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Research Article

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Abstract

Regrouping dairy cows is a common feature of dairy farm management. Cows are grouped based on lactation stage, age, milk yield and other factors. Regrouping cows during the dry period (from far-off area to close up area and from close up area to the main herd) brings new challenges. This is especially true for heifers who, after being confirmed gravid, may be grouped into a new pen with dried off cows. The aims of this study were to determine how grouping affects activity, nearest neighbour relationships and aggression, and how heifers' acclimatization to a new group differs from cows. Therefore, the hypotheses were that regrouping cows has less of an effect on older cows compared to heifers, and cows' individuality affects acclimatization to a new group. Aggression data were recorded using a video camera that was directed at the feed bunk, and activity was recorded with activity monitors that were attached around the right hind leg. Synchrony and distance to nearest neighbour were recorded, as was the cows' location on the first 3 d from the day they returned to the main herd. Motion index, mean number of steps and number of lying bouts were significantly higher after calving compared to the week before calving and the difference was higher amongst heifers compared to cows ($P < 0.001$). Both cows and heifers lay down more in the strawyard compared to cubicle housing ($P < 0.01$) and cows were more aggressive than heifers in both housing systems ($P < 0.001$ and $P < 0.05$, respectively). As hypothesized, heifers were more affected by regrouping and cows with more experience settled quicker to their new environment.

It is common practice on dairy farms to group cows according to lactation, milk yield, dietary requirements or reproductive status. On larger farms that practice year-round calving, cows may move from group to group as their status changes. With each regrouping, cows are exposed to new individuals or groups of individuals (Schirmann *et al.*, 2011), and cows may experience aggressive encounters at regrouping, as they attempt to establish their position in the hierarchy.

Dairy cow social structure has been described as a series of dominance relationships and social bonds characterized by aggressive and positive social interactions. Social interactions between cows depends on the space provided for them (Gibbons *et al.*, 2009) and, therefore, different housing systems may be expected to have an effect on the social behaviour and disruption of that behaviour. Calves and heifers are more vulnerable to group changes than older cows (Bøe and Færevik, 2003). Those heifers and calves who have had previous regrouping experience are known to fight less and establish dominance relationship faster than those with less experience (Raussi *et al.*, 2005).

Cows are often grouped during the dry period as well (Cook and Nordlund, 2004), which includes the transition period. The transition period is arguably the most critical time of a dairy cows' life cycle and is typically defined as three weeks before to three weeks after parturition (Huzzey *et al.*, 2005). During this vulnerable period cows experience physiological, nutritional and social changes which make them vulnerable to metabolic and infectious diseases (Huzzey *et al.*, 2005). Regrouping is inevitable in this period as typically the cow moves from a dry (non-lactating) group, through a calving group and then into the main lactating herd.

After calving, it is socially challenging for cows to be separated from their calf and introduced into a new pen with the main lactating herd, which is known to result in higher frequencies of antagonistic behaviour (Bak Jensen and Proudfoot, 2017). Much of this antagonistic behaviour occurs at the feed bunk and can affect feeding times and intakes (von Keyserlingk *et al.*, 2008; Bak Jensen and Proudfoot, 2017; Soonberg *et al.*, 2019). The most aggressive acts occur immediately after regrouping (Schirmann *et al.*, 2011). Heifers are

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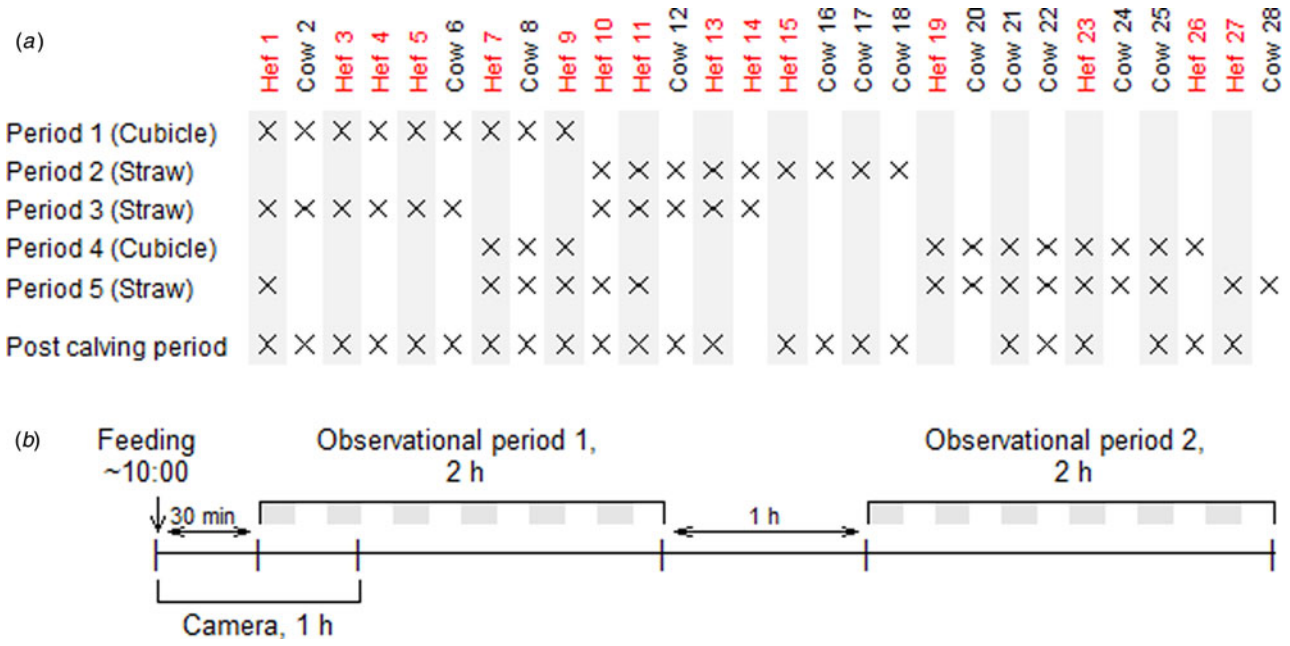

Fig. 1. (a) The distribution and co-presence of animals by study periods, heifers are presented in red and cows in black. (b) Time schedule at pre-calving periods, both two hours observational periods per day contained six 10-minutes observational sub-periods with 10-min gaps between them.

most affected by this situation, because of their lower hierarchy status than cows (Phillips and Rind, 2001).

Previous studies (McGilliard *et al.*, 1983; Schucker *et al.*, 1988; von Keyserlingk *et al.*, 2008) have investigated how group change affects lactating cows and how it affects dry cows' lying behaviour. The aim of this study was to determine how the movement from 'far-off' group (temporally further from calving and housed in cubicles) to a 'close-up' pre-calving group (7–10 d from expected calving and housed in a strawyard) affects the antagonistic behaviour initiated and experienced by dairy cows. The social integration of each cow was also assessed using nearest neighbour distance assessments and activity data. The effect of parity was also considered. It was hypothesized that regrouping of animals has less of an effect on older cows compared to heifers, and that cows' individuality plays a role in acclimatizing to a new group.

Materials and methods

Experimental farm

The study was conducted on Edinburgh University's Langhill Farm in early spring before turnout. The herd comprised 228 milking cows, and all were kept indoors through the winter. Management practice is for youngstock and low yielding cows to be turned out in May and return in October. High yielding cows stay indoors all year round.

Dry cows and heifers were housed in two separate groups. Cow that were between three weeks and 7–10 d of their expected calving date (referred to here as 'far-off' dry cows), were housed in a cubicle shed, with more than one cubicle/cow. Cubicles were bedded with mattresses and sawdust. The length of the feed bunk during the study was 15 m and the passageway width was 2.5 m. Between 7 and 10 d before calving, the cows and heifers (n = 10–18) were moved to a 'close-up' group in a strawyard, where they remained until calving. The strawyard dimensions were 11 m × 19 m and feed bunk length was 12 m. After calving, cow and calf were moved to an individual pen. After around 24 h the calf was removed and the cow entered the main milking herd after the next morning milking.

In the main herd cows were grouped into two groups: high yielding and low yielding cows. There were four pens, three of which were open, so cows could walk freely between them (the high yielding group), and one closed pen (the low yielding group). The dimensions of this house are 24 m × 84 m. They had cubicles bedded with mattresses cubicles (240) and sawdust bedding was laid down three times a week and raked twice a day during milking. A DeLaval milking system was used in a parallel milking parlour with 28 places and cows were milked twice a day at 05 : 00 and 15 : 00. Concentrate was given to each cow in the milking parlour according to their milk yield. Cows in the main herd were fed a total mixed ration, which consisted of maize, soya, barley, wheat, beet pulp, molasses, 1st cut silage and whole crop wheat. Cubicle housed cows (far-off dry) received straw and 2nd cut silage. Strawyard (close up) cows received straw, 1st cut silage, wholecrop and drycake. Water was available *ad libitum* from self-filling water troughs.

Experimental design

The study was carried out according to the animal care guidelines of the Animal Scientific Procedures Act of 1986 and was approved by the Animal Ethics Committee of SRUC.

Twenty-nine dry Holstein cows (n = 13) and heifers (n = 16) were selected for this trial based on their proximity to expected calving date at the time of the study (days to calving 24 ± 21). Seventeen of these animals were observed in the far-off cubicle pen, 27 of them in the strawyard and 23 while in the main herd. Not all cows moved together from one group to another. To assess the effect of group change, data were compared across five periods of 3 d duration (Fig. 1), two periods in the cubicle housing and three in the strawyard. One cow was eliminated from the study because she was found to be not gravid, and

three cows were diagnosed with milk fever after calving and were not included in the main herd observation period.

Regrouping

First regrouping took place a week before expected calving date from far-off cubicle area to close-up area. Second regrouping took place after calving from close-up or maternity pen to main herd.

Measurements

Aggressive behaviour was recorded to investigate changes in aggression and the consistency of individual cows' dominance behaviour after group change. Video cameras (Canon Legria) were attached on a metal pole with Manfrotto 'Magic Arms' above the far-off cubicle pen and the strawyard close-up pen directed at the feed bunk to record aggressive behaviour. Recording started in the morning when fresh feed was delivered, around 10 : 00, and lasted for one hour.

Observations of behaviour and of nearest neighbour distances were carried out for two two-hour periods with a one-hour break in the middle starting 30 min after feed delivery. All observations were made by one observer throughout the study period by noting down the position of an animal and that of its two closest neighbours after every 20 min. Nearest neighbour visual observations were recorded to see how group change affected animals' interactions with each other and to see if pairs stayed together throughout the study period, and if they changed when new cows/heifers were added to the group. The distance to the first two nearest neighbouring cows were scored in 0.5 m categories up to 2.5 m (i.e. <0.5, 0.5, 1, 1.5, 2, 2.5 and >2.5 m). Behaviours recorded were: posture (lying or standing), idling (doing nothing), ruminating, feeding and sleeping.

Observations of the main herd were made to assess the effect of the second group change on cows' behaviours. Visual observations started on the first day that the cow entered the main herd (on average 1–2 d after calving). In cases in which the cow joined the main herd 30 min after feed was delivered, the observations started on the following day. Cows were observed for one hour at 10-minute intervals and the area they were in was recorded every 10 min. Data recorded were animals' location classified as either at the feed bunk, in the passageway or elsewhere.

Fourteen cows had IceTags and 14 cows had IceQube (IceRobotics Ltd., UK) activity monitors attached around their right hind legs to record changes in activity parameters when the cow/heifer was introduced to a new group. Throughout the study period, activity monitors registered standing and lying times, numbers of steps and lying bouts and calculated motion index values (https://www.icerobotics.com/products/) for each cow (Kok *et al.*, 2015). Ice Tags were removed after the cow had spent two weeks in the main herd.

Statistical analysis

The mean numbers of aggressive actions performed by heifers against heifers, by heifers against cows, by cows against heifers and by cows against cows were calculated and these means were compared with *t*-tests followed by Bonferroni correction for multiple testing.

The two-way repeated measures analysis of variance was applied to test the statistical significance of the animals' age (heifers *vs.* cows), housing system (loose housed system with cubicle bedding *vs.* strawyard) and the age by system interaction effects on the percentages of times lying, standing, ruminating, feeding and idling, on the average distances to the first and to the second nearest neighbour, as well as on the average number of aggressive actions performed and received per hour. Model based means (*alias* least square means) were calculated and compared with the Tukey post-hoc test.

To assess the concordance between two housing systems, the correlation coefficients between the same variables (percentages of posture and behaviour, the average distances to the first and to the second nearest neighbour, and the number of aggressive actions performed and received per hour) registered on the same animals in the different systems were calculated.

To study the proximity network of animals, the nearest neighbour score between each pair of animals was calculated separately for each study period. The score with values in the interval from zero (two animals were not observed being neighbours of each other at all) to one (the closeness of two animals was maximum over all pairs of animals) was visually examined by constructing the circle network diagrams (chord diagrams). To study the concordance of the nearest neighbours at different periods, Pearson correlation coefficients between the nearest neighbour scores for animal pairs common to the compared periods were calculated.

In the post-calving period the percentages of posture and behaviour of heifers and cows were compared with Wilcoxon test.

The activity monitor measurements were divided into three time periods: measurements before calving, measurements on calving day and measurements after calving. A two-way repeated measures analysis of variance was applied to test the effect of time period, animals' age (heifers *vs.* cows) and their interaction.

The data were analysed using statistical software R 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria), except the repeated measures analyse of variance, which was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). More detailed descriptions of the statistical analyses can be found in the online Supplementary File.

Results

Aggressive behaviour

The mean number of aggressive actions per hour in cubicles was almost twice as high as in the strawyard, both among heifers and cows (Table 1). Overall, animals performed a mean of 6.58 (standard error ±1.19) aggressive acts per hour in cubicles and 4.13 ± 0.94 aggressive acts per hour in the strawyard, however, this difference was not significant ($P = 0.129$). The heifers performed 3.66 ± 1.05 and received 6.94 ± 0.73 mean aggressive acts per hour compared with cows, which initiated a mean of 7.04 ± 1.19 and received a mean of 2.38 ± 0.77 aggressive acts per hour. The differences between heifers and cows were significant ($P < 0.05$ and $P < 0.001$, respectively). Figure 2, which presents aggression by cows and housing type shows that animals kept in the strawyard were less aggressive. However, there was strong concordance between aggression in cubicles and the strawyard at the animal level; animals performing/receiving more aggression in cubicles also performed/received more aggression in the strawyard: $r = 0.68$ ($P < 0.01$) and $r = 0.53$ ($P < 0.05$). There were several individuals who were more frequently aggressors (cows) and several individuals who were more frequently recipients (heifers).

Table 1. Summary of observed variables at pre-calving periods on heifers and cows separately in loose house system with cubicle bedding and in straw yard

	Heifer		Cow		*P*-value[#]	
Variable	Cubicle (n = 9)	Straw (n = 14)	Cubicle (n = 8)	Straw (n = 13)	Parity	Place
Posture						
Lying, %	38.6 ± 4.4^{ab}	49.7 ± 3.5^{b}	31.4 ± 4.7^{a}	51.2 ± 3.7^{b}	0.489	**0.002**
Standing, %	61.4 ± 4.4^{ab}	50.3 ± 3.5^{b}	68.7 ± 4.7^{a}	48.6 ± 3.7^{b}	0.504	**0.002**
Behaviour						
Ruminating, %	31.2 ± 2.8	29.8 ± 2.2	31.3 ± 3.0	30.7 ± 2.3	0.849	0.708
Feeding, %	23.0 ± 2.6	21.2 ± 2.1	22.0 ± 2.7	21.9 ± 2.1	0.949	0.701
Idling, %	36.3 ± 3.2	39.2 ± 2.5	38.6 ± 3.3	36.7 ± 2.6	0.979	0.859
Neighbourhood*						
DistNN1, m	1.14 ± 0.06	1.00 ± 0.05	1.00 ± 0.06	1.04 ± 0.05	0.356	0.399
DistNN2, m	1.71 ± 0.08	1.51 ± 0.06	1.61 ± 0.08	1.51 ± 0.07	0.478	0.055
Aggression						
Aggressions per hour	4.91 ± 1.63^{ab}	2.42 ± 1.31^{a}	8.25 ± 1.73^{b}	5.83 ± 1.36^{ab}	**0.035**	0.129
Aggr. received per hour	7.69 ± 1.14^{a}	6.20 ± 0.92^{a}	3.21 ± 1.21^{ab}	1.55 ± 0.95^{b}	**<0.001**	0.161

The least square means (with standard errors) and factors' *P*-values according to the two-way repeated measures analysis of variance are presented, the least square means without common superscript letter are significantly different ($P < 0.05$, Tukey post-hoc test).
*DistNN1 and DistNN2 denote distances to the first and second nearest neighbours.
[#]Parity by place interaction effect was not significant for any variables (all $P > 0.05$).

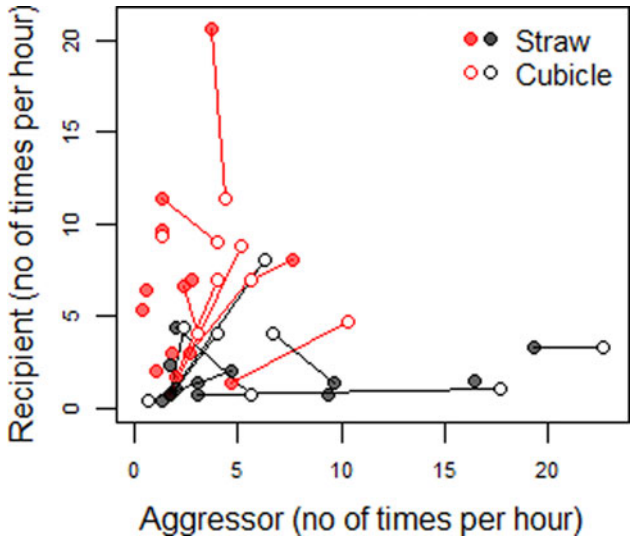

Fig. 2. The average number of times per hour the animal performed and received an aggression act. Animals present in both loose house system with cubicle bedding and straw yard have two points in the figure joined with line, animals present only in one system are notated with single dot. The most aggressive animals in right down corner of the figure as well as the animals receiving the highest number of aggressions in left upper corner of the figure are identified; heifers are in red and cows in black.

The aggression matrix (online Supplementary Fig. S2) shows that there were stable pairs of aggressors-recipients, and the absence of the target may have decreased the number of aggressive actions expressed by the aggressor of the pair. There was a high variation between animals. The mean number of aggressive actions by heifer against heifer was 1.22, cow against cow was 1.50 and by cow against heifer was 2.02. However, one heifer had a mean of 0.28 aggressive actions/hour against a single cow. There was no statistical difference between these means ($P > 0.05$, *t*-tests followed by Bonferroni correction for multiple testing).

Posture and behaviour comparisons in cubicle and strawyard areas

The animals lay down significantly more in the strawyard compared to in the cubicle pen (50.5% and 35.0%, respectively, $P < 0.01$). There was no difference between cows and heifers (Table 1). Correlation analysis of animals observed in both systems showed a weak and non-significant positive relationship between lying in the strawyard and lying in cubicles ($r = 0.34$, $P = 0.192$) at the individual level. Standing times were significantly higher in cubicles than in the strawyards ($P < 0.01$).

There were no differences between the two systems, nor between heifers and cows, for times spent ruminating, feeding or in idling behaviour (Table 1). These behaviours were also not correlated with the number of days before calving (online Supplementary Fig. S1B) and there was no significant tendency for animals ruminating, feeding or idling times to correlate across the two housing systems ($r = 0.24$, $r = 0.29$ and $r = 0.14$, respectively, all $P > 0.05$).

Nearest neighbour

The mean distance to the closest neighbour was about one metre in both loose housing systems, and there was no difference between heifers and cows (Table 1). The mean distance to the second nearest neighbour was 0.15 m longer in the cubicle house compared to the strawyard (1.66 and 1.51 m, respectively, $P = 0.05$).

The periods-by-period analyses of the nearest neighbour scores indicated that in each period there were several pairs of

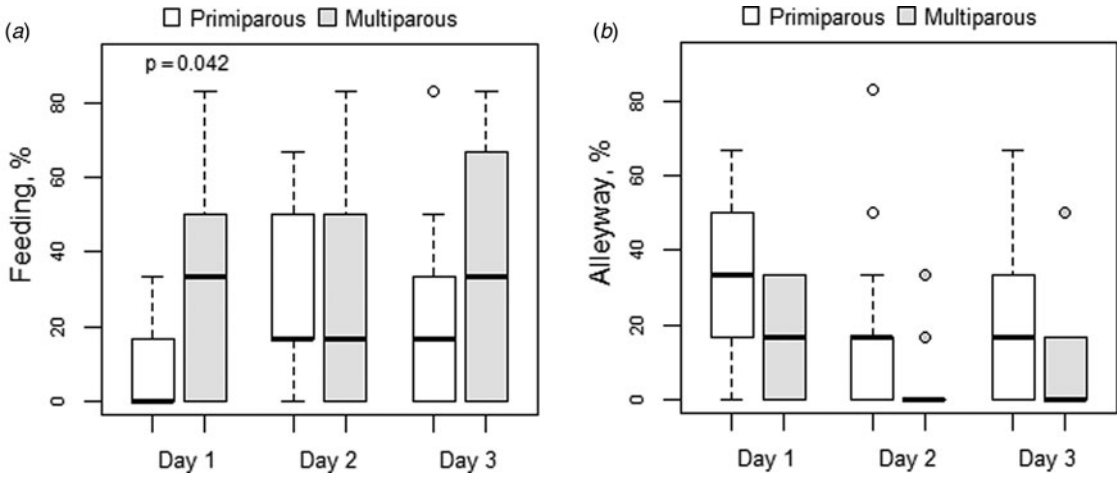

Fig. 3. The percentage of (a) feeding and (b) time spent in alleyway on the first 3 d after calving on primiparous and multiparous cows. The only significant difference in feeding behaviour on the first day in the main herd between primiparous and multiparous cows is denoted with *P*-value (Wilcoxon test).

animals that were observed more frequently close to each other (online Supplementary Fig. S3). However, these neighbours were not consistent. The weighted average of correlation coefficients measuring the concordance of the nearest neighbour scores between periods was only 0.18 (varying between −0.16 and 0.95). The highest correlation was estimated between periods 3 (straw) and 5 (straw) with common animals 1, 10 and 11. However, the concordance between these two periods does not indicate the closeness of these three animals to each other.

Observation in the main herd

After calving, the heifers were observed feeding less frequently compared to cows, especially on the first day after calving. The mean percentage of time spent located at the feed bunk for the heifers was 9.0%, compared with 31.7% for cows ($P < 0.05$, Wilcoxon test; Fig. 3a). For example the eldest cow (5th lactation) was observed feeding five times on the day she was sent to rejoin the main herd and feeding time decreased with each day. On average, heifers had one feeding time on the first day, except one heifer, who fed twice. Heifers were also twice as likely to be observed in the passageway compared with the cows, but this difference was not significant (Fig. 3b).

Activity before and after calving

The mean motion index values, as well the mean number of steps and number of lying bouts, were significantly higher after calving compared with the week before calving, and among heifers compared with older cows (all $P < 0.001$, Fig. 4a, c and d, online Supplementary Table S1). A week before calving animals lay for a mean of 5.0 h more per day than the week after calving ($P < 0.001$, Fig. 4b). However, there was no difference between the mean lying times of heifers and cows. After omitting two cows suffering from milk fever, all differences between time periods and age groups remained the same. Significant time period (pre/post calving) by age interactions had effects on the motion index, lying time and number of lying bouts ($P = 0.01$, $P < 0.05$ and $P < 0.05$, respectively). These results show that over time the heifers' motion index values and number of lying bouts increased more, and lying time decreased more, compared to cows.

Discussion

The results supported the hypothesis that it would be more problematic for a heifer to join a new group than for a cow, who has previously experienced the cycle. The study also showed that an animal's individuality plays a role in adjusting to a new group.

All cows spent more time lying in the strawyard compared to cubicles, as was previously observed by Fregonesi and Leaver (2001). No differences were observed between heifers and cows for comparative lying times in the strawyard or cubicle pen. Campler *et al.* (2018) found no difference in times spent lying in cubicles and a strawyard, although they observed more lying bouts in the strawyard. This is supported by Huzzey *et al.* (2005), who pointed out that when calving approaches, cows spend more time lying, and decrease the time spent in the feeding area. The proximity to calving may have also affected lying times in this study.

After calving heifers walked more and lay more frequently than cows. The same finding for walking was found in a study by Gonzalez *et al.* (2003). The increase in motion index and the decrease in lying bouts was greater for heifers than for cows. The process of calving and entering the main herd is a bigger challenge for heifers, as they have not experienced it before and it will take more time for their activity to stabilize. The animals all walked more and lay more frequently after calving than during the week before calving. Longer walking time is at least in part explained by the time spent walking to the milking parlour and back to their home pen twice a day post-calving. Cows were significantly more aggressive than heifers and received significantly fewer incidents of aggression. The same cows that were more aggressive in the strawyard were also more aggressive in the cubicles and likewise for the animals that were recipients of aggressive acts. Consistency of individual expression and the higher likelihood that heifers are the recipients of aggression has also been shown by Gibbons *et al.* (2009). Regrouping may increase cows' aggression towards heifers and heifers' aggression between each other (Campler *et al.*, 2018).

Heifers made fewer visits to the feed bunk than cows. The first few days of lactation are very important, and less time at the feed bunk means less food eaten and possibly a higher risk of negative energy balance and associated health, fertility and welfare problems. Even when they came to feed it was only for one or two

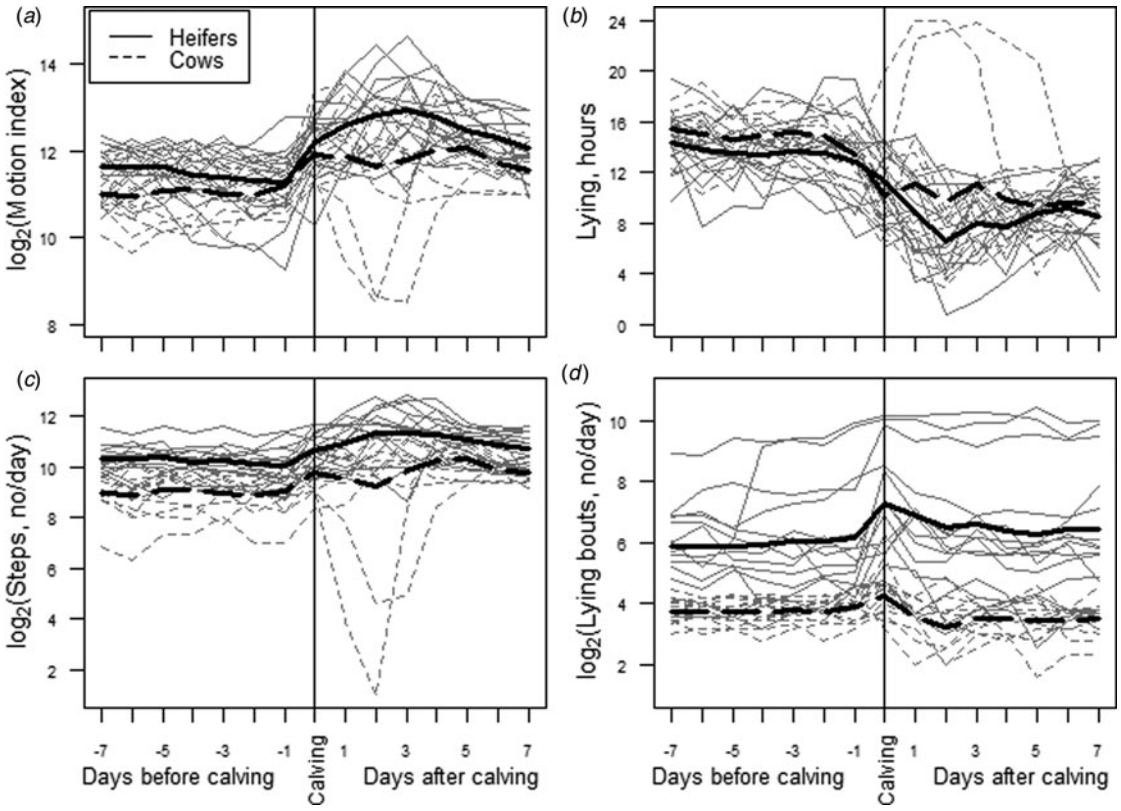

Fig. 4. Animals' behavioural characteristics 7 d before and after calving and at calving day measured with Ice Tag and Ice Qube sensors. Grey narrow lines denote single animals, black strong lines mark the average values by day separately for heifers and cows. For better fit the right skewed characteristics motion index, number of steps and number of lying bouts are binary logarithm transformed and in their scale one unit difference corresponds to two times difference in real values. Two cows with decreasing activity after calving suffered from milk fever.

occasions during the first hour after the morning milking. Not being able to get access to feed may have contributed to the high percentage of heifers who do not survive into the second lactation (e.g. Sherwin *et al.*, 2016). In contrast, one multiparous cow was seen feeding five times on the first day of relocation into the main herd. This could be explained by this cow being in her fifth lactation with many previous regrouping experiences in the same shed. Farm management should ensure that cows who have recently calved are given access to sufficient rest and feed by reducing social competition (Bak Jensen and Proudfoot, 2017). Postparturient cows' social environment may be improved by housing them in separate pens and smaller groups to reduce the level of competition (Cook and Nordlund, 2004; Bak Jensen and Proudfoot, 2017), or housing heifers separately from cows, although this may be impracticable on many farms.

When animals live together in one territory they very rarely space themselves randomly (Lindberg, 2001). Animals in this study were observed closer together in the strawyard than in the cubicles. There was some evidence that cows had individuals that they were preferentially closer to, although this was not consistent. This confirms work by Cooper *et al.* (2010) and Boyland *et al.* (2016), who also found evidence for preferential proximity to particular individual other cows. Boyland *et al.* (2016) and Gygax, *et al.* (2009) pointed out that cows might position themselves closer to other cows who are similar to them, for example, the same breed, weight or milk yield or share similar requirements and preferences.

In conclusion, we were able to support the main hypothesis that regrouping has a greater effect on heifers than on older cows. The results showed that regrouping affects heifers more than cows, and may have major impacts on cow nutritional status, productivity and welfare. Individual animal characteristics should be taken into account because, as shown from nearest neighbour and aggression data, cows changed their nearest neighbours when new animals arrived. Furthermore, individuals who fed and rested more in one housing system did the same in the other. Similar results for aggression observations were found: the more aggressive/subordinate cows in one housing system were also more aggressive/subordinate in the other housing system.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029921000066.

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