Lely A3 Astronaut AMS (Lely, Maassluis, the Netherlands). The cows had free access to water and *ad libitum* roughage, and they were provided with concentrates at the AMS. The farm comprised five different cow breeds, the majority being Holstein-Friesian, and had three pens for the lactating cows. Each pen for the lactating cows was equipped with at least one AMS and had the maximum capability of 110 cows. The stall stocking density was on average 52%. Cows were moved from either pen 1 to pen 2 or pen 2 to pen 3 depending on the stage of lactation.

Cows were identified by transponder collars (Qwes-HR tags, Lely), which were recognised in the AMS units, and they transmitted all the daily measurements including individual performance (such as milk yield, fat and protein content), activity, and rumination time to the on-farm computer using the "T4Cs" (Time for cows)" herd management software. These tags use radio frequency to transmit data and are used to monitor their rumination activity, overall physical activity, and health status of cows in addition to identifying them. As a result, they offer valuable insights into the health and well-being of individual cows and the entire dairy herd.

Experimental design

The cows on the farm were used to handling and selected individuals were regrouped weekly by staff personnel depending on the stage of lactation, pregnancy status, and nutrient requirements. The study did not interfere with the daily farm activities; hence, the identification and selection of the experimental cows were decided by the farm personnel. Cows ranged between their 1st and 7th calving. Between 3 and 5, cows were moved at a time from either pen 1 to pen 2 or pen 2 to 3 by the discretion of the farm personnel, but each relocation involved a mix of primiparous and multiparous cows. The list of the focal cows to be relocated was made available at least 2 days prior to regrouping by farm personnel. Each focal cow was identified with a unique mark on its back using non-toxic livestock crayon and coloured ribbons attached to the existing identification collars to differentiate the cows that had similar body colour.

The study involved direct visual observations of 52 lactating cows (primiparous, n = 17; multiparous, n = 35). Cows had detailed behaviour recorded by trained observers using an ethogram (Table 1). Due to the modelling approach detailed in the statistical analyses section, we estimated three domain activity states from the question dimension that the cow can either be interacting with other cows ("With Others") or be alone ("Alone") and if alone, whether she was feeding or not ("Feeding") following regrouping. Cows were observed in each pen for 4 h/day and observations were instantaneously scan-sampled at 2.5-min intervals continuously for 2 h in the morning and 2 h in the afternoon. This behaviour sampling method allowed the observers to examine a group of focal cows simultaneously (Mitlöhner et al., 2001). This gave time for each focal cow to be located and observed within the pen for

Table 1
Ethogram of cow behaviours observed on the day-prior (d-1), day-of (d0), day-after (d + 1), and 6-days after (d + 6) regrouping. The behaviours were recorded by scan sampling every 2.5 min during the study period. Behaviours were further grouped into 3 categories.

Behavioural state	Category	Description
ALONE	Rest/Lying	Cow stays in one place in the lying stall.
	Standing	Cow stands with no movement on 4 feet.
	Sleep	Cow assumes sleeping when laid down in one place with the neck relaxed and eyes closed.
	Locomotion	Cow moves from place to place within the pen.
	Milking	Cow enters the milking station/unit until it exits the unit.
	Queuing	Cow queues before the gate leading to the milking machine unit.
	Sniffing	Cow moves nose close to the object.
	Licking object	Cow licks item.
	Maintenance	Cow urinates or defecates.
	Self-grooming	• Cow engages in washing or smoothing its fur or hair using tongue or forelimbs (self-licking). • Cow rubs its body against object (e.g. grooming mechanical brush placed in each pen).
FEEDING	Eat	Cow puts head out over the fence to feed bunk (roughage).
	Drinking	Cow puts head into the drinking/water troughs.
WITH OTHERS[1]	Groom others/Allogrooming	Cow engages in smoothing or licking the fur/hair/head/body of another animal in its environment.
	Head-play/play	Cows rubbing heads against each other.
	Groom by others	Cow has its fur/hair/head/body smoothed by another animal in its environment.
	Fighting	Cow engages in head touching and continuously pushing (physical conflict) its own head against the head/body of another cow.
	Subordinate	The event which the cow is head butted by another cow and the recipient turns away.
	Dominant	Violent contact of the head or horn with another animal with the recipient turning away.

[1] With Others indicates social interactions with other cows.

each measurement. All observations took place from an aerial walkway over the pens where behaviours were easily identified and disturbance to normal behaviour was minimised. The measurements were taken on 4 different occasions: the day before regrouping (d–1), the day of regrouping (d0), one day after regrouping (d + 1), and 6 days after regrouping (d + 6). Before the official start of the experiment, three trainers were trained to conduct the direct observations and all the observers were familiar with the ethogram to assess the agreement between the observers. On the day of moving, the direct visual observations started an hour after the cows were introduced into the pen by three trained observers. The observers were not communicating with each other during the observations.

In addition, behavioural data related to the total daily activity (**ACT**) and rumination time (**RUM**) were collected continuously by accelerometer (Lely Qwes-HR tags) linked with the AMS. The acceleration data were captured and sent electronically in 2-h interval by recording the duration and the intensity of the cow movement and calculates a general activity index in 'activity units (u)'. These activity meters measure horizontal accelerations related to the upward movements of the cow's head and neck during walking and mounting behaviour (Elischer et al., 2013). The tags are also equipped with a microphone-based system capable of measuring the rumination time over a 24-h period as explained by (Reith and Hoy, 2012). Earlier work of Schirmann et al. (2009) had validated that HR-tag accurately recorded both ACT and RUM in lactating dairy cows.

Milk yield, fat, protein content and BW measurements

Milk yield (**MY**), fat, protein content, and BW data were retrieved from the herd management software "T4Cs" database. Milk fat and protein % were monitored through the Lely Milk Quality Control system.

Milk sampling and milk composition analyses

To determine the influence of the regrouping on the milk cortisol and oxytocin concentration, gross (total) energy, milk water, and DM content, milk samples were collected from the same 52 cows (n = 17 primiparous; 35 multiparous). The milk samples were collected directly from the *Lely Astronaut* AMS unit a day-prior (d-1), day-after (d + 1) and 6-days after (d + 6) over the same 2-h period each day. It was assumed that the effect of the regrouping will not be pronounced on the day of regrouping (d0); hence, no milk samples were collected on that day. For each sample, approximately 50 ml of milk was collected and stored in falcon tubes. The vials were stored on ice for a maximum of 6 h before they were processed in the laboratory. Milk samples were split and approximately 4 ml (in 2 aliquots) were then centrifuged at 10 000×g for 4 min at 4 °C and then centrifuged skim milk was stored at −80 °C pending further analysis whereas the remainder of the whole milk (∼40 ml) was used for the DM determination.

Water, DM content, and total energy (gross energy)

Milk samples were analysed for the DM and water content by placing them into an oven (Thermo Electron Corporation, Stabilitherm) at 60 °C for 10–14 days until their weight remained constant. Bomb calorimetry was used to determine the total energy (MJ/kg) in the milk samples using a Parr 6 200 Compensated Jacket Calorimeter.

Cortisol and oxytocin assays

A subset of 36 milk samples out of 52 were assayed. Commercial kits were used to measure milk cortisol concentrations (ng/ml) (EIA-EA65 kit; Oxford Biomedical research, Inc., Oxford, MI, USA, 2015) and milk oxytocin concentrations (pg/mL) (DetectX Oxytocin kit K048-H1; https://www.arborassays.com, MI, USA) in the clarified samples. The results were viewed using SoftMax Pro software (built-in 4PLC software). These hormonal kits had been used in other studies (Moscovice et al., 2021; Woolley et al., 2018). The intra- and inter-assay CV in cortisol and oxytocin pooled samples were 6.5 and 4.8%, and 8.9 and 10.1%, respectively.

Statistical analyses

All data were compiled using a Microsoft Excel file and data exploration, and statistical data analyses were carried out in the

'R' statistical software package (R Core Team, 2018) in RStudio (RStudio Team, 2016). The study investigated the number of occurrences and the duration of three behavioural states of the cows during the entire observational period relative to regrouping in each parity group (multiparous and primiparous). Fifty-two cows were observed (n = 19 968 observations).

Regrouping on behavioural dynamics

*Model development approach: hidden markov model **(HMM)** of behavioural states and activity budgets.* Data pre processing: we estimated the three domain activity states from the question dimension that the cow can either be interacting with other cows (State 1) or be alone (State 2) and if alone, whether she was feeding or not (State 3) following regrouping. This modelling approach helps to identify two ways by which behaviour may have been disturbed: short-term budget effect and long-term bout duration. The latter follows the adaptive behavioural models of Houston and McNamara (1999), in that individual's demands, and the environment in which individuals live, dictate the budget they can achieve. But that budget can be achieved in many different ways, i.e., the behavioural system can exist in different 'states' with different behavioural dynamics which all can achieve the same budget.

Initially, we examined the frequency/number of the behavioural state transitions observed, which resulted in expected heterogeneity in their occurrences. This variation could be partly driven by the cows' behavioural budgets. To be able to account for the temporal dependency of the behavioural observations and have an unbiased estimate of the budget given the replicates, a three-state time discrete-HMM was fitted to the data to estimate the 3×3 state transition *P* matrix (tpm) Q (below) of behavioural states over time using 'msm' function (multi state modelling, 'msm' package) in R (Jackson, 2011).

We assumed that any transition from one state to the next was possible and reasonable (plausible). To assess the influence of regrouping (time period relative to regrouping days: d-1, d0, d + 1, and d + 6) and parity (primiparous vs multiparous) on the cows' activity dynamics, a three-state time discrete-HMMs were fitted to describe the behavioural phenotypic changes over the three activity states using the multi state modelling assuming that the next state was solely dependent on the present/current state (Markov property).

Model selection: Five alternative model sets were developed to test the covariates' effect on the estimated state transition probabilities and activity states dynamics. The three-state models with (including an interaction term between regrouping and parity group) and without covariates were compared using the lowest Akaike Information Criterion (**AIC**) and maximum likelihood ratio test (lrtest.msm function, msm package) (Supplementary Table S1). To our best knowledge, there is no study that applied the HMM on the regrouping effect on the dairy cows' behavioural dynamics. Furthermore, the transition *P* matrix of the best model (lowest AIC value) was used to estimate the activity budgets (long-term budget effect, min per 4-h period) and average state bout duration (short-term budget effect, min per 4-h period) with their associated SEs for each state. The average bout duration ('sojourn.msm' function, msm package, the average time that the cow spends on each state before transition to the next state) and total time ('totlos.msm' function, msm package, min per 4-h period) between activity states estimated from the model were multiplied by 2.5 to get the length of stay/time until the next transition state in minutes. The results of the models with the covariates were reported as the minutes, and the significant effects of the independent variables on the behaviour and activity budgets between states were presented as confidence intervals (95%-CIs). The goodness-of-fit of the selected time-discrete Hidden Markov model was also assessed by plotting the expected prevalence percentage against the observed prevalence percentage.

$$Q = \begin{bmatrix} P_{11} & P_{12} & P_{13} \\ P_{21} & P_{22} & P_{23} \\ P_{31} & P_{32} & P_{33} \end{bmatrix}$$

In the 3×3 transition *P* matrix Q, the sum of the entries in each row is equal to one. For the matrix Q given above, where P_{12} is the *P* that the cow interacting with other cows (state 1) will be alone (state 2) in the next hour, P_{13} is the *P* that the cow interacting with other cows (state 1) will be feeding (state 3) in the next hour. P_{21} is the *P* that the cow that is alone (state 2) will be interacting with others (state 1) in the next hour, and so forth. All other transition probabilities are explained in the same manner.

Regrouping on activity, rumination time and milk yield and composition

Linear mixed model fitted using nlme package (Pinheiro et al., 2018) was used to determine whether regrouping and parity influenced several dependent variables, including MY and components (fat, protein, water, DM, gross energy, cortisol and oxytocin level), total daily activity counts and rumination time. The analysis included the fixed effects of sampling days before and after regrouping (day: d-1, d0, d + 1 and d + 6), parity and day*parity interaction and sampling day before and after regrouping as the repeated measure with the cow ID being the random effect. During the course of the experiment, 4 and 5 cows (n = 4 multiparous and 1 primiparous cows) activity and rumination time data, respectively, were missing on the sampling dates; therefore, they were excluded in the analysis.

The models with and without an interaction for each response variable were fitted and models' parameters were first estimated using the maximum likelihood method and compared, and then, the best model fit was selected based on the lowest AIC values and lower *P*-values. The selected model was then re-fitted to the data to estimate the parameters using the restricted maximum likelihood method. The model included the main effects of the regrouping days, parity and their interaction term. Furthermore, the postanalyses were performed using lsmeans function in "emmeans" package (Russell, 2020) in R with Tukey-adjusted least square means (**LSM**). As before, the regrouping effect was done by comparing the mean values of each group (d0, d + 1, and d + 6) against the values on the day-prior (d-1) to regrouping. The model residuals were assessed for normality and homogeneity of variance in R using diagnostic plots (histogram, residual plots against the fitted values and quantile–quantile plots). The variable that showed deviations from these assumptions was then transformed logarithmically. All the values in the study are reported as LSM and their associated standard errors (SEM) and the statistical significance of the means was declared at $P < 0.05$. All the plots were generated using the ggplot2 library.

Results

Hidden Markov modelling (Multi state modelling) on behavioural changes

A three-state HMM (Supplementary Table S1: Model E; AIC = 15 897.96, -2xLL = 332.79, df = 42, $P < 0.0001$) including an interaction term of the independent variables (regrouping*parity) significantly best explained the data compared to other models. Our findings indicate that the model selected with an interaction term between the covariates improved the fitted time discrete-HMM by 383. The model revealed that behavioural dynamics were significantly influenced by regrouping depending on the parity group.

Regrouping and parity on the behavioural dynamics and activity budget between behavioural states

Average bout duration (short-term budget effect)

The estimated average bout durations (minutes) of activity states are shown in Fig. 1A. The statistically significant difference between the regrouping days, depending on the parity groups on the states, was determined by no overlap in the 95%-CIs ($P < 0.05$). The results of the average bout duration comparison between the activity states over the 4-h observation period are presented as the estimate (minutes) ± standard error (SE) and the 95%-CIs. The findings indicated that regrouping had a significant impact on the behavioural dynamics and activity budgets between the activity states depending on the parity (goodness-of-fit, −2xLL = 333, df = 42, $P < 0.0001$).

Cows spent longer bouts alone than feeding, and the interacting with others state was the least expressed condition. On d-1, primiparous cows had a significantly longer average bout time engaged with others than multiparous (2.67 ± 0.46 vs 1.46 ± 0.15 min per bout). In the days following regrouping (on d + 1 and d + 6), primiparous cows were less likely to be engaged with others than d-1 compared to the multiparous cows. There was no difference between the parities on d0 or d + 1; however, by d + 6, multiparous cows spent longer bouts with others compared to primiparous (6.61 ± 0.84 vs 2.13 ± 0.47 min per bout). Moreover, the results of the model showed that no significant differences were found in the average bout duration between the activity states alone and feeding for both primiparous and multiparous cows on any day. However, on d0, cows spent significantly shorter average bout time than d-1 on the activity states alone (14.81 ± 0.96 min; 95%-CI: 13.05–16.80 vs 25.11 ± 2.22 min; 95%-CI: 21.12–29.85) and feeding (7.04 ± 0.49 min; 95%-CI: 6.13–8.07 vs 10.01 ± 0.94 min; 95%-CI: 8.33–12.04) but did not differ between parities and recovered to the pre regrouping levels (Fig. 1A). Similarly, multiparous cows conducted shorter average feeding bouts on d0 than d-1 (7.04 ± 0.49; 95%-CI: 6.13–8.07 vs 10.01 ± 0.94 min; 95%-CI: 8.33–12.04) but did not differ from the primiparous cows. There was no evidence that primiparous cows had shorter bout duration when feeding on d0 compared to their multiparous counterparts.

Total time spent (long-term budget)

Total time spent is the combination of all occurrences of an activity over the observation period. The results revealed that cows spent more total time alone than with others or feeding. Statistically significant differences in the total time cows were engaged with others were found in both primiparous and multiparous on d + 6 (Fig. 1B). On d0, the total time cows were engaged with others tended to increase but did not differ from their baseline. A significant reduction in the total time multiparous cows spent with other cows was observed on d + 1 compared to d0. However, on the days following regrouping primiparous tended to spend less total time engaging with other cows though they did not differ from their baseline values. Multiparous cows spent significantly more total time (21.34 min; 95%-CI: 16.37–27.33) engaged with others six days after regrouping than did the primiparous cows (7.64 min; 95%-CI: 5.15–11.13).

There was no difference observed in the total time cows spent alone or feeding between multiparous and primiparous cows at any time point. A significant decrease in the total time cows spent alone on d0 was observed. However, multiparous cows significantly reduced the total time spent alone from 179.54 min (95%-CI: 168.91–187.98) on d-1 to 160.37 min (95%-CI: 152.50–168.35) on the day or regrouping; d0, but it returned to baseline level on the days following regrouping.

Physical activity and rumination time behaviours

The cow's physical ACT was influenced by both regrouping ($F = 8.41$, $P < 0.001$) and parity ($F = 4.73$, $P = 0.035$) (Table S2). Daily ACT patterns across the sampling days can be observed in Fig. 2A. On average, primiparous cows were more active across all the days than multiparous cows (633 ± 26.1 vs 540 ± 19.3 μ/day). A significant increase in the activity in both primiparous and multiparous cows was found on the day of regrouping compared to the day before, 689 ± 28.7 and 582 ± 21.3 μ/day ($P < 0.001$), respectively (Fig. 3A).

In addition, regrouping had a significant effect on the RUM ($F = 50.14$, $P < 0.0001$) but both parity groups were similar ($F = 0.02$, $P = 0.88$) (Table S2). However, the effect of regrouping on the RUM was statistically significant depending on the parity ($F = 15.25$, $P < 0.0001$). Following regrouping, a significant decline in the RUM was more pronounced in the multiparous cows (< 31 min/day) than in primiparous cows (< 13 min/day) compared to d–1. On the contrary, multiparous cows significantly decreased their time spent ruminating on d0, but the values returned to the d-1 (baseline) values on the sixth day after regrouping (486 ± 19.6 min/day). This is an indication that while the multiparous cows quickly returned to baseline rumination levels, this was not the case for the primiparous cows (Fig. 3A and B).

Milk characteristics

Milk yield (kg/day)

There was no main effect of the regrouping on MY ($F = 0.75$, $P = 0.52$), but the main effect of parity was statistically significant, ($F = 6.04$, $P = 0.018$) (Table S2). However, we found a significant interaction between the effects of regrouping and parity on the MY ($F = 3.38$, $P = 0.020$). Primiparous cows produced 3.80 ± 2.42 kg (12.2%) ($P = 0.006$) MY less on d + 1 following regrouping compared to the baseline level, whereas multiparous cows did not change MY ($P = 0.72$) (Fig. 3C). Milk yield of primiparous cows returned to the level of the baseline on d + 6.

Milk composition and, milk cortisol and oxytocin concentration

The regrouping had a significant effect on the milk fat content, ($F = 10.62$, $P < 0.0001$), as did the effect of parity, ($F = 6.65$, $P = 0.013$) (Fig. 3D, Table S2). Generally, primiparous cows yielded significantly lower (3.77% vs 4.22%/d) fat content on average compared to the multiparous cows. A decrease in fat percentage for both primiparous and multiparous was observed in the days following regrouping and had not recovered by d + 6. Moreover, on d0, primiparous cows showed a significantly smaller percentage decrease of 3.23% in milk fat percentage compared to multiparous (6.13%).

Milk protein content was logarithmically transformed to meet the assumptions. Log milk protein percentage was significantly influenced by regrouping ($F = 2.99$, $P = 0.033$), but there was no significant effect of parity on milk protein percentage ($F = 1.83$, $P = 0.18$) (Table S2). On average, protein % for the multiparous cows (1.16% (3.20%)) was similar to that of primiparous (1.12% (3.06%)). The mean protein content on d-1, d0, d + 1 and d + 6 was 1.14 ± 0.01, 1.14 ± 0.01, 1.15 ± 0.01 and 1.13 ± 0.01%, respectively. A significant decrease in protein content was detected on d + 6 ($P = 0.015$) compared to the level on d-1 in both primiparous and multiparous cows (Fig. 3E). No differences in the log milk protein content were found on days d0 ($P = 0.70$) and d + 1 ($P = 0.86$) compared with baseline protein values.

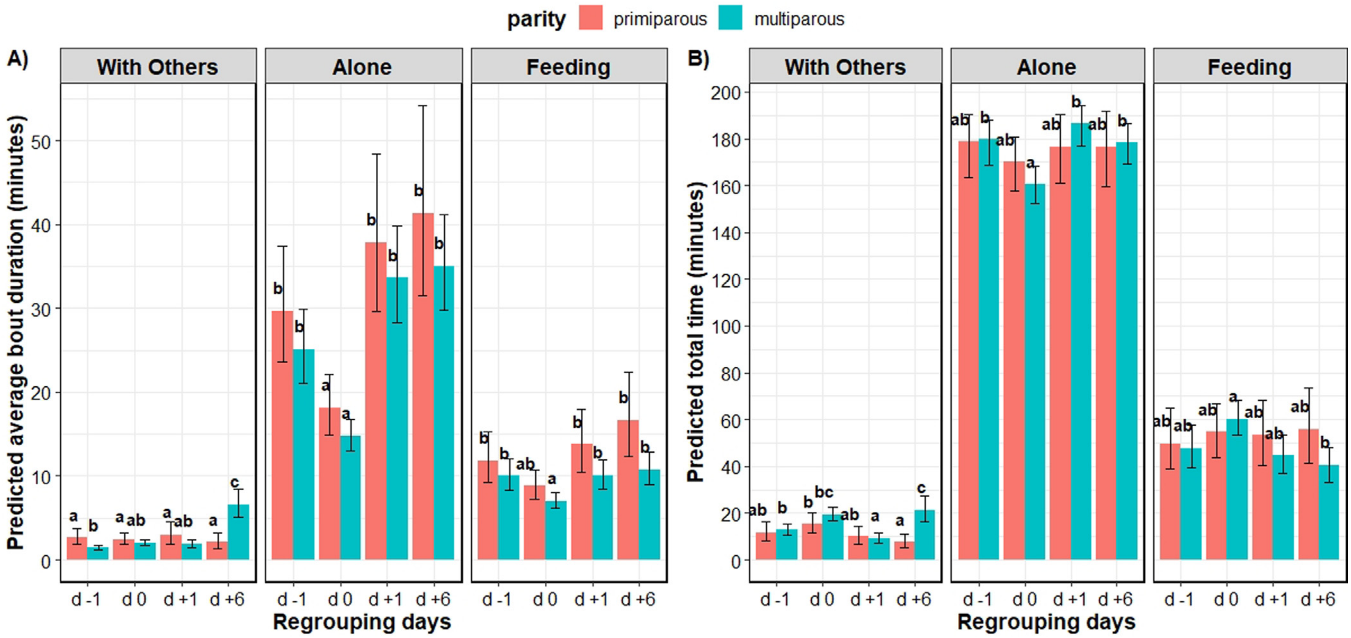

Fig. 1. Estimated average bout duration (mean sojourn time, minutes) and total time (minutes) spent between activity states during the 4 h on the days relative to regrouping (d-1: day-prior, d0: day-of regrouping, d + 1: day-after, d + 6: 6 days after regrouping) of lactating cows. Bars represent the parity group (see legend) whereas error bars represent 95% confidence intervals. Statistically significant differences in the average bout duration between regrouping days and parity are indicated as letters ([a,b,c]). Means that do not share a letter are statistically different between the regrouping days and between parity (primiparous vs multiparous) ($P < 0.05$).

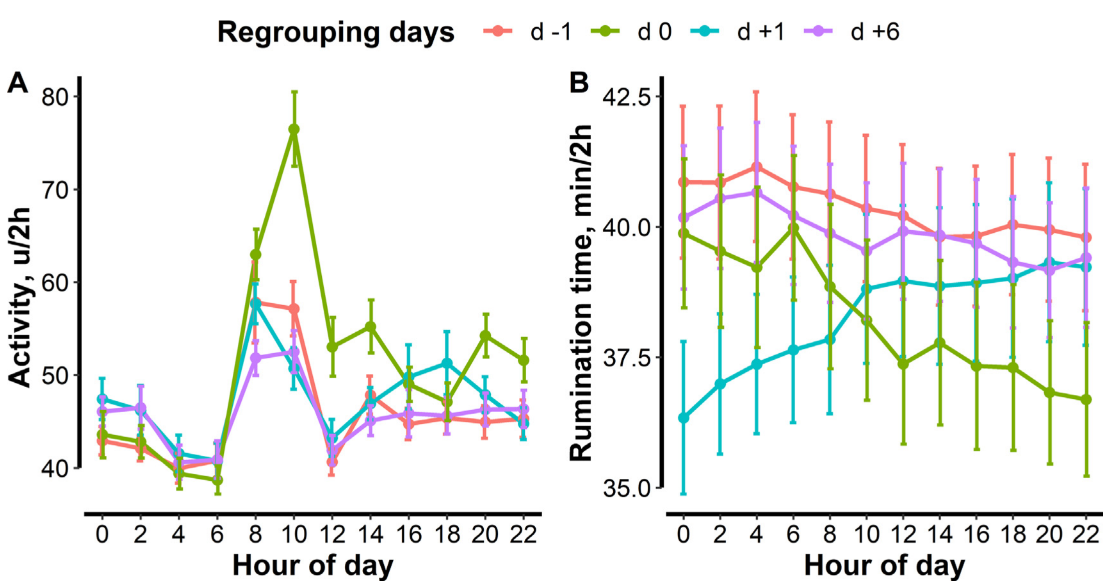

Fig. 2. Physical activity (A) (n = 48) and rumination time (B) (n = 47) dynamics of the lactating cows during the experimental periods. Coloured solid lines represent the experimental periods: d-1 = day-prior, d0 = day-of regrouping, d + 1 = day-after, d + 6 = 6 days after regrouping and the error bars represent the SE. The data were continuously measured by the Lely Qwes–HR tags in 2-h time blocks.

No significant effect of regrouping and parity ($P > 0.05$) was found in milk gross energy (MJ/day), DM (%), and cortisol concentration (ng/ml) (as illustrated in Table S3).

Square root milk oxytocin concentration was significantly affected by regrouping ($F = 3.33$, $P = 0.04$) and but not parity, $F = 1.31$, $P = 0.26$ (Table S3). A significant decrease in milk oxytocin concentration was detected on d + 1 compared to the level of the d-1 in both primiparous (11.3 ± 0.81 vs 12.2 ± 0.81 pg/mL) and multiparous (10.2 ± 0.62 vs 11.0 ± 0.62 pg/mL) cows ($P = 0.01$) (Fig. 3F). These both recovered by day + 6 ($P = 0.17$).

Discussion

This study investigated whether regrouping altered dairy cows' behaviour and impacted their oxytocin and stress levels, physical activity, rumination time, milk yield, and composition. Cows were expected to change their behaviour in order to cope in their new environment as a result of regrouping. The environment in which the animals live in, their daily demand, management, and cows' status, dictate their activity budget (von Keyserlingk et al., 2008). Under stressful events (in this study regrouping), cows'

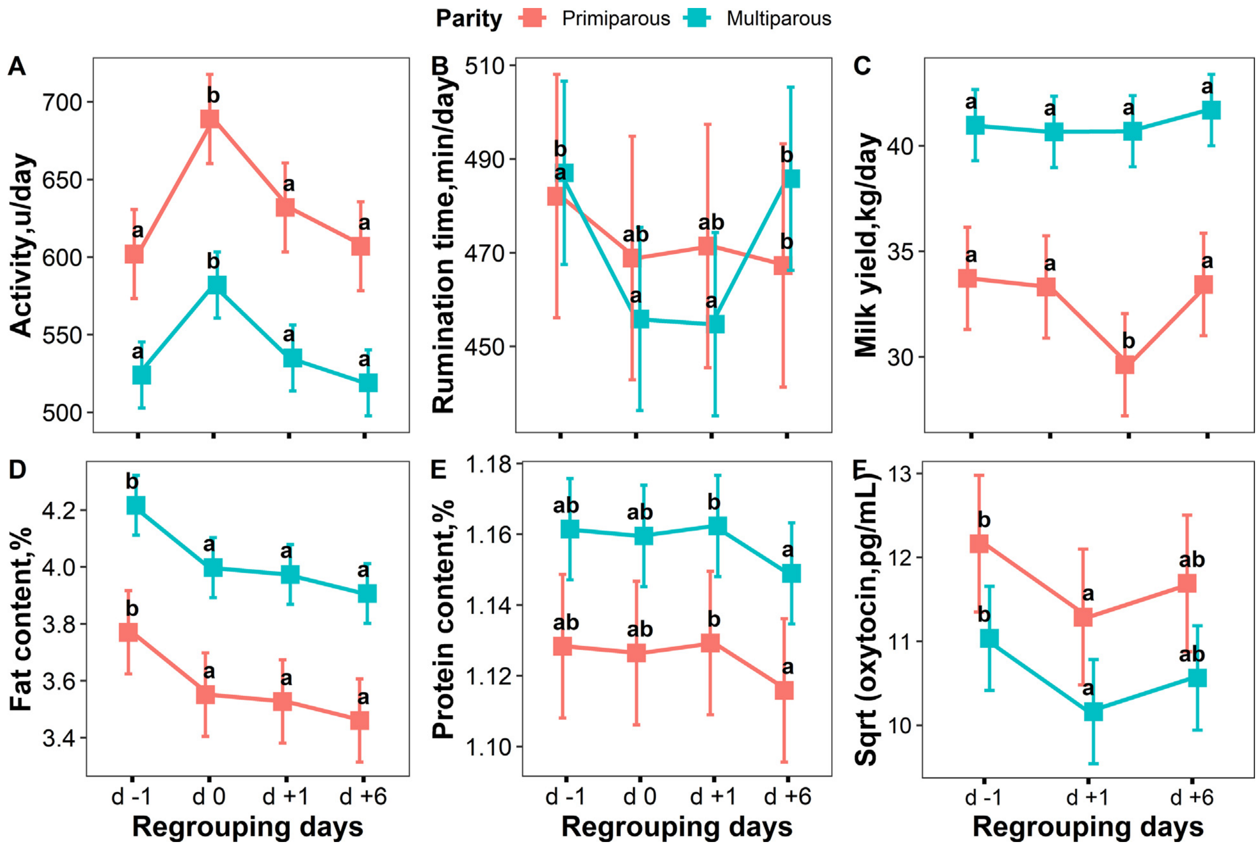

Fig. 3. Least square means (±SEM) of physical activity (A, u/day) (n = 48), rumination time (B, min/day) (n = 47), milk yield (C, kg/day) (n = 52), fat (D, %) (n = 52), log protein (E, %) (n = 52), and square-root Oxytocin (F, pg/mL) (n = 36) of the lactating cows during the experimental study periods. The plot illustrates the effect of regrouping (represented by the days relative to regrouping in the x-axis) and parity (represented by the coloured lines). Different superscripts ([a,b,c]) on the plot represent a significant difference ($P < 0.05$) among days within parity. There was a significant effect of parity ($P < 0.05$) on activity level (A), milk yield (C), and fat % (D) and a significant interaction, day× parity, on rumination time (B) and milk yield (C).

behavioural dynamics are challenged which could negatively influence their daily activity budgets (Christiansen et al., 2013; Lusseau, 2006) as they attempt to meet their daily physiological requirements as well as nutritional requirements.

As expected, the three-state (with others, alone, and feeding) time discrete-Hidden Markov Multi state model revealed that regrouping significantly influenced cows' behavioural dynamics and activity budgets between the activity states but the impact depended on the parity. Our findings are comparable to the earlier studies (Gutmann et al., 2020; Neave et al., 2017; Soonberg et al., 2021) which found that cows' behaviour differed between parities, namely primiparous and multiparous, during the transition period when introduced to a group of lactating cows after calving.

In general, the multiparous cows are older, and they are familiar with the regrouping practice, therefore, they may benefit from the previous experience of regrouping. Moreover, they are more likely to obtain a higher social position meaning they will not get displaced from the feeder etc. It seems that primiparous must adjust to be able to cope with the changing social environment which might also negatively influence their welfare.

Under a stable environment, dairy cows are able to form long dyadic relationships with other cows (Gutmann et al., 2015; Reinhardt et al., 1986). Disruptions of these bonds may have a negative impact on psychological and behavioural functioning (McNeal et al., 2014). Although the cows in the present study showed no stress responses, a decrease in oxytocin level was observed a day after regrouping and returned to baseline level 6 days after regrouping in both parity groups. Positive social interactions promote oxytocin secretion; therefore, a reduction on d0 may suggest fewer positive interactions. This finding accords with that of von Keyserlingk et al. (2008) that reported a decrease in social licking following regrouping; an indication of the consequences of regrouping on the social bonding between individuals (Sato et al., 1991).

The total time spent in both alone and feeding states returned to the baseline values from the day after regrouping for both primiparous and multiparous cows. These results are in agreement with the results of Broucek et al. (2017), Neave et al. (2017), and von Keyserlingk et al. (2008). In the present study, primiparous cows spent more time feeding on subsequent days to regrouping and this may suggest that as these cows had sufficient feeding stalls. Similarly, Gupta et al. (2008) found an increase in eating behaviour in regrouped steers. The social environment influences feeding behaviour, especially for indoor-housed cows (Nielsen, 1999).

Although our results revealed no regrouping effect on the cortisol concentration, a large individual variability in milk cortisol concentration between cows was observed and this could be an indication that the activation of the Hypothalamic-pirtuitary-adrenal axis (**HPA**) differs between individuals. Animals cope differently with stressors, and they first change their behaviour and then their physiological responses follow on (Moberg, 2000). Nogues et al. (2020) showed that individual cows used different strategies to cope with the social stress of regrouping. Contrary to the present results, other studies reported a significant effect of regrouping on the cortisol concentration. For instance, Mazer et al. (2020) observed greater faecal cortisol metabolites concentration in individually regrouped primiparous compared to the multiparous cows. Moreover, their findings revealed higher faecal cortisol metabolites in individually regrouped primiparous cows compared to primiparous cows that were moved with a partner. This suggests that the cows in the current study may not have experienced high levels of stress. This could be attributed to factors such as being moved in small groups.

However, the time of milk sampling following the action of stress may influence milk cortisol concentration (Caroprese et al., 2010). Verkerk et al. (1998) suggested that the milk cortisol reflects stress in lactating cows within 2–4 h before the sampling period. Moreover, according to Romero et al. (2015), there may be a delay of 1.5 h for both milk and blood cortisol following a severe stress action. We were not focused on the peak of stress response caused by the act of the farm staff collecting and moving the cattle but on the prolonged stress response of adjusting to the new surroundings. Based on these findings, the possible reasons for the lack of the treatment difference in our study in milk cortisol response could occur because the cows were not sampled on the day of regrouping and also moved with familiar partners, therefore they were much less stressed.

Regrouping not only influenced behaviour but also physiological changes. Earlier studies reported a negative impact of regrouping on production performance and traits in lactating cows (Hasegawa et al., 1997; Sevi et al., 2001; Sowerby and Polan, 1978). Behavioural disruptions could negatively influence animal productivity including decreased milk yield and feed intake (Cook and Nordlund, 2004) which ultimately negatively influence milk composition, mainly fat and protein content.

In the present study, we observed a significant reduction in milk yield of 3.80 ± 2.42 kg due to regrouping for primiparous cows the day after regrouping which returned to the maximum production level on the 6th day after regrouping. However, multiparous cows seemed to be adapted to the new environment; hence, no significant differences were observed across the days. These findings may be ascribed to the disruptions observed on the day of regrouping, for instance, increased standing time or physical activity level, which subsequently led to reduced time in rumination, followed by decreased milk yield. Moreover, in the current study, a reduction in oxytocin level was found a day after regrouping but returned to baseline level 6 days after regrouping. Similar to our findings, earlier studies by Bruckmaier et al. (1996); Bruckmaier et al. (1993) and Macuhová et al. (2002) reported disruptions in milk removal in primiparous cows when milked in unfamiliar surroundings due to inhibition of the oxytocin release from the anterior pituitary gland (Bruckmaier and Blum, 1998), however, one study (Sutherland et al., 2012) found an increase. The majority of these results suggest that the reduction in milk yield observed in primiparous cows could be explained by the decreased milk oxytocin level observed on the day after regrouping. It is possible that milk oxytocin level would have been partially increased the following day, but we did not observe this as there was no milk samples collected on the following day.

Although the study of Smid et al. (2019) found no effect of regrouping on milk production, suggested to be due to the lack of effect of the treatment on feeding behaviour, other earlier studies are consistent with our findings, but the reductions are to a lesser extent. For instance, Brakel and Leis (1976) reported a reduction of 3% in milk yield a day following regrouping compared to 12.2% for primiparous cows in the current study. However, it appears that the range is varied as Broucek et al. (2017) reported a significant reduction of 23.3% in milk yield a day following the transfer of cows. Reduction in milk yield may be more extreme in lower dominance rank primiparous (Hasegawa et al., 1997). Earlier studies indicated that the milk yield returned to baseline between 2 and 14-days following regrouping (Broucek et al., 2015; Hasegawa et al., 1997).

Moreover, regrouping and parity explained a significant reduction in fat but not protein percentage. Surprisingly, milk fat content decreased gradually after regrouping in both multiparous and primiparous cows and did not indicate a pattern of returning to the baseline level. It is difficult to explain this continuous decrease. Similarly, a decrease in average protein % was found in all cows on d + 6 compared to prior to regrouping. Multiparous cows yielded greater milk fat % than did primiparous. Although in the current study, the effect of the regrouping on the nutrition effect (feed intake/ DM intake) was not investigated since the study was carried out in a large dairy commercial herd, the reduction in milk yield and milk composition (fat and protein) could be explained by a reduction in the feed intake.

The present study showed that primiparous cows were more affected by the regrouping than multiparous cows; this could be due to factors such as their unfamiliarity with the practice, potentially holding the lower-ranking positions due to age and body size, having higher nutrient requirements for both growth and production, and potentially exhibiting greater lactation persistency (Grant and Albright, 2001).

Conclusions

The findings of the current study are of international relevance as they revealed that the commonly recognised practice of regrouping significantly disrupted behavioural dynamics and activity time budgets. This negatively influenced milk yield and composition, physical activity, and rumination time depending on the parity group. Milk yield, oxytocin level, physical activity, rumination time, and protein content did return to the baseline levels within 24 h. However, the fat content and time budgets, specifically cows' interactions with others, were still affected six-days after regrouping. Reduction in the average and total time cows spent alone and on feeding bout duration were observed on subsequent days to regrouping. Primiparous spent less time interacting with other cows after regrouping compared to the multiparous cows. This study showed that regrouping has an impact on the social environment/dynamics and well-being which in return influenced milk production, more especially in the primiparous cows.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101094.

Ethics approval

As the research simply observed the normal working practices of the commercial farm and did not manipulate the cows in any way, ethical approval was not required.

Data and model availability statement

None of the data was deposited in the formal repository. Data can be obtained upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

CRediT authorship contribution statement

J.L. Marumo: Writing – review & editing, Writing – original draft, Visualization, Investigation, Methodology, Funding acquisition, Formal analysis, Data curation. **D. Lusseau:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **J.R. Speakman:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **M. Mackie:** Resources. **A.Y. Byar:** Data curation. **W. Cartwright:** Data curation. **C. Hambly:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

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References

Arave, C.W., Albright, J.L., 1976. Social rank and physiological traits of dairy cows as influenced by changing group membership. Journal of Dairy Science 59, 974–981. https://doi.org/10.3168/jds.S0022-0302(76)84306-8.

Bobić, T., Mijić, P., Knežević, I., Šperanda, M., Antunović, B., Baban, M., Sakač, M., Frizon, E., Koturić, T., 2011. The impact of environmental factors on the milk ejection and stress of dairy cows. Biotechnology in Animal Husbandry 27, 919–927. https://doi.org/10.2298/BAH1103919B.

Bøe, K.E., Færevik, G., 2003. Grouping and social preferences in calves, heifers and cows. Applied Animal Behaviour Science 80, 175–190. https://doi.org/10.1016/S0168-1591(02)00217-4.

Boyland, N.K., Mlynski, D.T., James, R., Brent, L.J.N., Croft, D.P., 2016. The social network structure of a dynamic group of dairy cows: From individual to group level patterns. Applied Animal Behaviour Science 174, 1–10. https://doi.org/10.1016/j.applanim.2015.11.016.

Brakel, W.J., Leis, R.A., 1976. Impact of social disorganization on behavior, milk yield, and body weight of dairy cows. Journal of Dairy Science 59, 716–721. https://doi.org/10.3168/jds.S0022-0302(76)84263-4.

Broucek, J., Mihina, S., Uhrincat, M., Lendelova, J., Hanus, A., 2015. Impact of gestation and lactation stage on the dairy cow response following removal to unfamiliar housing and milking system. Italian Journal of Animal Science 14, 233–237. https://doi.org/10.4081/ijas.2015.3410.

Broucek, J., Uhrincat, M., Mihina, S., Soch, M., Mrekajova, A., Hanus, A., 2017. Dairy cows produce less milk and modify their behaviour during the transition between tie-stall to free-stall. Animals 7, 16. https://doi.org/10.3390/ani7030016.

Bruckmaier, R.M., Blum, J.W., 1998. Oxytocin release and milk removal in ruminants. Journal of Dairy Science 81, 939–949. https://doi.org/10.3168/jds.S0022-0302(98)75654-1.

Bruckmaier, R., Schams, D., Blum, J., 1993. Milk removal in familiar and unfamiliar surroundings - concentrations of oxytocin, prolactin, cortisol and beta-endorphin. Journal of Dairy Research 60, 449–456. https://doi.org/10.1017/s0022029900027813.

Bruckmaier, R., Pfeilsticker, H., Blum, J., 1996. Milk yield, oxytocin and beta-endorphin gradually normalize during repeated milking in unfamiliar surroundings. Journal of Dairy Research 63, 191–200. https://doi.org/10.1017/S0022029900031691.

Caroprese, M., Albenzio, M., Marzano, A., Schena, L., Annicchiarico, G., Sevi, A., 2010. Relationship between cortisol response to stress and behavior, immune profile, and production performance of dairy ewes. Journal of Dairy Science 93, 2395–2403. https://doi.org/10.3168/jds.2009-2604.

Christiansen, F., Rasmussen, M.H., Lusseau, D., 2013. Inferring activity budgets in wild animals to estimate the consequences of disturbances. Behavioral Ecology 24, 1415–1425. https://doi.org/10.1093/beheco/art086.

Cook, N.B., Nordlund, K.V., 2004. Behavioral needs of the transition cow and considerations for special needs facility design. Veterinary Clinics of North America: Food Animal Practice 20, 495–520. https://doi.org/10.1016/j.cvfa.2004.06.011.

Elischer, M.F., Arceo, M.E., Karcher, E.L., Siegford, J.M., 2013. Validating the accuracy of activity and rumination monitor data from dairy cows housed in a pasture-based automatic milking system. Journal of Dairy Science 96, 6412–6422. https://doi.org/10.3168/jds.2013-6790.

Grant, R.J., Albright, J.L., 2001. Effect of animal grouping on feeding behavior and intake of dairy cattle. Journal of Dairy Science 84, E156–E163. https://doi.org/10.3168/jds.S0022-0302(01)70210-X.

Gupta, S., Earley, B., Nolan, M., Formentin, E., Crowe, M.A., 2008. Effect of repeated regrouping and relocation on behaviour of steers. Applied Animal Behaviour Science 110, 229–243. https://doi.org/10.1016/j.applanim.2007.05.003.

Gutmann, A.K., Špinka, M., Winckler, C., 2015. Long-term familiarity creates preferred social partners in dairy cows. Applied Animal Behaviour Science 169, 1–8. https://doi.org/10.1016/j.applanim.2015.05.007.

Gutmann, A.K., Špinka, M., Winckler, C., 2020. Do familiar group mates facilitate integration into the milking group after calving in dairy cows? Applied Animal Behaviour Science 229,. https://doi.org/10.1016/j.applanim.2020.105033 105033.

Hasegawa, N., Nishiwaki, A., Sugawara, K., Ito, I., 1997. The effects of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behavior and adrenocortical response. Applied Animal Behaviour Science 51, 15–27. https://doi.org/10.1016/S0168-1591(96)01082-9.

Houston, A.I., McNamara, J.M., 1999. Models of adaptive behaviour: an approach based on state. Cambridge University Press, Cambridge, UK.

Hultgren, J., Svensson, C., 2009. Heifer rearing conditions affect length of productive life in Swedish dairy cows. Preventive Veterinary Medicine 89, 255–264. https://doi.org/10.1016/j.prevetmed.2009.02.012.

Jackson, C.H., 2011. Multi-state models for panel data: the msm package for R. Journal of Statistical Software 38, 1–28. https://doi.org/10.18637/jss.v038.i08.

Jensen, M.B., Proudfoot, K.L., 2017. Effect of group size and health status on behavior and feed intake of multiparous dairy cows in early lactation. Journal of Dairy Science 100, 9759–9768. https://doi.org/10.3168/jds.2017-13035.

Lusseau, D., 2006. Why do dolphins jump? interpreting the behavioural repertoire of bottlenose dolphins (Tursiops sp.) in doubtful sound New Zealand. Behavioural Processes 73, 257–265. https://doi.org/10.1016/j.beproc.2006.06.006.

Macuhová, J., Tancin, V., Kraetzl, W.-, Meyer, H.H.D., Bruckmaier, R.M., 2002. Inhibition of oxytocin release during repeated milking in unfamiliar surroundings: the importance of opioids and adrenal cortex sensitivity. Journal of Dairy Research 69, 63–73. https://doi.org/10.1017/S0022029901005222.

Marumo, J.L., Hambly, C., Lusseau, D., Speakman, J.R., 2020. Effect of regrouping lactating dairy cows on behavioural variability. In: Proceedings of the British Society of Animal Science Annual Conference, Advances in Animal Biosciences: The Challenge of Change 11, 30 March - 01 April 2020, Nottingham, UK, pp. 027. https://doi.org/10.1017/S2040470020000011.

Marumo, J.L., Hambly, C., Lusseau, D., Fisher, D.N., Speakman, J.R., 2021a. Environmental and social factors influence on milk yield in dairy cows. PhD thesis, University of Aberdeen, Aberdeen, Scotland, UK.

Marumo, J.L., Lusseau, D., Speakman, J.R., Mackie, M., Byar, A., Cartwright, W., Hambly, C., 2021b. Effects of regrouping of Scottish Holstein-Friesian cows on milk production, physical activity, rumination time and cortisol concentration in a robotic milking system. In: Proceedings of the British Society of Animal Science Annual virtual Conference, 12 - 15 April 2021, UK, p.121. https://doi.org/10.1016/j.anscip.2021.03.152.

Mazer, K.A., Knickerbocker, P.L., Kutina, K.L., Huzzey, J.M., 2020. Changes in behavior and fecal cortisol metabolites when dairy cattle are regrouped in pairs versus individually after calving. Journal of Dairy Science 103, 4681–4690. https://doi.org/10.3168/jds.2019-17593.

McNeal, N., Scotti, M.A.L., Wardwell, J., Chandler, D.L., Bates, S.L., LaRocca, M., Trahanas, D.M., Grippo, A.J., 2014. Disruption of social bonds induces behavioral and physiological dysregulation in male and female prairie voles. Autonomic Neuroscience: Basic and Clinical 180, 9–16. https://doi.org/10.1016/j.autneu.2013.10.001.

Mitlöhner, F.M., Morrow-Tesch, J.L., Wilson, S.C., Dailey, J.W., McGlone, J.J., 2001. Behavioral sampling techniques for feedlot cattle. Journal of Animal Science 79, 1189–1193. https://doi.org/10.2527/2001.7951189x.

Moberg, G.P., 2000. Biological response to stress: implications for animal welfare. In: Moberg, G.P., Mench, J.A. (Eds.), The biology of animal stress: basic principles and implications for animal welfare. CABI Publishing, New York, NY, USA, pp. 1–21.

Moscovice, L. R., Sobczak, B., Gimsa, U., Kanitz, E., 2021. Salivary oxytocin co-varies with parturition and nursing behavior in domestic pigs. bioRxiv, 2021.2006.2011.448025. Retrieved on 03 March 2023 from https://doi.org/10.1101/2021.06.11.448025.

Neave, H.W., Lomb, J., von Keyserlingk, M.A.G., Behnam-Shabahang, A., Weary, D.M., 2017. Parity differences in the behavior of transition dairy cows. Journal of Dairy Science 100, 548–561. https://doi.org/10.3168/jds.2016-10987.

Nielsen, B.L., 1999. On the interpretation of feeding behaviour measures and the use of feeding rate as an indicator of social constraint. Applied Animal Behaviour Science 63, 79–91. https://doi.org/10.1016/S0168-1591(99)00003-9.

Nogues, E., Lecorps, B., Weary, D.M., von Keyserlingk, M.A.G., 2020. Individual variability in response to social stress in dairy heifers. Animals 10, 1–10. https://doi.org/10.3390/ani10081440.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2018. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137, Retrieved on 09 March 2019 from: http://CRAN.R-project.org/package=nlme.

Proudfoot, K., Habing, G., 2015. Social stress as a cause of diseases in farm animals: current knowledge and future directions. The Veterinary Journal 206, 15–21. https://doi.org/10.1016/j.tvjl.2015.05.024.

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Raussi, S., Boissy, A., Delval, E., Pradel, P., Kaihilahti, J., Veissier, I., 2005. Does repeated regrouping alter the social behaviour of heifers? Applied Animal Behaviour Science 93, 1–12. https://doi.org/10.1016/j.applanim.2004.12.001.

Reinhardt, C., Reinhardt, A., Reinhardt, V., 1986. Social behaviour and reproductive performance in semi-wild Scottish Highland cattle. Applied Animal Behaviour Science 15, 125–136. https://doi.org/10.1016/0168-1591(86)90058-4.

Reith, S., Hoy, S., 2012. Relationship between daily rumination time and estrus of dairy cows. Journal of Dairy Science 95, 6416–6420. https://doi.org/10.3168/jds.2012-5316.

Romero, G., Restrepo, I., Muelas, R., Bueso-Ródenas, J., Roca, A., Díaz, J.R., 2015. Within-day variation and effect of acute stress on plasma and milk cortisol in lactating goats. Journal of Dairy Science 98, 832–839. https://doi.org/10.3168/jds.2014-8052.

RStudio Team, 2016. RStudio: Integrated Development Environment for R. Boston, MA, Inc, Retrieved on 30 October 2017 from: http://www.rstudio.com/

Russell, L., 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version, Retrieved on 10 April 2021 from: http://CRAN.R-project.org/package=emmeans.

Sato, S., Sako, S., Maeda, A., 1991. Social licking patterns in cattle (Bos taurus): influence of environmental and social factors. Applied Animal Behaviour Science 32, 3–12. https://doi.org/10.1016/S0168-1591(05)80158-3.

Schirmann, K., von Keyserlingk, M.A.G., Weary, D.M., Veira, D.M., Heuwieser, W., 2009. Technical note: validation of a system for monitoring rumination in dairy cows. Journal of Dairy Science 92, 6052–6055. https://doi.org/10.3168/jds.2009-2361.

Schirmann, K., Chapinal, N., Weary, D.M., Heuwieser, W., von Keyserlingk, M.A.G., 2011. Short-term effects of regrouping on behavior of prepartum dairy cows. Journal of Dairy Science 94, 2312–2319. https://doi.org/10.3168/jds.2010-3639.

Sevi, A., Taibi, L., Albenzio, M., Muscio, A., Dell'Aquila, S., Napolitano, F., 2001. Behavioral, adrenal, immune, and productive responses of lactating ewes to regrouping and relocation. Journal of Animal Science 79, 1457–1465. https://doi.org/10.2527/2001.7961457X.

Smid, A.C., Weary, D.M., Bokkers, E.A.M., von Keyserlingk, M.A.G., 2019. Short communication: the effects of regrouping in relation to fresh feed delivery in lactating Holstein cows. Journal of Dairy Science 102, 6545–6550. https://doi.org/10.3168/jds.2018-16232.

Soonberg, M., Kass, M., Kaart, T., Barraclough, R., Haskell, M., Arney, D., 2021. Effect of grouping on behaviour of dairy heifers and cows in the transition period. Journal of Dairy Research 88, 45–51. https://doi.org/10.1017/S0022029921000066.

Sowerby, M.E., Polan, C.E., 1978. Milk production response to shifting cows between intraherd groups. Journal of Dairy Science 61, 455–460. https://doi.org/10.3168/jds.S0022-0302(78)83620-0.

Sutherland, M.A., Rogers, A.R., Verkerk, G.A., 2012. The effect of temperament and responsiveness towards humans on the behavior, physiology and milk production of multi-parous dairy cows in a familiar and novel milking environment. Physiology and Behavior 107, 329–337. https://doi.org/10.1016/j.physbeh.2012.07.013.

Verkerk, G.A., Phipps, A.M., Carragher, J.F., Matthews, L.R., Stelwagen, K., 1998. Characterization of milk cortisol concentrations as a measure of short-term stress responses in lactating dairy cows. Animal Welfare 7, 77–86.

von Keyserlingk, M.A.G., Olenick, D., Weary, D.M., 2008. Acute behavioral effects of regrouping dairy cows. Journal of Dairy Science 91, 1011–1016. https://doi.org/10.3168/jds.2007-0532.

Woolley, C.E., Lachance, S., DeVries, T.J., Bergeron, R., 2018. Behavioural and physiological responses to pest flies in pastured dairy cows treated with a natural repellent. Applied Animal Behaviour Science 207, 1–7. https://doi.org/10.1016/j.applanim.2018.07.009.

Zelena, D., Haller, J., Halász, J., Makara, G.B., 1999. Social stress of variable intensity: physiological and behavioral consequences. Brain Research Bulletin 48, 297–302. https://doi.org/10.1016/S0361-9230(98)00176-2.