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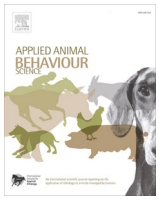

# Dairy cows housed both full- and part-time with their calves form strong maternal bonds

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ABSTRACT

Dairy cow and calf are typically separated shortly after calving preventing the formation of a maternal-filial bond. To allow some cow-calf contact, part-time contact during the first weeks is thought to be a feasible solution, but it is unknown if it weakens maternal bond, i.e., if maternal motivation is lower. This study aimed to investigate how different amounts of calf contact (full-time, part-time, and no contact) affect cows' maternal motivation. Using pneumatic push gates, we assessed cows' motivation to access their own calf using the maximum price paid (MPP) method. To mitigate frustration at high prices, cows could also access an unfamiliar calf at a constant low price. We expected that cows would access the unfamiliar calf when reaching the maximum price that they were motivated to pay to access their own calf. Following 48 h in a calving pen, cow-calf pairs were allocated to three different treatments: full-time (23 h contact/d, 28 pairs), part-time (10 h contact/d, 27 pairs), and no contact (0 h contact/d, 26 pairs). Approximately 40 d after calving, cows were trained to pass through each of two push gates: one leading to their own and one leading to an unfamiliar calf. The weight on the gate leading to the cows' own calf increased following each passing, while the gate leading to the unfamiliar calf remained light. Cows were tested once daily, until they failed to pass through the gate leading to their own calf on two consecutive days. MPP was analysed using a Cox's proportional hazards mixed effects model. Fewer no-contact cows than full- and part-time cows fulfilled the learning criteria. Furthermore, no-contact cows paid a lower maximum price compared to the two contact treatments, while the MPP of full- and part-time cows did not differ. Most cows remained in the start box if they did not pass the gate to their own calf, indicating that an unfamiliar calf could not substitute for their own calf at high prices. We conclude that cows with part-time calf contact form a maternal bond of similar strength to cows with full-time calf contact. Additionally, cows separated from their calf at 48 h after calving have a weaker maternal motivation at 40 days postpartum.

## 1. Introduction

Dairy cow and calf are commonly separated shortly after calving; however, this practice is increasingly criticized by consumers, who view it as detrimental to the animals' welfare (Busch et al., 2017; Placzek et al., 2021; Sirovica et al., 2022). One argument for early separation is to prevent the formation of a strong maternal-filial bond, thus reducing stress at separation (Flower and Weary, 2003; Neave et al., 2022). Another argument made by some farmers is that dairy cows are poor mothers and have little maternal motivation (Neave et al., 2022). This highlights uncertainty surrounding the strength of the maternal bond and the importance of maternal behaviours to dairy cows.

Providing animals with resources that are important to them is key to animal welfare (Dawkins, 2008). Such resources include opportunity to perform behaviours that constitute behavioural needs (Jensen et al., 2004; Jensen and Pedersen, 2007), defined as behaviours that animals are strongly motivated to perform irrespective of the external environment (Friend, 1989). Few studies have addressed the maternal motivation in dairy cows (Jensen, 2011; Johnsen et al., 2021; Wenker et al., 2020), and to our knowledge only Wenker et al. (2020) have previously

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quantified maternal motivation.

Keeping cow and calf together throughout the day and night can prove challenging to the farmer. Therefore, part-time cow-calf-contact systems have been suggested as alternatives to full-time contact (Bertelsen and Vaarst, 2023; Sirovnik et al., 2020). Previous studies have shown that cows with part-time calf contact spend less time grooming and nursing their calves than cows with full-time contact (Bertelsen and Jensen, 2023; Jensen et al., submitted). However, it is unclear whether such reduction in nursing and grooming also reduces maternal motivation compared to full-time cows. Therefore, the present study aimed to quantify dairy cows' maternal motivation and to investigate the effect of calf contact (either full-time, part-time, or none) on the motivational strength at 40 days postpartum. We hypothesised that cows with full-time calf contact are more motivated to regain contact with their calves than cows with part-time calf contact, and that cows with no calf contact would show the lowest motivation.

Different methods exist to quantify motivational strength. For undividable behaviours (i.e. behaviours that are devalued by interruption; Kruschwitz et al., 2008; Mason et al., 1998) the maximum price paid (**MPP**, also known as the reservation price) method is more feasible than e.g. establishing elasticity of demand functions, which requires that the animal works for repeated rewards (Jensen and Pedersen, 2008; Kirkden et al., 2003). In the MPP method, the amount of work an animal has to perform (i.e., the price it has to pay) to access a given resource (for long enough to perform an uninterrupted bout of the behaviour in question) is gradually increased (e.g., in daily test sessions) until the animal is no longer willing to 'pay the price', i.e., the cost of access exceeds the animal's motivation to access the resource. This method allows for comparison of motivational strength of a certain behaviour in different situations, e.g. across experimental treatments, but does not allow us compare motivational strength of different behaviours (Jensen and Pedersen, 2008). Similar methods have been suggested to estimate offsprings' attachment to their mother (Gubernick, 1981). Animals can work in different ways, for instance by pressing bars or levers, or by pushing through weighted gates. Push gates with increasing weight have previously been used to measure MPP in studies on cattle (Tucker et al., 2018; Wenker et al., 2020). Once the MPP is reached, and the required price exceeds the animal's motivation to access opportunity to perform the behaviour in question, the animal may become frustrated (Tucker et al., 2018). This frustration may be due to lack of alternative options and thus a sense of loss of control in the situation, which can be perceived as stressful (Englund and Cronin, 2023; Leotti et al., 2010). A secondary aim of this study was therefore to appraise a novel take on the MPP method to avoid frustration at high 'prices'. The cows were presented with a choice between two resources: their own calf and an unfamiliar calf. The price for accessing their own calf increased as in a traditional MPP test, while the price of accessing the unfamiliar calf remained constant and low. We hypothesised that cows would choose the unfamiliar calf when they reached their maximum price and that the unfamiliar calf could, at least to some extent, function as a substitute (Hursh, 1980) for the cows' own calves.

## 2. Materials and methods

The study was conducted at the Danish Cattle Research Centre, Aarhus University (Tjele, Denmark), from September 2021 to August 2022. All animal procedures were approved by the Danish Animal Experiments Inspectorate in accordance with the Danish Ministry of Environment and Food Act No. 474 (May 15, 2014).

### 2.1. Housing and contact treatments

Eighty-four Danish Holstein-Friesian cows (*Bos taurus taurus*) and their calves were enrolled in the study. The cow-calf pairs were enrolled over seven blocks with 12 pairs in each block. Enrolment took place over two weeks before the experimental period began. All cows calved in individual calving pens, and cow and calf remained together in the calving pen for approximately 48 h postpartum (range 42–66 h). Behavioural responses to separation are increased when cows and calves are kept together for 4 days compared to 24 h (reviewed in Jensen, 2018) indicating that maternal bond strength increases with time together. Nevertheless, cows are able to recognise their calves for 12 h postpartum after just 5 min of contact (Hudson and Mullord, 1977). Due to pen availability, cow-calf pairs were kept in the calving pens for approximately 48 h, which appear to be enough time for them to form a selective bond to each other (Bertelsen and Jensen, 2023; Jensen et al., submitted). Enrolment criteria included unassisted calving, healthy cow and calf, no twin-birth, and unassisted suckling observed within the first 48 h. Following the time in the calving pens, pairs were allocated to one of three different cow-calf-contact treatments balanced for time of birth and cow parity (two primiparous and two multiparous cows per treatment per block when possible, always at least one primiparous cow): full-time contact (23 h contact per day, cow and calf together and only separated when the cows were away to be milked in the milking parlour, 28 pairs), part-time contact (10 h contact per day, cow and calf together during the day between morning and afternoon milking, but separated during the night, 27 pairs), and no contact (cow and calf separated within 48 h of birth, 26 pairs). Cow-calf pairs were allocated to treatments two pairs at a time, and allocation order rotated across the blocks on a predetermined rotational basis. None of the enrolled cows had previous experience with raising their calves for more than 12–24 h postpartum.

Three cow-calf pairs were excluded from the study before the experimental work began, resulting in n=81 experimental cows. Two calves (one no-contact, block 1, and one part-time, block 4) had to be euthanized due to disease and one cow (no-contact, block 2) was euthanized due to an injury unrelated to the experiment. Part-time pairs comprised of 12 primiparous cows and 15 multiparous, with 13 heifer calves and 14 bull calves. Full-time pairs comprised of 11 primiparous cows and 17 multiparous, with 17 heifer calves and 11 bull calves. No-contact pairs comprised of 10 primiparous cows and 16 multiparous, with 14 heifer calves and 12 bull calves.

No-contact cows were housed in a free-stall pen holding up to 12 cows at a time. No-contact calves were housed in groups of four in straw-bedded pens (3.0×3.0 m) in a separate barn from their dams (Fig. 1). Full- and part-time cow-calf pairs (herein referred to as contact pairs) were housed in groups of four pairs in straw-bedded pens (7.5×9.0 m) fitted with two calf creeps (3.0×3.0 m and 1.5×1.5 m, Fig. 1) in the same barn as the no-contact calves. Full-time cows spent both day and night with their calves in these pens, while part-time cows were housed in a free-stall pen adjacent to the no-contact cows during the night (from 1600 to 0500 h); part-time calves remained in the straw-bedded pen day and night, preventing visual, olfactory, and, possibly, auditory contact

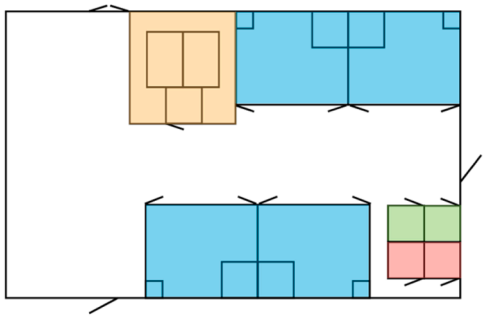

**Fig. 1.** Schematic drawing of the barn set-up. Four contact pairs were housed together in deep-bedded pens with two calf creeps (blue). No-contact calves were housed in pens of four calves (green), while no-contact cows were housed in a separate barn (not pictured). Before training and testing, contact calves were held in holding pens (red), and all cows were trained and tested in the arena (orange).

between dams and calves during the nightly separation. Cows were milked twice daily at 0500 and 1530 h for full- and part-time cows, and at 0530 and 1600 h for no-contact cows. All cows were fed TMR (approximately 50:50 concentrate to roughage ratio) *ad libitum* and had access to water from self-willing bowls or troughs. All calves had *ad libitum* access to concentrate from bowls, hay from racks, and water from self-filling bowls. Contact calves could access the cows' TMR, while no-contact calves were fed TMR from bowls. No-contact calves were fed milk *ad libitum* from a teat bucket twice daily (at 0630 and 1700 h), while contact calves only obtained milk from the cows; they were not fed any supplementary milk.

### 2.2. Test apparatus

Training and testing for maternal motivation took place in an arena located in the same barn as the contact cows' home pens (Fig. 1 and Fig. 2). The arena consisted of a start box, two reward pens, and two return walkways. The start box was connected to each reward pen via a 'push gate'. The floor of the arena was concrete covered in saw dust, which was replaced if soiled between each cow. The walls of the arena were built from standard barn fixtures fitted with plastic boards. The cows were not able to look over the division between the reward pens, but they could see outside of the arena. The 'push gates' were made from metal pipes, and the resistance on the gates was controlled using hydraulics. The corresponding weight for each increase in bar of pressure was measured and controlled throughout the experiment (Table 1; see SM1 for description of the measuring procedure). No feed or water was available in the arena.

To minimise discomfort while interacting with the push gates, two modifications to the gates were made: 1) the gates were padded using rubber tires (diameter = 21 cm; Figs. 3) and 2) a switch released the pressure on the gate once it was pushed more than half-way open. This prevented the cow from experiencing pressure on her abdomen as she passed through; she would instead experience pressure on her shoulders and the back of her neck, while she pushed the gate open.

### 2.3. Training

Training took place Thursday and Friday of the fifth week of the study and Monday of the sixth week. For some cows, Tuesday of the sixth week was included as well. Calves were on average 40.5 ± 6.4 d old on the first training day. The order of which each treatment group was trained and tested was balanced across blocks. A flow chart outlining the training steps can be found in SM2, Fig. 1.

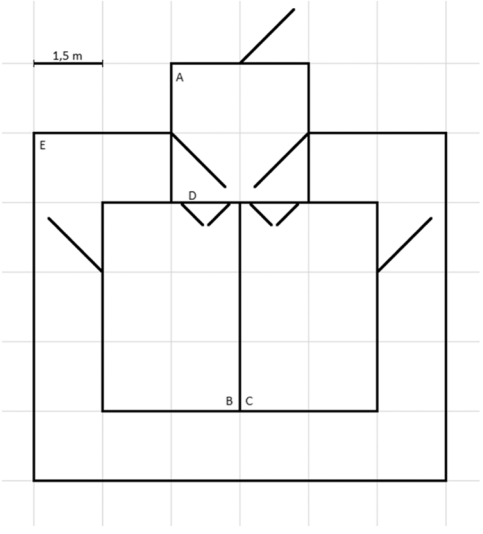


**Fig. 2.** Schematic drawing of the test arena. A: the start box, where the cow is lead to and from where she must choose between the two calf pens. B and C: tether points inside reward pens, where either the cow's own or an unfamiliar calf is tethered. D: push gates. The cow can see the calves through each push gate. E: return walkway, from which the cow (and calves, when released from their tethers) can return to the start box from the calf pens.

**Table 1**
The corresponding weight for each pressure level, i.e., the highest level of resistance the cows would experience on each pressure level, before the gates would release the pressure. The procedure for recording corresponding weights is described in SM1.

| Pressure (bar) | Weight (kg±std) |
|---|---|
| 1 | 29.9±0.9 |
| 2 | 42.7±0.9 |
| 3 | 55.8±0.7 |
| 4 | 68.9±0.6 |
| 5 | 82.1±0.8 |
| 6 | 95.9±1.1 |
| 7 | 111.2±0.4 |
| 8 | 124.6±1.2 |
| 9 | 139.8±1.2 |
| 10 | 151.9±1.5 |


**Fig. 3.** A photo of one of the push gates. The perceived width (W) between the two parts of the gate was 11 cm when fully closed, 52 cm when half-way open, and 67 cm when fully open.

#### 2.3.1. Training to pass push gates

Cows were randomly allocated to be first trained to access their own calf or an unfamiliar calf. The unfamiliar calf was always chosen from the no-contact treatment and was the same for the individual cow throughout training and testing. No-contact calves would function as unfamiliar calves for up to three different cows. Both full- and part-time cows were separated from their calves for 1 h before training of the first cow of the treatment. The length of the deprivation therefore varied for each cow but was at least 1 h. Deprivation increases motivation (Jensen et al., 2004), and pilot tests revealed that cows showed little motivation to access their calf without prior deprivation. Deprivation was initiated at 07:00 at the earliest, part-time cow-calf pairs had at least 1.5 h of contact following reunion after nightly separation. During the deprivation period, the calves were kept in a holding pen away from their home pen but in the same barn (Fig. 1). The calves had access to concentrate,

hay, and water during the deprivation period. No-contact cows, until initiation of training, had not seen their own calf since separation at 48 h postpartum.

Before a training session began, a calf (either own or unfamiliar) was tethered by a halter and an approximately 100 cm long rope in one of the reward pens (randomly allocated side, B or C in Fig. 2, calf placement remained consistent for the individual cow throughout training and testing). The other reward pen was empty, and the push gate leading to the empty pen was shut and blocked with a board. The cow was then led into the start box (A in Fig. 2) and the gate was closed behind her. The training sessions on the first two days consisted of four steps: 1) the push gate (D in Fig. 2) leading to the calf was completely open (the width of the opening 67 cm, Fig. 3); 2) the push gate was half-way open (width of opening 52 cm); 3) the push gate was shut completely (width of opening 11 cm) but had no resistance; and 4) the push gate was shut completely and had a resistance of 0.5 bar. The calf in the reward pen was visible to the cow while standing in the start box. For each step, the cow had two minutes to walk through the gate on her own accord. If she did not do this, she was encouraged to go through by gently touching/clapping her body with one hand. Either way, she received two minutes of calf contact as a reward; the calf remained tethered throughout the training session. If she was encouraged to walk through the gate, the step was repeated; if not, she progressed to the next step. Each step was repeated a maximum of five times, before proceeding to the next. The training day ended when the cow had passed through the gate with 0.5 bar of pressure, or when approximately 30 min had passed since training was initiated. Step 1–4 was repeated the following day, this time using the other calf (either own or unfamiliar, depending on which calf the cow was trained on the first day) and reward pen.

For the cow to meet the learning criterion of the first two days, she had to walk through a closed gate with 0.5 bar of pressure at least once on her own initiative, independently of which calf (own or unfamiliar) she accessed. If this did not happen during the first two days, a third day was included for this training step. On this third day, the cow resumed training with her own calf from the last step she passed. If she did not pass 0.5 bar on this extra day, she was excluded from testing due to not passing the first learning criterion within the predetermined deadline.

#### 2.3.2. Training to make a choice

When the cows had met the first learning criterion, they progressed to the next training step. Again, calves were separated 1 h before the training of the first cow of each treatment. During this training session, the cows first entered a simple preference test, which was then followed by a free choice. The aim of the simple preference test was to record the immediate preference of the cow, as well as informing her that she now could choose between the two calves; therefore, both own and unfamiliar calf were tethered in their respective reward pens. Both push gates were shut and set to 0.5 bar resistance. The cow was then led to the start box and had two minutes to pass through one of the two gates. If she did not make a choice, she was encouraged to go through a randomly predetermined gate. For the initial preference test, the cow was led directly back to the start box immediately after her choice. The gate she first chose was now blocked, and the cow again had two minutes to pass through the opposite gate (i.e., forced choice), before she was encouraged to pass through this gate. She was then again immediately, without any reward time, led back into the start box.

Directly after this initial preference and forced choice test the cow had her first free choice. The aim of the first free choice test was for the cow to experience a test situation. Both gates were set to 0.5 bar, and the cow had 2 minutes to walk through either of the gates. When she had walked through a gate and thus chosen a calf to access, her chosen calf was released from its tether through a quick release mechanism operated from a distance. The cow now received 4 minutes of reward time, before cow and (own) calf were led back to their home pen (no-contact cows were led home without their calf, and the no-contact calves were led back to their own home pen). It was recorded which calf, if any, was the cow's choice in the first free choice. The cow passed training if she passed through one of the two gates (and thus chose a calf) within the 2 minutes, and testing began the following day. If the cow did not choose a calf within 2 minutes, she was permitted one extra day to pass this training step. If she did not meet the learning criterion on this day either, she was not included for testing.

As part of a concurrent study, the cows had previously been trained in a visual discrimination task and tested for their judgement bias to assess their affective states (Neave et al., 2023), but this was not expected to affect the training or testing of the cows in the present study.

### 2.4. Testing: Maximum price paid (MPP)

Eighteen cows (22.2% of cows enrolled for training, Table 2) did not pass training, and three cows (two full-time and one part-time, block 5) were excluded during the test due to minor injuries unrelated to the testing procedure. MPP data was therefore collected from n=60 cows. These cows were 24 part-time (11 primi- and 13 multiparous; 10 heifer and 14 bull calves), 25 full-time (11 primi- and 14 multiparous; 17 heifer and 8 bull calves), and 11 no-contact cows (5 primi- and 6 multiparous; 8 heifer and 3 bull calves). MPP testing took place from the day after the cow passed training (earliest Tuesday of the sixth week of the study) until, at the latest, Friday the following week. Thus, there was a maximum of 10 testing days in total.

All testing took place in the morning, between 0800 and 1100 h. Contact cows were separated from their calves for 1 h prior to testing of the first cow in their treatment group. Cows were always tested in the same order. After the deprivation period, two calves (own and unfamiliar) were moved to the testing arena, where they were tethered in the side opposite to the gate in their respective reward pen (B and C, Fig. 2). The push gates were closed and the gate leading to the unfamiliar calf was set to 0.5 bar, while the gate leading to own calf was set to 1.0 bar. The cow was then fetched from her home pen and led into the start box (A, Fig. 2). The gate was closed behind her, and she then had two minutes to walk through one of the two gates. If the cow entered the reward pen with her own calf, the calf was released from its tether using a quick release system, and cow and calf had undisturbed contact for four minutes. Afterwards, they were led back to their home pen. The next day, the pressure on the gate leading to own calf was increased by 1.0 bar (see Table 1 for corresponding weights in kg), while the gate leading to the unfamiliar calf remained on 0.5 bar throughout testing. If the cow stayed in the start box for the entire two minutes, she was led back to the home pen without her calf. Her own calf was kept in the reward pen for five minutes before it too was herded back to the home pen; this was to ensure that the cow was only rewarded when she passed through a gate. If the cow entered the reward pen holding the unfamiliar calf, the calf was released from its tether using a quick release system, and cow and calf had undisturbed contact for four minutes. Afterwards, the cow was led back to her home pen. Her own calf was kept in the reward pen for an additional five minutes, before it too was moved back to the home pen.

The cow had two chances on each level of pressure; if she did not successfully enter the pen holding her own calf, the next day she would get another opportunity on the same pressure point. For instance, if a cow failed on 4.0 bar on the fourth testing day, she would get another

**Table 2**
Training success across the treatments. Cows were trained over three to five days, depending on how quickly they met the learning criteria. To pass training and continue to the testing phase, the cows had to meet two learning criteria: 1) passing a closed gate with 0.5 bar of resistance and 2) making a choice between their own and an unfamiliar calf.

| Treatment | Passed training | Failed training |
|---|---|---|
| No-contact | 11 | 15 |
| Part-time | 25 | 2 |
| Full-time | 27 | 1 |

chance at 4.0 bar on the fifth testing day. If she passed this, she continued to 5.0 bar on the sixth testing day; however, if she failed twice in a row (i.e., either chose the unfamiliar calf or remained in the start box), she was excluded from further testing, and we interpreted this as her having reached her maximum price. A flow chart outlining the testing procedure can be found in SM2, Fig. 2. For each cow that failed to reach her own calf, we recorded whether she chose the unfamiliar calf or remained in the start box. Additionally, we recorded whether she first attempted to push through the gate (defined as shoulders touching the gate) to reach her own calf. No cows were tested on pressures higher than 10.0 bar, as cows were due to enter another study following the end of the seventh week of the study. Two cows passed through the gate with a resistance of 10.0 bar.

At each testing day, the cows' choices were noted, and the latency from entering the start box until the cow passed through the gate to her own calf was recorded. Furthermore, it was recorded whether the calf (both own and unfamiliar, depending on her choice) suckled the cow or not. This was intended as an additional indicator of bond strength.

### 2.5. Statistical analysis

All statistical analyses were performed in R (R Core Team, 2022). *P*-values $<0.05$ were considered significant, and values of $0.05 \leq P \leq 0.1$ were considered tendencies.

First, the effects of treatment on whether the cows passed training or not were analysed. Pairwise $X^2$ tests were used to compare no-contact to full- and part-time cows, respectively. For the comparison between full- and part-time contact, a Fisher's exact test was used, as expected values were lower than 5. Additionally, the cows' preferences in the first free choice were analysed using Fisher's exact tests to compare between treatments, and binomial tests were used to determine preferences within treatments.

Second, MPP was analysed using a Cox's proportional hazards mixed effects model and the packages coxme (Therneau, 2022) and survival (Therneau, 2023). MPP was analysed using the Surv-function (Therneau, 2023) with time set to the highest pressure level successfully passed. Cows passing through 10.0 bar were censored to 0, while all other cows were censored to 1. The survival probability was initially modelled as a function of treatment (full- vs. part-time vs. no-contact), cow parity (primiparous vs. multiparous), calf sex (heifer vs. bull), calf age at first training day, position of own calf (left vs. right), the 2-way interaction between treatment and parity, and the nested random effects of block and pen. The interaction between treatment and parity was not significant and was therefore left out of the final model. The fit of the model was confirmed by assessing the significance of the integrated log-link test (using the summary function in R) and by testing the proportional hazards assumption (using the cox.zph function in R, Therneau, 2023). To graph the survival curves, a secondary model was fitted using the coxph function (Therneau, 2023) with the combined block and pen set as cluster.

Third, we assessed whether it was equally likely for cows to choose the unfamiliar calf versus remain in the start box. Cows' choice of the unfamiliar calf (yes or no) was analysed using binomial tests. Only cows that had passed 1 bar of pressure and who had two consecutive fails (i.e., reached their maximum price before testing ended) were included in these analyses (n=43). Fisher's exact tests were used to analyse whether there was a relationship between cows attempting to reach their own calf (defined as the cows' shoulders touching the gate leading to own calf) and the choice they eventually made. i.e., remaining in the start box or reaching the unfamiliar calf.

Finally, the likelihood of cows on any of the treatments to nurse their calves during the reward period was assessed using a $X^2$ test between full- and part-time cows, and Fisher's exact tests for the comparisons between the no-contact cows and each of the two contact treatments, due to expected values lower than 5.

## 3. Results

### 3.1. Training

Fewer no-contact cows passed training (42.3%) compared to full-time cows (96.4%; $X^2$=16.4, df=1, $P$<0.001) and part-time cows (92.6%; $X^2$=13.2, df=1, $P$<0.001, see Table 2). No difference in training success between full- and part-time cows was detected ($P$=0.61). Sixty-seven cows reached the first free choice training step. There was no difference between full- and part-time cows in their initial preference between own and unfamiliar calf (none of the contact cows chose to remain in the start box; 22 full-time cows (81.5%) chose their own calf and 5 chose the unfamiliar; 20 part-time cows (80.0%) chose their own calf and 5 chose the unfamiliar). Fewer of the no-contact cows chose their own calf (8 no-contact cows (53.3%) chose their own, 3 (20.0%) chose the unfamiliar, 4 remained in the start box) compared to full- ($P$=0.012) and part-time cows ($P$=0.021). Both full-time and part-time cows preferred their own calf to the unfamiliar (full-time: probability and CI: 0.81 (0.62–0.94), $P$<0.001; part-time: 0.80 (0.59–0.93), $P$<0.001). No preference could be detected for no-contact cows.

### 3.2. Maximum price paid

The mean MPP was 1.3 ± 1.2 bar for no-contact cows, 5.1 ± 2.8 bar for part-time cows, and 4.4 ± 2.6 bar for full-time cows. Two cows passed through 10.0 bar (2 part-time cows from block 1 and 5). The Cox' hazard ratio did not differ between full- and part-time cows. However, no-contact cows had a higher hazard rate, and thus a higher probability of reaching their MPP earlier, than both part- and full-time cows (ratio and CI: 7.69 [3.38–17.47] vs. 0.51 [0.35–0.76] and 0.83 [0.57–1.21], respectively, $X^2$=24.6, df=2, $P$<0.001). The two fitted models (mixed effects Cox's hazard and Cox's hazard cluster model) showed similar results; Fig. 4 shows the survival curves based on the cluster model. In addition to treatment, there was a tendency for the hazard ratio to increase with calf age, i.e., MPP was lower when calves were older ($X^2$=3.5, df=1, $P$=0.06).

In 95 of the total 390 test events (24.4%), cows did not make a choice within the two-minute threshold. In 35 events (9.0%), the cows chose the unfamiliar calf, and in 260 events, the cows chose their own calf. In four of the cases of choosing own calf, the latencies were not properly registered due to stopwatch errors, resulting in data from 256 test

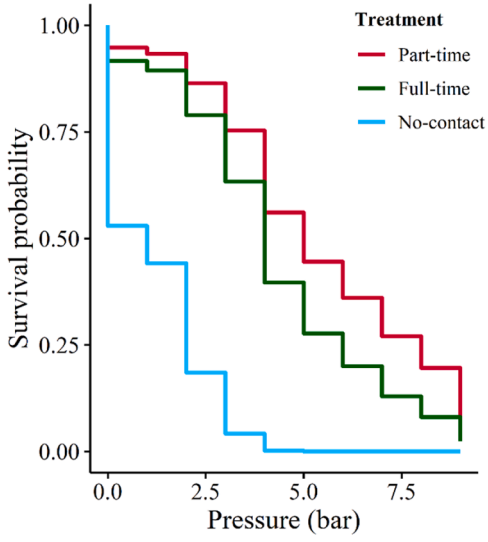


**Fig. 4.** Survival curves for the three treatments. No-contact cows (blue, 11 tested cows) had a significantly higher hazard ratio compared to full-time (green, 27 tested cows) and part-time (red, 25 tested cows) treatments, reflecting a lower survival probability as pressure increases.

events. In 222 (86.7%) of these test events, latency to walk through the gate was one minute or less, while 34 of 256 latencies were of more than one minute.

### 3.3. Choice of lower price alternative – the unfamiliar calf

When cows failed to push through the gate to their own calf, they were less likely to utilise the alternative option of contact to the unfamiliar calf than to remain in the start box; for both first and second fail, probability of choosing the unfamiliar calf was less than 0.5 ($P<0.001$). The unfamiliar calf was chosen in nine out of the 43 first fails (probability of choosing unfamiliar calf and CI: 0.21 [0.10–0.36]) and in eight out of the 43 second fails (probability and CI: 0.19 [0.08–0.33]). Furthermore, it was also registered whether the cows attempted to enter the pen with their own calf before they failed (Table 3). It was expected that cows may choose the unfamiliar calf after first attempting to reach their own calf; however, we did not find support for this relationship. Five of 43 cows (11.6%) chose the unfamiliar calf in both their first and second fail, while 31 of 43 cows (72.1%) remained in the start box for both fails. Seven of 43 cows switched between the two options, with four cows choosing the unfamiliar calf on the first fail and then choosing to remain in the start box on the second fail, and three cows doing the opposite.

### 3.4. Nursing

Only 3 full-time and 3 part-time cows nursed their calves one or more times during testing, so no statistical analyses were performed. Nursing took place in a total of 13 out of 260 test events (seven for part-time, six for full-time).

## 4. Discussion

There was no difference in the maximum price paid that cows from the two contact treatments were willing to pay to access their calves. Additionally, there was no difference in the number of full- and part-time cows who passed training. Trainability also can be interpreted as a measure of motivation (Sankey et al., 2010), supporting the result of the motivation test. Part-time cow-calf contact has been suggested as an alternative to full-time contact (Bertelsen and Vaarst, 2023), so an urgent question is whether a reduction in daily contact also weakens the mother-filial bond after it has been formed. Behavioural observations of the same animals used in the current study found that part-time cows spent less time nursing and grooming their calves compared to full-time cows (Jensen et al., submitted). A previous study using a similar approach to measure maternal motivation indicates that nursing is important for the formation and preservation of the maternal bond among cows with part-time contact to their calves (Wenker et al., 2020). We had therefore hypothesized that part-time cows would have a weaker maternal bond than full-time cows, but we found no support for this hypothesis.

**Table 3**
The use of alternative options in the cows' first and second fail to reach their own calf. Included are only cows that passed 1 bar of pressure, and who had two consecutive fails to reach their own calf. Also shown is whether the cows first tried to access their own calf (touched the gate with their shoulders) before they chose either the unfamiliar calf or to stay in the start box.

| Alternative choice | Tried own calf | Did not try own calf | Total |
|---|---|---|---|
| First fail | | | |
| Chose unfamiliar calf | 2 | 7 | 9 |
| Stayed in start box | 13 | 21 | 34 |
| Total | 15 | 28 | 43 |
| Second fail | | | |
| Chose unfamiliar calf | 1 | 7 | 8 |
| Stayed in start box | 3 | 32 | 35 |
| Total | 4 | 39 | 43 |

Fewer no-contact cows met the learning criteria in the training phase compared to cows on the two contact treatments, and those non-contact cows that did meet the learning criteria had a lower MPP than cows from the two contact treatments. Thus, cows with only 48 h of calf contact did not appear to be maternally motivated 40 days postpartum. Furthermore, no-contact cows showed no preference among the two calves in the first free choice and may not have been able to recognise their own calf at the time of testing. The longer cow and calf are kept together, the stronger are the response to separation (reviewed by Jensen, 2018), which could indicate a stronger maternal-filial bond (Gubernick, 1981). The strength of no-contact cows' motivation may have been stronger had they spent more time with their calves after calving, or had they been tested at an earlier point. We encourage future studies to investigate these aspects.

Only two cows pushed through the gates at the maximum price, and thus only these two may have been motivated to pass through even heavier gates than the maximum of 10 bar (equivalent to 152 kg) for this test. A previous study reported cows passing through push gates with a weight of up to 224 kg for access to lie down (Tucker et al., 2018), illustrating that Holstein dairy cows are able to push more weight than the maximum possible in this study. We increased the weight on the gates in increments of 1.0 bar and possibly these increments may have been too high and discouraged cows in our study to proceed. With a longer testing period, weight increments could have been smaller, and the maximum weight reached might have been higher. We cannot rule out that this may have increased sensitivity of the test. However, the two cows that pushed through the gates at highest possible price in this study were part-time cows, strengthening the suggestion that part-time cows' maternal motivation is not different from that of full-time cows.

Part-time cows experienced a proportionally longer daily period of calf-contact deprivation than full-time cows, which may have increased their maternal motivation. Both contact treatments were separated for 1 hour before testing, which for full-time cows corresponded to 1/23 of their daily contact time and for part-time cows to 1/10 of the daily contact time. However, we chose this approach as a way of standardizing the length and timing of deprivation for both treatments. Furthermore, part-time cows had at least 1.5 h of undisturbed calf contact where they could reunite with and nurse their calves between morning milking and the temporary separation. Within this time period, they were able to reach a similar amount of time spent nursing to full-time cows (Jensen et al., submitted). Therefore, we do not expect the proportionally different lengths of temporary deprivation to have affected our results. However, future studies could investigate this by comparing the effect of proportionally standardised deprivation periods to that of a consistent period across treatments. Additionally, a longer deprivation period could increase motivation (Jensen et al., 2004); this could also be investigated in future research.

The layout of our experiment might have affected the trainability and motivation of the no-contact cows. Training and testing took place in the barn holding the contact pens (Fig. 1), meaning that full- and part-time cows were trained and tested in a more familiar environment, while the training and testing environment was initially unfamiliar to the no-contact cows. Ideally, all cows would have been trained and tested in an environment either novel or familiar. However, we provided slow-learning cows with extra training days, and thus allowed for additional habituation to the environment. In a concurrent study, cows required on average 2–3 days to habituate to an arena in an unfamiliar barn before training could proceed (Neave et al., 2023). The extra training days provided in the current study were therefore likely sufficient to allow the no-contact cows to habituate to the testing arena.

With our novel experimental set-up, we aimed to provide cows some control over the test situation, which could mitigate possible frustration and aversiveness of the test (Englund and Cronin, 2023; Leotti et al., 2010). We hypothesised that interacting with the unfamiliar calf could potentially provide some outlet for cows' maternal motivation, and that

they would therefore choose this option when the price to reach their own calf became higher than what they were motivated to pay. This hypothesis, however, assumes a degree of substitutability (Hursh, 1980) between cows' own calf and an unfamiliar calf. As only few cows utilised the unfamiliar calf option, it appears that substitutability was very low. This is supported by previous findings that nursing and grooming almost exclusively happen between the dam and her own calf (Johnsen et al., 2015), and findings that cows more easily adopt foster calves when their own calf has been removed shortly after calving (Kent, 2020). That only few cows utilised the unfamiliar calf option suggests that performing maternal behaviours towards a calf other than her own does not substitute for caring for her own calf. Anecdotally, cows choosing the unfamiliar calf appeared frustrated; some vocalised and looked over the fence for their own calf, while others pushed and headbutted the unfamiliar calf. Additionally, one cow ran from her home pen to the test arena and straight through the gate to the unfamiliar calf two days in a row; she did not show much interest in the unfamiliar calf and instead repeatedly vocalised. Our interpretation of these anecdotal observations is that some cows were indeed highly motivated to reach their own calf but confused by the choice of two calves. Placing the gates on opposite sides of the start box or further apart from each other could possibly make the choice clearer.

Another way to avoid possible frustration could be to reduce the time spent in the start box. In 87% of the successful trials, the cow passed through the gate within one minute of entering the start box. However, the time in the start box should not be too short either; the remaining successful trials would not have counted as successful if the cut-off was at one minute. We expect that two minutes was a suitable period for cows to make their decision, and that cows that did not pass the gate to their own calf within this period would not have done so at all. Alternatively, cows could be provided with the option to end the trial before the two minutes were up; this approach has successfully been used in judgement bias tests (Hintze et al., 2018). Behavioural observations of cows in set-ups with and without the option of choice could indicate cows' degree of frustration. In the current study, the waiting time did not exceed two minutes; risk of frustration may be higher in closed-economy tests, where access to the recourse is only available by 'paying the price' (Tucker et al., 2018).

Overall, our results show that cows with both part-time and full-time calf contact are similarly motivated to reunite with their calf in a 'maximum price paid' test apparatus, suggesting they formed similarly strong maternal bonds. As part-time contact may be more feasible for some farmers than full-time contact (Bertelsen and Vaarst, 2023), this is a positive finding regarding animal welfare. Access, even somewhat limited, to highly motivated resources is correlated with positive animal welfare (Boissy et al., 2007; Broom, 1998; Mellor, 2015). On the other hand, the repeated separations experienced by cow and calf is expected to be somewhat stressful (Neave et al., 2022; Roadknight et al., 2022), and our concurrent research did indeed find a more negative affective state in part-time compared to full-time cows in a judgement bias test (Neave et al., 2023). Furthermore, the ethical implications of limiting access to a highly motivated resource need considering. The effect of part-time contact on cow and calf welfare thus requires more research to ensure that the needs of both cows and calves can be met, while also considering the feasibility of dairy production. Furthermore, the maternal motivation and affective states of cows separated from their calves shortly after calving deserves investigation.

## 5. Conclusion

In conclusion, our study shows that cows do form strong bonds to their calves and express maternal motivations, both when housed with full- and part-time calf contact. However, no-contact cows did not appear to have maintained a maternal bond with their calf when separated 48 h after birth and tested 40 days later. An unfamiliar calf did not appear to be a suitable alternative to the cow's own calf when costs became high.

## CRediT authorship contribution statement

**Jensen Emma Hvidtfeldt:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Bateson Melissa:** Conceptualization, Supervision, Writing – review & editing, Methodology. **Neave Heather W.:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Rault Jean-Loup:** Conceptualization, Writing – review & editing. **Jensen Margit Bak:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2024.106182.

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