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Differences between single and paired heifers in residency in functional areas, length of travel path, and area used throughout days 1–6 after integration into a free stall dairy herd

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ABSTRACT

The integration of heifers into free stall dairy herds is a frequent management procedure, but little systematic research has been conducted on its effect on cow behavior. Previous studies mainly focused on aggressive interactions, but it is also of interest how integration affects the spatial distribution of both the cows in the herd and the integrated heifers. In the present study we integrated a single and a pair of heifers on each of six Swiss working farms in a balanced order. Using an automatic tracking system, we recorded the positions of all the cows and of the integrated heifers at 1 min intervals for six continuous 24 h periods. From these data we calculated the proportion of time the animals spent in the activity area, at the feed rack and in the lying cubicles, their average path length and the area of the barn that they used. We then compared the behavior of the integrated heifers with that of the cows in the introductory weeks. We also compared the behavior of the cows recorded in the control weeks directly preceding the integration and in the introductory weeks. For evaluation we used linear mixed-effects models. Singly integrated heifers spent a higher proportion of time in the activity area (0.29 vs. 0.14; $P < 0.001$) and a lower proportion of time in the lying area (0.40 vs. 0.53; interaction with day, $P = 0.011$) than the cows, whereas the heifers of the pairs mainly spent a lower proportion of time in the feeding area than the cows (0.23 vs. 0.32; interaction with day, $P = 0.044$). Average path length was longer for the integrated heifers soon after introduction but approached the values of the cows later on (interaction with day, $P = 0.012$). The total barn area used by a given animal was largest in the cows and was reduced in heifers integrated singly or in pairs (cows: 341/373 m^2, pairs: 306 m^2, single heifer: 333 m^2; $P = 0.055$). Cows were little influenced in their space use by the integration of a single or pair of heifers. In summary, the behavior of the singly integrated heifers differed more markedly from that of the cows than the behavior of the heifers introduced in pairs during the introductory week. We would therefore recommend integrating pairs rather than single heifers into herds of dehorned dairy cows to ease their integration.

1. Introduction

The integration of heifers into herds of dairy cows is a frequent management procedure for replacing older or sub-optimally productive cows. Given cow replacement rates between 10% and 30% (average maximum parity between 10 and 3), 10–30 single heifers need to be integrated in an average year given a herd size of 100 cows (Mark and Rasby, 2004). In larger herds or with smaller maximum parity, the number of heifers to be integrated is even higher. Typically, heifers are integrated some time before their first parturition to habituate them to the cows

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in the herd and to management procedures such as being driven together before milking and individually entering milking stalls. The process of integration itself is often associated with overt aggression and more subtle avoidance situations (Knierim, 1999; Menke et al., 2000; Sato et al., 1990). In addition, feed intake of the integrated animals can be disrupted over a short period of time (von Keyserlingk et al., 2008) and milk production can be decreased (Sowerby and Polan, 1978) when shifting producing cows between production groups. Similar effects are found if groups of calves and cows are newly mixed (Kondo and Hurnik, 1990; Kondo et al., 1984; Phillips and Rind, 2001) but that situation may not be comparable to the one where few heifers to be introduced are confronted with a stable herd because (1) the heifers are in the minority and (2) may have the disadvantage of younger age and less experience.

Though aggression and changes in feeding patterns can be viewed as substantial consequences of the integration of heifers, the process of integrating dairy heifers has hardly been investigated scientifically (Bøe and Færvik, 2003) to derive recommendations on how the process can be eased. Knierim (1999) reported that heifers greatly increased their lying time from the first to the fourth day after integration, independently of whether they were integrated as single animals or in triplets. At the same time, the number of social interactions and the number of agonistic interactions decreased. In horned cows, Menke et al. (2000) found that the integration of a single heifer led to fewer aggressive interactions compared to the synchronous integration of three heifers. Sowerby and Polan (1978) did not find a dependence of loss in milk production on the proportion of cows shifted when cows were shifted within different groups of the same herd. These previous studies either solely focused on the integrated animals and on (aggressive) social interactions, or at most used average values for the herd members. In addition, these studies were either conducted using one single experimental herd with consecutive integrations (Knierim, 1999; Sato et al., 1990) or using a design that might include a temporal bias (Menke et al., 2000).

The goal of the study was to determine how much heifers and cows used different functional areas of the barn (i.e. areas in which they performed specific behavior patterns such as lying or feeding) after heifers had been introduced into cow herds of mixed parity. We recorded space use data after integrating single and pairs of heifers into dairy herds on six Swiss working farms by means of an automatic position measurement system (Gygax et al., 2007). We were able to continuously record the positions of all the cows and the integrated heifers at 1 min intervals. Using the data collected with this automatic system, we calculated the proportion of time the animals spent in different functional areas (activity area, feeding area, lying area), the average length of their travel paths and the amount of area they used in the barn. We did so for six continuous days of a control week and the six first days after integrating the heifers. We expected that integrated heifers would spend less time in the feeding and lying area due to competition and that their travel paths and area use either increased due to displacements or decreased due to their movement being restricted. Due to a dilution effect, we expected that heifers in the pairs would be less affected than singly integrated heifers.

2. Materials and methods

2.1. Animals, farms and experimental design

The study was performed on six Swiss working farms with mixed parity cows (Table 1). On all farms, lying cubicles were provided at an animal:cubicle ratio of 0.73 to

Table 1
Characteristics of the herds and farms investigated as well as data availability. cS: Control week before single heifer was integrated, iS: integration of single heifer, cP: control week before pair of heifers was integrated, iP: integration of pair of heifers.

	A	B	C	D	E	F
Herd size and composition						
cS/iS	24/25	24/25	26/27	29/30	30/31	44/43
cP/iP	22/24	25/27	24/26	30/32	31/33	43/45
Breeds[a]	BS	BS	Mixed	Mixed	BS	BS
Parity[b]	4.0 ± 0.5	2.6 ± 0.4	4.2 ± 0.5	3.7 ± 0.4	9.9 ± 6.4	3.3 ± 0.3
DIM[b]	179 ± 61	144 ± 62	174 ± 57	175 ± 54	236 ± 51	144 ± 47
Barn and management						
Barn area (m^2)	193	243	195	319	230	340
Floor material[c]	Rubber	Slatted	ma	Concrete	Slatted	ma
Feeding regime[d]	$2\times$	$2\times$	2x	$1 \times$ TMR	$2\times$	$1 \times$ silage
Access to exercise yard	None	None	Temporary	ad lib.	None	ad lib.
Use of pasture	Until 17 h	Until 17 h	None	Mornings	Mornings	None
Proportion of available data corrected for system failures and time on pasture[e]						
cS	94, 67–99	57, 36–67	89, 67–97	85, 67–100	94, 83–100	64, 0–74
iS	92, 56–100	76, 0–92	88, 59–97	73, 0–100	90, 0–100	68, 0–77
cP	89, 31–96	76, 49–85	87, 72–94	75, 44–100	91, 39–100	63, 41–74
iP	93, 0–100	84, 62–100	92, 0–96	73, 44–100	86, 72–94	74, 0–89

[a] BS = mainly Brown Swiss, mixed = mainly Red and Black Holstein on farm C, Brown Swiss and Red Holstein on farm D.
[b] Mean ± standard error of the mean.
[c] ma = mastic asphalt, slatted floor made out of concrete.
[d] Cows were fed maize silage twice daily; TMR: total mixed ration.
[e] Median and range of the proportion of available values per animal and week compared with the total number of measurement intervals.

1.03 (number or animals divided by number of lying cubicles) and an animal:feeding place ratio of 0.79 to 1.03 (number of animals divided by the number of feeding spaces at the feed rack each designed for use by a single animal) in a single roof-covered barn. All cows on the farms were dehorned. Each farm was visited for 7 weeks. In week 1 an automatic tracking system (see below) was set up and was again dismantled in week 7. Weeks 2 and 5 served as control weeks during which the behavior of the cows in the herd was observed. In weeks 3 and 6 a single or a pair of heifers was integrated on the first day and both the cows and the integrated heifers were tracked immediately. A single heifer was integrated in week 3 and a pair in week 6 at three of the farms, and at the other three farms, a pair of heifers was integrated in week 3 with a single heifer integrated in week 6. All the integrated heifers were foreign to the cows, though the heifers in the pair had been kept together before integration. They grew up together since shortly after their birth, potentially with some short breaks due to the fact that they were not always re-grouped at exactly the same time. The integrated heifers were in the median 24 months old (range 18–36) and on day 159 into their pregnancy (range 50–258) at the time when they were integrated. Heifers were introduced to the barn when cows were absent, i.e. fixed in the feed rack during feeding time or on pasture. Heifers had thus at least 15 min to explore the barn without facing social interaction. Week 4 was without observations and was used as further habituation time for the cows and the heifer(s) integrated in week 3. An observational week consisted of six continuous 24 h periods. We thus observed a total of 144 days: (2 control weeks + 2 weeks of integration) $\times$ 6 days $\times$ 6 farms.

On four farms (B, C, D, E), herd composition regarding cows was stable from at least 3 days before the first introduction of a heifer onwards. On farm A, one cow was removed 5 days into the experiment owing to health problems unrelated to the experiment and this animal's data were excluded from all analyses. On farm F, herd composition remained unchanged from one day before the start of the experiment onwards. At this time, two cows were removed form the herd (start of dry period) and were replaced by three others which were due to calve shortly. These three cows were, however, familiar with the rest of the herd and had been away from the herd for their dry period (maximum 42 days). On farm F, two more cows had to be removed in the final week of the experiment owing to health problems unrelated to the experiment. Consequently, their data was not taken into account for that week.

2.2. Automatic recording of locations

The behavior of the cows was tracked within the barn using an automatic recording system (Gygax et al., 2007). In principle the system evaluates locations (of single cows) at a frequency of 300 Hz. In our situation, values received within 1 s were immediately averaged and stored. The transponders on the cows' necks were set to actively transmit for 10 continuous seconds every minute. The first three of these values were discarded due to the fact that the positions could be estimated with increasing precision within this time. The remaining values were again averaged such that a two-dimensional position estimate was collected every minute for each individual cow and heifer. Taking the time the animals spent on pasture and in the exercise yard into account, data availability ranged between 57% and 94% per animal and week implying that, on average, at least one data point was available in the barn every second minute for each animal (Table 1). On 9 of the 144 24 h observation periods (6%), the LPM failed to deliver any location estimates. On an additional 22 days (16%) either half of the 24 h period was missing or the amount of data was markedly reduced throughout the complete day to about 50% of the potential data. These 31 days were excluded from analysis and the analysis is therefore based on the remaining 113 days (78%). In addition, data of single cows was excluded on a day to day basis if the total amount recorded was less than 4 h (240 data points).

2.3. Variables measured

All variables were calculated per cow/heifer and observation day. The area use within the barn was differentiated for three functional areas. A cow was considered to be in the feeding area if her transponder on the neck was within 1 m of the feed rack. She was appointed to the lying area if her transponder was within the area of the lying cubicles. Cubicles were arranged in several rows. The remaining area within the barn which cows used to circulate between the feed rack, the lying cubicles and the water trough was defined as the activity area. The proportion of observations in the feeding area, the lying area and the activity area per total number of observations in the barn for a given animal and day were calculated.

In addition, the average path length was calculated as the sum of all distances connecting the estimated positions in their temporal sequence using straight lines divided by the number of observations of a given cow/heifer and day, resulting in the average path length between positions tracked at 1 min intervals. The barn area used was estimated in m^2 by applying a 95% minimum convex polygon to all the observed location estimates of a given cow/heifer and day (Calenge, 2006). Total barn area corresponded to the sum of the functional areas, i.e. the sum of the activity, feeding and lying area (Table 1).

2.4. Statistical analyses

The proportion of time spent in the activity area, in the feeding area and in the lying area as well as the average length of travel path and the area used served as response variables in two linear mixed-effects models each (Pinheiro and Bates, 2000). The proportion of time spent in the different areas summed to one and thus the models based on these proportions were mutually dependent and only to be interpreted in combination. To account for the assumptions of normality and homoscedasticity in the errors, the proportions of time spent in the different functional areas were logit-transformed and the travel path and area used were log-transformed. Some few outliers which were very likely to have accrued due to measurement error or a small amount of available daily data were excluded (details below). The assumptions were checked using a graphical analysis of residuals.

Model 1: Behavior of integrated heifers. The first series of models focused on the differences in behavior of the singly integrated heifers and the heifers in pairs, at the same time using the data on the cows as a comparative basis. Type of animal (factor with three levels: cow, singly integrated heifer, heifers of the pair), days (course of time: continuous) and their interaction were included as fixed effects. The interaction was dropped from the model if it did not reach significance. Experimental treatment (number of integrated heifers) nested within individual animal nested within farms were included as random effects to account for the dependence in the data due to the experimental design. 0, 4 (2.4‰), 1 (0.6‰), 3 (1.8‰) and 1 (0.6‰) outlier(s) were excluded from a total of 1697 animal days for the proportion of time in the activity area, the feeding area, the lying area, the travel path length and the barn area used, respectively.

Model 2: Changes in the behavior of the cows. The second series of models investigated the changes within the cows in the herds due to the integration of heifers. The explanatory variables type of week (factor with three levels: before integration, after integration of a single heifer and after integration of a pair of heifers) and days (course of time: continuous) and their two-way interaction were included in the models as fixed effects. The interaction was omitted from the model if it did not reach significance. To account for the hierarchically nested data structure, the observation week nested in the experimental treatment (number of integrated heifers) nested within individual cow nested within farms were used as the random effect. 1 (0.3‰), 8 (2.5‰), 7 (2.2‰), 4 (1.3‰) and 1 (0.3‰) outlier(s) were excluded for statistical analysis from a total of 3180 animal days for the proportion of time in the activity area, the feeding area, the lying area, the travel path length and the barn area used, respectively.

To account for a non-linear temporal pattern of the response variables over the course of the observational week without imposing an a-priori shape, the days were included as natural splines with three degrees of freedom of the continuously numbered days (1–6). These three degrees of freedom reflect the maximum number of knots in the splines that could be modeled based on the available observations per animal and week.

The study design for the two model approaches was fully crossed in that all types of animals (singly integrated heifers, heifers integrated in pairs, cows; model 1) and all types of weeks (control, after integration of single heifer, after integration of heifer pair; model 2) were available for all farms. In addition, all animals were in principle observed throughout the complete 6-day period of each observational week. Day was thus a within-subject variable with respect to the individual cows and type (either type of animal, model 1; or type of week, model 2) a within-subject variable with respect to the farms. Type can also be seen as a between-subject variable with respect to the individuals in model 1 and a repeated-measures variable with respect to the cows in model 2. These aspects of the experimental design were accounted for by the inclusion of the random effects as stated above. This means that the number of sampling days provided the starting point for the degrees of freedom for the variable time and the number of animals for the variable type of animal (model 1), the number of cows times the number of observational weeks for the variable type of week (model 2). As usual for hierarchical models, degrees of freedom available for interactions corresponded to those of that variable included in the interaction which was on a lower hierarchical level, i.e. for the interaction of type and day the number of sampling days was relevant. By setting up the model as described, the temporal pattern across the days of each observational week were estimated on a per individual basis and then compared for consistency across types of animals (model 1) or types of weeks (model 2). The sample of six farms was too small to include farm-level variables and we restricted our analysis in respect to farms by including farm as a random effect. Thus, we accounted for the differences of farms, though we do not try to identify their source, and also accounted for the hierarchical structure of the data in all our models.

The available data set was somewhat unbalanced in that data was not available for all cows and days. The restricted maximum-likelihood estimation used in our evaluations can safely deal with such imbalance (Pinheiro and Bates, 2000). As we only considered data from the barn, not all daily estimates and not all estimates of cows from different barns were based on the same number of location estimates. We would expect that this leads to random variation in the measured variables and would thus make it more difficult to actually find systematic patterns. The patterns presented can thus be viewed as strong because they were detectable in spite of the variable number of location estimates per animal and day. Averaging across e.g. all animals of the same type per observational week and farm can not be recommended (Bates, personal communication) because averaged values vary less than the individual values and thus differences of averages on a higher hierarchical level (farm) are exaggerated if the variability on a lower level (such as the cows) was not modeled explicitly. Thus, averaging as an approach to hierarchically structured data needs to be viewed as anti-conservative.

3. Results

3.1. Use of functional areas

In the week after introduction of the heifer(s), an increasing proportion of time was spent in the activity area by the cows, the heifers of the pairs and the singly integrated heifers (Table 2 and Fig. 1a; $F_{2,182} = 23.27$, $P < 0.001$). All these types of animals showed a similar temporal pattern in this functional area with some systematic variations in time (Fig. 1a; $F_{3,1326} = 7.06$, $P < 0.001$). Cows spent larger proportions of their time in the feeding area than singly integrated heifers. Heifers of the pairs spent even less time in that area, though their time there approached the values of the cows towards the end of the observational week (Table 2 and Fig. 1a;

Table 2
Average values and 95% confidence intervals of the use of functional areas (activity, feeding, lying), travel path length and area used in control weeks and weeks where either a single or a pair of heifers was introduced. Means and CI were calculated on the transformed data and then back-transformed. Values are means ± SE of the farm means, i.e. of the means across all values of heifers/cows of the same type on a given farm which in turn are means across all days of the observational week.

	Use of functional areas (proportion)			Average path	Area used
	Activity area	Feeding area	Lying area	length (cm/min)	(m^2)
Control week					
Cows	0.14, 0.01–0.20	0.31, 0.26–0.37	0.52, 0.42–0.62	147, 101–214	378, 253–566
Introductory week single heifer					
Cows	0.14, 0.09–0.20	0.31, 0.26–0.37	0.53, 0.44–0.61	140, 84–235	373, 224–620
Heifer	0.29, 0.17–0.46	0.27, 0.21–0.33	0.40, 0.23–0.60	197, 114–339	333, 188–588
Introductory week heifer pairs					
Cows	0.16, 0.09–0.25	0.32, 0.30–0.35	0.49, 0.39–0.60	134, 98–184	341, 224–520
Heifer of pair	0.22, 0.14–0.31	0.23, 0.17–0.30	0.53, 0.38–0.67	155, 121–200	306, 179–525

interaction: $F_{6,1316} = 2.16$, $P = 0.044$). Heifers of the pairs and cows spent similar proportions of their time in the lying area, but singly integrated heifers spent reduced proportions of time in that area at the beginning of the observational week. Singly integrated heifers increased the time spent in the lying area towards the end of the week, but did not reach the values of the cows nor those of the heifers in pairs (Table 2 and Fig. 1a; interaction: $F_{6,1319} = 2.77$, $P = 0.011$).

Differences in use of functional areas by the cows between control weeks and introductory weeks with single or pairs of heifers were hardly discernible overall (Table 2). Compared to the control weeks prior to the integration of heifers, cows were slightly less often in the

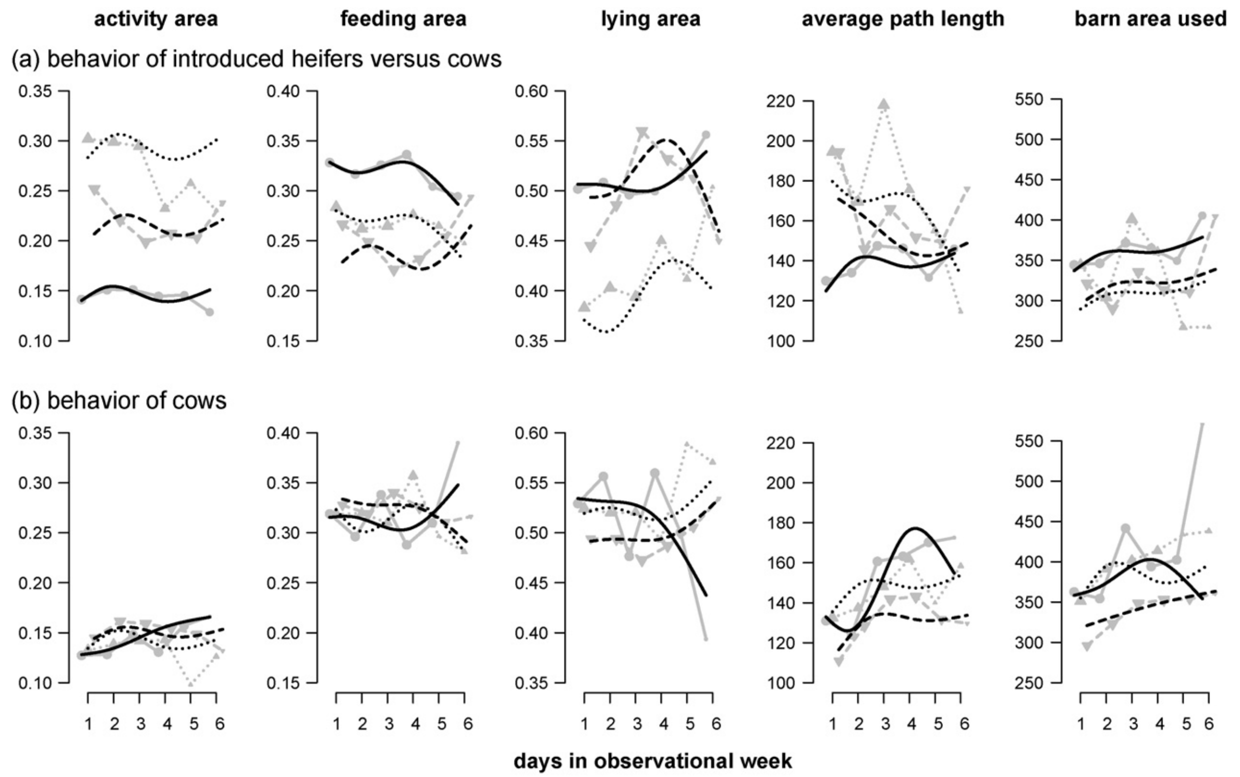

Fig. 1. Proportion of the time budget spent in the three functional areas, average path length between positions at 1 min intervals (cm) and size of the barn area used (m^2) of (a) the singly integrated heifers (dotted lines), the pair of heifers (dashed lines) and the cows (solid lines) during the introductory week and of (b) the cows in the weeks prior to (solid lines) and after the introduction of single (dotted lines) and pairs of heifers (dashed lines). Model estimates are given in black. Averaged data are given in gray (●): (a) cows, (b) cows before the introduction of heifers; (▲): (a) singly integrated heifers, (b) cows after introduction of single heifer; (▼): (a) pairs of integrated heifers, (b) cows after introduction of pairs of heifers). Averages were calculated using the logit transformation for the time budget and the logarithm for average path length and barn area used and were then back-transformed. In the case of cows, individual values were averaged per farm and averages of these farm-averages are shown. Symbol size is proportional to the number of farms with data available on a given day.

activity area early in the introductory week but more often so later in the introductory weeks, with little differences between weeks after the integration of a single or a pair of heifers (Fig. 1b; interaction: $F_{6,2484} = 10.65$, $P < 0.001$). Cows spent a slightly higher proportion of time in the feeding area at the beginning of the week after adding a pair of heifers to the herd compared to control weeks. Later in the week this proportion increased towards the end of the control and decreased towards the end of both introductory weeks. There was little difference between the control week and the week after integrating a single heifer early in the week (Fig. 1b; interaction: $F_{6,2477} = 15.17$, $P < 0.001$). An inverse pattern was found for the lying area (Fig. 1b; interaction: $F_{6,2478} = 18.66$, $P < 0.001$).

3.2. Average path length and barn area used

In the weeks after integration of the heifer(s), the average path length was more or less constant for the cows, whereas it decreased from higher values at the beginning of the week to comparable values towards the end of the week for the heifers integrated as pairs and – more slowly – for the singly integrated heifers (Table 2 and Fig. 1a; interaction: $F_{6,1317} = 2.73$, $P = 0.012$). Barn area use increased for all types of animal throughout the introductory weeks (Fig. 1a; $F_{3,1325} = 7.96$, $P < 0.001$). It was largest for the cows and was clearly reduced for the singly integrated heifers and slightly less so for the heifers of the pairs (Fig. 1a; $F_{2,182} = 2.96$, $P = 0.055$), a pattern inversed for singly integrated and pairs of heifers in the weekly mean values (Table 2).

The average path length of the cows increased greatly in the control weeks prior to the integration of heifers. In the introductory weeks their average path length increased more slowly and was longer if only a single heifer was integrated compared to a pair of heifers (Table 2 and Fig. 1b; interaction: $F_{6,2481} = 25.3$, $P < 0.001$). The area used by the cows fluctuated on a similar level for the control weeks and the introductory week of the single heifer. In contrast, it was on a markedly lower level at the beginning of the introductory week with the pair of heifers but approached similar values by the end of that week (Table 2 and Fig. 1b; interaction: $F_{6,2484} = 4.01$, $P < 0.001$).

4. Discussion

Singly integrated heifers spent more time in the activity area and accordingly reduced their time in both the feeding and the lying area compared to the cows. This potentially led to reduced resting times, an increased duration of time spent standing and to decreased feed intake. von Keyserlingk et al. (2008) observed similar effects in cows that were newly introduced singly into stable groups of 11 cows. The heifers of the pairs spent a similar proportion of time in the lying area compared to the cows, whereas their time in the feeding area was reduced at the beginning of the introductory week and approached similar values to those of the cows by the end of that week. This pattern of use of the feeding area potentially indicates a short-term reduction of feed intake in the heifers of the integrated pairs. In contrast to our study, Knierim (1999) found no differences in feeding and lying times between singly integrated heifers and three heifers integrated at the same time. However, her results may not be representative for dairy herds in general, as she made all her observations on a single herd. The pattern we found, by contrast, was an average over six farms and thus more easily generalisable to further farms. Similarly to our results, Knierim reported a clear increase in lying time from day 1 to day 4 for the integrated heifers.

The average path length for all the integrated heifers was higher at the beginning of the week of integration than the path length of the cows, implying that the heifers moved more, possibly while avoiding cows. By the end of the introductory week, the average path length of the heifers was similar to those of the cows for heifers both introduced singly and in pairs, but the latter seemed already to have approached the values of the cows after half a week, indicating that they habituated faster to the new situation. The size of the barn area used increased throughout the introductory weeks for both the cows and the heifers, but by the end of the week the latter still showed a restricted area of use compared to the former.

Overall, pairs of heifers seemed to integrate into a herd more easily than single heifers in that they spent less time in the activity area during the introductory week than singly integrated heifers, increased their time in the feeding area to the levels of the cows, spent a similar amount of time in the lying area as the cows and approached the average path length of the cows more quickly than singly integrated heifers. This is in contrast to the recommendation to integrate single rather than triplets of heifers based on agonistic encounters (Menke et al., 2000) whereas Knierim (1999) found no clear differences in integrating single or triplets of heifers overall.

Except for the average path length, singly integrated heifers and pairs of integrated heifers did not seem to have adjusted to the new surroundings in the first 6 days that we observed. By the end of the introductory week they spent more time in the activity area and less time in the feeding and lying area, and used a smaller area of the barn compared to the cows. Thus they obviously needed more time to learn about their novel social and non-social surroundings. Sato et al. (1990) estimated that it takes 30–45 days until heifers are completely integrated into herds socially. Though our habituation time was only about half this length after the first integration on each farm (2 weeks: first week of integration with observation, second week with break in observations), it was clearly longer than the 4–6 days used by Menke et al. (2000). This might partly account for the differences in their results and those reported here.

The cows were more influenced by the integration of a pair of heifers compared to the integration of a single heifer. They increased their time in the feeding area and decreased their time in the lying area early in the introductory weeks of the pairs. Moreover, compared to control weeks, cows had reduced average path lengths by the end of the introductory week and a smaller total area used at the beginning of the same week when pairs of heifers was added to the herd. These differences regarding the behavior of the cows in the control weeks and their behavior after the integration of single or pairs of heifers as reflected in the statistical interactions may have been heavily influenced by the temporal patterns found throughout the control weeks. Obviously these patterns were so strong and similar across the farms investigated that they were picked up statistically. It remains unclear, though, what caused these patterns.

The differences in use of different barn areas between singly integrated heifers and heifers integrated as a pair were in the range of about 10, 5 and 10% for the activity, feeding and lying areas respectively (Table 2 and Fig. 1a). These differences were much smaller between the cows after the integration of a single and a pair of heifers (<5% in all areas, Table 2 and Fig. 1b). Cows reduced their average path length between 1 min sampling points by about 20 cm after the integration of a pair of heifers compared to integrating a single heifer, whereas singly integrated heifers had increased average path length between 1 min sampling points by up to about 50 cm in the second half of the introductory week (Table 2 and Fig. 1a and b). There was little difference in barn area used by singly integrated heifers and those integrates as a pair (Table 2 and Fig. 1a). Whereas cows had reduced the area of barn use soon after the integration of a pair of heifers, this leveled out to pre-integration values by the end of one week (Table 2 and Fig. 1b). Overall, the differences between singly integrated heifers and the heifers integrated as a pair were much larger than the differences in space use behavior between cows after integration of a single and a pair of heifers.

Compared to previous studies, we used a specific baseline for each of our integrations in our experimental design. In addition, we used a balanced design such that half of the farms might have had a small carry over effect from integrating a single and the other half from integrating a pair of heifers. This effect was nevertheless so small that we could find systematic differences between integrating a single heifer and a pair of heifers.

5. Conclusions

In conclusion, pairs of heifers seemed to integrate into a herd more easily than single heifers. In contrast, cows in the herd were more strongly influenced by the integration of pairs of heifers in our study but with much smaller absolute differences. In the light of the large differences between singly integrated heifers and those integrated in pairs and the reduction of necessary integrations per herd and year if several heifers are introduced at once, it can be recommended that pairs of heifers rather than single heifers are integrated into herds of dehorned dairy cows given the observed herd sizes of up to 50 cows.

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