

## Calf-directed affiliative behaviour of dairy cows in two types of cow-calf contact systems

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### ABSTRACT

There is an interest in alternative rearing systems that allow for prolonged cow-calf contact (CCC). Yet, a better understanding of cows' affiliative behaviour in those systems is needed. We evaluated the effect of type of CCC on calf-directed affiliative behaviour in dairy cows. Cows were permitted to have either: i) partial contact (PC) with their calf; calves were housed in a pen adjacent to the cow area allowing limited physical contact on initiative of the dam but no suckling ( $n = 18$ ), or ii) full contact (FC) with their calf including suckling; calves were housed together with the dams in a free stall barn ( $n = 20$ ). Proximity and physical contact between the cow and her own calf were recorded between 0 and 48 h postpartum in an individual maternity pen, and from 1 to 5 weeks postpartum in a free stall barn. Data were analysed with generalized linear models, except for behaviour with excess of zero-valued data where a Kruskal Wallis test was used. Principal component analysis (PCA) was carried out to identify consistency of behaviour in the maternity pen and free stall barn. After parturition, latency to onset of allogrooming did not differ among treatments ( $\text{mean} \pm \text{SE}$ ,  $8 \pm 3$  min,  $P = 0.39$ ). Throughout the first 48 h postpartum, no treatment differences were found in percentage of observed time spent allogrooming the calf (PC:  $7.7 \pm 1.3\%$ , FC:  $9.5 \pm 1.5\%$ ), standing in proximity ( $\leq 1$  m radius) (PC:  $22.9 \pm 2.1\%$ , FC:  $21.2 \pm 2.1\%$ ), or lying in proximity (PC:  $30.5 \pm 4.3\%$ , FC:  $32.5 \pm 3.2\%$ ) ( $P > 0.10$ ). However, in the following 5 weeks, relative to PC cows, FC cows spent more time on average in close proximity to their calf ( $10.9 \pm 0.1\%$  versus  $3.1 \pm 0.4\%$ ,  $P < 0.001$ ), and on allogrooming ( $2.1 \pm 0.2\%$  versus  $0.5 \pm 0.1\%$ ,  $P < 0.001$ ). PCA revealed four components (explaining 76% of the variance). Lying in close and standing in far proximity in the maternity pen loaded (positive, negative, respectively) onto component 1, whereas physical contact and standing in close proximity in the free stall barn loaded negatively onto component 2. Standing in close proximity in the maternity pen loaded onto component 3, and standing 1–2 m near the calf in the free stall barn loaded onto component 4. Our results indicate that, in comparison with FC, PC decreases the expression of calf-directed affiliative behaviours in dairy cows, except in the 48 h following parturition. The partial CCC set-up limited the calf's accessibility, whereas calves in full CCC could initiate contact as well. Nonetheless, large inter-individual differences in calf-directed affiliative behaviour were found that lacked consistency.

### 1. Introduction

Cattle are known to form long-lasting social bonds in small stable groups (Bouissou et al., 2001; Reinhardt and Reinhardt, 1981). A social bond can be defined as a preferential mutual, affectionate relationship characterized by, among others, spatial proximity, synchronised behaviour, and allogrooming (Bouissou et al., 2001; Newberry and

Swanson, 2008). These affiliative behaviours are primarily of a positive nature, provide opportunities for social support in challenging situations, and are accompanied by specific calming and rewarding physiological reactions (Newberry and Swanson, 2008; Rault, 2012). Hence, allowing for the formation and maintenance of social bonds between cattle, also under commercial conditions, is considered important in ensuring their welfare (EFSA, 2009).

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Generally, the first established social bond in life is the bond between mother and young, which is essential for survival of a new-born calf under natural conditions (Newberry and Swanson, 2008). The mother's affiliative behaviour is essential for bonding, which is driven by complex internal processes (e.g. hormones around parturition) and external factors (e.g. presence of a new-born), and is known to be affected by maternal experience (Olazábal et al., 2013; von Keyserlingk and Weary, 2007). However, on most commercial dairy farms, calves are separated from the dam within 24 h postpartum; a practice which raises public concern (Busch et al., 2017; Hötzel et al., 2017; Ventura et al., 2013).

Alternative systems, where dairy cows and their calves can bond by staying in contact for a prolonged period of time, are receiving interest from various stakeholders due to the opportunities it may provide for increasing the social acceptance of the dairy sector, for example by allowing the expression of natural behaviours, and better responding to consumer demands (Beaver et al., 2019; Brombin et al., 2019). These so-called cow-calf contact (CCC) systems can differ in the type of physical contact allowed between the dam and her calf, and are generally described as full or partial CCC systems (Sirovnik et al., 2020). Full CCC (i.e. unrestricted physical contact including suckling) typically involves keeping calves together within the herd, which allows cow-calf pairs to express affiliative behaviours, such as licking each other, suckling, and resting in contact (Johnsen et al., 2016; Wagenaar and Langhout, 2007). Yet, dairy producers that allow full CCC may face several challenges, such as loss of saleable milk, stress at separation, milk ejection disturbances, hygiene issues, and a poor human-calf relationship (Johnsen et al., 2016). One way to overcome those challenges is to reduce the duration of daily contact via part-time CCC systems, in which cow and calf have contact only during specific moments of the day (e.g. half-day or only briefly after milking) (Sirovnik et al., 2020). However, those systems are considered as labour intensive (Johnsen et al., 2016). Another option would be to allow partial CCC, where contact can be restricted by preventing suckling and limiting the physical contact (e.g. housing the calf behind a fence adjacent to the dams or using an udder net for the dam) (Sirovnik et al., 2020). Calves' nutritional independence from the dam seems to reduce stress at separation (Johnsen et al., 2018) and minimize loss of saleable milk due to suckling (De Passillé et al., 2008), whereas limiting physical contact could benefit practicability of CCC while still allowing for social interaction among cow and calf. Nevertheless, suckling is considered to be the most important and common care-giving behaviour in cattle (Lévy, 2016; von Keyserlingk and Weary, 2007). Recent work has shown that the social bond seems to grow stronger when suckling is allowed, as suckled dams showed an increased motivation to reunite with their own calf compared to non-suckled dams (Wenker et al., 2020). However, a better understanding of the social interactions in various CCC systems is needed before recommendations for specific systems can be made (Meagher et al., 2019).

Therefore, the objective of the present study was to evaluate the extent to which the type of CCC alters calf-directed affiliative behaviour of dairy cows over time. It was hypothesised that full CCC cows would show more affiliative behaviours towards their calf than partial contact cows in the weeks following calving, and that this effect would be less profound in the hours succeeding parturition.

## 2. Materials and methods

This study was conducted at the Knowledge Transfer Centre in Zegveld (the Netherlands) from February 2019 to July 2020. All applicable international, national, and/or institutional guidelines for the care and ethical use of animals were followed. The experimental design was approved by the Central Committee on Animal Experiments (The Hague, the Netherlands; approval number 2017.D-0083).

### 2.1. Animals and treatments

Thirty-eight Holstein Friesian cows were included in this study with a parallel group design. Cows were included at calving if they gave birth to a single heifer calf without substantial calving difficulties or health problems. In order for calves to have an similar aged peer, every two cows that calved successively were assigned to the same treatment, and to have either: i) partial contact (PC) with their calf; calves were housed in a pen adjacent to the cow area allowing physical contact on initiative of the dam but no suckling ( $n = 18$ ), or ii) full contact (FC) with their calf including suckling; calves were housed together with the dams in a free stall barn ( $n = 20$ ). The mean parity of PC cows was 3.5 ranging from 1 to 6 versus a mean parity of 2.5 for FC cows that ranged from 1 to 7. Treatment order for every set of two cows was randomized. CCC was allowed for 10 weeks. In the present study, we report data from cow-calf pairs during the first 5 weeks postpartum. However, this study was part of a large longitudinal experiment that followed the animals for longer: cows were studied until 12 weeks postpartum and calves were studied up to 6 months of age.

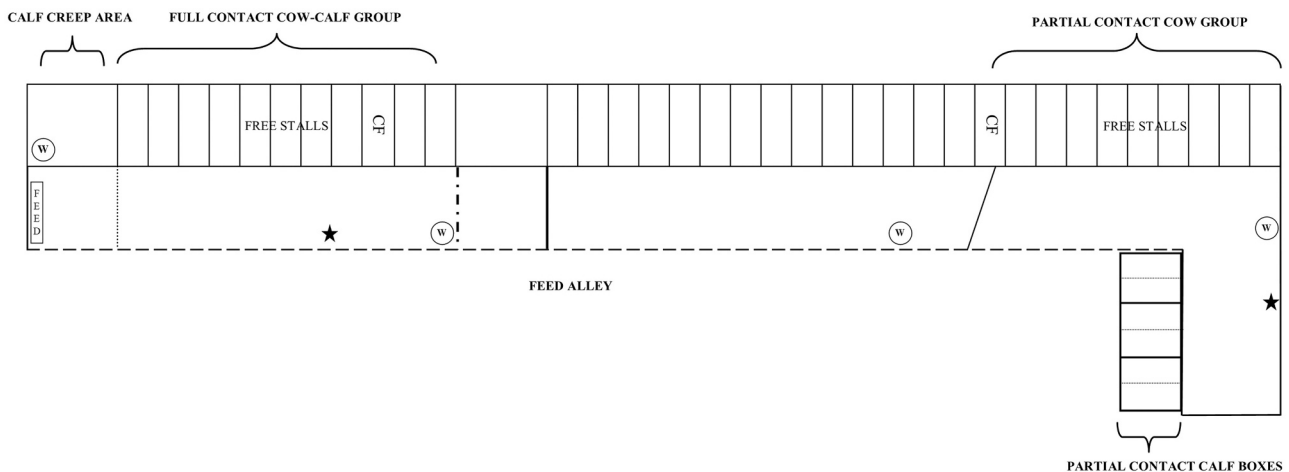
### 2.2. Calving management

Based on signs of imminent calving, cows were moved into an individual indoor straw-bedded maternity pen (3.0 m wide  $\times$  5.1 m long). Cows that were about to calve were video-monitored and the calving was assisted if necessary. Despite farm staff's regular checks of calving signs, seven cows (two in the PC group and five in the FC) calved in the dry-cow pen resulting in the calf being born on the slatted floor. Those cow-calf pairs were still included in the trial, but behaviours were only scored once a cow-calf pair was present in the maternity pen. Immediately after birth, navels were dipped with 2% iodine. Calves were briefly separated from the dam in order to measure the birth weight on a full-body calf scale (Type 8700, Welvaarts, the Netherlands). PC calves were placed in a cuddle-box (consisting of four plywood plates of 1.2 m wide  $\times$  0.8 m high) inside the maternity pen. The cuddle-box (see [supplementary material S1](#) for an illustration) prevented suckling, while still allowing tactile, visual, audible, and olfactory contact, and was placed in one of the corners across from the feeding rack. The cow could lick and sniff her calf when the calf was standing or lying, by moving her head into the box to reach the calf. When the barn temperature was below 10 °C, calves were provided with a heating lamp.

Cows in the maternity pen were milked twice daily with a mobile milking machine (Mini-milker, Kurtsan, Turkey). All calves were bottle fed with on average 3 L of colostrum from their own mother within 2 h after birth. Calves in the PC treatment group received an additional 2 L colostrum by bottle at 8 – 12 h, as well as at 20 – 24 h after birth. After the first colostrum meal by bottle (to standardize first colostrum consumption), FC calves were allowed to suckle the remaining colostrum directly from the dam's udder. Camera footage confirmed farm staff's suspicion that seven FC calves (but none of the PC calves) suckled their first colostrum before farm staff could bottle-feed them, however 4 of them did still receive some colostrum by bottle. Both PC and FC calves stayed with their dam in the maternity pen for about 72 h, after which they moved to designated group pens in the free stall barn.

### 2.3. Housing and feeding

The PC and FC cow-calf groups were housed inside a free stall barn in two dynamic group pens, one for each treatment (Fig. 1). All experimental cows were milked twice a day at approximately 08:00 h and 18:00 h in the milking parlour with a five-point open tandem side and 11 side-by-side places. Cows were fed grass silage (early spring cuttings) once a day at approximately 09:30 h. Feed was pushed automatically (MoovPro, JOZ, the Netherlands) to the feeding rack 8 times a day. Additionally, cows could eat up to 10 kg of concentrates per day that were provided partly in the milking parlour and by an individual



**Fig. 1.** The full contact (FC) and partial contact (FC) cow-calf groups were housed in two dynamic group pens inside a free stall barn. The free stall barn was naturally ventilated with open sidewalls and had perlite-bedded lying stalls (1.1 m × 3.0 m). The alley was covered with rubber flooring and cleaned 8 times a day by an automated scraper. Within the FC group pen, FC calves had access to a calf creep area (3.3 m × 4.8 m) with a straw-bedded lying area (3.3 m × 1.9 m), water bucket, hay and concentrates. A metal bar hindered cows to access this area. PC calves were housed individually (1.0 m × 1.6 m) in a straw-bedded calf box for the first two weeks, after which they were pair housed in the same box (2.0 m × 1.6 m) with their similar-aged peer. The calf boxes were placed behind a wall (1.2 m high) to limit physical contact and prevent suckling. The area behind the wall where cows could stand to interact with their calf was 2.5 m × 6.0 m. W = water, CF = individual concentrate feeder, ★ = automated brush.

concentrate feeder. In both group pens cows had access to an automated brush (swinging cow brush, DeLaval, the Netherlands). When the barn temperature was below 10 °C, all young calves were fitted with a calf jacket for the first three weeks of life.

PC calves were kept in a straw-bedded calf box (Topcalf Duo-Flex, Schrijver, the Netherlands) behind a wall (1.2 m high) adjacent to the PC cow group pen. This set-up allowed for visual, auditory, olfactory, and limited tactile contact between cow-calf pairs (Fig. 2). Cows could move their head over the wall and when the calf was standing on the other side, cow-calf pairs could sniff and lick each other. One calf box could house two calves individually (1.0 m × 1.6 m), but also offered the opportunity to pair house them (2.0 m × 1.6 m) by removing the partition wall in the middle of the box. PC calves were housed

individually for the first two weeks, after which they were pair housed with their similar-aged peer in the same box. In each calf box ad lib water, hay and concentrates (Topfok Kalf, de Samenwerking, the Netherlands) were provided as soon as the calf moved into the free stall barn (at 3 days of age). The PC group never exceeded more than six cow-calf pairs. Calves in the PC group were provided bulk tank milk in individual teat buckets following a fixed feeding schedule (see [Supplementary material S2](#)) after all three colostrum meals were consumed. Milk was provided around 08:00 h, 13:00 h, and 18:00 h. Bulk tank milk was heated up to 41 °C using a milk taxi (Milchtaxi 2.0, Holm & Laue, Germany) before being fed to the calves.

FC calves were housed together with the dams in the FC group pen, but had access to a calf creep area (inaccessible for the dams). The calf



**Fig. 2.** Partial cow-calf contact set-up. Calves were housed in a calf box (individually for the first two weeks and pair-housed afterwards) behind a 1.2 m high wall, cows could move their heads over the wall to lick and sniff the calf.

creep area provided them with a straw-bedded lying area and ad lib water, hay and concentrates from the day the new-born calves moved into the free stall barn. The FC group never exceeded more than eight cow-calf pairs. Except when cows were milked, FC calves could suckle their dams and, if allowed, other dams.

#### 2.4. Behavioural observations

Four neighbouring maternity pens were equipped with two cameras each (Hikvision; Model DS-2CE16H5T-ITE). Cameras were installed in bird's eye view across from each other above the pens. The behaviour of PC and FC cow-calf pairs was video recorded during the first 48 h after parturition. From these recordings (in the maternity pen) the behaviours described in Table 1 were continuously monitored at 3-min intervals during five observation periods (i.e. 0–4 h, 12–14 h, 22–24 h, 36–38 h, 46–48 h postpartum) by one of the two observers using The Observer (XT 14) software (Noldus Information Technology, the Netherlands). By observing 3 min and skipping the subsequent 3 min, 50% of each observation period was watched. Due to technical problems, no recordings were made of eight cows (three from the PC group and five cows from the FC group) in the maternity pen and were therefore excluded from this dataset.

Two similar cameras were used for both the PC and FC group pen inside the free stall barn to record cow behaviour. For the PC group pen, the cameras were placed in bird's eye view across from each other to have a front and back view of the calf-boxes. In the FC group pen, two cameras were installed onto the barn ceiling, in bird's eye view, and adjacent to each other to visualize the complete pen. In the free stall barn, the behaviours described in Table 2 were monitored by two observers when calves were approximately 7 days (week 1), 21 days (week 3), and 35 days (week 5) of age using The Observer software. There was one observation day for each week and this occurred on Sunday in order to reduce behavioural disturbances due to activities of farm staff as much as possible. For each observation day in the free stall barn, behavioural recordings between 04:00–22:00 h were analysed by one of the two observers using instantaneous sampling with a 3-min sampling interval. As mentioned earlier, in each treatment group two new born calves that were close in age were paired as similar aged peers. Focal sampling was applied to each of the two calves (and their dams) on observation days based on the birth date of the first calf.

**Table 1**

Description of recorded behaviours during the first 48 h in the maternity pen for both FC (full contact) and PC (partial contact) cow-calf pairs (adapted from Jensen, 2011 and Johnsen et al., 2015). Measurement unit: seconds.

| Behaviour        | Definition   | Modifier   |
|------------------|--|--|
| Lying            | Dam is lying on her sternum or side, head may be rested or raised  | Distance between any part of the dam's body (except her tail) and her calf: $\leq 1$ m, $> 1$ m          |
| Standing         | Dam's body supported by four legs, standing upright  | Distance between any part of the dam's body (except her tail) and her calf: $\leq 1$ m, $> 1$ m          |
| Feeding          | Dam's head through feeding rack, above the feed or taking grass silage into mouth  | Distance between any part of the dam's body (except her tail) and her calf: $\leq 1$ m, $> 1$ m (for FC) |
| Allogrooming     | Dam's muzzle in close proximity ( $< 5$ cm) of calf, or in contact with calf's body to lick, sniff or rub any body part; could be scored simultaneously with nursing |  |
| Nursing          | Calf's muzzle is under the dam's belly in the udder area (for FC)  |  |
| Other activities | Dam is engaged in any other behaviour not listed above (e.g. drinking, social interaction other cows)  |  |

**Table 2**

Description of recorded behaviours during the five weeks postpartum in the free stall barn for FC (full contact) and PC (partial contact) cow-calf pairs. Measurement unit: number of scans.

| Behaviour                     | Definition   | Modifier                    |
|-------------------------------|--|-----------------------------|
| <b>Proximity<sup>a</sup></b>  |  |                             |
| 0–1 m                         | Distance between any part of the dam's head and her calf $< 1$ m                       | Cow posture: lying/standing |
| 1–2 m                         | Distance between any part of the dam's head and her calf 1–2 m                         | Cow posture: lying/standing |
| $> 2$ m                       | Distance between any part of the dam's head and her calf $> 2$ m                       | Cow posture: lying/standing |
| <b>Contact</b>                |  |                             |
| No contact                    | No physical contact between the dam and her calf                                       |                             |
| Physical contact <sup>b</sup> | Dam's muzzle in close proximity ( $< 5$ cm) of calf's body or in contact with the body | Calf: own/alien calf/both   |
| Udder contact                 | Dam stands while the calf's muzzle is under the dam's belly in the udder area (for FC) | Calf: own/alien calf/both   |

<sup>a</sup> For PC cow-calf pairs proximity was estimated from the wall (at the calf's pen) to the dam's head. When a FC calf was lying the calf creep area, proximity was estimated from the metal bar to the FC dam's head.

<sup>b</sup> For FC cows physical and udder contact could occur simultaneously, but if there was udder contact and physical contact at the same time, this was scored as udder contact

For all video observations, both the intra-observer agreement and inter-observer agreement were calculated (kappa coefficient  $> 0.90$  and  $> 0.85$ , respectively).

#### 2.5. Data handling & statistical analyses

All statistical analyses were performed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC), treating the cow-calf pair as the experimental unit. Residuals of all outcome variables (i.e. in the maternity pen: latency to onset of allogrooming and proportion of observed time spent on allogrooming, lying in close proximity, standing in close proximity; in the free stall barn: proportion of observed time spent on allogrooming, standing in 1–2 m proximity, standing in  $< 1$  m proximity) were visually assessed for normality.

##### 2.5.1. Data handling for maternity pen observations

In total 13 PC and 10 FC cow-calf pairs were included for the analysis of the latency to onset of allogrooming in the maternity pen. Latencies were missing for 15 cow-calf pairs, as 2 PC and 5 FC calves were born in the dry-cow pen, and video footage of the first 4 h for 3 PC and 5 FC calves was missing due to technical problems (see supplementary material S3). Latencies were calculated as the time from birth to the onset of the behaviour within the first 4 h postpartum and were log transformed to normalize the data.

Descriptive analyses of the proportion of observed time spent on affiliative behaviours in the first 48 h were derived by summarizing the durations that a behaviour was registered for all observed time periods divided by the total observed time that an animal was visible. These behaviours were thus expressed as proportions of observed time. This method allowed us to also include the calves born in the dry-cow pen that were observed once they were moved to the maternity pen and therefore had a shorter observation period (Barrier et al., 2012), resulting in data of 16 PC and 15 FC cow-calf pairs being analysed. Due to the technical problems, there was no video material available for 2 PC and 5 FC calves (see supplementary material S3).

##### 2.5.2. Statistical analysis for maternity pen observations

Generalized linear model analyses were performed to analyse the observed behaviours in the maternity pen (i.e. latency to onset of allogrooming, proportion of observed time spent allogrooming, lying in close proximity, and standing in close proximity) using the PROC GLM procedure. The systemic part of the model (referred to as model 1)

consisted of the following fixed effects:

$$\mu + \text{Treatment}_i + \text{Batch}_j + \text{Parity}_k + (\text{Treatment}_i \times \text{Parity}_k) \quad (1)$$

Here,  $\mu$  was a base level and  $\text{Treatment}_i = \text{type of CCC}$  ( $i = \text{partial contact, full contact}$ ),  $\text{Batch}_j = 16\text{-week time period in which a calf was born}$  ( $j = 1, 2, 3, 4$ ), and  $\text{Parity}_k = \text{parity of the dam}$  ( $k = \text{primiparous or multiparous}$ ) were main effects. Batches were defined retrospectively to control for seasonal differences and varying group sizes in the two pens over time. Hence, the duration of the experiment was split up into batches of 16 weeks based on calving dates, so that every treatment was represented in a batch and batches represented the various seasons. Since parity is known to have an effect on cow's affiliative behaviour (von Keyserlingk and Weary, 2007), parity and the two-way interaction between parity and treatment were included in the statistical model. For the analysis of behavioural data expressed as proportions of time, the (logistic regression) model comprised a multiplicative dispersion factor with respect to the binomial variance function. Analyses of logistic models were based on maximum quasi likelihood with overdispersion parameters estimated from Pearson's generalized chi-square statistic (McCullagh and Nelder, 1989). An interaction was considered not significant when  $P \geq 0.05$ . Interactions that were not significant were excluded from the analysis. For all fixed effects either F-tests in analysis of variance or quasi likelihood ratio tests in logistic models were used, and significance was declared at  $P < 0.05$ .

### 2.5.3. Data handling for free stall barn observations

Due to technical problems with the video recordings in the free stall barn, 4 PC and 8 FC cow-calf pairs had one or more observation days missing (see supplementary material S3). Hence, 14 PC and 12 FC cow-calf pairs with a complete series of data for all weeks were included. Descriptive analyses of the observed time spent on affiliative behaviours in the free stall barn were derived by summarizing the total amount of scans that a behaviour was scored divided by the total number of scans. The total number of scans was corrected for the scans that cows were being milked. For FC cows, the proportion of time spent standing near their calf ( $\leq 1$  m) was corrected for the proximity due to nursing by subtracting for each week the number of scans scored as udder contact from the total number of scans scored as standing in close proximity.

### 2.5.4. Statistical analysis for free stall barn observations

Since the proportion of time spent in contact with any calf in the free stall barn contained an excess of zero-valued data, treatment differences were analysed using a non-parametric Kruskal-Wallis test.

The proportion of time spent in proximity to the own calf was analysed with a generalized linear mixed model for repeated measures, using the PROC GLIMMIX procedure. The systemic part of the model comprised the following fixed effects:

$$\mu + \text{Treatment}_i + \text{Batch}_j + \text{Parity}_k + \text{Week}_l + (\text{Treatment}_i \times \text{Parity}_k) + (\text{Treatment}_i \times \text{Week}_l) \quad (2)$$

in the same notation as before in model 1, and additionally  $\text{Week}_l = \text{age of calf expressed in weeks}$  ( $l = 1, 3, 5$ ) as main effect. The model also included two-way interactions between treatment and parity, and between treatment and week. The random part of the model contained random animal effects. For the animal effects, a first-order autoregressive model (based on the actual distance between time points) was adopted to introduce correlation in the model between repeated measurements on the same animal. Similar to the analysis of behavioural proportions obtained in the maternity pen, this model comprised a multiplicative dispersion factor with respect to the binomial variance function. Again, an interaction was considered not significant when  $P \geq 0.05$ . Interactions that were not significant were excluded from the analysis. For all fixed effects either F-tests in analysis of variance or quasi likelihood ratio tests in logistic models were used, and significance was declared at  $P < 0.05$ .

### 2.5.5. Principal component analysis of cow behaviour

To examine patterns of intercorrelations between calf-directed affiliative behaviours, and to assess whether cows were consistent in their behaviour across the two contexts (i.e. after parturition in the maternity pen versus the following weeks in the free stall barn), a principal component analysis (PCA; Jolliffe, 2002) was carried out. Eight behavioural parameters (expressed as proportions of time) were included in a PCA, five parameters that were recorded in the maternity pen, and three parameters that were recorded in the free stall barn. In the maternity pen: lying  $\leq 1$  m distance from the calf, lying  $> 1$  m distance from the calf, standing  $\leq 1$  m distance from the calf, standing  $> 1$  m distance from the calf pen, and allogrooming in the maternity pen. In the free stall barn: standing within 1 m from the calf, standing between 1 and 2 m from the calf, and physical contact with the calf. Calf-directed affiliative behaviours of the free stall barn were averaged for the three observation days. Those eight behaviours were selected as most relevant, because all cows could express these behavioural responses regardless the treatment group. In total 31 cows (i.e. 16 PC and 15 FC cows; see supplementary material S3) were included in the PCA, as for those animals data was available in both the maternity pen and free stall barn. PCA was performed on residuals of an analysis of variance model with treatment and parity as fixed effects. This allowed us to examine the correlation structure adjusted for treatment and parity, thereby focusing on covariation of behaviours within treatment and within parity, i.e. due to individual differences. Residuals of proportions were obtained using a logistic regression model comprising a multiplicative overdispersion factor with respect to the binomial variance function. After extraction, principal components were scaled by their standard deviations (square roots of associated eigenvalues) and subjected to varimax rotation. According to the Kaiser criterion, factors with eigenvalues larger than 1 were retained for further consideration. Loadings higher than (+/-) 0.50 were considered for interpretation.

## 3. Results

### 3.1. Affiliative behaviours in the maternity pen

After parturition, the latency to start allogrooming did not differ between PC and FC cows (mean  $\pm$  SE in min,  $7.5 \pm 2.8$  min,  $P = 0.39$ ) (Table 3). Throughout the first 48 h postpartum, time spent allogrooming the calf averaged approximately  $8.6 \pm 1.0\%$  and did not differ among PC and FC cows ( $P = 0.17$ ). Similarly, no significant differences were found between PC and FC cows for the time spent in close proximity to their calf, as all cows spent on average  $22.1 \pm 2.7\%$  of the time standing and  $31.5 \pm 1.0\%$  of the time lying within 1 m radius to their calf ( $P = 0.10$ ,  $P = 0.67$ , respectively) (Table 3). Detailed results regarding the effects of batch and parity on affiliative behaviour in the maternity pen are summarized in supplementary material S4.

**Table 3**

Behavioural observations in the first 48 h postpartum in the individual maternity pen. Response parameters are shown separately for the two treatments (partial cow-calf contact, full cow-calf contact). Latencies are shown as minutes (mean  $\pm$  SE) after birth, and the times that cows spent in proximity ( $\leq 1$  m radius) to the calf and allogrooming the calf are shown as proportion of time (mean  $\pm$  SE in %) of total time visible. For the number of included observations for each behaviour see supplementary material S3.

| Behaviour                   | Treatment       |                | F    | P value |
|-----------------------------|-----------------|----------------|------|---------|
|                             | Partial contact | Full contact   |      |         |
| <b>Latency</b>              |                 |                |      |         |
| Allogrooming                | 10.2 $\pm$ 4.6  | 4.1 $\pm$ 2.3  | 0.78 | 0.39    |
| <b>Proportion of time</b>   |                 |                |      |         |
| Standing in close proximity | 22.9 $\pm$ 2.1  | 21.2 $\pm$ 2.1 | 2.83 | 0.10    |
| Lying in close proximity    | 30.5 $\pm$ 4.3  | 32.5 $\pm$ 3.2 | 0.18 | 0.67    |
| Allogrooming                | 7.7 $\pm$ 1.3   | 9.5 $\pm$ 1.5  | 2.01 | 0.17    |

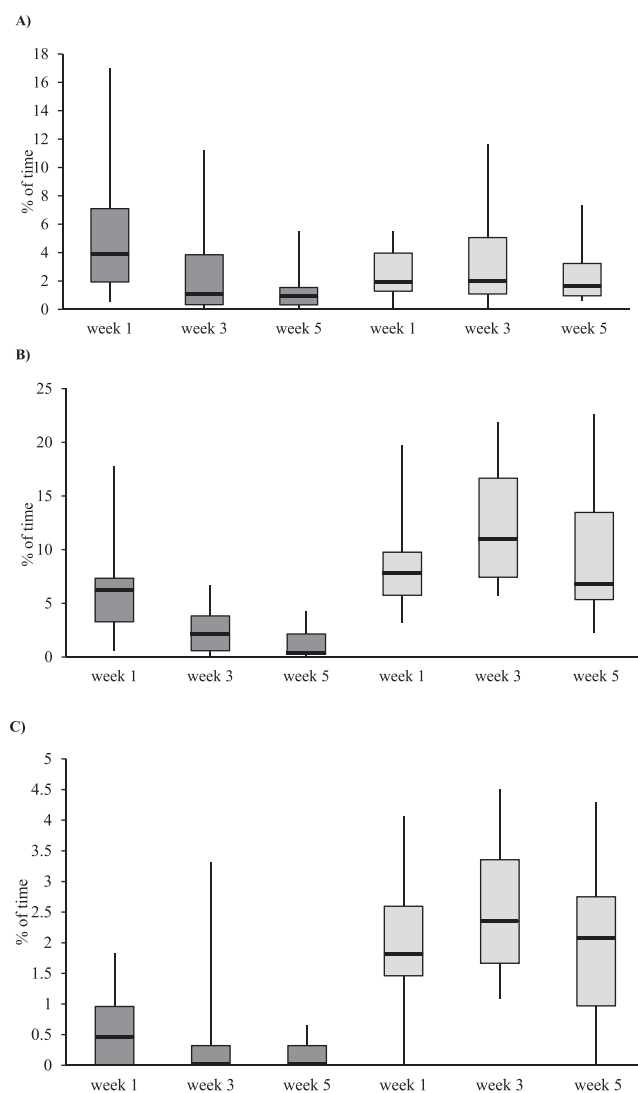
### 3.2. Affiliative behaviours in the free stall barn

In the free stall barn, FC cows spent on average  $2.8 \pm 0.4\%$  of the time (mean proportion  $\pm$  SE) standing in 1–2 m proximity to their calf versus  $3.1 \pm 0.5\%$  for PC cows. Only in the first week treatment differences were found ( $P = 0.045$ ). Overall, individual levels of this behaviour ranged from 0% to 17.0% for PC cows and from 0% to 11.7% for FC cows. PC cows spent more time standing in 1–2 m proximity to their calf in the first week postpartum ( $5.5 \pm 1.3\%$ ) compared to the third ( $2.7 \pm 0.9\%$ ;  $P = 0.02$ ) and fifth week postpartum ( $1.3 \pm 0.4\%$ ;  $P = 0.001$ ), whereas FC cows showed a more steady pattern over time (week 1:  $2.4 \pm 0.5\%$ , week 3:  $3.4 \pm 1.1\%$ , week 5:  $2.3 \pm 0.6\%$ ;  $P > 0.31$ ) (Fig. 3A).

Moreover, FC cows spent on average  $10.9 \pm 0.1\%$  of the time standing within 1 m proximity to their calf compared to  $3.1 \pm 0.4\%$  for PC cows. Treatment differences were found in week 3 and 5 ( $P < 0.001$ ). Inter-individual variation for the time spent in close proximity ranged from 0% to 17.8% for PC cows and 2.2–22.6% for FC cows. As shown in

Fig. 3B, PC cows spent more time standing in close proximity to their calf in the first week postpartum ( $6.3 \pm 1.2\%$ ) compared to the third ( $2.2 \pm 0.6\%$ ;  $P < 0.001$ ) and fifth week postpartum ( $1.3 \pm 0.4\%$ ;  $P < 0.001$ ). However, no difference between week 3 and week 5 was found ( $P = 0.18$ ). In contrast, FC cows showed an increase in standing close to the calf in the third week ( $12.8 \pm 1.7\%$ ,  $P = 0.01$ ) compared to the first ( $8.5 \pm 1.2\%$ ) and fifth week ( $8.5 \pm 1.6\%$ ) (Fig. 3B). Detailed results regarding the effect of batch and parity on the observed time spent in proximity are summarized in [supplementary material S4](#).

In addition, FC cows spent on average more time in physical contact with their calf ( $2.1 \pm 0.2\%$ ) compared to PC cows ( $0.5 \pm 0.1\%$ ;  $P < 0.001$ ). Among individuals, time spent in physical contact with the own calf ranged from 0% to 3.3% for PC cows and 0–4.5% for FC cows. Throughout time, both PC and FC cows showed a rather stable pattern in their average time spent in physical contact with their own calf ( $P = 0.16$ ,  $P = 0.50$ , respectively) (Fig. 3C). No treatment differences were found for the average time spent in physical contact with an alien calf ( $0.1 \pm 0.0\%$ ,  $P = 0.63$ ).



**Fig. 3.** Calf-directed affiliative behaviour at three different calf ages expressed by dairy cows that were allowed to have either partial contact (dark grey) ( $n = 14$ ) or full contact (light grey) ( $n = 12$ ) with their calf in the free stall barn. Results are shown separately for **A**) time spent standing within 1–2 m proximity to the own calf, for **B**) time spent standing in close proximity ( $< 1$  m radius) to the own calf, and for **C**) time spent in physical contact with the own calf. The boxplots show median (bold horizontal line within the box), 25th and 75th percentile (top and bottom of box), and range (tips of vertical whiskers).

### 3.3. Cross-situational consistency of affiliative behaviours

The PCA of the residuals of calf-directed affiliative behaviours expressed in the maternity pen and the free stall barn revealed four principal components with eigenvalues  $> 1$  which accounted for 76% of the variance of the data (Table 4). Notably, behaviours recorded in the maternity pen consistently loaded on different factors than behaviours recorded in the free stall barn (Table 4). Factors 1 and 3 were determined by affiliative behaviours expressed in the maternity pen, whereas factors 2 and 4 were dominated by behaviours exhibited in the free stall barn. Factors 1 and 3 summarized different types of proximity of the cow to the calf in the maternity pen, related to lying and standing close to the calf, respectively. Measures of proximity in the free stall barn exclusively loaded on factor 2 (Table 4).

## 4. Discussion

The objective of this study was to evaluate the effect of different types of CCC on calf-directed affiliative behaviour of dairy cows. We focused on the dam's affiliative behaviour towards her calf, as partial CCC was created by preventing the calf to roam freely among the cows, which resulted in a cow-driven CCC system. Our results showed that the type of CCC did not affect cows' behaviour in the hours succeeding parturition. However, in the following weeks FC cows spent a higher

**Table 4**

Loadings<sup>a</sup> on the first four components extracted by principal component analysis (PCA), after varimax rotation, of residuals<sup>b</sup> of cow's affiliative behaviours towards their own calf ( $n = 31$ ), and the eigenvalues and proportions of total variation explained by each component.

| Variable                         | Location        | PC1          | PC2          | PC3          | PC4         |
|----------------------------------|-----------------|--------------|--------------|--------------|-------------|
| Lying in $\leq 1$ m from calf    | Maternity pen   | <b>0.94</b>  | 0.02         | 0.08         | 0.13        |
| Lying in $> 1$ m from calf       | Maternity pen   | <b>-0.55</b> | -0.20        | <b>-0.66</b> | -0.28       |
| Standing in $\leq 1$ m from calf | Maternity pen   | -0.16        | 0.03         | <b>0.92</b>  | -0.01       |
| Standing $> 1$ m from calf       | Maternity pen   | <b>-0.82</b> | 0.06         | 0.12         | 0.06        |
| Allogrooming                     | Maternity pen   | 0.04         | 0.10         | 0.10         | 0.13        |
| Standing in 0–1 m proximity      | Free stall barn | -0.10        | <b>-0.86</b> | -0.22        | 0.16        |
| Standing in 1–2 m proximity      | Free stall barn | 0.08         | 0.06         | 0.04         | <b>0.94</b> |
| Physical contact                 | Free stall barn | 0.27         | <b>-0.64</b> | 0.49         | -0.27       |
| Eigen values                     |                 | 2.36         | 1.47         | 1.23         | 1.04        |
| Variance explained (%)           |                 | 29.52        | 18.41        | 15.37        | 13.02       |

<sup>a</sup> Loadings greater than 0.50 are indicated in bold.

<sup>b</sup> Residuals from an analysis of variance model with treatment and parity as fixed effects.

proportion of time performing affiliative behaviours towards their calf than PC cows.

At parturition the expression of affiliative maternal behaviour is controlled by endocrine mechanisms (Lévy, 2016). Licking of the new-born calf is considered essential in establishing a mother-young bond (von Keyserlingk and Weary, 2007). After birth, all experimental cows accepted and interacted with their calf. The cuddle box in the maternity pen did not seem to hinder PC cows to interact with their calf, as both CCC groups showed similar calf-directed affiliative behaviour in the first 48 h postpartum. In contrast, Green et al. (2020) recently showed that postpartum fence-line separation from the calf elevated stress behaviour in the dams reflected by increased alertness to the calf and high-frequency calls. In our study the average time spent in close proximity or allogrooming did not differ among the groups, so there was no indication of increased alertness towards the calf for the PC cows. Although we did not document vocalisations, the elevated stress levels found by Green et al. could be the result of the design of the fence-line. The fence-line only allowed occasional physical contact between the dam and her calf, whereas in our cuddle-box the calf was easily accessible.

In the weeks following parturition, the preference for the related calf plus the times spent in close proximity to and in contact with the own calf suggest that a bond was formed between the cow-calf pairs regardless of the type of contact (Bouissou et al., 2001; Gubernick, 1981). In the first week, PC cows spent a larger proportion of the observed time in 1–2 m proximity to their calf than FC cows. This may have been the result of the automated brush that was positioned 2.5 m across the PC calf boxes. Fresh cows are known to use the brush frequently in the first week postpartum and this usage decreases over time (Mandel and Nicol, 2017). Nonetheless, for PC dams the proportion of observed time spent in less than 1 m proximity to and in physical contact with their calf was lower and gradually decreased over time, in contrast to FC dams that showed rather steady patterns throughout time. Dairy cattle have been classified as a ‘hider species’ where the mothers actively seek contact throughout the first days of life while the new-born hides itself (Lent, 1974). In the succeeding weeks the initiative to make contact shifts towards the calf (Jensen, 2011; Tucker, 2009). The higher levels of calf-directed behaviour in FC cow-calf pairs in the free stall could be the result of contact initiated by the FC calves that were roaming free in contrast to PC calves that were restrained in their calf pen and could not actively seek out the dam. Since FC cow-calf pairs could also spend time lying together in close proximity (besides the reported time spent standing in close proximity), their actual total time spent in close proximity may have been even higher than described in this study. Previous work found that affiliative behaviours did not differ among suckled and non-suckled cow-calf pairs (Johnsen et al., 2015). Those pairs had half-day contact and were observed only in the two hours following reunion. Contrary to the current study, Johnsen et al. (2015) housed both treatments in the same group pen in which calves roamed freely, allowing all calves to be the initiator of contact as well. We suggest for future studies to identify the contact-initiator in CCC systems and include calves’ affiliative behaviour as well. Our partial CCC set-up was designed to meet some major concerns of dairy producers. By housing PC calves aside the cow herd, suckling and direct contact with manure of adult cows was prevented, while it allowed for individual feeding of calves and certain cow-calf interactions. However, in cases where the PC calf was lying in the back of the calf pen, this pen limited PC cows to interact with their calf as they could not reach the calf. Therefore, PC cows may have received less reinforcement to socially interact compared to FC cows that could more easily make contact with their calf (Meagher et al., 2019).

Possibly, the combination of those factors affected the cow-calf bond and reduced the PC dams’ affinity with her calf. Nevertheless, recent descriptive work of Johnsen et al. (2021) showed that limiting physical contact in a cow-driven full CCC system did also affect the cow’s affiliative behaviour. In that particular study the cows had to pass selection

gates to access a meeting area for social interactions (including suckling) with their calf, and were allowed to have either free access or limited access depending on a successful milking in the automatic milking system. Numerically the limited group showed less successful visits and a lower duration of allogrooming their calf compared to the unlimited group, although they did not differ in suckling duration (Johnsen et al., 2021). This indicates that limiting physical contact in cow-driven CCC systems affects the cow’s affiliative behaviour in various ways. On the other hand, oxytocin is known to be an important hormone involved in social bonding (Carter et al., 1992; Kendrick, 2000) and suckled dams have been found to have higher oxytocin levels in response to suckling/milking than non-suckled dams (Lupoli et al., 2001). In addition, suckled dams showed an increased motivation to reunite with their own calf compared to non-suckled dams, which indicates that the social bond seems to grow stronger when suckling is allowed (Wenker et al., 2020). Therefore, the greater expression of calf-directed affiliative behaviours in FC dams may also have been the result of the suckling opportunity that strengthened the mother-young bond.

Nevertheless, the current results show large variations in calf-directed affiliative behaviour between individual cows regardless of the CCC treatment, which implies that certain cows express a greater interest in being near or interacting with their calf than others. Since animal welfare relates to the quality of life as experienced by the individual animal (Winckler, 2019), individual responses should not be overlooked when investigating pure group mean responses (Richter and Hintze, 2019), especially since interactions with offspring are suggested to have a positive hedonic value for mammalian mothers (Olazábal et al., 2013). Individual differences in maternal care among cattle have been previously described and are known to be affected for example by breed (Le Neindre, 1989; Le Neindre and Sourd, 1984), cow’s body condition and calf characteristics (i.e. sex, birth weight) (Stěhulová et al., 2013), parity (Edwards and Broom, 1982; Vandenheede et al., 2001), and received maternal care as calf (Le Neindre, 1989). Furthermore, individual maternal differences also seem consistent throughout lactation (Dwyer, 2008; Stěhulová et al., 2013). Interestingly, the present study showed that cows were not consistent in their affiliative behaviours (in terms of proximity to their calf) across context, i.e. in the maternity pen and the free stall barn. PCA clearly showed that affiliative behaviours expressed in the maternity pen loaded on different factors than affiliative behaviours expressed in the free stall barn. This means, for example, that cows spending a relatively long time in close proximity to their calf in the maternity pen did not necessarily show the same behavioural pattern in the free stall barn. In addition, the loading pattern (i.e. the extent to which behavioural variables correlated with a component) obtained after PCA seemed to suggest that different types of proximity may exist, for example, proximity determined either by standing or lying close (within 1 m) to the calf in the maternity pen (see Table 4, PC1 and PC3), or proximity defined in terms of standing close (within 1 m) or less close (between 1 and 2 m) from the calf in the free stall barn (see Table 4, PC2 and PC4). Collectively, these findings could imply that different affiliative behaviours (exhibited in different contexts) are driven by different motivational states. Behaviour is conceptually organized in so-called motivational systems that are each activated by specific motivational states with different underlying neurobiological systems (Koolhaas et al., 1997). Generally, animals adapt their behaviour to satisfy different motivations and to perform optimally in a given situation/environment, so maternal animals do not necessarily act according to fixed patterns but make decisions based on contextual information, emotional and internal states (including multiple motivations) (Olazábal et al., 2013). Possibly, the motivation to stand between 1 and 2 m from the calf in the free stall barn may have been controlled by a different motivational state than standing within 1 m from the calf; perhaps the former involves the motivation to be close to another resource in the barn (e.g. automated brush or drinker) at the same time, whereas the latter involves predominantly maternal traits. Similarly, being in close proximity in the maternity pen may be driven

by a different neurochemical brain state compared to standing in close proximity in the free stall barn (Koolhaas et al., 1997). Overall, individual differences and independent dimensions underlying those differences give insight into the complexity and variety of the animals' behaviour.

A limitation of the current study is that technical problems with the cameras and digital video recorder resulted in missing data for several cow-calf pairs. However, those problems occurred randomly, so missing observations arose by chance which still allowed for an unbiased comparison between the treatment groups. More research is needed to identify other factors or traits underlying cows' variation in calf-directed affiliative behaviour. Moreover, further assessment of the effect of full and partial CCC systems on stress responses at unanswered contact attempts or when debonding (i.e. weaning and separation phase) is recommended.

## 5. Conclusion

This study shows that, except for the hours succeeding parturition, type of cow-calf contact (CCC) affects the expression of calf-directed affiliative behaviour in dairy cows. Partial CCC resulted in less calf-directed affiliative behaviours compared to full CCC, except in the 48 h following parturition. This may be due to the fact that the partial CCC set-up limited the accessibility of the calf or because in the full CCC set-up calves could also initiate contact. Moreover, large inter-individual differences were found and the expression of calf-directed affiliative behaviour in the free stall barn could not be predicted based on the behavioural responses expressed in the maternity pen.

## Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.applanim.2021.105461](https://doi.org/10.1016/j.applanim.2021.105461).

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