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Mother rearing of dairy calves: Reactions to isolation and to confrontation with an unfamiliar conspecific in a new environment

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ABSTRACT

The aim of this study was to test the effects of mother rearing on behavioural and physiological stress reactions of calves in challenging situations. Thus, we compared mother-reared and artificially reared calves that were kept in the same group but with varying contact with adults. Mother-reared calves (*Mother*) were suckled and had unrestricted contact with their mothers and also with the cow herd in the cubicle barn; artificially reared calves were fed milk up to 16 kg per day and animal via an automatic milk feeder (*Automat*). At the age of 43 days, the calves were separated from the group for 15 min (isolation test; *Mother*: $n = 16$; *Automat*: $n = 16$), and at 90 days of age, they underwent a social confrontation test with an unfamiliar calf in an arena for 20 min (*Mother*: $n = 11$; *Automat*: $n = 11$). Data were analysed using ANOVA and GLMM. In the isolation test, *Mother* calves showed more ($P < 0.05$) escape behaviour and tended to be more vigilant ($P < 0.1$). Concerning physiological parameters, no differences were detected in the mean heart rate over 15 min of isolation, but the increase in salivary cortisol concentrations 5 min after the end of the test tended to be lower in *Mother* calves than in *Automat* calves ($P < 0.1$). During confrontation, *Mother* calves showed less frequently solitary play behaviour (i.e. mainly locomotor play) than *Automat* calves ($P < 0.05$) but initiated more frequently social play when no cow was present adjacent to the test arena ($P < 0.05$). The results suggest that mother-reared calves showed higher motivation to rejoin their mothers and/or herd and tried to cope more actively with being isolated. In addition, in the confrontation test *Mother* calves seemed to be socially more active and more attentive to their social environment, but less motivated for locomotor play possibly due to the much larger space available to them in the cow barn.

1. Introduction

The social environment early in life can have short- and long-term effects on behavioural, immune and endocrine development, as shown in different species (mice/rats: Veenema, 2009, 2012; guinea pigs: Kaiser et al., 2003; Kaiser and Sachser, 2001; pigs: Kanitz et al., 2009; Tuchscherer et al., 2004; primates: Veenema, 2009; ruminants: Napolitano et al., 2008; Roth et al., 2009; humans: Heim and Binder, 2012). The mother, as the primary social bonding partner, is an important component of the social environment (Newberry and Swanson, 2008; von

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Keyserlingk and Weary, 2007). Complete deprivation from maternal care, i.e. separation from the mother, can cause increased behavioural and physiological responses in challenging situations as well as reduced immune responses and lead to stereotypic behaviour in the short-term, i.e. during infancy (Latham and Mason, 2008; Napolitano et al., 2008; Weary et al., 1999), but also to deficits in social learning and stereotypic behaviour in the long-term, i.e. in adulthood (Bastian et al., 2003; Latham and Mason, 2008; Lévy et al., 2003).

In dairy production, social deprivation in early life is common. Calves are usually separated from the mother shortly after birth, fed by bucket or an automatic milk feeder and often kept individually with limited social contact, at least for some weeks before being group-housed. In contrast, a strong mother-young bond develops under natural conditions; calves suckle their mother around 8–10 months and live in a diverse social environment with other calves and adult animals (Reinhardt and Reinhardt, 1981).

Previous research on the effects of early social environment in cattle had mostly focused on the comparison between animals reared in groups of calves and animals reared individually, i.e. without contact with the mother or other adult animals. For example, group-reared calves fed earlier from concentrate, were less fearful in open-field tests, had a higher social rank after grouping and had increased play and competitive success (Broom and Leaver, 1978; Duve et al., 2012; Jensen et al., 1997; Veissier et al., 1994; Warnick et al., 1977) compared to individually reared calves.

More recently, the effects of contact with the mother on behaviour, health and stress reactivity were investigated. For example, Duve et al. (2012) found that contact to the mother decreased the response to restraint stress when compared to calves reared singly or in pairs but without contact to the mother. Furthermore, calves left with their dams stood up earlier after birth (Lidfors, 1996), tended to have less diarrhoea in the first 2 weeks of life, gained more weight and were socially more active at 6 weeks of age (Weary and Chua, 2000) compared to single-reared calves separated from their dam shortly after birth. With respect to social behaviour, calves reared with their mother for 2 weeks were socially more active when confronted with an unfamiliar conspecific in a test arena at 6 weeks of age, e.g. they exhibited more licking, sniffing and rubbing (Flower and Weary, 2001), which may indicate that they were less fearful (Krohn et al., 1999) than singly reared calves. In addition, rearing with the mother, i.e. being nursed by the dam and restricted or unrestricted contact with the dam, prevented behavioural disorders such as cross-sucking and tongue rolling (Fröberg and Lidfors, 2009; Roth et al., 2009).

To our knowledge, only one experiment studied possible effects of rearing with the mother and other adult cows in comparison to group-reared calves without contact with the mother on behavioural and physiological stress responses to challenging situations (Roth, 2008). Calves reared with the mother for 12 weeks were threatening and fighting more often than artificially group-reared calves when integrated into a group of older unfamiliar calves (Roth, 2008). These two rearing groups also differed from each other in an isolation test. Furthermore, at the age of 11 weeks mother-reared calves showed higher cortisol responses after administration of ACTH as compared to group-reared calves separated from their mother within 1 day after birth (Roth, 2008), which was interpreted by the authors as a sign of lower chronic stress level (Mormede et al., 2007).

However, in the study by Roth (2008), the calves in the two rearing systems markedly differed in weight due to a difference in milk consumption. There, calves reared without mother could drink a maximum of 8 kg milk per day, while mother-reared calves could suckle their mothers, and milk consumption was assessed to be up to 16 kg milk per day (Barth et al., 2009). Thus, the calves differed not only with respect to social environment but also in nutritional status and body development (Roth et al., 2009), which may have influenced social behaviour as well as physiological responses.

Thus, our aim was to test potential effects of mother rearing on reactivity to social isolation and to confrontation with an unfamiliar calf. We compared mother-reared calves, i.e. calves that had access to the cow barn and thus suckled their mother and had nearly unlimited voluntary contact not only with the mother but also with other cows or heifers of the herd, to calves that were separated from the mother on the first day of life and housed in groups. To eliminate nutritional influences, calves without contact with the mother were offered up to 16 kg milk per day, equating the assessed consumption of milk by mother-reared calves in previous studies (Barth et al., 2009). According to this a similar nutritional status in both rearing treatments was given. We investigated behavioural reactions as well as heart rate and cortisol in saliva. We expected mother-reared calves to show a higher motivation to contact the social partners in the isolation test and a higher social activity in the social confrontation test. Furthermore, we expected a lower stress reactivity to these challenges in both tests.

2. Animals, materials and methods

2.1. Animals, housing and management

The study was carried out between October 2009 and April 2010 with 39 calves of two breeds, Black-and-White German Holstein (GH; $n = 16$) and German Red Pied (GRP; $n = 23$), at the Institute of Organic Farming (TI; Federal Research Institute for Rural Areas, Forestry and Fisheries) in Trenthorst, Germany. Since 2004, two dairy cow herds had been kept separately according to breed (50 cows each) in two identical parts of an open-sided barn with cubicle loose housing. Each part of the dairy barn offered a total space of 785 m^2 and was divided into three areas (for the scheme of the barn, see Wagner et al., 2012): (i) a largely unroofed feeding area (total 43 m × 4 m) with a feeding fence, (ii) a walking area (43 m × 3 m) providing access to transponder-controlled concentrate feeders and to the (iii) roofed lying area with 50 cubicles in total divided by an alley (39 m × 3 m). All dairy cows were milked twice a day. For calving, the cows were brought into individual calving pens adjacently to the cow barn and the calf area. The cows stayed in the calving pens together with their calves for 1–5 days after calving, depending on rearing (see Section 2.2). Afterwards, the calves of both treatments were kept in one calf group per herd in identical pens, located adjacently to the cow barn (group 1 – on the GH side; group 2 – on the GRP side). Thus calves of the two treatments shared the same calf pens and formed a dynamic calf group per side with all calves staying there until weaning at the age of 12 weeks and leaving thereafter. Both calf groups comprised calves of different ages (1–12 weeks old) and group composition changed regularly. At the beginning of the experiment the two calf groups were composed of (older) non-experimental animals with increasing proportion of experimental animals of both treatments during the course of the experiment.

Each pen for the calves was subdivided into a resting area (deep-bedded straw area, 13 m^2) and a running area (rubber-coated and bare concrete floor, 54 m^2). This corresponds to a total individual space allowance of 6 ± 3 m^2 per calf (min–max 4–17), depending on the actual size of the calf group in the calf area (ranging from 3 to 19 in group 1 and from 5 to 19 in group 2). According to rearing (see Section 2.2), the calves had transponder-controlled access either to the cow barn via selection gates or to the milk feeder (for the amount of milk, see Section 2.2; FA Förster-Technik GmbH, Engen, Germany). All calves had access to a transponder-controlled concentrate feeder (FA Förster-Technik GmbH, Engen, Germany) providing up to 1.5 kg concentrate per day for each calf. Silage, hay and water were available ad libitum for all calves in the calf area.

2.2. *Rearing treatments*

The rearing conditions for calves of the two rearing treatments differed in the first 12 weeks of life. Calves were allocated to the two treatments balanced for their mothers' number of lactations (primiparous or multiparous) but otherwise in sequence of their birth alternating the two treatments. Due to the dynamic group composition, all calves had older and younger peer calves (experimental or non-experimental calves) during the 12 weeks of rearing treatment. Group size was on average 13 ± 5 animals. Group composition regarding number (mean (min–max)) of calves in the different treatments was *Mother* 4 (1–6) and *Automat* 8 (0–13) and non-experimental 1 (0–3) for group 1 and *Mother* 6 (1–10) and *Automat* 4 (0–7) and non-experimental 3 (0–10) for group 2.

2.2.1. *Mother rearing (Mother: n = 19)*

Calf and mother stayed together in the calving pen for the first 5 days *post partum*, and the calf was suckled by its mother. The cow was milked in the milking parlour twice a day starting with the first milking following calving (max. 12 h after calving). Within 6 h after birth (all except 3 calves within 8 h; mean ± SD, 2 ± 2.5 h), calves were bottle-fed 1 l of colostrum. Thereafter calves were observed and assisted for sucking the dam if necessary. On the sixth day, the calf was moved into the calf area and taught to use the selection gate. Through this gate the calves had free access to the cow barn and thus to their mothers and cow herds, except during the provision of fresh straw for bedding (about 30 min) in the cow barn twice a week. During milking times, the calves had only access to the lying area in the cow barn and no contact with their mothers or the cow herd. The calves were weaned abruptly on day 90 (min–max 86–95) by moving to another building out of visual and auditory contact to their former peers or the cow herd.

2.2.2. *Artificial rearing (Automat: n = 20)*

Within 6 h after birth (mean ± SD, 2 ± 1.7 h), all calves were bottle-fed 1 l of colostrum. The calves were separated from their mothers within the first 12 h after birth. Cows were brought to the milking parlour at the first milking time following their calving and went back to cow herd thereafter; calves were moved into the calf area. There the calf was bottle-fed 5 days four times daily (milk amount increased from 4 to 6 kg per day and animal). After the first 5 days, they had access to the automatic milk feeder providing a maximum milk portion of 8 kg per day/animal, which was increased to 16 kg per day/animal on day 27. Calves were fed a minimum of 4 and a maximum of 8 meals per day (one meal: maximum 4 kg; Förster Technik, 1996). *Automat* calves had no access to the cow barn. The calves were weaned abruptly on day 90 (min–max 87–95) by moving them from the calf group to another building out of visual and auditory contact to their former peers.

2.3. *Test procedures*

The calves were subjected to two different test situations at two different ages. Around the age of 44 days (min–max 39–49) calves were tested in an isolation test for 15 min and at the test-day the order of calves was assigned randomly. According to this, three animals in maximum were tested per test-day. At this age, *Mother* calves had a weight of 86 ± 9 kg, and *Automat* calves weighed 84 ± 12 kg. Furthermore, the distribution of sex was on rearing treatment for female: *Mother*: $n = 9$, *Automat* $n = 7$ and for male: *Mother* $n = 7$, *Automat* $n = 9$ and on breed was the distribution for female: GH $n = 8$, GRP $n = 8$ and for male: GH $n = 4$, GRP $n = 12$, in the isolation test. Around the age of 90 days (min–max 86-95) calves were tested in a confrontation test. The tested pairs were selected by same age, unfamiliarity and treatment, according to this, 11 pairs were tested in total and in maximum two pairs were tested per test-day (for more details see Section 2.3.2). At that time, the mean weight of the *Mother* calves was 139 ± 19 kg and of the *Automat* calves 143 ± 20 kg. Furthermore, the distribution of sex was on rearing treatment for female: *Mother*: $n = 9$, *Automat*: $n = 7$ and for male: *Mother*: $n = 2$, *Automat* $n = 4$ and on breed was the distribution for female: GH: $n = 8$, GRP $n = 8$ and for male: GH $n = 1$, GRP $n = 5$, in the confrontation test.

2.3.1. *Isolation test*

Between 9:00 and 12:00 h, the experimenter entered the calf group and approached the experimental calf. The calf was gently held while saliva was sampled for measurement of cortisol (for details, see Section 2.5.1). Heart rate monitors were fitted to the calf (for details, see Section 2.5.2). Heart rate measurement was started, and the calf was moved immediately to the isolation box by being gently and patiently pushed. The isolation box measured

1.4 m × 1.4 m and had solid wooden walls of 1.5 m height. The box was located 3 m from the calf area. Thus, the tested calf had no visual but auditory contact with other animals. After closing the isolation box, the test started and lasted for 15 min. The experimenter opened the box, approached the calf and took another saliva sample. Then the calf was moved back to the group and was released. In the calf group, the last saliva sample was taken 5 min after the end of the test. During restraint in the isolation box, the calf was videotaped (Sony Handycam DCR-HC47) to analyse its behaviour. When a calf tried to escape the box vigorously, the test was stopped. These animals (*Mother*: $n = 2$; *Automat*: $n = 3$) were excluded from further analysis. During the tests, the selection gates to the cow barn were closed for all calves (maximum 1 h).

2.3.2. Confrontation test

Two identical test arenas (9 m × 3 m each) were constructed at the GH or GPR side, one close to each of the two calf areas (about 2 m) and adjacent to the calving pens, in an area unfamiliar to the calves. For each test, one calf from the GH side (group 1) was confronted with an unfamiliar calf of the other treatment and from the group of the GRP side (group 2). Thus, for one of the two calves to be tested the distance and duration of movement was longer while the other calf was moved only a short distance (shorter: *Mother* $n = 5$, *Automat* $n = 6$ or GH $n = 4$, GRP $n = 7$; longer: *Mother* $n = 6$, *Automat* $n = 5$ or GH $n = 5$, GRP $n = 6$). By largely balancing of the rearing treatments over the two test arenas/sides, a potential bias due to the longer distance between the rearing treatments could be excluded. During testing the calving pens were locked and covered by a plastic curtain, sometimes with presence of a cow in the calving pen (no: $n = 6$; yes: $n = 16$). To prevent visual contact between test calf and the calf group close by, the latter were restrained in a part of the calf area (lying plus feeding area; the running area was inaccessible during testing). Thus, only auditory contact was possible.

When performing the tests, first the heart rate equipment was applied to the calf in its respective home pen (for detailed procedure, see Section 2.5.2) between 9:00 and 12:00 h in the morning. When the calf from the other calf area (i.e. with a longer distance from its home pen to the test arena and thus a longer distance to move) arrived in front of the test arena, the calf from the adjacent calf group was immediately moved to the test arena. Both calves were moved by pushing them gently and patiently. After the second calf had entered the test arena the door was closed. Three minutes later the behavioural observation started, because observers were identical with the persons handling the experimental calves and had to get into the observation position. The observers' upright position was directly in front of the test arena on the opposite side of the entrance, where they were visible for the tested calves. Twenty minutes after the start of observation the test ended and calves were moved back into their home pens. In all cases, one calf from the *Mother* treatment was confronted with one calf from the *Automat* treatment of the other breeding group (groups 1 and 2), thus unfamiliar to each other. Both calves had to be in the same age, unfamiliar to each other and of the opposite treatment. Additionally each calf was tested only once. Therefore only data from 22 out of 39 calves (tested in 11 pairs) could be analysed. If more than one calf pair could be built at 1 day, pairs were built randomly. Data from both calves were used in the analyses to get sufficient sample size.

2.4. Behavioural observations

Behaviour was recorded with the software Observer® (5.0, Noldus, NL) with continuous focal-animal behaviour sampling (Martin and Bateson, 1993) from videotapes in the isolation test and by direct observation in the confrontation test. For the isolation test one observer and for the confrontation test two observers recorded the behaviour. In the confrontation test, both calves were observed at the same time, and social interactions were measured by identifying both initiator (I) and receiver (R). For definitions of behavioural parameters for individual behaviour (isolation and confrontation test) and social behaviour (confrontation test only) see Table 1. Frequency (FR) in events or interaction per test duration and total duration (TD) in percent (%) of test duration for the isolation test and in seconds (sec) for the confrontation test were recorded. For further analysis, the social behaviours threatening, butting, pushing away and fighting were summarised as aggressive behaviour. In addition, the proportion of received aggression to all aggressive interactions was computed (rate of received aggression divided by all aggressive interactions).

2.5. Physiological measurements

2.5.1. Adrenocortical activity

Saliva for cortisol analysis was sampled during the isolation test. Saliva was sampled directly after the experimenter had entered the calf area (Cort0, baseline), directly after the 15 min of isolation (Cort1) and 5 min later, when the calves were back in their groups (Cort2, Section 2.3.1). Saliva was collected using an absorbent cotton (Salivette®, Sarstedt AG, Nürnbrecht) held by an arterial forceps that was inserted into the calf's mouth for 10 s. The samples were stored in the freezer (−20 °C) to prevent drying until analysis. For the analysis, the Salivette® tubes were defrosted at room temperature, and saliva was removed from the absorbent cotton by centrifugation (10 min at 2500 × *g*). Afterwards, aliquots of the saliva samples were analysed directly using a cortisol enzyme immunoassay, as described by Palme and Möstl (1997).

For statistical analysis, difference to baseline (Cort0) – both after 15 min of isolation (Cort_change_1-0) and 5 min after the end of isolation (Cort_change_2-0) – were calculated.

2.5.2. Heart rate

Heart rate was measured during both tests non-invasively using the Polar® system (Elektro Oy, Kempele, Finland; S810 monitor and T61 transmitter). One day before the test, animals were shorn on the left side of the chest, right behind the elbow, and habituated to the equipment. A chest belt with two integrated electrodes and a transmitter was fitted around the torso, directly behind

Table 1
Description of the behaviour during isolation (Iso) and confrontation test (Confron); frequency (FR) and total duration (TD).

		Iso	Confron
Individual behaviour			
Vigilance	Head position at an angle above the horizontal to the withers, ears upright	FR, TD	–
Head normal	Head position horizontal or at an angle below the horizontal to the withers or above the horizontal to the withers but without ears upright	FR, TD	–
Exploration	Sniffing or licking on floor or wall	FR, TD	FR, TD
Walking	More than two steps with forelegs	FR, TD	FR, TD
Self-grooming	Licking and scratching the own body	FR, TD	FR, TD
Vocalisation	Any vocalisation of the calf	FR	FR
Elimination	Defecation and micturition	FR	FR
Escape	Attempts to escape; jumping against and trying to get over the wall	FR	
Solitary play	Comprising *locomotor play* (i.e. gallop, leap, jump, buck, turn) and *object play* (i.e. butting bars in a playful manner), according to Jensen et al. (2000)	–	FR
Social behaviour; recording initiator (I) and receiver (R)			
Licking	licking at the body of the other calf	–	FR
Social play	I standing in front of R, and both calves putting their heads against head/neck of the other in a playful manner without power	–	FR
Aggression			
Threatening	I adopting a typical threat posture (presenting the forehead with inclined head) without touching R, and R moving away or changing the position	–	FR
Butting	I butting R with its head, but R staying in place	–	FR
Pushing	I pushing R with head, resulting in R moving away or changing the position		FR
Fighting	I standing in front of R, and both calves putting their heads against each other forcefully	–	FR

the forelegs, using enough electrode gel to ensure contact. Additionally, a horse blanket belt, with an extra pocket to hold the polar monitor during measurement, was fitted over that belt. Interbeat interval data were recorded and downloaded to a computer. The recorded intervals were corrected for measurement errors (artefacts) by use of the software Polar Version 4.03.050. For both tests, the mean heart rate (beats/min) over the entire testing time was calculated for the 15 min (HR0-15) of isolation and for the 20 min (HR0-20) of the confrontation test, accordingly.

2.6. Statistical analysis

All statistical analyses were carried out with the software package PASW Statistics, Version 17. In the results section, the original data are presented in box plots and estimated values can be found in the tables.

2.6.1. Isolation test

For analysing behavioural parameters and mean heart rate, we performed an ANOVA with rearing (*Mother*/*Automat*), sex (female/male), breed (GH/GRP) and the two-way interactions with rearing (rearing × breed; rearing × sex) as fixed factors, and for heart rate analysis, weight was included as covariate, because its known to be associated with this parameter (Hagen et al., 2005; von Borell et al., 2007). In the ANOVA, interactions were excluded from the models in a stepwise manner when $P > 0.10$. To verify the assumption of the models, residuals were checked for normal distribution using a Shapiro–Wilk test and visually for homogeneity of variance. For frequency of vocalisation, elimination and escape behaviour, as well as for duration of self-grooming, we used square-root or $\log_{10}$ transformation to get normally distributed residuals and homogenous variances. For frequency of self-grooming, the assumption of the model was not fulfilled, even after transformation (normal distribution of residuals), so we used Mann–Whitney *U* test for analysing rearing effects only.

For saliva cortisol, we first conducted repeated-measures ANOVA with the same fixed factors as mentioned before. However, the model assumptions (normal distribution of residuals) were not fulfilled, even after transforming the response variables. Therefore, we used separate ANOVA analyses with the differences of adrenocortical activity to baseline (Cort_change_1-0 and Cort_change_2-0) as response variables. Fixed factors and procedure were the same as for the behavioural variables.

There were missing values due to technical problems: behavioural parameters are missing for one animal from *Mother* and for one from the *Automat* treatment. Moreover, heart rate measurements for one animal from the *Automat* treatment are lacking, and for two animals there are no weight measurements. In addition, 5 animals (*Mother*: $n = 2$; *Automat*: $n = 3$) were not included in the analysis because they tried to escape from the box vigorously, so the test was stopped after 5–9 min (mean 7.5 min) due to a high risk of injury.

2.6.2. Confrontation test

First we carried out a GLMM with pair as repeated factor modelling the potential dependency of reactions of the two calves tested as pairs. Based on the strength of correlation and more specifically the Akaike information criterion (AIC), it was decided if the GLMM was used or, instead a simpler ANOVA without the random pair effect was calculated. To gain an intuition, notice that ANOVA models tended to give a lower AIC value than GLMM, for correlations within pairs that were lower than 0.40. In these situations, we calculated ANOVAs, otherwise a GLMM was used. We used rearing (*Mother*/*Automat*), sex (female/male), breed (GH/GRP), distance to test arena (short/long) and presence of cow in calving pen next to test arena (yes/no) as fixed factors. Additionally, for heart rate and social behaviour, weight was included as covariate as weight is known to be associated with these parameters (Bouissou, 1972; Hagen et al., 2005; Landaeta-Hernández et al., 2013; von Borell et al., 2007) but not with the others.

With the GLMM and ANOVA, factors were excluded stepwise from the models if they had a $P > 0.1$. If effect of breed, sex, distance to test arena or presence of cow in calving pen was $P < 0.1$, a two-way interaction with rearing was included in the model and presented in results when $P < 0.1$.

To verify the model assumptions, residuals were checked for normal distribution using a Shapiro–Wilk test and homogeneity of variance was checked visually. For frequency of vocalisation and elimination a square-root transformation was used to get normally distributed residuals and homogenous variances. For frequency of licking, the model assumptions (normal distribution of residuals) were not fulfilled even after transformation, so we used the Mann–Whitney *U* test for analysing rearing effects only. There are missing values due to technical problems: for one animal of the *Automat* treatment, heart rate was not measured correctly.

3. Results

3.1. Isolation test

3.1.1. Behaviour

Mother calves ($n = 16$) were observed more often with a normal position of the head ($P = 0.014$; Table 2) and tended to be vigilant more often ($P = 0.071$; Table 2) than *Automat* calves ($n = 16$). For the duration of these two behaviours, we found an interaction between rearing and breed (Table 2, head normal (TD): $P = 0.011$; vigilant (TD): $P = 0.095$): GH *Mother* calves showed shorter normal position of the head and tended to show longer vigilant behaviour than GH *Automat* calves; in GRP no differences were detected. Furthermore, calves of the *Mother* group tried to escape from the isolation box more often than *Automat* calves ($P = 0.031$; Table 2). No other rearing effects were confirmed (Table 2).

Regarding sex effects, females explored significantly less often than males (mean ± SE, female: 17 ± 2 events/15 min; male: 22 ± 2 events/15 min; $F_{1,32} = 4.63$, $P = 0.040$). There were no further effects of sex on any of the other behavioural parameters. Furthermore, we found an interaction between rearing and sex ($P = 0.054$; Table 2): female *Mother* calves showed two times more vocalisations than female *Automat* calves, while in male calves it was the opposite – within treatments there was a difference only in *Mother* calves with females vocalising four times more than males. Concerning breed effects, no effects were found in the isolation test.

3.1.2. Heart rate

We found no differences in mean heart rate between the two rearing treatments over the 15 min of isolation ($F_{1,31} = 2.48$, $P = 0.128$; Table 2). Moreover, no effects of breed, sex or weight were found.

3.1.3. Adrenocortical reactivity

Concerning absolute values of saliva cortisol, 24 calves had a higher level of saliva cortisol directly after the test (Cort1) compared to baseline (Cort0), whereas in 10 tested calves (*Mother*: $n = 7$; *Automat*: $n = 3$) a decrease was found. With regard to difference to baseline, no significant difference directly after the isolation test between rearing treatments was detected (Cort_change_1-0; $F_{1,34} = 2.39$, $P = 0.133$; Fig. 1a). Nevertheless, 5 min after the end of the test, the difference tended to be lower in *Mother* calves than in *Automat* calves (Cort_change_2-0; $F_{1,34} = 3.28$, $P = 0.080$; Fig. 1b). Furthermore, the difference was significantly higher in females (Cort_change_2-0) than in males (female: 0.31 ± 0.01 ng/g; male: −0.004 ± 0.012 ng/g; $F_{1,34} = 4.48$, $P = 0.043$).

3.2. Confrontation test

3.2.1. Behaviour

Calves from the *Mother* treatment showed less solitary play behaviour during the confrontation test than calves from the *Automat* treatment ($P = 0.001$, Table 3). Furthermore, we found interactions between rearing and sex (self grooming (TD): $P = 0.087$) or presence of a cow in a calving pen close to the test arena (initiated social play (FR): $P = 0.005$; Table 3): male *Mother* calves tended to self-groom more often than male *Automat* calves; in females no differences were detected. When no cow was present, *Mother* calves initiated significantly more social play behaviour than *Automat* calves, whereas no difference existed when a cow was present (Table 3).

No further rearing effects were found (Table 3), but other factors influenced the calves' behaviour or heart rate in the confrontation test. GH-calves explored less (GH: 23 ± 3 events/20 min; GRP: 30 ± 3 events/20 min; $F_{1,22} = 6.23$, $P = 0.033$), tended to walk longer (GH: 152 ± 3 s; GRP: 103 ± 24 s; $F_{1,22} = 3.71$, $P = 0.083$) and showed solitary play behaviour less often (GH: 3 ± 1 events/20 min; GRP: 4 ± 1 events/20 min; $F_{1,22} = 5.47$, $P = 0.043$) compared to GRP-calves. When a cow was present in the adjacent calving pen, calves tended to self-groom more often than when no cow was present (yes: 3 ± 0.4 events/20 min; no: 1 ± 1 events/20 min; $F_{1,22} = 4.39$, $P = 0.050$).

3.2.2. Heart rate

During the confrontation test heart rate of *Mother* and *Automat* calves did not differ ($P = 0.547$, Table 3). Calves had a higher heart rate when the distance to the test arena was longer compared to a shorter distance to the arena (short: 118 ± 6 beats/min; longer: 141 ± 6 beats/min; $F_{1,22} = 7.80$, $P = 0.012$).

4. Discussion

The present study gives some evidence that the rearing of dairy calves with contact with their mothers and the whole cow herd via access to the cow barn affects behavioural and physiological stress reactions of the calves, as compared to artificially reared calves of a comparable nutritional status. As expected, in the social isolation test, *Mother* calves showed a higher motivation to re-establish

Table 2
Results of ANOVA for behaviour (*Mother*: $n = 16$; *Automat*: $n = 16$) and for heart rate (*Mother*: $n = 17$; *Automat*: $n = 14$) during 15 min isolation test. Furthermore, the interactions with $P < 0.1$ of rearing with breed (GH: *Mother*: $n = 7$, *Automat* $n = 5$; GRP: *Mother* $n = 9$, *Automat* $n = 11$) or sex (female: *Mother* $n = 9$, *Automat* $n = 7$; male *Mother* $n = 7$, *Automat* $n = 9$) on the outcome variables during isolation test. The table shows the estimated mean ± standard error (SE) and back transformed means in brackets of frequency (FR; in events/15 min), total duration (TD; in %) and heart rate (beats/min).

			Mean ± SE		*F*	*df*	*P*
			Mother	*Automat*			
Vigilant (FR)	Rearing		15 ± 1	12 ± 1	3.53	1.32	**0.071**
Vigilant (TD)	Rearing × breed	GH	31 ± 6	16 ± 7	3.00	1.32	**0.095**
		GRP	31 ± 5	35 ± 5			
Head normal (FR)	Rearing		22 ± 1	17 ± 2	6.88	1.32	**0.014**
Head normal (TD)	Rearing × breed	GH	33 ± 5	50 ± 6	7.55	1.32	**0.011**
		GRP	32 ± 4	24 ± 4			
Exploration FR)	Rearing		21 ± 2	19 ± 2	0.49	1.32	0.488
Exploration (TD)	Rearing		33 ± 4	37 ± 4	0.59	1.32	0.448
Walking (FR)	Rearing		18 ± 2	17 ± 2	0.19	1.32	0.669
Walking (TD)	Rearing		17 ± 2	19 ± 2	0.31	1.32	0.584
Self grooming (FR)	Rearing		2 ± 0.4	1 ± 0.4			0.323
Self grooming[b] (TD)	Rearing		1.34 ± 0.27 (0.1)	0.92 ± 0.28 (1)	1.19	1.32	0.284
Vocalisation[a] (FR)	Rearing × sex	Female	4.85 ± 0.79 (24)	2.47 ± 0.90 (6)	4.06	1.32	**0.054**
		Male	0.90 ± 0.90 (1)	2.02 ± 0.87 (4)			
Elimination[a] (FR)	Rearing		1.00 ± 0.15 (1)	1.29 ± 0.16 (2)	1.58	1.32	0.219
Escape[a] (FR)	Rearing		1.50 ± 0.28 (2)	0.59 ± 0.29 (1)	5.14	1.32	**0.031**
Heart rate	Rearing		154 ± 4	144 ± 5	2.48	1.31	0.128

[a] Square root transformation.
[b] $\log_{10}$ transformation.

contact with their social partners. Although *Mother* calves showed lower cortisol responses to the isolation test, no significant difference was found in heart rate. In the social confrontation test, a higher social activity reflected in more frequently initiating social play when no cow was present close to the test arena was found. On the other hand, *Mother* calves exhibited less locomotor play compared to *Automat* calves. Against our hypotheses we did not find a reduced stress reactivity in *Mother* calves in the confrontation test as reflected in the results of heart rate.

4.1. Isolation test

Mother calves showed more escape attempts. This may be interpreted as a higher motivation to rejoin social partners (higher sociality) or as differences in reaction patterns when in a stressful situation or a combination of both (Erhard and Schouten, 2001). In the study by Roth (2008), no escape attempts were observed despite comparable test conditions with respect to the isolation box, duration of test and age of calves. Kilgour et al. (2006) described escape attempts in an open-field test and defined them as signs of general agitation. In our study, it seems that mother-reared calves showed more effort to rejoin the mother, herd or calf group, which is in accordance with results found by Napolitano et al. (2002, 2008) and Sevi et al. (1999) in lambs: In an open-field test, they observed more flight attempts, less ambulatory behaviour and shorter latency to first movement in lambs with longer contact with the mother; the authors concluded that a higher motivation to contact the dam was the result of the strong bond to the mother. *Mother* calves had not only been separated from

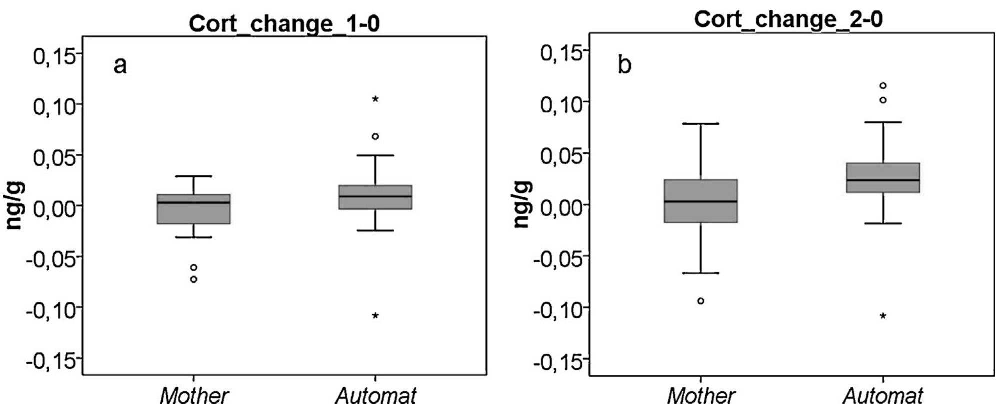

Fig. 1. Box plots of the change in cortisol concentration (ng/g) in saliva from baseline to (a) the end of the isolation test (Cort_change_1-0) and (b) 5 min after isolation (Cort_change_2-0, right) for *Mother* calves ($n = 17$) compared to *Automat* calves ($n = 17$). Graphs are based on the original, non-transformed data. Data are presented as box-and-whisker plots, with boxes representing the first and third quartiles, the central bar being the median, and the whiskers extending to the minimum and maximum values; dots represent outliers and stars extremes.

Table 3
Results of GLMM or ANOVA for behaviour (*Mother*: $n=11$; *Automat*: $n=11$) and for heart rate (*Mother*: $n=11$; *Automat*: $n=10$) during 20 min confrontation test. Furthermore, the interactions with $P<0.1$ of rearing with sex (female: *Mother*: $n=9$, *Automat* $n=7$; male: *Mother* $n=2$, *Automat* $n=4$) or presence of a cow (yes: *Mother*: $n=8$, *Automat* $n=8$; no: *Mother* $n=3$, *Automat* $n=3$) on the outcome variables during confrontation test. The table shows estimated mean ± standard error (SE) and back transformed means in brackets of frequency (FR, in events/15 min), total duration (TD, in s) and heart rate (beats/min).

			Mean ± SE		*F*	*df*	*P*
			Mother	*Automat*			
Exploration (FR)	Rearing		24.69 ± 3.19	28.19 ± 2.58	2.18	1.22	0.175[a]
Exploration (TD)	Rearing		516.11 ± 55.39	593.16 ± 51.85	2.54	1.22	0.142[a]
Walking (FR)	Rearing		15.64 ± 2.00	12.91 ± 2.00	0.93	1.22	0.347[b]
Walking (TD)	Rearing		136.78 ± 26.59	118.81 ± 22.49	0.53	1.22	0.486[a]
Self-grooming (FR)	Rearing		1.80 ± 0.53	1.53 ± 0.53	0.15	1.22	0.704[b]
Self-grooming (TD)	Rearing × sex	Female	10.98 ± 5.58	12.80 ± 6.64	3.50	1.22	**0.087**[a]
		Male	45.25 ± 10.36	21.01 ± 8.30			
Vocalisation[c] (FR)	Rearing		3.74 ± 0.70 (14.00)	2.09 ± 0.70 (4.37)	2.80	1.22	0.110[b]
Elimination[c] (FR)	Rearing		1.22 ± 0.14 (1.49)	1.05 ± 0.14 (1.10)	0.67	1.22	0.424[b]
Solitary play (FR)	Rearing		1.72 ± 0.57	5.35 ± 0.94	22.20	1.22	**0.001**[a]
Licking (FR)	Rearing		0.27 ± 0.20	0.36 ± 0.20			0.748[d]
Initiated social play (FR)	Rearing × cow presence	Yes	0.75 ± 0.50	1.75 ± 0.50	10.34	1.22	**0.005**[b]
		No	5.00 ± 0.81	1.67 ± 0.81			
Initiated aggression (FR)	Rearing		5.60 ± 2.07	3.60 ± 1.62	2.39	1.22	0.153[a]
Received aggression (FR)	Rearing		4.36 ± 1.46	6.82 ± 2.09	2.66	1.22	0.134[a]
Proportion of received aggression (%)	Rearing		33.58 ± 9.75	47.45 ± 9.75	1.01	1.22	0.326[b]
Heart rate (beats/min)	Rearing		127.16 ± 5.56	132.10 ± 5.81	0.38	1.21	0.547[b]

[a] GLMM.
[b] ANOVA.
[c] Square root transformation.
[d] Mann–Whitney *U*.

the calf group but also from their primary bonding partner, the dam. The dam is described as the first bonding partner and plays an important role for calves (von Keyserlingk and Weary, 2007; Newberry and Swanson, 2008).

Furthermore, lambs with longer mother contact vocalised more often in open-field tests (Napolitano et al., 2008; Sevi et al., 1999). Our results confirm these observations in part, because only female *Mother* calves tended to vocalise more often than female *Automat* calves, but no differences were found in male calves. Vocalisations are performed especially after separation from a bonding partner (Boivin et al., 2000). Although the total duration of contact of calves with their mothers decreases with age (Kiley-Worthington and de la Plain, 1983), the bond remains strong until weaning and even later on (Veissier et al., 1990). It seems that female *Mother* calves had a higher motivation to re-establish contact with social partners and probably especially with their first primary bonding partner, the dam (Reinhardt, 1980).

In addition, *Mother* calves tended to be vigilant more often and, in case of GH, also longer. This also supports the notion that *Mother* calves try to re-establish contact with the dam or peers in order to receive auditory information (hearing calls from the dam). One function of vocalisation in cattle is to contact social partners (Reinhardt, 1980; Watts and Stookey, 2000; Watts et al., 2001). On the other hand, more vigilance could be a sign of a higher level of fear (Welp et al., 2004). The general function of vigilant behaviour is to scan the environment for information, be it about potentially dangerous environmental features (e.g. a dog or aversive handler nearby, as in the study by Welp et al., 2004) or about conspecifics in the social environment (Boissy and Dumont, 2002; Cameron and du Toit, 2005). As we also observed a normal position of the head more often in *Mother* calves, we assume that vigilance is triggered largely by the search for auditory contact with conspecifics and is not a sign of a higher fear level in *Mother* calves.

This interpretation also corresponds with other results for behaviour. More walking and elimination are commonly recorded variables in open-field or isolation tests that are interpreted to reflect the level of fear (Forkman et al., 2007 review). However, we found no differences between rearing groups in these behaviours and, with respect to these parameters, no indication of more or less fear in *Mother* calves than in *Automat* calves. Moreover, exploration and self-grooming did not differ between the rearing groups. Thus, despite seeming differences in social motivation, isolation from social partners and being restrained in a small box in a novel environment is a stressful situation for calves of both rearing treatments (Boissy and Le Neindre, 1997; Færevik et al., 2006; Herskin et al., 2004, 2007).

Although the mean heart rate of *Mother* calves was numerically higher than that of *Automat* calves, no significant differences were detected. The slightly higher heart rate could be (partly) due to the greater number of escape attempts, as more physical activity could lead to a higher heart rate (Hagen et al., 2005). Similarly, Roth (2008) found a higher mean heart rate in mother-reared calves in a 15-min isolation test. The author attributes this to higher physical activity. Furthermore, mother-reared calves in the study by Roth (2008) were heavier, and weight can affect heart rate (Hagen et al., 2005; von Borell et al., 2007 review). In our study, cortisol in saliva increased in all except 10 (*Mother*: $n=7$; *Automat*: $n=3$) calves from baseline to the time directly after isolation and even more 5 min later. The increase in the level of salivary cortisol tended to be lower in *Mother* calves than in *Automat* calves in the sample 5 min after the test (Cort_Change_2-0). Thus, it seems that adrenocortical activation was lower in calves of the *Mother* group than of the *Automat* group.

The findings of the isolation test suggest that *Mother* calves were competent to react adequately to an unfamiliar isolation challenge. They experienced the separation from the mother and peers as stressful, as indicated by the increase in saliva cortisol, and tried to overcome the situation by attempts to rejoin (escape attempts) or get into (auditory) contact with the mother and peers (vigilance). Adrenocortical activation was lower in *Mother* calves, suggesting lower stress levels compared to *Automat* calves.

In addition to rearing effects, we found that females explored less often and showed a higher increase in the level of salivary cortisol compared to males, indicating higher levels of stress in females.

4.2. Confrontation test

Solitary play behaviour was composed of locomotor and object-play behaviour, but the latter was observed extremely rarely and thus contributes negligibly to solitary play. *Mother* calves showed solitary play behaviour less frequent, which could be either an indicator for lower welfare during the test (Jensen et al., 1998; Mintline et al., 2013) or indicate that the need for locomotor play is more satisfied compared to *Automat* calves. Calves of the *Automat* treatment had only the calf area available, whereas calves of the *Mother* treatment additionally had access to the cow barn. *Mother* calves often conducted locomotor play in the alleys of the cow barn, especially when cows stayed outside this area during milking (personal observation). Jensen and Kyhn (2000) tested calves from home pens with different space allowance (1.3, 2.2, 3.0 or 4.0 m^2 per animal) in an open-field test. Calves with less space allowance in their home pen showed more play behaviour than the others in the test. However, *Automat* calves had in average a space allowance of 6.35 m^2per animal (at minimum 4, up to 17 m^2 per calf) which was in comparison to Jensen and Kyhn (2000) very generous. In addition, the structure of the space can affect the locomotor play behaviour, in that more running was observed in larger and longer spaces (Mintline et al., 2012). The alleys of the cow barn in our study offered a long run (39 m), while in the calf area it was 8.5 m length at maximum. Our test arena was also longer than wide (9 m $\times$ 3 m each) which might have elicited running behaviour especially in *Automat* calves that were deprived of long empty spaces.

Self-grooming is interpreted partly contradictory. On the one side it is seen as a behavioural need of cattle and therefore an increase may reflect better welfare (Boissy et al., 2007; Bolinger et al., 1997). On the other side, increased self-grooming can occur as displacement behaviour in situations of motivational conflict (Herskin et al., 2004; Jensen, 1995). In our study, the interaction found in the duration of self-grooming between rearing and sex should be interpreted very cautiously, because in the *Mother* treatment only two males were represented and in addition only a tendency was detected. Aside from rearing effects, self-grooming was seen more often when the cow was present than when not. The presence of a cow close to the test arena, although they separated by plastic curtains, seemed to have influenced the calves' behaviour to a certain extent.

Mother calves numerically vocalised more often than *Automat* calves in the confrontation test which corresponds to the isolation test where female *Mother* calves vocalised more often. Sample size in the confrontation test was smaller than in the isolation test which might have contributed to the lack of a statistically confirmed difference.

Concerning social behaviour, 12-week-old mother-reared calves initiated more aggressive interactions (fighting and butting) and were receivers of aggression less often during the first 60 min after being moved into a new group than calves reared without mother contact (Roth, 2008). In contradiction to this study we found no differences in initiated or received aggressive interactions of *Mother* and *Automat* calves in the confrontation test. However, in the study of Roth (2008), mother-reared calves were heavier than calves reared without mother due to a restricted milk allowance in the latter. Weight is positively associated with social status (Reinhardt and Reinhardt, 1975; Sambraus, 1978) and differences in weight can contribute to differences in social interactions and social success in adult cattle (Bouissou, 1972; Landaeta-Hernández et al., 2013). In our study, the weight of calves of the two rearing groups did not differ. Additionally we confronted calves with one calf in an environment novel for both animals, whereas in the study of Roth (2008) calves were integrated into an established group of calves. Also Flower and Weary (2001) found only numerically higher levels of butting in calves separated from their mother later (after 2 weeks) as compared to calves separated after the first day after birth. This is in agreement with our *Mother* calves numerically initiating more and receiving less agonistic interactions than *Automat* calves.

Socio-positive behaviours such as social licking and social play were not recorded by Roth (2008). We found no differences in initiated social licking, which occurred very rarely (median (min–max): *Mother* 0 (0–2), *Automat* 0 (0–2)). This may be explained by the fact that usually calves are mostly licked by the mother and less by other calves (Le Neindre, 1993). In addition, the calf in the test situation was unfamiliar. In cattle, social licking can be mostly observed with social partners (Boissy et al., 2007; Wasilewski, 2003). Flower and Weary (2001) found only a numerical difference between calves separated after 1 or 14 days from the mother with the latter initiating more licking. These calves also rubbed the unfamiliar calf more often and showed more tail wagging when oriented towards the other calf. This is likely to indicate social play behaviour, as we also saw in our study: *Mother* calves initiated more social play behaviour when no cow was present. In contrast for the *Automat* calves the presence of a cow seems to play no role and thus no difference was found in social play when a cow was present. Calves play with the mother, but the primary partners for social play are other calves and rarely other adult cows (Reinhardt, 1980;

Kiley-Worthington and de la Plain, 1983). Possibly the cow distracted the *Mother* calves from their confrontation partner thus reducing their social play. Also in the cow barn *Mother* calves especially played at times when adult cows were not present (see above). Contact with the mother and with other adult animals during rearing gave the calves more opportunities for social experiences (Arnold and Taborsky, 2010; D'Andrea et al., 2007), which might enhance social (playful) activity even with unfamiliar calves. Furthermore, social play behaviour is essential for the development of social behaviour in young animals for later life and can even prepare for unexpected situations (Bekoff, 2001; Spinka et al., 2001; Van den Berg et al., 1999).

Roth (2008) found a higher heart rate in mother-reared calves in a social confrontation test compared to calves without contact with the mother, which was explained by higher social activity. In our study, no difference in heart rate was detected. However, the physical activity might have obscured potential emotionally caused differences in heart rate. In comparison to our study, in the study by Roth (2008) test duration was longer, there were differences in weight between the rearing treatments, and the tested calves were confronted with a group of unfamiliar and older calves, which might explain the differences in the results. Moreover, stress was increased when animals had been moved a longer distance and for a longer time prior to the confrontation challenge, which was reflected by a higher mean heart rate. The greater distance to the home pen (on the other side of the barn, i.e. out of visual contact with the home pen area and auditory contact with the dam and peers) might have contributed to a higher level of stress in the calves. Calves that had been moved only a short distance could possibly hear their pen mates and thus might have received social support and felt more secure (Rault, 2012 review).

Aside from rearing effects, GH-calves explored less frequently, tended to walk longer and showed more solitary play behaviour compared to GRP-calves. It seems that the GH-calves were more active than the GRP-calves. The two breeds might differ in their behavioural strategy to cope with the confrontation. Differences between breeds in reactivity to a stressful situation have been described before (Boissy and Le Neindre, 1990, 1997).

4.3. General discussion

Although we investigated a relatively small number of animals given the influencing factors present in the confrontation test, we found some clear differences between the rearing treatments. Moreover, the enriched environment with contact to the mother and other adult cows in the rearing of *Mother* calves entailed in both tests more social activity/involvement, observable in the isolation test with more contacting and trying to rejoin the social partners as well as in the confrontation test where they were socially more active. As we discussed before, we assume that *Mother* calves showed less locomotor play in the test situation because this behaviour had been satisfied due to the larger space allowance in the cow barn. More non-social and social play and additional social experiences with other adult animals could be advantageous as a preparation for unexpected or competitive situations later in life (Arnold and Taborsky, 2010; Bekoff, 2001; D'Andrea et al., 2007; Spinka et al., 2001; Wagner et al., 2012). For future prospects, if *Mother* and A*utomat* calves indeed differ in the amount of play behaviour in the home environment and in physiological indicators of welfare is currently investigated.

5. Conclusion

Even though *Mother* calves were found to suffer from stress during isolation, they were able to react adequately to the challenging situation. The results suggest that mother-reared calves showed higher motivation to rejoin their mothers and/or herd and cope more actively with the isolation test. As well in the confrontation test *Mother* calves seem to be socially more active and more attentive to their social environment. Furthermore, it seems that rearing with contact to the mother by giving access to the whole cow herd enables the calves to satisfy their need for locomotor play by providing space of sufficient dimensions, pointing at better welfare.

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