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Integration into the dairy cow herd: Long-term effects of mother contact during the first twelve weeks of life

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ABSTRACT

The objective of this study is to investigate the long-term effects of mother rearing on the ability to cope with the challenge of integration into the cow herd shortly before first parturition. Four groups of heifers with different levels of contact with their mothers during the first twelve weeks of life were compared. Two rearing groups were fed via an automatic milk feeder two (A2, $n = 5$) or six times (A6, $n = 5$) a day. The animals in these groups were compared to two treatment groups that were either kept separate from the cow herd and with limited contact with their mothers (M2, $n = 9$) or permanent access to the herd and their mothers through selection gates (MP, $n = 7$). At the age of 25 ± 0.2 months, heifers were integrated individually into the cow herd and observed for 33 h. Social and other behaviour of each heifer was recorded by continuous behaviour sampling, and the identity of the nearest neighbour was determined by time sampling every 5 min. Additionally, faecal samples were taken for measurement of adrenocortical activity. For statistical analysis ANOVA was performed with treatment and breed as fixed factors and herd size, except for social behaviour as covariate. For post hoc testing, heifers were allocated to groups according to contact with the mother (M2 and MP; *mother*) i.e. suckling by the mother compared to automatic feeding (A2 and A6; *automat*) and according to contact with the herd (MP; *permanent suckling* vs. M2, A2, A6; *no herd*). The treatment groups tended to differ in the frequency of self grooming ($p = 0.102$), with *mother*-heifers tending to self-groom more often than *automat*-heifers ($p = 0.109$). *Permanent suckling*-heifers performed self-grooming more often than *no herd*-heifers ($p = 0.048$). Concerning social behaviour, the treatment groups tended to differ in submissive behaviour ($p = 0.062$), with *mother*-heifers being submissive more often than *automat*-heifers ($p = 0.023$) and *permanent suckling*-heifers more often than *no herd*-heifers ($p = 0.055$). The increase in the concentration of faecal cortisol metabolites after the first two days of integration tended to be different between the treatment groups ($p = 0.088$). In our nearest neighbour analysis, one MP-heifer and one M2-heifer could clearly identify their mothers in a herd of 50 cows after 2 years of separation ($p < 0.05$, over chance level). This suggests that rearing with contact with the mother in the first twelve weeks, even if very limited, may have an effect on later behaviour and may lead to possibly enhanced social skills in dairy heifers.

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1. Introduction

The integration of dairy heifers into the cow herd around the time of their first parturition is a common management practice. This kind of regrouping is associated with behavioural changes, stress and loss in production (for review Bøe and Færevik, 2003). It was observed that cows showed less feeding behaviour and a decline in milk production after regrouping (Grant and Albright, 2001; Phillips and Rind, 2001; von Keyserlingk et al., 2008). Heifers after regrouping were shown to have a lower milk yield after regrouping than non-regrouped heifers, and there was, in high-ranking animals only, a significant increase in serum cortisol in response to ACTH challenge was found two weeks after regrouping (Hasegawa et al., 1997). Furthermore, shorter lying durations and reduced allogrooming were reported on the first day after regrouping (Hasegawa et al., 1997; Knierim, 1999; von Keyserlingk et al., 2008).

However, the level of stress during integration of heifers may vary depending on management practices, such as the number of regrouped animals at a time (single vs. pair or group; Knierim, 1999; Menke et al., 2000; Neisen et al., 2009; O'Connell et al., 2008) and time of integration (Lawson, 1999; O'Connell et al., 2008), although results are sometimes contradictory. As well housing conditions such as space allowance are likely to influence the level of heifers' stress during integration as the amount of total space is negatively related to the frequency of agonistic interactions and injuries in dairy cows and heifers (e.g. Menke et al., 1999; Fregonesi and Leaver, 2002).

Together with environmental conditions, the previous experiences of regrouped animals can affect their behaviour and the ability to cope with social and non-social challenges (Mendl, 2001). For example, calves without experience of regrouping occupied lower social ranks than experienced animals when mixed with each other (Veissier et al., 1994). In addition to direct regrouping experiences, differences in the social environment and thus in social experience during early life were also shown to have an effect on future behaviour and ability to cope with different social challenges in cattle (Le Neindre, 1989b; Le Neindre and Sourd, 1984) and other species (mice: Parfitt et al., 2004; guinea pigs: Sachser et al., 1998; fish: Arnold and Taborsky, 2010; sheep: Romayer and Bouissou, 1992; monkeys: Bastian et al., 2003). However, later coping abilities with regrouping were rarely investigated (mice: D'Andrea et al., 2007; Kikusui et al., 2004; cattle: Le Neindre, 1989b). In cattle, early separation from the dam can affect later social behaviour. Heifers reared by a foster cow were shown to be socially more active and to have clearer social structures than heifers reared individually for 10–12 weeks (Le Neindre and Sourd, 1984). Moreover, there is evidence that cows reared by a foster cow show more maternal behaviour, such as prolonged licking and suckling of their calves, than cows reared in isolation (Le Neindre, 1989a). Additionally, in an open field test, isolated-reared animals were shown to be less active and to have a higher respiratory rate than animals reared by foster cows (Le Neindre, 1989b). Furthermore, it was observed that, in Salers but not in Friesian breeds, animals reared by foster cows dominated isolated-reared ones on pasture (Le Neindre, 1989b).

In dairy production, separation from the dam shortly after birth is a common management practice. Mother rearing, i.e. suckling by the mother until weaning at about three months (in dairy milk production) or up to nine months of age (in beef cattle), may not only reduce welfare problems in calves (Roth, 2008; Roth et al., 2009) but also stress in heifers during their integration into the herd shortly before their first parturition. Thus, we were interested in the long-term effect of mother rearing in dairy cattle. To the authors' knowledge, no investigations of the long-term effects of mother rearing in dairy cattle were conducted. What is more, there are only a few studies (Fröberg and Lidfors, 2009; Roth, 2008; Roth et al., 2009; Vaarst et al., 2001) that examined mother- vs. group rearing in dairy cattle. These studies investigated the short-term effects of rearing. Calves reared with their mothers for the first eight weeks show more resting, less solid feed intake and less non-nutritive oral behaviour than group reared calves (Fröberg and Lidfors, 2009). Furthermore, calves with contact with the mother in the first twelve weeks were shown to gain more weight, to have fewer signs of chronic stress in an ACTH-challenge test at the age of eleven weeks and to be more socially active in a social confrontation test at the age of 13 weeks than animals reared in a group of calves without contact with the mother from the second day of life (Roth, 2008; Roth et al., 2009). We used the animals of these latter experiments to compare heifers reared by their mothers with heifers reared artificially in a group in the first twelve weeks of life on their later ability to cope with regrouping into the cow herd. We hypothesised, that those heifers with contact with the mother would show lower stress reactivity and cope better with this social challenge. Consequently, mother rearing could result in improved welfare of animals.

2. Materials and methods

2.1. Animals, housing and management

The experiment was conducted between October 2008 and May 2009 with two dairy cow herds separated by breed (one Black-and-White German Holstein, one German Red Pied) at the Institute of Organic Farming of the Johann Heinrich von Thünen-Institut (VTI; Federal Research Institute for Rural Areas, Forestry and Fisheries) in Trenthorst, Northern Germany. German Holstein cows (GH) had an average milk yield of 7621 kg/lactation, with 4.34% fat and 3.07% protein averages (in 2009); German Red Pied cows (GRP; double purpose type), on the other hand, had an average milk yield of 6157 kg/lactation, with 4.52% fat and 3.31% protein averages (in 2009). Throughout the experiment the herd sizes varied from 45 to 50 (GH herd) and 34 to 45 (GRP herd), respectively, throughout the experiment due to calving, culling and the integration of new animals. Animals were not dehorned, thus all except eight old cows in the GRP herd had horns. Since 2004 the two herds had been kept separately in two identical parts of an open-sided barn with cubicle loose housing. Each part offered a total space of 785 m^2. This corresponds to a total individual space allowance of 15.7–17.5 m^2 in the GH herd and 17.5–23.1 m^2 in the Red Pied herd, depending on the

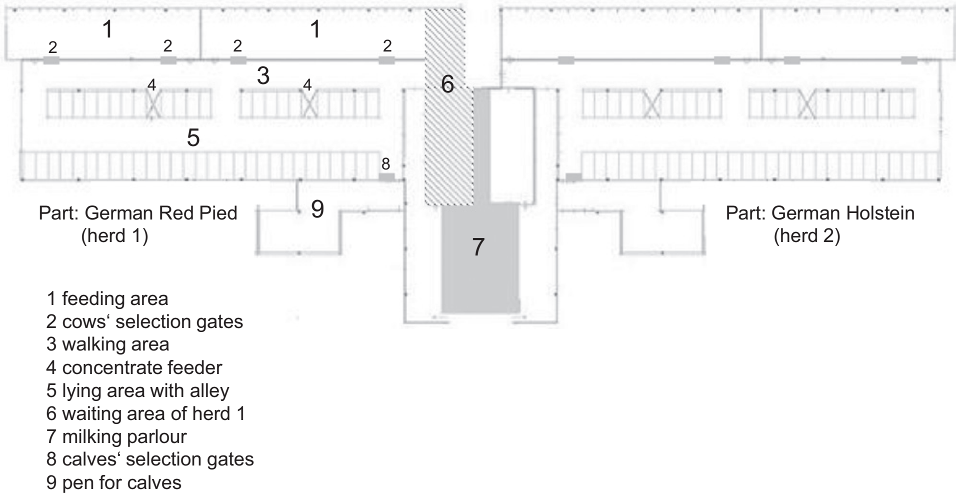

Fig. 1. Scheme of the barn for both herds, milking parlour and calf area. Numbers describe one part (herd 1) of the cow barn. On the right, the barn is shown as available outside milking times; on the left, the waiting area is shown.

actual size of the herd. Fig. 1 shows a scheme of the barn including the two parts for each herd separated by the milking parlour. Each part was divided into three areas: (i) a largely unroofed feeding area with a feeding rack, (ii) a walking area (43.3 m × 3.1 m) providing access to transponder-controlled concentrate feeders and to the (iii) roofed lying area through three short alleys (2.7 m × 2.5 m per alley). This area contained two rows of cubicles (total 50 cubicles) divided by an alley (39.0 m × 3.0 m). The feeding area (total 43.3 m × 4.1 m) was additionally separated into two divisions and could be entered through transponder-controlled selection gates. The individual cows had access to one of the feeding areas, depending on milk yield and stage of lactation.

Cubicle floor was composed of straw and cow dung, forming a compact mattress (straw mattress) and thus resembling deep-litter bedding. The cows were milked two times a day, starting at 5:15 and 15:45 h (duration about 2 h per milking). After milking, the lying area was closed, and the animals were restrained in the feeding rack until 9:00 and 18:00 h, respectively. A mixed ration (silage and concentrate feed) was provided once per day during afternoon milking. To prevent animals from entering the feeding area while fresh feed was provided, the selection gates were closed between 15:00 and 16:00 h.

Calves stayed in the calving pens together with their mother for 1–5 days after calving, depending on treatment (see Section 2.2.1). Afterwards, calves were kept in one calf group per herd in identical pens located adjacently to the cow barn (see Fig. 1, number 9). Each pen was subdivided in a resting and a running area (for details Roth et al., 2009). According to the treatment (see Section 2.2.1), calves had transponder-controlled access to either their mothers or to the milk feeder (FA Förster-Technik GmbH, Engen, Germany). Silage, hay, concentrate and water were available ad libitum. Calves of both herds/breeds were weaned after twelve weeks and then moved into a single joined calf group until the age of seven months. Thereafter, all experimental animals were kept successively in each of three groups of young cattle, differing in the age of the group members (age 7–16 months; 16 months until successful insemination; pregnant heifers, max. age 31 months). Group size varied between 25–30 animals in the younger group (7–16 months) and 30–40 animals in the two older groups. All groups of young cattle were housed in a barn with deep-bedded loose housing during winter and pastured during summer.

2.2. *Experimental design and procedure*

2.2.1. *Treatment*

In total, 26 heifers of both breeds (GH $n = 12$, GRP $n = 14$) were included in the experiment. These heifers belonged to four treatment groups that different rearing condition during the first twelve weeks of life. They had been part of a study on short-term effects of contact with the mother, where male and female calves were investigated (in total 57 calves – GH $n = 25$, GRP $n = 32$; Roth, 2008; Roth et al., 2009). From these calves 26 female calves reached the stage of being integrated into the cow herd. The sample size given in the following description refers to these animals used in our experiment. Table 1 gives an overview on sample size for treatments and breeds. Calves were allocated to the four treatment groups according to the mother's lactation number and season – in sequence of their birth, i.e. the different treatment groups were studied in parallel. Two treatment groups (*automat*, A) were fed similar number of whole milk via an automatic milk feeder at different feeding intervals without contact to the mother or herd. Calves were separated and moved to the calving area in the first 24 h after calving. They were bottle fed the first five days. Afterwards they were fed up to 8 l whole milk twice (actual frequency of milk intake 2.3 ± 0.2, portion size: 4 l; A2, $n = 5$), or up to six times (actual frequency of milk intake 4.9 ± 0.2, portion size: 1–2 l; A6, $n = 5$) a day via an automatic milk feeder (for distribution of animals over the two breeds see Table 1).

Table 1
Overview of rearing conditions of the different treatment groups in the first twelve weeks of life. Number of heifers in the different treatment groups, source of milk, contact with the herd, different breeds German Red Pied (GRP) and German Holstein (GH) and the number of mothers whose were present in cow herd at time of integration.

Treatment	*n*	Source of milk	Contact with herd	GRP	GH	Mothers present
2 times daily (A2)	5	Automat	No herd	3	2	2
6 times daily (A6)	5			2	3	4
Restricted suckling (M2)	9	Mother		4	5	7
Permanent suckling (MP)	7		Permanent suckling	5	2	5

The calves in the two other treatment groups (*mother*, M) were housed together with the mother in the calving pen for the first five days, afterwards the mother was moved back to the herd and the calves were moved into the calf group. Then they had either restricted contact with their mothers, i.e. mothers were moved twice a day (before milking) in a part of the calf area (closed for the other calves at that time) and calves were allowed to stay with and suckle their mother for 15 min (M2, $n = 9$) or calves had unrestricted contact with their mother, i.e. they could enter the cow housing through transponder controlled selection gates and thus had permanent access to their mother and to other adult cows (MP, $n = 7$). Calves in all four treatment groups shared the same calving area and thus formed one calf group per breed with all calves staying there until the age of 12 weeks and leaving thereafter. For further details, see Roth (2008) and Roth et al. (2009). Table 1 summarises the distribution of animals over treatments and breeds.

After the first twelve weeks of life, female animals in the different treatment groups were, as described in Section 2.1, kept together according to age until their integration into the cow herd, whereas, male animals were sold and left the dairy farm after the first twelve weeks. At the time of the present experiment the 26 heifers were aged between 24 and 28 months (25 ± 0.2 months) and had a mean weight of 627 kg ± 46 kg (A2: 642 ± 31, A6: 610 ± 53, M2: 623 ± 60, MP: 632 ± 33). In the majority of cases (see Table 1), mothers were present in the herd at the time of the integration of the heifers.

2.2.2. Procedure of integration

The individual integration of the experimental heifers into the cow herd of the respective breed took place between 28 and 34 days before expected parturition (20–44 days before actual parturition). On the day of integration, the experimental heifer was weighed at around 7:30 h and then transported by trailer from the straw yard or pasture to the cow barn. At 9:00 h the heifer was moved from the trailer into the cow barn. Until 9:30 h, the rest of the cow herd was restrained in the feeding rack, so that the heifer could explore the cubicle barn undisturbed from cows during the first 30 min. The heifers were gathered together with the cows during milking time and had to pass through the milking parlour. If they did not pass through the selection gates, until half an hour after leaving the milking parlour during the first evening milking, i.e. around 18:30 h of the first day, they were assisted by farm staff members and lead to the feeding rack.

2.3. Data recording

2.3.1. Behavioural observations

The observation of each focal animal started immediately after the heifer had entered the cow barn at 9:00 h and lasted until the afternoon milking of the next day, i.e. 33 h later. The observation time was divided into seven sessions. In total, three observers were designated for fixed sessions.

The behaviour and the location of the focal animal was observed directly by continuous focal-animal behaviour sampling (Martin and Bateson, 1993) and recorded on Husky®, FC-PX5 using the software Observer® (3.0, Noldus, NL). If behaviour, e.g. lying was not observed within the 33 h of observation, the latency for this behaviour was set equal to the total observation time. Social interactions were measured by identifying both initiator (I) and receiver (R) and herd size was determined by relating the number of observed social interactions to the number of potential interaction partners, i.e. the number of interactions was divided by herd size. The definitions of behavioural parameters were subdivided into individual behaviour and social behaviour (Table 2). Furthermore, for the analysis of social behaviour, aggressive interactions (see Table 2) were summarised into initiated aggression (if heifer was initiator) and received aggression (if the heifer was the receiver of that behaviour).

Additionally, the nearest neighbour was recorded by time sampling every 5 min (Martin and Bateson, 1993) to find out whether heifers recognised their mothers and if present, sought proximity to them. During the observation, kinship between cow and heifers was unknown to the observer.

2.3.2. Adrenocortical activity

For assessing adrenocortical activity, we measured cortisol metabolites in faeces. Faecal cortisol metabolites are excreted with a delay of about 12 h in cattle. Thus the measured concentrations reflect glucocorticoid production about 10–12 h ago (Palme et al., 1999).

All samples were taken between 7:30 and 8:30 h on the different days and thus reflect the evening before sampling. Faeces was sampled at day 0 (day of integration), days 1, 2, 4 and 7. On day 0 faeces were sampled when the heifers were restrained in the weighing scale, on the following days while heifers being restrained in the feeding rack. For further analysis we used the faecal sample taken shortly before integration (day 0) as a baseline. Furthermore, we assumed that the faecal samples from the two days after integration would reflect the period of highest stress levels

Table 2
The definitions of behavioural parameters were subdivided into individual behaviour and social behaviour. We recorded frequency (F), total duration (TD) and latency (L) of the different behavioural elements.

Description of recorded behaviours	
Individual behaviour	
Lying in cubicles (F, TD, L)	Resting and sleeping behaviour, chest and abdomen touch the ground in cubicles
Lying in alley (F, TD, L)	Resting and sleeping behaviour, chest and abdomen touch the ground in alleys or on the floor in the feeding area
Feeding (TD)	Muzzle in the feed or chewing of feed
Self-grooming (F, TD)	Licking or scratching their own body
Exploration (F, TD)	Smelling or licking of objects in the barn environment
Social behaviour	
Submissive (F, TD)	Head position below horizontal to the withers, external ears pendulous
Aggressive interaction (aggression) summarised by	
Push away (F)	I pushing R with her head, resulting in R moving away or changing the position
Displacing (F)	I pushing R with her head, resulting in R moving away or changing the position and I pushing R again away
Butting (F)	I butting R with her head, but R remains
Chase up (F)	I pushing a lying R, resulting in R standing up
Fight (F)	I touches forehead or horn basis of R, and both animals push each other forcefully
Threat (F)	I adopts a typical threat posture (presenting the forehead with inclined head) without touching R, and R moves away or changes the position or remain
Socio-positive interaction summarised by	
Social licking (F)	I licks R (except anogenital area)
Head play (F)	I touches forehead or horn basis of R, and both animals rub or push gently each other without force

(days 1 and 2). To examine the duration of increased stress levels after integration, we also took samples on days 4 and 7.

A sample from one animal taken on day 7 was excluded from analysis due to high values (Patel et al., 1996), caused by premature parturition.

Faecal samples were taken directly from the rectum of the heifer, were frozen within 10 min and stored at −20 °C until further analysis. For extraction, the faeces were thawed, and 0.5 g of the sample were weighted and supplemented with 4 ml of methanol (80%) and 1 ml of water. Concentrations of 11,17-dioxandrostanes were measured by an 11-oxoaetiocholanolone enzyme immunoassay (EIA), as described by Palme and Möstl (1997).

2.4. Statistical analysis

All statistical analyses were carried out with the software package PASW Statistics, Version 17. In a first step, we calculated an ANOVA (analysis of variance) with the fixed factors treatment (A6, A2, M2, MP) and breed (GH, GRP) for all variables. For all parameters except for social interactions, the herd size was added as covariate. During ANOVA breed and herd size were stepwise excluded from the models if they had no significant or tendency ($p < 0.1$) effect. All behavioural variables were transformed into ranks in order to make the inference robust against outliers and other deviations from the normal distribution. However, due to special structure (e.g. lying in cubicles contained many ceros) of frequency of submissive behaviour (submissive, F) and total duration of lying in alleys (lying in alleys, TD) we used a Kruskal–Wallis test in this case.

For statistical analysis of adrenocortical activity, we did not carry out a repeated measurement analysis. Since we expect the effect to vary both with the individual and the point of observation, such a model would be too complex given very low sample size per treatment group. Thus, in view of the multiple testing issue we focussed on the contrasts which were most interesting to us. As with paired *t*-tests, we considered the differences to "baseline" to get independent observations and to remove the component of variance associated with the individual animal. We think that the faecal samples from the two days after integration (day 1 and day 2) best reflect the period of highest stress levels. Therefore, we calculated the differences from baseline (day 0) to the day of integration (day 1, Cort_Change0_1) and to the day after integration (Cort_Change0_2).

We carried out post hoc tests for those response variables, where treatment had an influence with an error probability of 10% ($p < 0.1$) in the ANOVA or Kruskal–Wallis and used Mann–Whitney *U* tests. Significant results may not only arise by differences in means, but also by other differences in distribution. To reduce the number of post hoc tests and thus multiple testing we aggregated the individuals into two different group allocations. On the one hand, according to contact with the mother, i.e. suckling by the mother compared to automatic feeding (*mother* vs. *automat*), on the other hand, according to contact with the herd (*permanent suckling* vs. *no herd*) for overview see Table 1.

To test the difference between treatment groups in passing the selection gates for the first time with or without help we used the Pearson Chi2 test (help yes/no).

For each experimental heifer, it was tested whether her mother was her nearest neighbour more often than would be expected by chance according to the following formula:

$$Z\text{-value} = \frac{\hat{p} - p_0}{\sqrt{p_0 * (1 - p_0)}} * \sqrt{n}$$

$$p\text{-value} = 1 - \Phi(Z\text{-value})$$

where $\hat{p}$ is the proportion of mother as nearest neighbour, p_0 the proportion of mother as nearest neighbour under the null model of randomly chosen positions, and n is the total number of scans.

With all variables presented in boxplots, tables and in the text, we used the original non-transformed values, except for lying in alleys F and L, were we present estimated values for breed effects.

3. Results

3.1. Behaviour

3.1.1. Individual behaviour

Only four animals (1 A2, 1 A6, 2 MP) laid down before the first evening milking, i.e. within 6 h after the start of the integration. Another eight heifers (1 A2, 5 M2, 2 MP) laid down within 8 h after evening milking and feeding time until around 24:00, when the majority of cows were lying. In the whole observation period of 33 h, lying time was quite short: for all animals, the median was only 8.6% of observation time, i.e. about 3 h, with the longest lying time being 8.3 h and the shortest only 12 min. Regarding the location of lying, three animals did not lie down in cubicles (2 A2, 1 M2) and eleven did not lie down in alleys (2 A2, 2 A6, 5 M2, 2 MP) within the 33 h of observation (latency was set equal observation time). No significant differences (Fig. 2a–c) were observed with respect to frequency (F = 2.073; df = 3,26; p = 0.133), latency (F = 1.620; df = 3,26; p = 0.213) and total duration (F = 0.636; df = 3,26; p = 0.600) of lying in cubicles or in alleys (frequency: F = 0.460; df = 3,26; p = 0.713; latency: F = 0.466; df = 3,26; p = 0.710; total duration: p = 0.750).

Besides, herd size had a significant influence on the frequency (Correlation Pearson = −0.119; F = 5.978; df = 1,26; p = 0.024) and latency of lying in alleys (Correlation Pearson = 0.340; F = 6.020; df = 1,26; p = 0.023). Furthermore, breed tended to influence the frequency (estimated values, mean ± std error; GH: 18.16 ± 2.85 events/33 h; GRP: 9.79 ± 2.51 events/33 h; F = 3.426; df = 1,26; p = 0.079) and latency (GH: 9.31 ± 2.82 h; GRP: 17.10 ± 2.49 h; F = 3.005; df = 1,26; p = 0.098) of lying in alleys.

With respect to feeding, only ten animals (2 A2, 1 A6, 5 M2, 2 MP) fed within the first 6 h until the first milking time after integration. The total duration of feeding did not differ significantly between the treatment groups (F = 1.063; df = 3,26; p = 0.385; Fig. 2d). To get into the feeding area, the heifers had to pass through transponder-controlled selection gates. After milking, most heifers passed through the gates on their own; eight heifers did not do so within half an hour after passing through the milking parlour, i.e. 8 h after integration (2 A2, 1A6, 4 M2, 1 MP). The treatment groups did not differ with respect to passing through selection gates with or without help (Chi2 = 2.155; df = 3,26; p = 0.541).

Concerning self-grooming, the treatment groups differed in frequency (F = 2.328; df = 3,26; p = 0.102, Fig. 2e), but not in duration (F = 1.052; df = 3,26; p = 0.389, Fig. 2f). According to post hoc tests *mother*-heifers tended to self-groom more often (*mother*: 61 (25–214) events/33 h; *automat*: 45 (15–88) events/33 h; p = 0.109) and permanent-heifers performed self-grooming significantly more often than heifers reared without contact with the herd (*permanent suckling* 82 (25–214) events/33 h; *no herd*: 47 (15–126) events/33 h; p = 0.048).

No significant differences were found between the treatment groups in the duration of exploration (A2: 4.29 (2.14–6.29) events/33 h, A6: 2.90 (2.24–5.40) events/33 h, M2: 3.92 (1.86–5.25) events/33 h, MP: 3.06 (1.95–4.97) events/33 h, F = 1.001; df = 3,26; p = 0.411). With the exception of frequency and lying in alleys, none of the recorded individual behaviours were influenced significantly by either breed or herd size.

3.1.2. Social behaviour

Regarding aggressive behaviour, the number of initiated aggressive interactions ranged from one to 276 events per 33 h, while received aggression ranged between 193 and 514 events. As to socio-positive behaviour, we only observed a total of 86 events in all animals. While on 74 occasions the experimental heifer was licked by a resident cow, it initiated licking only 12 times. Head play was never observed. The treatment groups did not differ in aggressive (initiated: F = 1.741; df = 3,26; p = 0.188; received: F = 0.140; df = 3,26; p = 0.935; Fig. 3a and b) or socio-positive behaviour (initiated: A2: 0 (constant) interactions/33 h, A6: 0 (0–0.02) interactions/33 h, M2: 0 (0–0.08) interactions/33 h, MP: 0 (0–0.04) interactions/33 h, F = 0.983; df = 3,26; p = 0.419; received: A2: 0.0833 (0–0.28) interactions/33 h, A6: 0 (0–0.04) interactions/33 h, M2: 0.04 (0–0.27) interactions/33 h, MP: 0.046 (0–0.25) interactions/33 h, F = 1.490, df = 3,26; p = 0.245). Submissive postures were observed between five and 67 times in all heifers. The frequency tended to differ between treatment groups (p = 0.062, Fig. 3). In post hoc tests *mother*-heifers showed submissive postures significantly more often than *automat*-heifers (*mother*: 0.69 (0.26–1.53) interactions/33 h; *automat*: 0.36 (0.12–0.97) interactions/33 h; p = 0.023); heifers in the permanent suckling group tended to show more submissive postures than heifers without contact with the herd (*permanent suckling*: 0.39 (0.12–1.24) interactions/33 h; *no herd*: 0.80 (0.43–1.53) interactions/33 h; p = 0.055). Again, no significant influence of breed on social interactions was found.

3.2. Mother as nearest neighbour

By providing information on kinship relations, it could be revealed that for one heifer with permanent contact and one heifer with restricted contact the mother was found to be the nearest neighbour above chance level (p < 0.05) and for another heifer with restricted contact in tendency (p = 0.06). Moreover, it was observed that the above mentioned heifer in the permanent suckling group tried to suck on her mother. The percentage of having the mother as nearest neighbour did not differ between treatment groups (F = 2.019; df = 3,26; p = 0.158, Fig. 3d).

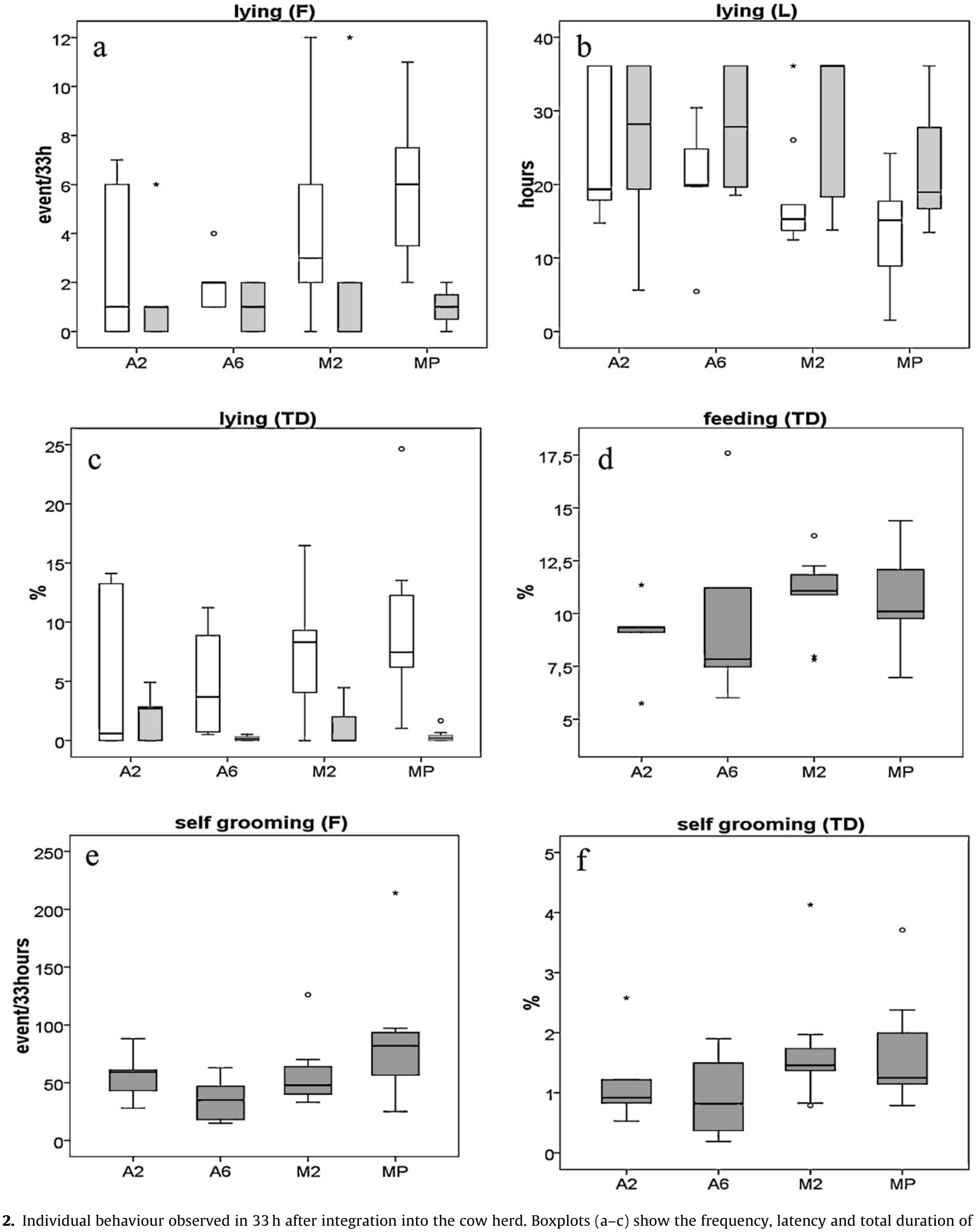

Fig. 2. Individual behaviour observed in 33 h after integration into the cow herd. Boxplots (a–c) show the frequency, latency and total duration of lying (light = lying in cubicles, grey = lying in alleys), (c) total duration of feeding, (d) frequency and (e) total duration of self grooming of heifers of the four treatment groups, two times daily automat (A2, *n* = 5), six times daily automat (A6 *n* = 5), two times daily suckling by mother (M2 *n* = 9) and permanent contact to the mother and herd (MP *n* = 7). Data are presented as box-and-whisker plots, with boxes representing the first and third quartiles, the central bar the median and whiskers the minimum and maximum before being outliers; dots represent outliers and stars extremes.

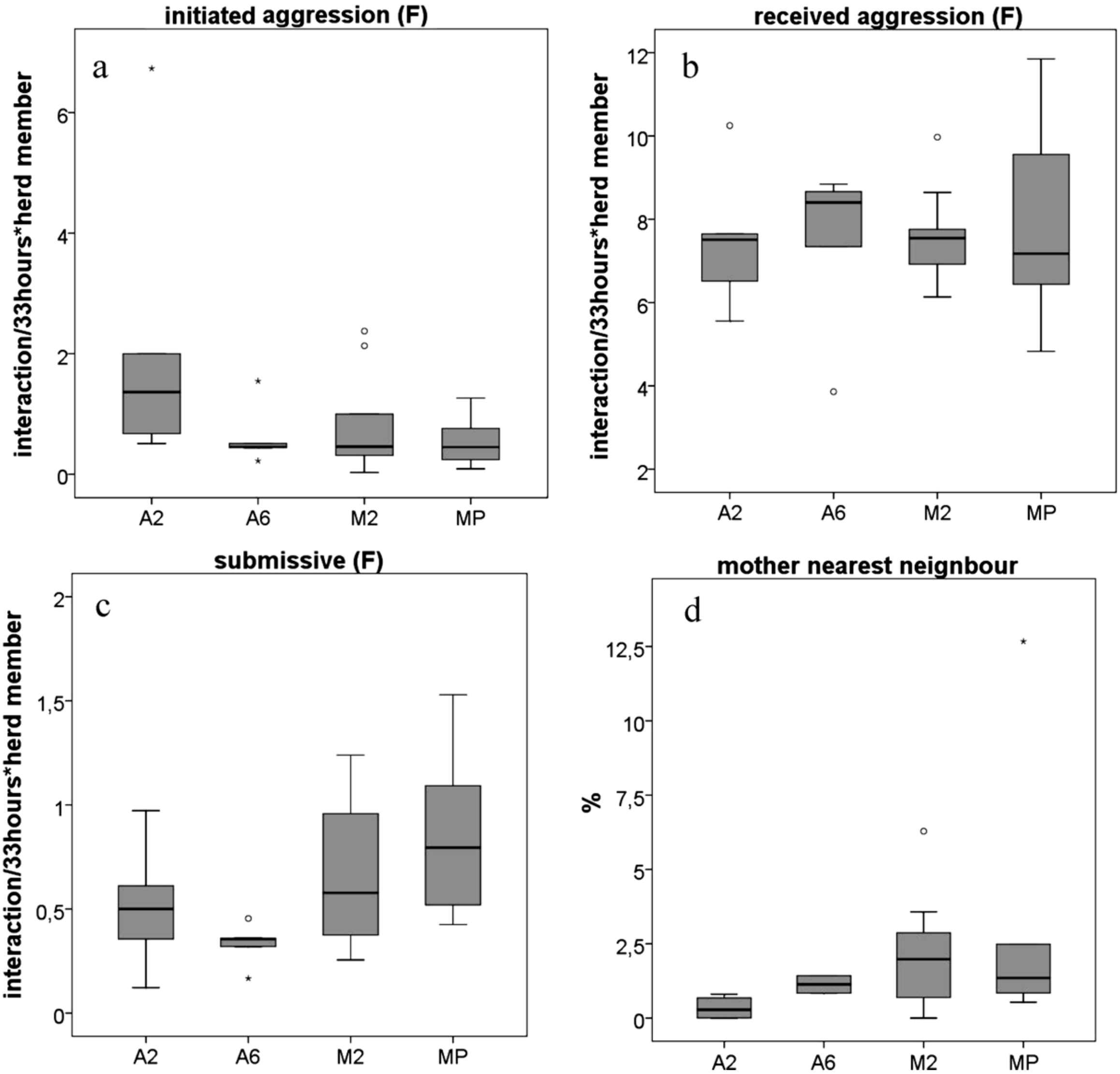

Fig. 3. Social behaviour observed in 33 h after integration into the cow herd. Boxplots of the frequency of initiated aggressive interactions (a), received aggressive interaction (b) and submissive postures (c) of heifers from different treatment groups two times daily automat (A2, $n = 5$), six times daily automat (A6 $n = 5$), two times daily suckling by mother (M2 $n = 9$) and permanent contact to the mother and herd (MP $n = 7$). Further, boxplot of percentage of mothers being the nearest neighbour (d) of heifers with 2 times access to the automat (A2, $n = 4$), six times to the automat (A6, $n = 2$), two times suckling by the mother (M2, $n = 7$) and permanent access to mother and herd (MP, $n = 5$).

3.3. Adrenocortical activity

In all experimental heifers ($n = 26$) faecal cortisol metabolite concentrations from the first sample (day 0) ranged from 92 to 459 ng/g (222 ± 97 ng/g). On the other days (days 1, 2, 4 and 7 on average), concentrations ranged from 147 to 592 ng/g (287 ± 110 ng/g). In Fig. 4a, the course over time is presented for all heifers in the different treatment groups. In most of the heifers, cortisol metabolite concentration increased from the baseline (day 0) to day 1. In three animals, a remarkable decrease (154 ng/g for 1 M2 and 91 and 225 ng/g, respectively, for 2 MP) was detected, in four other animals, there was a negligible change (<10 ng/g). The heifer with the largest decrease in cortisol metabolite concentration was the one trying to suck on her mother. However, no treatment effect was detected on Cort_change0_1 ($F = 1.325$; df = 3,23; $p = 0.293$, Fig. 4b). In contrast, the treatment groups tended to differ in the difference to baseline to day 2 (Cort_change0_2; $F = 2.522$; df = 3,26,; $p = 0.088$, Fig. 4b). In post hoc tests for Cort_change0_2, no significant differences were detected between *mother*- and *automat*-heifers (*mother*: 41.91 (−269.94–334.62) ng/g; *automat*: 59.84 (−65.23–460.24) ng/g; $p = 0.363$) nor between heifers in the permanent suckling group and heifers without contact with the herd (*permanent suckling*: 26.07 (−269.94–225.94)

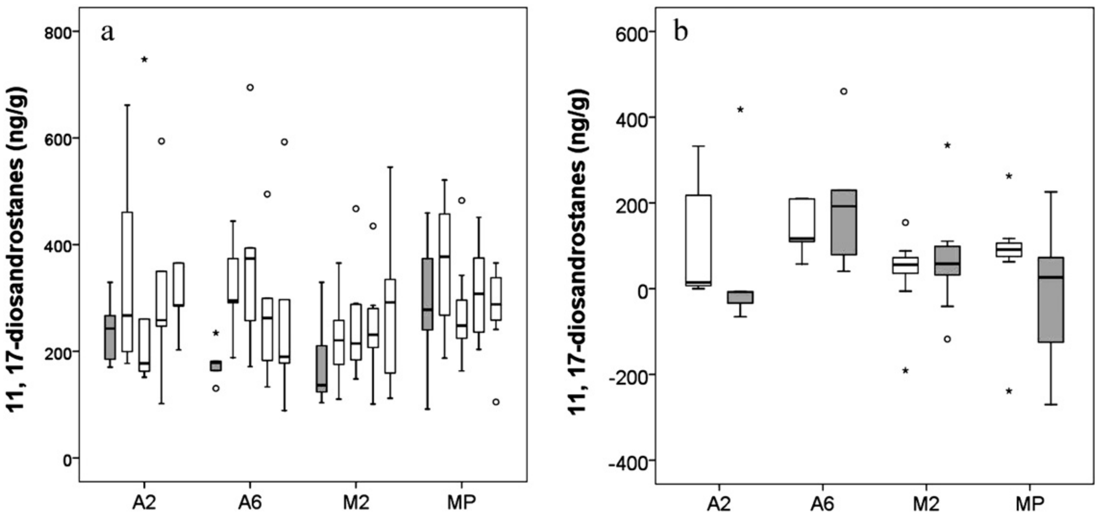

Fig. 4. Boxplot (a) shows the concentration of glucocorticoid-metabolites (11, 17-dioxandrostanes, ng/g) in faeces of heifers of the different treatment groups (A2, $n = 5$; A6, $n = 5$ except day 7 $n = 4$, M2, $n = 9$; MP, $n = 7$) over time, first boxplot demonstrate baseline (light grey; day 0), second 1. day of integration (day 1), third 2. day after integration (day 2), fourth 4. day after integration (day 4) and fifth the 7. day (day 7) after integration, respectively. Boxplot (b) shows the differences to baseline from day 1 (light; Cort_change0_1) and day 2 (grey; Cort_change0_2) of cortisol metabolites for the different treatment groups (A2, $n = 5$; A6, $n = 5$; M2, $n = 9$; MP, $n = 7$).

ng/g; *no herd*: 58.41 (−117.59–460.24) ng/g; $p = 0.209$). Again, there were no significant effects of breed and herd size.

4. Discussion

The results confirm again that the integration into the cow herd is highly challenging for heifers, involving reduced lying times, many agonistic interactions and, mostly, increased levels of cortisol. The results also demonstrate for the first time that there are indications that contact with the mother as compared to group rearing in the first twelve weeks of life can have an effect on the later behaviour of heifers during integration into the cow herd. Heifers reared with contact with their mothers performed self-grooming and submissive head postures more often during integration. A higher frequency and lower latency to lie and longer duration of feeding in animals reared with contact with the mother could not be confirmed by an ANOVA. In addition, cortisol increase differed between the treatments.

Sample size was very small, especially in the two automat groups (A2, A6). We expect this to affect the power of the statistical tests, i.e. we might not have found differences, where there are some, while the type I errors are still guaranteed assuming that the model assumptions are approximately satisfied (Zar, 2009). Taking this into account, our preliminary study gives interesting suggestions on long term effects. Our investigations of long-term effects of rearing systems were based on a previous experiment where short term effects on challenge response and behaviour could be confirmed (Roth, 2008). In our view, a comparison with long term effect is valuable although the results must be interpreted very carefully. Strictly speaking: we could not prove many of the results although this may be due to the low power caused by our small samples. At least the reported numbers and box-plots give an idea about the magnitude of the effects.

4.1. Individual behaviour

The duration of lying of dairy cows in stable groups is around 10–12 h per day (24 h) in cubicle housing (Dechamps et al., 1989; DeVries and von Keyserlingk, 2005; Krohn and Munksgaard, 1993). In our study, most of the heifers did not lie down on the first day of integration, i.e. in the first 15 h after integration and half of the animals were lying down for less than 3 h in total during the whole observation period of 33 h, with the shortest lying time being only 12 min. This is in line with previous studies where reduced lying time after regrouping was recorded (Hasegawa et al., 1997; Knierim, 1999; von Keyserlingk et al., 2008). Cows have a strong motivation to lie down, so that lying has the highest priority over eating and social contact (Munksgaard et al., 2005). Thus a markedly reduced lying time points to a highly challenging situation and reduced welfare of heifers. In our study, we found no significant differences between the treatment groups, although there seemed to be, as visualised in the boxplots (Fig. 2a and b) a numerical trend for a higher frequency and lower latency of lying in cubicles in mother reared animals.

Lower space allowance and a higher level of competition for lying places lead to less and later lying (Fregonesi et al., 2007). In the same direction, we found, the higher the herd size was, the less often and later the animals laid down in alleys. Concerning breed effect, GH tended to lie down more often and earlier in alleys than GRP. While differences between breeds in reactivity to a stressful situation exist (Boissy and Le Neindre, 1990, 1997), it is not possible for us to disentangle breed and herd effect. So far no study has compared German Holstein with German Red Pied animals.

Less than half of the heifers fed before the evening milking on the first day, and feeding durations were very short, even when the definition of feeding is taken into account. It is known that the regrouping of cows reduces feeding (Phillips and Rind, 2001; Schirmann et al., 2011; von Keyserlingk et al., 2008). In our study, all animals were restrained in the feeding rack after milking, so that heifers could, in most cases, enter the feeding area and feeding rack without displacement. Again, we found no significant difference in the total duration of feeding between the treatment groups, but numerically duration was longer in mother reared heifers. It is possible that the use of self-locking feed barriers had contributed to this. All heifers in the different treatment groups had no experience with the self-looking feed barrier.

Furthermore, we found no differences in the numbers of animals passing through the selection gates to the feeding area without being moved by a human. We had expected that permanent suckling-heifers would have been better able to use the selection gates due to their earlier experience as calves. However, the selection gates for the calves were shaped differently than those for cows, which might have been the reason why one heifer in the permanent suckling-group did not pass through the gates without help. Another explanation might be the lack of motivation to get into the feeding area, possibly to avoid encounters with cows. However, feeding motivation should have been quite high after 8 h without feeding (Schütz et al., 2006).

In a novel environment test, Le Neindre (1989b) observed more exploration behaviour in animals reared by a foster cow than in animals reared without a cow. This suggests that stress levels are lower in animals reared by a foster cow (Forkmann et al., 2007). In our study, by contrast, no differences in exploration were found. The challenges of regrouping in cubicle housing, which mainly arise from social challenges and spatial structures, differ largely from those in a novel arena test. What is more, permanent suckling-heifers might have remembered the surroundings of their first 3 months of life, which could have reduced the level of motivation to explore. This is why a possible lower stress level may not have shown up in more exploration.

In our study heifers with contact with the mother tended to self-groom more often than heifers without. Admittedly, the interpretation of self-grooming is not straightforward. On the one hand, self-grooming behaviour is considered a behavioural need of cows and its performance a sign of good welfare (Boissy et al., 2007; Bolinger et al., 1997). In a stressful situation, one could expect a decrease in the behaviour according to stress level (Boissy et al., 2007). On the other hand, self-grooming behaviour may occur as displacement behaviour in situations of motivational conflict (Herskin et al., 2004; Jensen, 1995). The exact performance (duration, intensity of scratching or licking) and the region for self-grooming (hard or easy to reach) may be of further help to distinguish between displacement activity and comfort behaviour (Duncan and Wood-Gush, 1972). Such details were not recorded in our study and might merit detailed consideration in future research. Other studies on grouping did not observe self-grooming or found no differences between animals in mixed and unmixed groups (Phillips and Rind, 2001).

4.2. Social behaviour

The number of agonistic interactions increases markedly on the first day when unfamiliar animals are mixed (Menke et al., 2000; Knierim, 1999; Veissier et al., 1994) which is due to the need to re-establish the social order (Kondo and Hurnik, 1990). Heifers newly integrated into a herd of cows often occupy only low social status (Bøe and Færevik, 2003) and thus become mainly receivers of aggression rather than initiators (Gibbons et al., 2009; Mülleder et al., 2003), which is in line with our study: heifers were receivers of aggressive interaction 193–514 times in the 33 h of observation, corresponding to 6–16 times per hour on average, while they initiated aggression only 24.5 times. No effect of rearing could be confirmed. Le Neindre and Sourd (1984) reported that heifers reared by foster cows displayed more agonistic behaviour than animals reared without cow contact and that, as a consequence, animals reared by a foster cow dominated the others. Higher dominance was maintained after first parturition by Salers but not by Friesian cows (Le Neindre, 1989b). In contrast to Le Neindre (1989b), all calves in our study were housed in groups and were not isolated during the first 12 weeks. In addition, in their study, a new herd was formed that consisted exclusively of experimental animals, so that the two rearing groups were tested against each other, i.e. animals of similar age and weight. In marked contrast to this, in our study, heifers were integrated in an existing cow herd. In this herd, many animals were superior in weight and strength, and the chance of occupying a high social status was small. These differences in study design might have contributed to the different findings on conflict behaviour.

However, *mother*-heifers showed more submissive postures. A strategy of indicating subordinate status through submissive behaviour may be beneficial in case of a small chance of winning fights and of gaining dominance. This may reduce the amount of aggression received. Although a reduction could not be confirmed, indicating submission may give heifers better access to resources without higher aggression, as suggested e.g., by trends of earlier lying activity and longer duration of feeding. Thus, it seems that the mother reared animals in our study were better able to adopt appropriate social behaviour in social challenges, as was shown in other species (Arnold and Taborsky, 2010; D'Andrea et al., 2007). Supported by results for individual behaviour, it appears that mother reared heifers can integrate more easily into the dairy cow herd.

In agreement with earlier studies, socio-positive behaviour (social licking) was observed much less frequently than agonistic interactions (Mülleder et al., 2003). No differences were detected in socio-positive behaviour (social licking) between heifers with different rearing experience. However, social licking was rarely observed in a stressful situation such as regrouping (Bøe and Færevik, 2003; Boissy et al., 2007; von Keyserlingk et al., 2008) as was the case in our experiment. Later observations after integration would be interesting for further studies.

4.3. Mother as nearest neighbour

Our results suggest that in a herd of about 40 cows at least some animals can identify their mothers after two years of separation. Actually, one of the *permanent suckling*-heifers tried to suckle on her mother; the same animal also laid down beside her mother very early (about 2 h after integration). It is evident that this heifer received social support in the form of the presence of a bonded individual in the challenging situation (Wiepkema and Schouten, 1990). In general, the presence of familiar conspecifics reduces the reactivity in response to stress (Boissy and Le Neindre, 1990; Færevik et al., 2006; Veissier and Le Neindre, 1992). Furthermore, learning ability can improve by social buffering (Boissy and Le Neindre, 1990). Due to the low sample size, we were not able to analyse and confirm social support in other animals, but this would be interesting to look at in further studies.

In addition, our findings indicate a strong bond with the mother and a good memory, at least in a few cases. After weaning and the birth of another young, the mother-offspring bond still exists (Newberry and Swanson, 2001; Reinhardt, 1980; Veissier et al., 1998). In beef cattle, less agonistic behaviour was observed at the feeding place in a group of mothers and their young than in a group of unrelated animals; the young had been separated from the dam until at least one year before reunion and observation (Swanson and Stricklin, 1985).

4.4. Adrenocortical activity

The concentration of faecal glucocorticoid metabolites in cattle reflects adrenocortical activity about 10–12 h before sampling (Palme et al., 1999, 2000; Möstl et al., 2002). Thus, in our study, the evening before regrouping (baseline value) was compared to the evening of the day of regrouping and to the evening of the next day. In most heifers, concentrations of faecal cortisol metabolites increased after regrouping compared to the baseline value of day 0, which is a sign of the activation of the HPA axis. However, a remarkable decrease was found in three of the *mother*-heifers but in none of the *automat*-heifers. While the treatments did not differ in the increase in faecal cortisol metabolite concentration on the day of integration, probably due to the very high stress levels of nearly all animals, they tended to differ in the increase on the second day (Cort_Change0-2). Individual differences in stress reaction to grouping were also observed by Hasegawa et al. (1997). In their study, cortisol response to ACTH injection was higher on day 14 after regrouping in formerly dominant heifers but not in middle-ranking or subordinate animals. The authors concluded that the dominant heifers had already recovered from regrouping. We have no data on the social status of heifers before or after integration, but in previous research (Le Neindre, 1989b) differences between rearing groups were found, which may have contributed to differences in cortisol responses between heifers with contact with the *mother*- and *automat*-heifers. Furthermore, the reunion with the mother may have alleviated the stress of integration – at least in animals showing a definite recognition of their mothers (see nearest neighbour). The *permanent suckling*-heifer who clearly recognised her mother had the largest decrease in cortisol metabolite concentrations. Finally, a general difference in stress reactivity, which had developed in the first 12 weeks of life, may exist. Heifers in the *automat* groups showed reduced blood cortisol responses to ACTH challenge at an age of 12 weeks, which is a sign of higher levels of chronic stress (Roth, 2008). If these differences continue to exist until the time of integration, a lower cortisol release in stressful situations due to regulatory differences may lead to lower cortisol rise during stress despite higher levels of stress experienced psychologically.

4.5. General discussion

In our study, the treatment groups differed only in rearing during the first 12 weeks of life but had equal conditions after weaning, with all treatment groups being kept together for two years. Nevertheless, there is some evidence of differences in the animals' reactions to the integration into a cow herd suggest long-term effects of mother rearing on the ability to cope with social challenges. This is striking given the long time span between rearing and the small number of animals in our study. Although these differences were few and small, it is interesting to observe that the main differences were found between *mother*- and *automat*-heifers, suggesting that there was, already after a short period of time, an effect of interacting with and suckling on the mother. Provided the fact that *mother*-heifers drank much more milk than *automat*-animals (see Section 2 and Roth et al., 2009), the question remains to what extent the nutritional aspect and related physiological stress reactions contribute to the results relative to the social aspect of contact with the mother. Future studies that differentiate between nutritional and social aspects and involve a greater number of animals are necessary and planned.

5. Conclusion

Our results suggest a long-term effect of contact with the mother during the first twelve weeks of life on behaviour and adrenocortical response during integration into the cow herd, even if this contact with the mother is very limited. The results also indicate a strong bond between mother and daughter and the chance of later recognition, at least in some individuals.

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