Applied Animal Behaviour Science 253 (2022) 105694

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Comparing gradual debonding strategies after prolonged cow-calf contact: Stress responses, performance, and health of dairy cow and calf

Margret L. Wenker[a,b], Cornelis G. van Reenen[a,b], Eddie A.M. Bokkers[a,*], Kirste McCrea[c], Daiana de Oliveira[c], Kristin Sørheim[d], Yanran Cao[e], Rupert M. Bruckmaier[f], Josef J. Gross[f], Gerrit Gort[g], Cynthia M. Verwer[h]

[a] Animal Production Systems group, Wageningen University & Research, PO Box 338, 6700 AH Wageningen, the Netherlands
[b] Livestock Research, Wageningen University & Research, PO Box 338, 6700 AH Wageningen, the Netherlands
[c] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Box 7068, 750 07 Uppsala, Sweden
[d] Norwegian Centre for Organic Agriculture (NORSØK), Gunnars veg 6, 6630 Tingvoll, Norway
[e] Norwegian University of Science and Technology, Department of Biological Sciences, Kompasset 271, Ålesund, Norway
[f] Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, 3012 Bern, Switzerland
[g] Biometris, Wageningen University & Research, PO Box 16, 6700 AA Wageningen, the Netherlands
[h] Louis Bolk Institute, Kosterijland 3-5, 3981 AJ Bunnik, the Netherlands

A R T I C L E I N F O

Keywords:
Mother-bonded rearing
Weaning
Separation
Suckling
Sensor data
Animal welfare

A B S T R A C T

We assessed effects of two-step debonding strategies in calf rearing systems with different types of prolonged cow-calf contact (CCC) on stress responses, health and performance of dairy cows and calves. Forty-eight Holstein Friesian cow-calf pairs had either: 1) full contact including suckling, where contact was reduced before weaning via fence-line separation at day 49 (FC-FS) (n = 10); 2) full contact, where contact was reduced at day 56 by fitting calves with a nose-flap (FC-NF) (n = 10); 3) partial contact (calves were housed in a pen adjacent to the cow area allowing physical contact on initiative of the dam but no suckling), where contact was reduced before weaning by moving the calf box from the wall to prevent physical contact at day 49 (PC-BW) (n = 6); 4) partial contact, where contact was reduced the week after weaning by moving the calf box away from the wall at day 63 (PC-AW) (n = 12); 5) no contact (calves were removed from dam directly after birth and housed in a calf barn), calves were weaned at day 56 (NC) (n = 10). Between weeks 7–10, we assessed physiological stress parameters, weight gain, and the health status of calves, plus general activity patterns based on accelerometer sensor data of cow-calf pairs before, during and after the debonding interventions. Additionally, calves were subjected to four consecutive behavioural tests (i.e. open field, novel object, voluntary human approach and involuntary human approach test) prior to permanent separation at day 70 and their behavioural responses were assessed via video recordings to assess fearfulness. Machine-harvested milk yields of cows were evaluated during weeks 6–12. Data were analyzed with (generalized) linear mixed models. Throughout the debonding period, FC-NF calves had an impaired growth rate ($P = 0.02$). In weeks 6–9, FC-FS and FC-NF cows had lower machine-harvested milk yields than PC-BW, PC-AW, and NC cows ($P \leq 0.01$). We found no differences in responsiveness of calves to behavioural tests, except that NC calves exhibited more solitary play events compared to PC and FC calves in the novel object test ($P = 0.002$). Overall, our results imply that calves with partial CCC showed low stress responses to debonding, whereas abrupt weaning with a nose-flap during full contact seemed most stressful. Machine-harvested milk yield of FC cows seemed to recover once calves were weaned. More research into strategies to improve the process of debonding is warranted.

1. Introduction

Separating the calf from the dam shortly after birth is a routine practice on commercial dairy farms that differs from natural settings where the calf is raised by the dam (Whalin et al., 2021) and is perceived as contentious by the public (Busch et al., 2017; Ventura et al., 2013).

* Corresponding author.
E-mail address: eddie.bokkers@wur.nl (E.A.M. Bokkers).

https://doi.org/10.1016/j.applanim.2022.105694
Received 14 January 2022; Received in revised form 27 June 2022; Accepted 29 June 2022
Available online 30 June 2022

Hence, some farmers have developed rearing systems that allow for prolonged cow-calf contact (CCC) including suckling (Johnsen et al., 2016; Vaarst et al., 2020). One major welfare challenge in those so-called full CCC systems is the moment of debonding that is generally accompanied by strong signs of distress in both cow and calf (Flower and Weary, 2001; Veissier et al., 2013).

In commercial CCC systems calves are generally weaned and separated from the dam at 8–12 weeks of age (Sirovnik et al., 2020), which is known to be more stressful for both the dam and the calf compared to weaning at the natural weaning age of about 8 months (de Souza Teixeira et al., 2021; Lambertz et al., 2015; Stěhulová et al., 2017). This relatively early weaning gives calves a shorter time period to learn to live independently from the mother and on a diet of solid feed only (Enríquez et al., 2011), even though this weaning age is comparable to standard rearing systems in Europe (Marcé et al., 2010). Moreover, when calves are abruptly separated from the dam after prolonged CCC, two stressful events occur at the same time: calves are weaned off of milk (i.e. need to become nutritionally independent) and lose contact with the dam (i.e. need to become socially independent) (Newberry and Swanson, 2008).

Abrupt separation after prolonged contact is thus highly stressful and should be avoided at all times, which has led to the development of gradual two-step debonding strategies (see review by Enríquez et al., 2011). Two-step debonding methods encourage calves to become nutritionally independent before separation and can, for instance, be implemented by using anti-suck devices (i.e. nose-flap that abruptly prevents suckling but allows all other forms of social interaction (Loberg et al., 2008)) or fence-lines (i.e. reduces physical contact and suckling across a fence and is generally applied for a certain period prior to complete separation (Johnsen et al., 2015a)). These two-step debonding methods seem to reduce stress-responses around weaning in beef cattle at 6–7 months of age (Haley et al., 2005; Price et al., 2014) and in dairy cattle at 8–10 weeks of age (e.g. fence-line separation in Johnsen et al., 2015a; nose-flap in a foster cow system in Loberg et al., 2007). Perhaps, because those gradual debonding strategies contain elements of natural weaning (i.e. the calf can no longer suckle milk although other forms of physical contact still occur). Yet, to our best knowledge no work has attempted to compare these two-step debonding methods in dam-reared calves with full CCC to identify which strategy can best minimize the adverse effects of breaking the bond after prolonged contact (Jensen, 2017).

Since calves' nutritional independence from the dam appears to reduce stress at separation (Johnsen et al., 2018), another interesting management strategy to explore is the allowance of partial CCC. A partial CCC system prevents suckling by permitting limited physical contact (Sirovnik et al., 2020). Moreover, it allows for a gradual weaning schedule, as calves can be fed manually by the farmer or via an automatic milk feeder. Recently, we showed that cows in a partial CCC system still show calf-directed affiliative behaviour but to a lesser extent than cows in a full CCC system (Wenker et al., 2021). In addition, we found an increased motivation of cows to reunite with their calf when full CCC was allowed compared to partial CCC, which implies that the mother-offspring bond might be less comprehensive in dairy cows that are not suckled (Wenker et al., 2020). The next step is to examine the animals' stress responses to weaning and separation when partial CCC is allowed.

Therefore, the objective of this study was to assess the effect of two-step debonding strategies in two types of cow-calf contact systems on stress parameters, health, and performance of dairy cow and calf in comparison to standard practice (i.e. early separation after birth and gradual weaning schedule). To this end, we examined responses of cow-calf pairs with either partial or full CCC subjected to two different debonding strategies by limiting the amount of contact either before the calf was fully weaned (i.e. preventing physical contact for partial CCC one week before weaning or implementing fence-line contact for full CCC the week before weaning) or after the calf was fully weaned (preventing physical contact for partial CCC the week after weaning or fitting calves a nose-flap at weaning for full CCC). We hypothesized that cow-calf pairs with partial contact would show a lower stress response to the two-stage debonding strategies, as their responses were expected to be more similar to cows and calves that were managed according to the standard practice after early separation than full CCC. In contrast, cow-calf pairs with full contact were hypothesized to show a stronger stress response to debonding than the other groups, especially the nose-flap strategy calves compared to the fence-line strategy.

2. Materials and methods

The experimental design was approved by the Central Committee on Animal Experiments (The Hague, the Netherlands; approval number AVD4010020174307). The study was conducted at the Knowledge Transfer Centre in Zegveld (the Netherlands) from February 2019 to July 2020. All applicable international, national, and institutional guidelines for the care and ethical use of animals were followed.

2.1. Animals and treatments

Forty-eight Holstein Friesian cows were included in the experiment with a parallel group design. Cows were allowed to have either: 1) full contact with their calf including suckling (calves were housed together with the dams in a free stall barn) and contact was reduced before weaning via fence-line separation from day 49 onwards (FC-FS) ($n = 10$), 2) full contact with their calf including suckling and to wean the calves suckling was prevented by fitting calves with a nose-flap (FC-NF) from day 56 onwards ($n = 10$), 3) partial contact with their calf (calves were housed in a calf box adjacent to the cow area allowing physical contact on initiative of the dam but no suckling) and contact was reduced before the calf was fully weaned by moving the calf box 0.5 m from the wall to prevent physical contact from day 49 onwards (PC-BW) ($n = 6$), 4) partial contact with their calves and contact was reduced the week after the calf was fully weaned by moving the calf box away from the wall at day 63 (PC-AW) ($n = 12$), or 5) no contact with their calf (calves were removed directly after birth and the calf was housed in a separated calf barn) and calves were gradually weaned via a feeding schedule and no longer received milk from day 56 onwards as reference group (NC) ($n = 10$). At 70 days of age, cow-calf pairs with partial and full CCC were permanently separated. Only female calves were included for this experiment; therefore, cows were inseminated with sexed semen. In order to have a similar aged peer for calves throughout the experiment, every two cows that calved successively were assigned to the same treatment. In each block of six successive calvings, every set of two cows was randomly assigned to one of the three CCC treatments (i.e. no contact, partial contact, full contact). Subsequently, specific debonding strategies within the CCC groups were assigned to every set of two cows in alternating order. The mean parity was 2.7 (range: 1–5) for NC cows, 3.8 (range: 2–5) for PC-BW cows, 3.3 (range: 1–6) for PC-AW cows, 2.0 (range: 1–4) for FC-FS cows, and 3.0 (range: 1–7) for FC-NF cows. The PC cow-calf pairs were unevenly distributed due to a twin birth that led to exclusion of two PC-BW calves and two PC-BW calves that were assigned to the wrong treatment by mistake (i.e. PC-AW). A visual overview of all treatments and related events over time can be found in Fig. 1.

2.2. Housing and feeding

Based on signs of imminent calving, cows were moved into an individual indoor straw-bedded maternity pen (3.0 m wide × 5.1 m long). After birth, all full contact (FC) calves had access to their dam and could move freely inside the maternity pen, whereas all partial contact (PC) calves were placed in a cuddle-box (consisting of four plywood plates of 1.2 m wide × 0.8 m high) inside the maternity pen that prevented suckling, while still allowing tactile, visual, audible, and olfactory

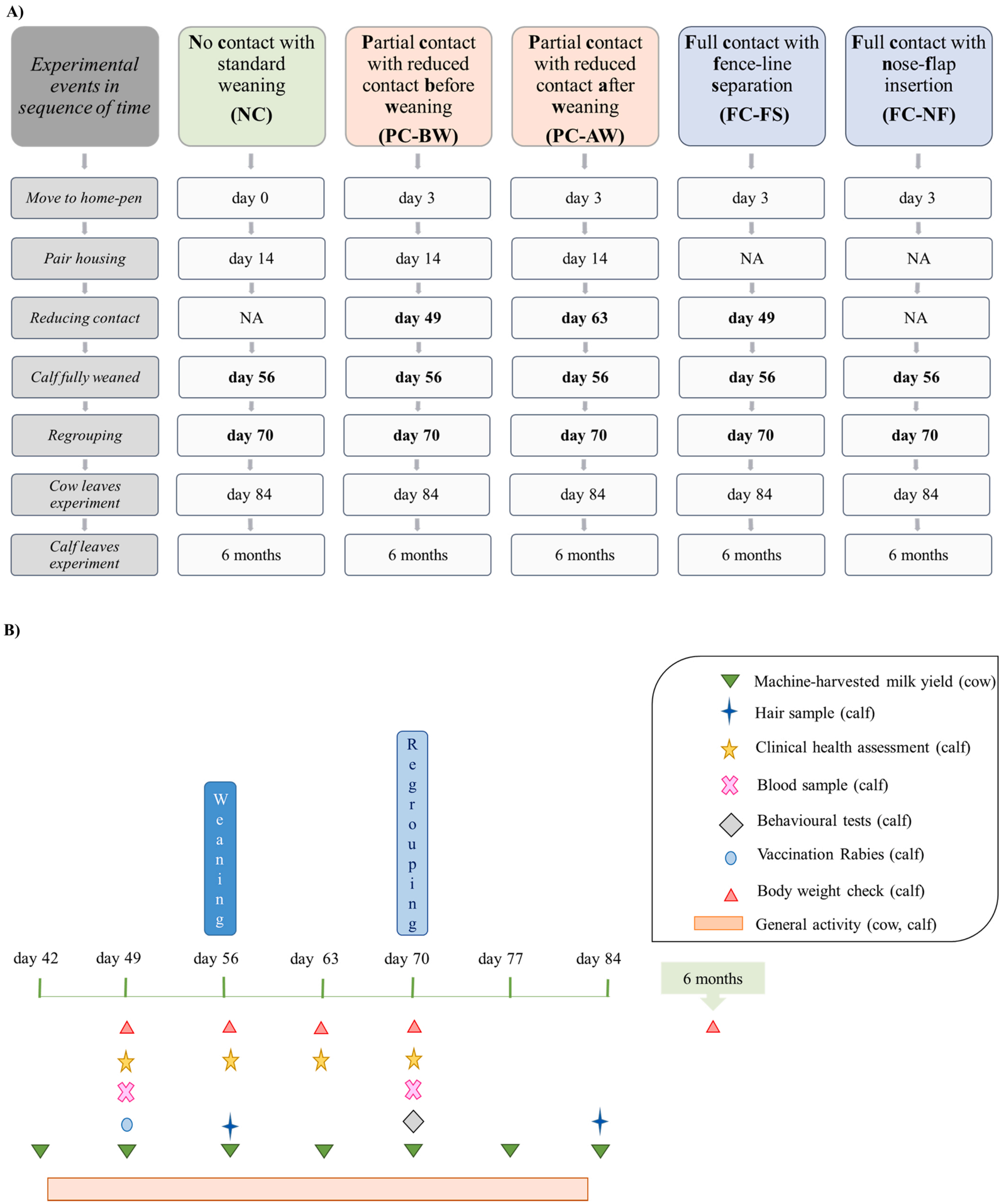

Fig. 1. Trial overview with A) experimental events in sequence of time per treatment group, and B) animal-based measures collected during the experiment.

contact (see Wenker et al., 2021 for an illustration). This meant that all PC cows could lick and sniff their calf when the calf was standing or lying, by moving their head into the box to reach the calf. NC calves were removed from the dam within 1.5 h after birth (median: 6 min, range: 1–63 min) and placed in an individual straw-bedded calf box (1.0 m × 1.6 m; Topcalf Duo-Flex, Schrijver, the Netherlands) in an indoor calf barn. The birth weight of the newborn calf was measured on a full-body calf scale (Type 8700, Welvaarts, the Netherlands). All calves were bottle fed with on average 3 L of colostrum from their own mother within 2 h after birth. After the first colostrum meal by bottle, all FC calves were allowed to suckle the remaining colostrum directly from the dam's udder. Calves in the NC and both PC treatment groups received an additional 2 L colostrum by bottle feeding at 8–12 h, as well as at 20–24 h after birth. More details on calving management can be found in Wenker et al. (2021). All NC cows moved to the designated group pen in the free stall barn after the second postpartum milking. All FC and PC cow-calf pairs stayed in the maternity pen for about 72 h, after which they were moved to designated group pens in the free stall barn.

Inside the free stall barn, experimental cows were housed in dynamic group pens (i.e. FC-FS and FC-NF in one group pen, PC-BW and PC-AW in one group pen, and NC in one group pen) (see Supplementary Material S1 for an illustration). Because calves and their dams were enrolled in sequence of their birth throughout the experimental period of 1.5 years, dynamic treatment groups were formed with all calves staying there until they moved to the young stock barn at age of 70 days. Hence, each treatment group comprised calves of different ages (1–10 weeks old) and group composition changed regularly. The free stall barn was naturally ventilated with open sidewalls and had perlite-bedded lying stalls (1.1 m × 3.0 m). The closed floor was covered with rubber and cleaned 8 times a day by an automated scraper. All experimental cows were milked twice a day at approximately 08:00 h and 18:00 h in the milking parlor with a five-point open tandem side and 11 side-by-side places. Cows were fed with 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 was provided partly in the milking parlor and partly by an individual concentrate feeder. When the barn temperature was below 10 °C, all young calves were fitted with a calf jacket for the first three weeks of life.

All FC calves were housed together with the dams in the FC group pen and had access to a calf creep area (inaccessible for the dams due to a vertical metal pipe). The calf creep area (3.3 m × 4.8 m) provided them with a straw-bedded lying area (3.3 m × 1.9 m) and free access to water plus solid feed (i.e. hay and concentrates) from the day the newborn calves moved into the free stall barn. Throughout the suckling period, FC calves could freely suckle their dams throughout the day (except when cows were milked) and, if allowed, other dams as well. The FC group never exceeded more than eight cow-calf pairs.

All PC calves were kept in a straw-bedded calf box on-wheels (Topcalf Duo-Flex, Schrijver, the Netherlands) behind a wall (1.2 m high) adjacent to the PC cow group pen (see Wenker et al., 2021 for an illustration). This set-up prevented suckling, direct contact with manure of adult cows, and housing of calves within the cow herd, while it allowed for individual feeding of calves, as well as visual, auditory, olfactory, and limited tactile contact between cow-calf pairs. 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. All 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). Calves in the PC group were provided bulk tank milk in individual teat buckets following a fixed feeding schedule (Table 1) 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. The PC group never exceeded more than six cow-calf pairs.

NC calves were individually housed for the first two weeks in a straw-bedded calf box (identical to those of the PC calves) in an indoor naturally ventilated calf barn, after which the partition was removed and they were pair housed in the calf box with their similar-aged peer. Each calf was provided with ad lib water, hay and concentrates (Topfok Kalf, de Samenwerking, the Netherlands) from 3 days of age onwards, and was fed bulk tank milk according to the same feeding schedule as PC calves (Table 1).

Table 1
Fixed feeding schedule that allowed for gradual weaning for each individual calf fed bulk tank milk and having either no contact and partial contact with their dam.

Week of age	Number of meals per day	Amount of milk per meal (L)
1	3	2.5
2	3	3
3	3	3.5
4	3	3.5
5	2	3.5
6	2	3
7	2	2
8	1	1

2.3. Weaning and regrouping

All experimental calves were fully weaned at 56 days of age. The fixed feeding schedule (Table 1) for NC and all PC calves allowed for gradual weaning and they received no more milk after day 56. In order to separate the moment of loss of milk from the moment of loss of physical cow-contact, the boxes of PC calves were moved away from the wall either the week before (after day 49) or the week after (after day 63) weaning occurred.

To initiate gradual weaning for FC-FS calves, we used a fence-line that consisted of three metal bars that allowed the calf to stick its head through the fence (see Supplementary Material S2 for an illustration) and suckle on initiative of the dam (i.e. the cow had to position herself next to the fence in order for the calf to have udder access) once it was placed behind the fence-line at day 49. At 56 days of age, the fence was closed off to prevent suckling by adding an extra metal bar in between the two lowest bars of the fence, which prevented the calf from sticking its head through. Behind the fence-line, calves had access to a straw-bedded lying area (3.0 m × 2.0 m) and ad libitum water, hay, and concentrates (see Supplementary Material S1).

In the FC-NF treatment group, calves were fitted with a nose-flap (Quiet Wean, JDA Livestock Innovations, Canada) to prevent udder access (i.e. abrupt weaning off of milk) at 56 days of age (see Supplementary Material S2 for an illustration). Those calves stayed in the cow pen until permanent separation at 70 days of age took place. The nose-flap still allowed calves to drink water and eat solid feed in the calf creep area (see Supplementary Material S1) and was removed when calves moved to the young stock barn.

All calves were regrouped at 70 days of age in a young stock barn. Each pair of calves was introduced in groups of maximum six calves in straw-bedded group pens (3.0 m × 4.0 m). At 4 months of age, calves moved to a larger group pen (3.0 × 10.0 m) and were followed up to 6 months of age. All dams remained in the initial group pen until lactation week 12, after which they left the experiment and moved back into the commercial herd.

2.4. Data collection

2.4.1. Performance

Between the age of 49–70 days, the body weight of calves was measured once a week using a full-body calf weighing scale (Type W8700, Welvaarts, the Netherlands). At 6 months of age, calves' absolute body weight was measured again using a full-body cow scale (Type 8700, Welvaarts, the Netherlands).

Machine-harvested milk yield of experimental cows was automatically collected using AgroVision dairy farm management software (AgroVision B.V., the Netherlands) between weeks 6–12.

2.4.2. General activity

Ear-tag accelerometer sensors (CowManager sensor, Agis Automatisering B.V., the Netherlands) were used in both cows and calves to track their general activity patterns. Sensors were attached to a radio-frequency identification ear-tag. Calves received their accelerometer sensor in the left ear at 3–4 weeks of age, when they were sedated for dehorning procedures. Cows were already equipped with an accelerometer sensor in their right ear for farm management purposes. The ear-tag based motion sensors contain a 3-dimensional accelerometer with proprietary software algorithms. They provided hourly measurements recorded in minutes for time spent eating, ruminating, highly active, active, and inactive, mutually exclusive times. Sensors were previously validated for activity, eating, and ruminating in adult dairy cows (Bikker et al., 2014; Pereira et al., 2017) and for inactivity, feeding, and ruminating in 6-week-old calves (Hill et al., 2017). Given that no studies validated the time spent in high activity (Stygar et al., 2021), this behaviour was excluded from the analysis.

2.4.3. Clinical health check

During the weekly weighing moments, calves were clinically examined using a standardized health scoring system (see Supplementary Material S3). The health scoring system was adapted from Renaud et al. (2017) to evaluate the respiratory system (nasal discharge, ocular discharge, coughing), fecal consistency, navel inflammation, and rectal temperature on a 4-point scale.

2.4.4. Plasma cortisol and serum IgG concentrations

To measure plasma indicators of stress (Sheriff et al., 2011) and serum indicators of humoral immunity (Ochsenbein, 1999), blood samples (9 mL) of calves were taken via jugular venipuncture at 49 and 70 days of age. Calf age at the actual sample moment could deviate from the intended 49 and 70 days of age (range: 43–55 days of age at the first time point and 64–77 days of age at the second time point), as the majority of calves was sampled during the weekly health and growth assessments. We followed this approach to reduce the handling of calves, as the animals' response to humans was also studied in this experiment. Blood was collected into different vacutainer tubes (Vacuette, Greiner BioOne, Austria). EDTA-plasma samples for cortisol analyses were stored at 4 °C for a maximum of 2 h, whereas serum samples were stored at room temperature for 1 h prior to processing. All samples were centrifuged for 15 min at 3000 rpm and 4 °C, and were stored at − 20 °C until further processing.

Plasma cortisol concentrations were measured in 91 out of 96 samples using a radioimmunoassay (RIA) (Schwinn et al., 2016). Plasma cortisol was analyzed by extracting 0.1 mL plasma with 1 mL absolute ethanol. After mixing the tubes on a vortex mixer, the protein precipitate was sedimented by centrifugation at 1500*g* for 20 min at 4 °C. Supernatants were decanted into fresh tubes, evaporated to dryness, and reconstituted in 0.5 mL PBS (0.14 M sodium chloride and 0.01 M sodium phosphate, pH 7.0) containing 0.1 % gelatin. A standard curve was run in duplicate by adding cortisol at concentrations between 0.25 and 100 ng/mL. Upon addition of 0.1 mL diluted antiserum and 0.1 mL [1, 2–3H] cortisol (78 Ci/mmol), each tube was mixed and incubated at 4 °C for 15 h. Separation of the free hormone from the bound hormone was achieved by adding 0.4 mL of a 0.75 % dextran-coated charcoal suspension. After 4 min, tubes were centrifuged (2800*g*, 15 min, 4 °C) and 0.7 mL was pipetted from the supernatant and mixed with scintillation fluid for radioactivity counting. The intra-assay CV was 9.7 % and the inter-assay CV was 6.3 %.

IgG concentrations were measured in 87 out of 96 serum samples with indirect enzyme-linked immunosorbent assay (ELISA) against bovine IgG. Wells were coated for 1 h with affinity-purified sheep anti bovine IgG-heavy chain (Cat. No. A10–118A-13, Bethyl Laboratories, United States of America) diluted 1:100 in coating buffer (0.05 M carbonate-bicarbonate, pH 9.6, Merck KGaA, Germany). Plates were washed 6 times with 50 mM TRIS 0.14 M NaCl (Merck KGaA, Germany), incubated for 1 h in the same buffer (blocking), and then washed 6 more times. After the 6th wash, 24 mg/mL of bovine reference serum (Cat. No. RS10–103–5, Bethyl Laboratories, United States of America) or diluted calf sera were added to each well, and the plates were incubated for 1 h. Wells were then washed 6 times, 100 mL of sheep anti bovine IgG-heavy chain (1:120,000) conjugated to horse-radish peroxidase (HRP) (Cat. No. A10–188 P-30, Bethyl Laboratories, United States of America) was added, and plates were incubated for 1 h. After incubation, plates were washed 6 times and tetra methyl benzine (TMB) (SanBio B.V., the Netherlands) was added. Reactions were stopped after 15 min with 0.2 M H_2SO_4 (Merck KGaA, Germany) and the optical density at 450 nm was determined with an automated plate reader. The standard curve was generated by means of a 4–parameter curve fit and the IgG concentrations in the test samples were quantified by interpolating their absorbance from the standard curve generated in parallel with the samples.

2.4.5. Hair cortisol

In addition to plasma indicators of stress, we measured hair cortisol in calves which is assumed to be able to reveal more long-term stress (Burnett et al., 2015; Heimbürge et al., 2019). Hair samples of calves were collected on days 56 and 84, as those time points reflect the level of hair cortisol metabolites between day 49 and 77 (González-de-la-Vara et al., 2011). Again, calf age at the actual sample moment could deviate from the intended age (range: from 50 to 66 days of age at the first time point and 78–93 days of age at the second time point). Samples were collected from the tip of the tail by carefully clipping 2–3 cm of the tail hair with surgical scissors as close to the skin as possible (Burnett et al., 2015). The hair samples were stored in individually identified zip-lock plastic bags, which were kept at − 20 °C until further processing. In total 64 out of 96 samples were mechanically cleaned and defatted with 5 mL n-hexane/isopropanol. Samples were dried overnight at room temperature. The dried samples were cut into small fragments approximately 1–2 mm long with scissors. Individual 100 mg aliquots from each of the samples were milled at 30 Hz with 3 mm beads for 5 min using a TissueLyserII (Qiagen, Germany). The milled hair samples were placed in a glass test tube along with 5 mL of methanol, and the tubes were incubated at 50 °C for 18 h. After centrifuging, the liquid in the tubes was transferred to another glass vial and evaporated to dryness in a stream of nitrogen. The remaining residue was dissolved in 200 μL of Neogen extraction buffer. Extraction of all hair samples (0.5 g each) was performed with 100 % methanol, after which hair cortisol metabolites were determined using a Neogen cortisol kit (Product no. 402710, Neogen, United States of America).

2.4.6. Vaccination challenge

All calves were vaccinated with an inactivated Nobivac Rabies vaccine (1 mL intramuscular; WBVR, the Netherlands) at 49 days of age to evaluate the humoral immune response to a viral vaccination. Blood was collected via jugular venipuncture at 70 days of age. After incubation at room temperature for 1 h, blood samples were centrifuged for 15 min at 3000 rpm and 4 °C. Sera were stored at − 20 °C until analysis. Serological responses of 43 out of 48 calves to the Rabies vaccine were analyzed by Wageningen Bioveterinary Research using the fluorescent antibody virus neutralization test (Cliquet et al., 1998) (Reference number 00–14–0871, Wageningen Bioveterinary Research, the Netherlands). Serological titers were converted to international units (IU)/mL.

2.4.7. Behavioural tests

Prior to moving the experimental calves to the young stock barn at 70 days of age (range: 64–79 days of age), calves were subjected to four behavioural tests applied in consecutive order (adapted from van Reenen et al., 2005, 2009; Duve et al., 2012 and Lecorps et al., 2018) to assess fearfulness (Forkman et al., 2007; van Reenen et al., 2009). The order of the tests was: Open Field Test (OFT, 4 min), Novel Object Test

(NOT, 4 min), and a Human Approach Test (HAT) consisting of an voluntary (2 min) and involuntary approach phase (2 min). All tests took place in the same straw-bedded experimental area (7 m long, 3 m wide test pen) between 09:30 h and 12:30 h, and animals were tested individually in a predetermined random test order. The focal animal was taken from its home pen to the test pen in a calf transporter by two experimenters. The 12-min test began once the calf entered the test pen and the door was closed. For the OFT, calves entered the test pen that was empty and unfamiliar. After 4 min OFT, the NOT started by dropping a novel object (black-white umbrella) over one of the fences enclosing the test arena (see Supplementary Material S4). After 4 min NOT, the umbrella was removed by pulling it with the attached wire over the fence out of the arena. Subsequently, the calf was given 1 min to habituate to the removal of the novel object, where after the HAT started. One of the two (familiar) experimenters entered the test pen and stood immobile in the middle of the side-fence enclosing the test area (see Supplementary Material S4) for 2 min, while avoiding eye contact with the calf. Next, the experimenter started to move (i.e. one step per second) and actively approach the calf with one arm stretched out attempting to stroke the calf (even when it retreated) for 2 min. After the HAT, the behavioural tests were finished and the calf was loaded on the trailer for transportation to the young stock barn and the next test animal was brought up to the test pen. Behaviour during the tests was recorded using a camera (Hikvision, model DS-2CD2145FWD-IS combined with the Hikvision DS-2FP2020 microphone) positioned 3 m above the pen. Previous work in (dairy) cattle suggested that individual differences in latencies to approach humans or objects as well as differences in locomotion and vocalizations during novel object, response to human, and open field tests were all associated with differences in fearfulness (Boissy and Bouissou, 1995; de Passillé et al., 1995; Grignard et al., 2000; Hemsworth et al., 1996; van Reenen et al., 2009). Hence, for the OFT, time spent in locomotion, time spent in contact with the wall and floor, time spent vigilant, and frequency of vocalizations and solitary play were recorded. For the NOT and HAT, both latency to first contact with the object (NOT)/human (HAT) and time spent in contact with the object (NOT)/human (HAT) were recorded. Details with respect to the ethogram can be found in Supplementary Material S5. Due to technical problems video footage was available for 39 out of 48 calves (hence, NC: $n = 7$, PC-BW: $n = 6$, PC-AW: $n = 10$, FC-FS: $n = 8$, FC-NF: $n = 8$)). All videos were continuously observed by one trained observer (intra-observer agreement accepted at kappa > 0.85) who was blind to treatments, using the software Mangold Interact® (Program Version 18.1.4.4).

2.5. Data handling & statistical analyses

All statistical analyses were performed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC), treating the animal as the experimental unit. Residuals of all variables were visually checked for normality and homogeneity of variance, and response variables were log-transformed when needed.

2.5.1. Calf performance

Birth weight was added as covariate for the analysis of calf performance only.

Average daily gain (ADG) in calf body weight was calculated over the 3-week period between weeks 7–10 by dividing the difference in body weight between week 7 and 10 by the difference in age in days between the measurement moment in week 7 and 10. A linear mixed model analysis was performed using the PROC GLIMMIX procedure. The systematic 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, μ was a base level and Treatment_i = type of CCC and corresponding debonding strategy (i = FC-FS, FC-NF, PC-BW, PC-AW, NC), Batch_j = 16-week time period in which a calf was born (j = 1, 2, 3, 4), Parity_k = parity of the dam (k = primiparous or multiparous), and a two-way interaction between treatment and parity. Batches were defined retrospectively to control for seasonal differences and varying group sizes in the treatment groups over time. Hence, the duration of the experiment was split up into batches of 16 weeks based on calving dates and they represented the various seasons. Interactions that were not significant ($P \geq 0.05$) were excluded from the model. In addition, the model comprised a random effect for the interaction between treatment and batch. For all fixed effects, approximate F-tests were used (Kenward and Roger, 1997) and significance was declared at $P < 0.05$. Subsequent pairwise comparisons were made according to the Tukey method.

Calves' absolute body weight between weeks 7 and 10 were analyzed with a linear mixed model for repeated measures (PROC GLIMMIX). The systematic part of the model (referred to as model 2) consisted of 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 (model 1), and additionally Week_l = calf age in weeks (l = 7, 8, 9, 10) as main effect and a two-way interaction between treatment and week. Random calf effects were introduced to handle repeated measurements for calves. Further procedures were identical to model 1.

Calves' absolute body weight at 6 months of age was analyzed with a linear mixed model identical to model 1 and its corresponding procedures.

2.5.2. Cow performance

Continuous data for cows' machine-harvested milk yield were analyzed with a linear mixed model identical to model 2 and its corresponding procedures, except that fixed effect Week_l now included lactation weeks (l = 6, 7, 8, 9, 10, 11, 12).

2.5.3. General activity

For both cows' and calves' behavioural sensor data, the hourly output data from the CowManager sensor system were summarized into daily measures of general activity. Only data with 18 or more hourly recordings within 24 h were included in the analysis for the reason that not all hourly measurements were successfully being transmitted through the router to the coordinator, which resulted in missing data points. The proportions of time spent inactive, active, eating and ruminating were calculated by dividing the total number of minutes of recorded behaviour by the total number of minutes recorded per day. Since a few sensors malfunctioned during the experiment, data was available for 39 out of 48 cows and 38 out of 48 calves (see Table 2). A baseline before any debonding interventions took place was calculated based on the average proportion of time spent inactive, active, eating or ruminating, between days 39–44 (i.e. age for calves, days in milk for cows). The behavioural responses after debonding interventions took place were based on the average proportion of time spent on all behaviours between days 0–4 after reducing contact, weaning, and regrouping (Johnsen et al., 2015b). Additionally, the average proportion of time spent on all behaviours after all interventions took place (i.e. between days 7–11 after regrouping) was calculated.

A generalized linear mixed model (fitted with PROC GLIMMIX) with an overdispersed binomial distribution and logit link function with multiplicative dispersion factor was used to analyze behavioural sensor data (i.e. proportion of time spent inactive, active, eating and ruminating). The systematic part of the model comprised the following fixed effects:

$$\mu + \text{Treatment}_i + \text{Batch}_j + \text{Parity}_k + \text{Time Period}_m + (\text{Treatment}_i \times \text{Parity}_k) + (\text{Treatment}_i \times \text{Time Period}_m) \quad [3]$$

Table 2
Behaviour of cows and calves during debonding expressed as proportion of time based on ear-tag accelerometer sensors, A) effect of debonding phase (mean ± SE [95 % CI]) on cow and calf behaviour, and B) interaction between treatment and debonding phase (LS means ± SEM [95 % CI]) in three types of cow-calf contact systems with different debonding strategies on cow behaviour.

A. Effect of debonding phase[a]	Before interventions	Reduced contact	Weaning	Regrouping	After interventions	F-value	P-value Time period
Calves							
Inactive	0.39 ± 0.01 [0.23–0.56][a]	0.34 ± 0.01 [0.18–0.47][ab]	0.35 ± 0.01 [0.24–0.46][b]	0.29 ± 0.01 [0.15–0.43[c]	0.30 ± 0.00 [0.22–0.42][c]	16.87	< 0.001
Eating	0.11 ± 0.00 [0.05–0.19][a]	0.10 ± 0.01 [0.02–0.20][a]	0.11 ± 0.00 [0.03–0.19][a]	0.16 ± 0.01 [0.06–0.27][b]	0.18 ± 0.01 [0.08–0.30][c]	31.73	< 0.001
Cows							
Inactive	0.24 ± 0.01 [0.13–0.37][a]	0.25 ± 0.01 [0.14–0.41][abc]	0.24 ± 0.00 [0.14–0.37][ab]	0.25 ± 0.00 [0.17–0.35][bc]	0.26 ± 0.00 [0.16–0.39][c]	3.32	0.01

B. Interaction Treatment × Debonding phase[b]	Before interventions	Reduced contact	Weaning	Regrouping	After interventions	P-value Treatment × Time period
Cows						
Eating						0.03
NC[c]	0.38 ± 0.06 [0.26–0.52][a]	N.A.[d]	0.33 ± 0.05 [0.22–0.47][b]	0.30 ± 0.05 [0.20–0.43][b]	0.30 ± 0.05 [0.20–0.43][b]	
PC-BW[c]	0.16 ± 0.05 [0.07–0.31]	0.17 ± 0.06 [0.08–0.34]	0.18 ± 0.06 [0.08–0.34]	0.21 ± 0.07 [0.10–0.38]	0.18 ± 0.06 [0.08–0.34]	
PC-AW[c]	0.30 ± 0.06 [0.18–0.45]	0.28 ± 0.06 [0.16–0.43]	0.31 ± 0.06 [0.18–0.47]	0.28 ± 0.06 [0.16–0.43]	0.30 ± 0.06[0.18–0.46]	
FC-FS[c]	0.33 ± 0.07 [0.20–0.50][ab]	0.33 ± 0.07 [0.20–0.50][ab]	0.35 ± 0.07 [0.21–0.52][a]	0.31 ± 0.06 [0.18–0.46][b]	0.31 ± 0.06 [0.18–0.47][b]	
FC-NF[c]	0.35 ± 0.06 [0.23–0.49][a]	N.A.[d]	0.32 ± 0.05 [0.22–0.46][ab]	0.32 ± 0.05 [0.21–0.45][ab]	0.31 ± 0.05 [0.20–0.44][b]	

[a] Different subscript letters indicate significant differences between time periods (P < 0.05).
[b] Different subscript letters indicate significant differences between time periods within a treatment group (P < 0.05).
[c] NC: no contact, standard weaning (n = 8); PC-BW: partial contact, reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall (n = 3); PC-AW: partial contact, reducing contact after weaning by moving partial contact calves from the wall at day 63 (n = 11); FC- FS: full contact, reducing contact (at day 49) by placing full contact calves behind the fence-line (n = 8); FC-NF: full contact, reducing contact at weaning by fitting full contact calves a nose-flap at day 56 (n = 9).
[d] No contact calves were only subjected to weaning and regrouping; There was no reduced contact phase for FC-NF calves, as contact was only reduced at the moment the nose-flap was inserted to wean the calf.

in the same notation as in model 2 by replacing $Week_l$ for Time $Period_m$, at which Time $Period_m$ = 4-day period after a debonding intervention (m = baseline before interventions, reduced contact, weaning, regrouping, after debonding interventions). The model also included two-way interactions between treatment and parity, and between treatment and time period. As random effect the model included the interaction between treatment and batch. Random animal effects were introduced in the model to handle correlation between repeated measurements on the same animal. All further procedures were identical to model 2.

2.5.4. Health scores

Prior to statistical analyses, calves were classified for having clinical symptoms of respiratory issues (i.e. 'yes' when they had a composite respiratory score ≥ 4 based on the sum of ocular discharge, nasal discharge, cough score) and diarrhea (i.e. 'yes' when fecal score ≥ 2) for each week (adapted from (McGuirk, 2008)). Subsequently, the number of weeks classified as having clinical symptoms for each health deficit was summed per calf. Therefore, a calf that was observed 2 out of 4 weeks with clinical respiratory symptoms and 1 out of 4 weeks with clinical symptoms for diarrhea, would get an outcome of 2 for respiratory problems and 1 for diarrhea.

The prevalence of calves classified at least once with clinical symptoms for diarrhea or respiratory issues were analyzed using a Fisher's exact method for pairwise comparisons. A generalized linear mixed model with an overdispersed binomial distribution and logit link function was fitted to analyze the number of weeks that calves classified with clinical symptoms for respiratory issues and diarrhea. As random effect the model included the interaction between treatment and batch. The systematic part was identical to model 1 and its corresponding procedures.

2.5.5. Physiological stress responses

Differences between week 10 (end of debonding strategies) and week 7 (start of debonding strategy) (delta, Δ = day 70 – day 49) were calculated for serum IgG and plasma cortisol concentrations. Additionally, differences in hair cortisol values were calculated between week 12 and week 8 (delta, Δ = day 84 – day 56).

Linear mixed model analyses identical to model 1 and its corresponding procedure were performed for Δ serum IgG, Δ plasma cortisol, Δ hair cortisol, and Rabies IgG responses. Additionally, the difference in days between the calf's age at the two sample moments was added as co-variate among the fixed effects.

2.5.6. Behavioural tests

Latencies (i.e. latency to first approach/contact novel object and human) were analyzed with a linear mixed model identical to model 1, and additionally calf age as co-variate among the fixed effects. The exact calf age during the tests could deviate from the intended age of 70 days, as calves were regrouped together with their similar aged peer directly after the behavioural tests. In case a calf did not approach/touch the novel object or the human at all, the maximum latency for that calf was set to the total test duration.

Generalized linear mixed model analyses with a Poisson distribution and log link function with multiplicative dispersion factor were used for behaviours expressed as frequency (e.g. vocalizations), whereas an overdispersed binomial distribution with logit link function was used for behaviours expressed as proportion of time (e.g. proportion of time spent walking). The systematic part of the model consisted of the same fixed and random effects as model 1, and additionally calf age as co-variate. Again, all further procedures were identical to model 1, except that a Bonferroni correction method was applied for multiple testing (i.e. for all 19 outcome parameters of the fear tests).

2.5.7. Power analysis

Sample size sufficiency was studied by a power study for one of the behavioural activity parameters (i.e. inactivity in calves) and one of the health parameters (i.e. occurrence of diarrhea). By simulating new responses based upon the fitted models in R (version 4.05), we found a power of 0.94 for the test of main effects of treatment for inactivity, and a power of 0.59 to detect significant differences among the treatments (using $\alpha = 0.05$) for the occurrence of diarrhea, given the effects found in the data. We conclude that for the behavioural parameter power was sufficient (for differences as found in the data), but for the health parameter power was relatively low.

3. Results

3.1. Performance of cow-calf pairs

For calves' absolute body weight, an interaction was found between treatment and week ($P = 0.001$). Overall, calves' absolute body weight increased over time, except for FC-NF calves between week 8 and 9 and PC-BW calves between week 9 and 10 (Fig. 2A). Notably, FC-FS and FC-NF calves weighted on average, respectively, 8 and 14 kg more than PC-BW, PC-AW, and NC calves at week 7 ($P > 0.12$). In terms of ADG from weeks 7–10, FC-NF had a lower mean ADG (± SE) (0.64 ± 0.08 kg/d) compared to FC-FS calves (0.79 ± 0.05 kg/d), PC-BW calves (0.81 ± 0.08 kg/d), PC-AW calves (0.91 ± 0.06 kg/d), and NC calves (0.88 ± 0.07 kg/d) ($P = 0.02$). At 6 months of age, no significant difference in absolute body weight was found among treatment groups (FC-FS calves: 183.6 ± 13.3 kg, FC-NF calves: 213.5 ± 9.5 kg, PC-BW calves: 194.0 ± 7.1 kg, PC-AW calves: 191.8 ± 11.3 kg, NC calves: 211.4 ± 6.9) ($P = 0.56$).

For cows' daily milk yield, an interaction was found between treatment and week ($P < 0.001$), as FC-FS and FC-NF cows had a lower machine-harvested milk yield in week 6 until 9 compared to PC-BW, PC-AW, and NC cows. From week 10 onwards machine-harvested milk yield no longer significantly differed between any of the treatment groups (Fig. 2B).

3.2. General activity patterns of cow-calf pairs

For calves' general activity pattern, an interaction between treatment and time period was found for the proportion of time spent active and ruminating ($P \leq 0.002$) (Fig. 3). These data should be interpreted cautiously, as the number of calves in some groups was relatively small. The proportion of time spent active was higher in FC-FS and FC-NF calves compared to PC-BW, PC-AW, and NC calves in the 4-day period before interventions took place, whereas in the period after weaning FC-NF spent a larger proportion active compared to FC-FS and NC calves (Fig. 3A). The proportion of time spent ruminating was lower in FC-FS and FC-NF calves in the 4-day period before interventions took place, after reducing contact, and after weaning compared to PC-BW, PC-AW, and NC calves (Fig. 3B). Furthermore, the overall proportion of time spent inactive decreased over time for all calves ($P < 0.001$) (Table 2A), and tended to be higher for FC-NF calves compared to FC-FS, PC-BW, and PC-AW calves ($P = 0.08$) (Supplementary Material S6). The average proportion of time spent eating increased from the moment of weaning until the week after regrouping ($P < 0.001$) (Table 2A). Besides, the

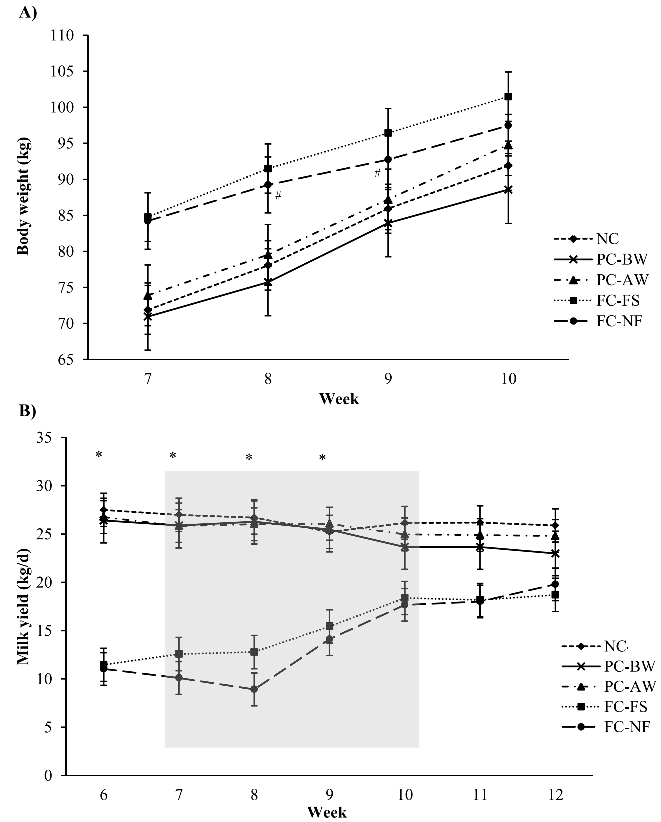

Fig. 2. Interactions between treatment and week for animal performance in three types of cow-calf contact with different debonding strategies systems for A) growth of calves represented by absolute body weight (kg) from week 7–10 (LS means ± SEM), and B) milk production (kg/d) of cows between week 6–12 (LS means ± SEM). The light grey box indicates the debonding period between week 7–10. NC: no contact, standard weaning; PC-BW: partial contact, reducing contact before weaning; PC-AW: partial contact, reducing contact after weaning; FC-FS: full contact, reducing contact before weaning via fence-line separation; FC-NF: full contact, reducing contact at weaning via nose-flap insertion. Asterisks at specific time points indicate significant treatment differences ($P < 0.05$), whereas the # represents a significant effect of time within the FC-NF treatment.

overall proportion of time spent eating tended to differ between treatment groups ($P = 0.07$) (Supplementary Material S6).

The effect of treatment on general activity patterns of cows depended partly on the time period, as reflected by a significant interaction term treatment by time for the proportion of time spent eating ($P = 0.03$) (Table 2B). Again, our small sample size requires cautious interpretation of these results. Overall, the proportion of time spent inactive increased over time ($P = 0.01$) (Table 2A). No significant differences between treatment groups were found for the proportion of time spent active and ruminating ($P > 0.32$) (Supplementary Material S6).

3.3. Calves' health status and physiological stress-responses

The number of weeks that calves were classified with clinical symptoms for respiratory symptoms between weeks 7–10 did not differ significantly among treatment groups (mean weeks ± SE, FC-FS: 1.1 ± 0.5; FC-NF 0.9 ± 0.4; PC-BW: 0.7 ± 0.3; PC-AW: 0.7 ± 0.2; NC: 0.1 ± 0.1) ($P = 0.12$). Similarly, number of weeks that calves were scored with clinical symptoms for diarrhea did not significantly differ among treatment groups (FC-FS: 1.4 ± 0.3; FC-NF: 1.6 ± 0.4; PC-BW: 0.5 ± 0.3; PC-AW: 1.0 ± 0.30; NC: 0.9 ± 0.4) ($P = 0.37$). Prevalence of calves classified with clinical symptoms for diarrhea and respiratory issues can be found in Table 3.

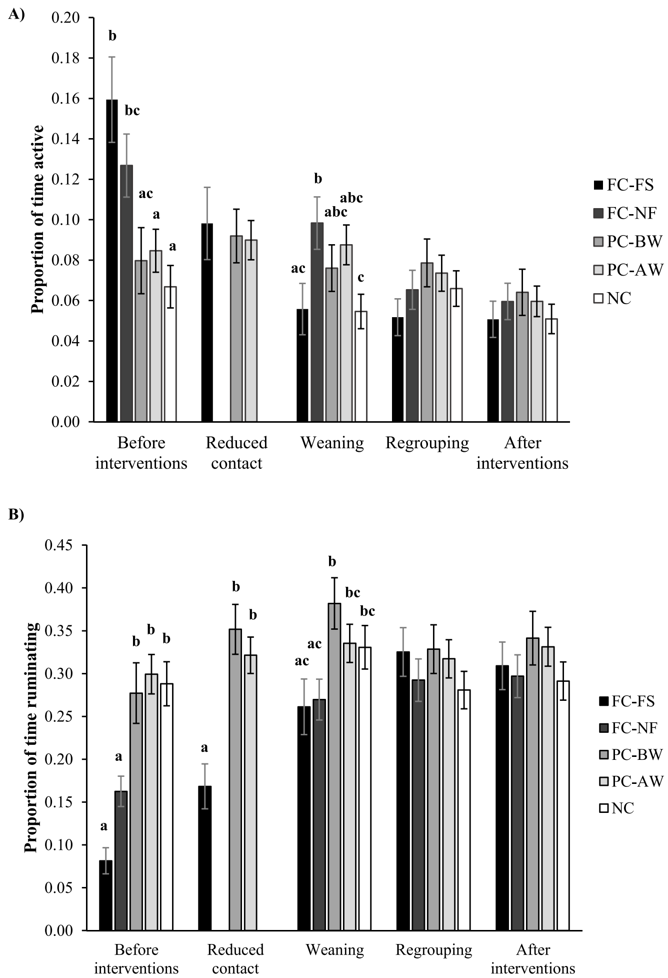

Fig. 3. Interaction between treatment and time period for calf behaviour in three types of cow-calf contact systems with different debonding strategies for A) proportion of time spent active (LS means), B) proportion of time spent ruminating (LS means). NC: no contact, standard weaning; PC-BW: partial contact, reducing contact before weaning; PC-AW: partial contact, reducing contact after weaning; FC-FS: full contact, reducing contact before weaning via fence-line separation; FC-NF: full contact, reducing contact at weaning via nose-flap insertion. Different subscript letters within a time period indicate significant differences ($P < 0.05$) between treatment groups.

No significant treatment differences were found for delta plasma cortisol levels, delta serum IgG concentrations (both Δ = day 70 – day 49), and Rabies IgG response to vaccination challenge (Table 3). Additionally, no significant treatment differences were found for delta hair cortisol concentrations (Δ = day 84 – day 56) (Table 3).

3.4. *Calf behaviour in behavioural tests*

In the OFT, the proportion of time spent walking, running, standing, vigilant, or in contact with the wall or floor did not differ significantly between treatments (Table 4). In addition, no significant differences between treatment groups were found for the frequency of solitary play and vocalizations during the OFT (Table 4).

In the NOT, NC calves showed a larger frequency of solitary play compared to FC-FS, FC-NF, PC-BW, and PC-AW calves ($P = 0.002$) (Table 4). No significant differences among treatment groups were found regarding the latency to first approach or first contact with the object, the proportion of time spent vigilant, and vocalizations during the NOT (Table 4).

During both the voluntary and involuntary approach phase in the HAT, no significant differences among treatment groups were found regarding the latency to first contact with the human and the proportion of time spent in contact with the human (Table 4).

4. Discussion

Weaning and separation after prolonged CCC in farm settings is often imposed at younger ages and more abruptly than in natural settings, which can be stressful for cow-calf pairs at times (Sirovnik et al., 2020; Weary et al., 2008; Whalin et al., 2021). The ear-tag accelerometer system exhibited that PC-BW and PC-AW calves showed no explicit distress responses when debonding interventions took place, as their behaviour appeared to be similar to NC calves. For PC calves, the loss of contact with the dam was not linked to loss of milk, and possibly the fixed feeding schedule with gradual milk reduction minimized PC-BW and PC-AW calves' weaning distress (Khan et al., 2011). Moreover, PC calves could not freely initiate contact from their calf boxes, as the current partial CCC system was cow-driven. Given that our previous work showed that partial contact reduced cows' affiliative behaviour towards their calves in the weeks following parturition, the PC calves might have been more socially independent from the dam (Wenker et al., 2021). Hence, it could be argued that a different mother-young bond is established when partial contact without suckling is allowed, which eventually mitigated the debonding process. This argument is supported by findings of Johnsen et al. (2018) that reported a smaller amount of vocalizations in response to separation for calves in a partial CCC system where suckling was prevented by using udder nets compared to calves with full CCC. Nevertheless, in the present study the behavioural responses of cows to debonding after prolonged CCC were less distinctive than those of calves. We could not detect clear differences in cow behaviour during debonding with various two-step debonding strategies following prolonged CCC compared to cows without CCC. This might indicate that debonding with two-step methods mitigated cows' distress responses, which would be in line with previous work (Loberg et al., 2007; Ungerfeld et al., 2016). However, given the small number of animals included in the current experiment, as well as the fact that the ear-tag accelerometer sensor used in this study described rather general (e.g. active, inactive) than detailed (e.g. number of steps, duration of lying bouts) behavioural patterns, future research is required to enhance our understanding of behavioural stress responses of cows to two-step debonding after prolonged CCC.

Results from the behavioural tests seem to suggest that debonding strategies did not affect calves' level of fearfulness, although the absence of significant differences in behavioural responses when exposed to challenging conditions could have also been the result of the rather small sample sizes. Perhaps, our findings are more related to type of CCC rather than the weaning and separation method, given that previous studies also reported no differences in fearfulness between calves reared without CCC or with full CCC during an OFT and NOT at 14 days of age (Santo et al., 2020) or 65 days of age (Buchli et al., 2017). Individual calves differed substantially in their behaviour responses during the different behavioural tests, which is in agreement with other studies that reported large individual differences in fearfulness that were stable over time, related to personality traits (Lecorps et al., 2018; (Van Reenen et al., 2004, 2005)), and were linked to mood-states (Lecorps et al., 2018). Notably, we found no behavioural differences between treatment groups during the HAT, which suggests that calves reared with prolonged CCC may not always be more "wild" or afraid of humans as has been described by farmers (Neave et al., 2021; Vaarst et al., 2020). This might be explained by the fact that in the present study all calves were frequently handled during weekly health and growth assessments, although other factors such as calf manager behaviour (Calderón-Amor et al., 2020) and calf personality also affect animal's reactivity to humans (Waiblinger et al., 2006). Interestingly, NC calves showed more solitary play during the NOT compared to the other treatment groups. This finding corresponds to work from Wagner et al. (2013) in which calves reared without CCC exhibited more solitary play during an

Table 3
Results of debonding strategies in three types of cow-calf contact systems on A) physiological stress responses of calves (mean ± SE) (Δ = difference between the start- and end of interventions for serum IgG and plasma cortisol concentrations in blood (i.e. Δ = day 70 – day 49) and hair cortisol concentrations (i.e. Δ = day 84 – day 56)) and B) prevalences of calves classified at least once with clinical symptoms of diarrhea and respiratory issues between day 49 to day 70.

A.		***Physiological stress responses***					
	No contact	Partial contact		Full contact			
	(n = 10)	BW[a] (n = 6)	AW[a] (n = 12)	FS[a] (n = 10)	NF[a] (n = 10)	F-value	P-value
Blood							
Δ IgG (mg/mL)	4.82 ± 3.27	7.19 ± 5.04	4.77 ± 0.71	1.80 ± 2.52	6.80 ± 1.57	0.76	0.58
Δ Cortisol (ng/mL)	0.68 ± 0.45	0.76 ± 0.48	0.85 ± 0.35	-1.52 ± 1.18	-1.10 ± 0.63	1.18	0.40
Rabies IgG (IU/mL)	0.89 ± 0.24	1.52 ± 0.95	2.15 ± 0.71	1.74 ± 0.54	1.05 ± 0.30	1.07	0.44
Hair							
Δ Cortisol (ng/g)	0.68 ± 1.12	-3.88 ± 6.16	0.23 ± 1.47	-0.14 ± 1.92	1.16 ± 2.55	0.90	0.50
B.		***Health variables***					
	No contact	Partial contact		Full contact			
	(n = 10)	BW (n = 6)	AW (n = 12)	FS (n = 10)	NF (n = 10)		
Diarrhea[b] (%)	50.0^{ab}	33.3^{a}	58.3^{ab}	90.0^{b}	90.0^{b}		
Respiratory issues[c] (%)	10.0	50.0	50.0	50.0	40.0		

Different subscript letters within a row indicate significant differences (P < 0.05) between treatment groups.

[a] BW: reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall; AW: reducing contact after weaning by moving partial contact calves from the wall at day 63; FS: reducing contact (at day 49) by placing full contact calves behind the fence-line; NF: reducing contact at weaning by fitting full contact calves a nose-flap at day 56.

[b] For each treatment: (Number of calves with at least once feces score ≥ 2 (based on a 0–3 scale) divided by total number of calves) times 100.

[c] For each treatment: (Number of calves with at least once lung score ≥ 4 (i.e. sum of scores for ocular discharge, nasal discharge, and cough based on a 0–3 scale) total number of calves) times 100.

Table 4
Calf behaviour during the behavioural tests at 70 days of age in ascending order (i.e. A–D). Definitions of behavioural measures can be found in Supplementary Material S5.

	No contact	Partial contact		Full contact			
	(n = 7)	BW[a] (n = 6)	AW[a] (n = 10)	FS[a] (n = 8)	NF[a] (n = 8)	*F-value*	*P-value*
A. *Open field test (4 min)*							
Walking (% of time)	30.79 ± 3.27	24.92 ± 4.79	31.04 ± 2.80	46.9 ± 5.59	35.28 ± 6.56	1.54	1.00
Running (% of time)	15.22 ± 7.18	17.02 ± 6.49	10.49 ± 3.02	0.07 ± 0.07	0.32 ± 0.26	1.67	1.00
Standing (% of time)	52.62 ± 6.92	57.57 ± 7.29	57.88 ± 3.61	49.91 ± 5.59	63.14 ± 6.61	0.21	1.00
Contact with wall/floor (% of time)	20.04 ± 3.40	16.35 ± 3.19	21.05 ± 2.71	37.28 ± 5.36	33.50 ± 5.02	5.99	1.00
Vigilant (% of time)	13.01 ± 2.52	20.80 ± 3.11	15.03 ± 2.70	11.25 ± 3.47	13.24 ± 3.81	0.99	1.00
Vocalizations (frequency)	23.26 ± 18.20	10.25 ± 8.48	21.42 ± 10.12	0.17 ± 0.17	6.25 ± 3.36	0.89	1.00
Solitary play (frequency)	11.40 ± 4.34	10.67 ± 3.84	6.30 ± 1.71	0.17 ± 0.17	0.13 ± 0.13	2.57	1.00
B. *Novel object test (4 min)*							
Latency first close approach (s)	3.50 ± 0.87	75.50 ± 35.44	141.11 ± 32.77	71.17 ± 24.45	129.40 ± 45.69	2.01	1.00
Latency first contact with object (s)	144.40 ± 56.72	231.50 ± 1.73	193.5 ± 28.19	179.83 ± 23.12	134.60 ± 43.15	0.55	1.00
Contact with object (% of time)	6.55 ± 4.33	0.42 ± 0.42	0.60 ± 0.47	4.44 ± 1.58	4.96 ± 2.39	1.75	1.00
Vigilant (% of time)	19.43 ± 3.72	25.12 ± 2.61	29.09 ± 5.34	24.49 ± 6.29	19.06 ± 4.69	1.17	1.00
Vocalizations (frequency)	8.25 ± 4.23	3.00 ± 1.55	4.75 ± 1.52	0.83 ± 0.48	8.67 ± 3.33	0.78	1.00
Solitary play (frequency)	4.40 ± 1.91^{b}	1.00 ± 0.82^{a}	0.50 ± 0.27^{a}	0.17 ± 0.17^{a}	0.00 ± 0.00^{ab}	9.34	0.002
C. *Voluntary phase human approach test (2 min)*							
Latency first close approach (s)	27.25 ± 13.52	86.60 ± 20.30	56.00 ± 18.94	94.33 ± 16.81	75.40 ± 24.14	1.16	1.00
Latency first contact with human (s)	24.80 ± 11.18	74.60 ± 23.73	62.50 ± 17.88	103.67 ± 15.36	99.50 ± 17.90	0.96	1.00
Contact with human (% of time)	67.12 ± 11.52	23.21 ± 12.78	23.37 ± 10.56	1.39 ± 1.39	1.23 ± 1.23	0.62	1.00
D. *Involuntary phase human approach test (2 min)*							
Latency first contact with human (s)	3.25 ± 1.31	8.33 ± 3.14	8.20 ± 1.78	23.20 ± 1.85	14.17 ± 5.91	3.08	0.73
Contact with human while walking (% of time)	11.33 ± 3.23	18.61 ± 3.41	13.83 ± 2.62	8.89 ± 2.13	6.90 ± 2.76	1.65	1.00
Contact with human while standing (% of time)	46.00 ± 16.23	42.92 ± 11.52	63.83 ± 4.15	45.00 ± 12.81	45.87 ± 13.64	1.02	1.00

Different subscript letters within a row indicate significant differences (P < 0.05) between treatment groups.

[a] BW: reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall; AW: reducing contact after weaning by moving partial contact calves from the wall at day 63; FS: reducing contact (at day 49) by placing full contact calves behind the fence-line; NF: reducing contact at weaning by fitting full contact calves a nose-flap at day 56.

isolation test compared to calves with full CCC. The increased solitary play may reflect activity rebound due to the increased space allowance in the test arena compared to the confined home pen (Jensen, 1999). Calves with full CCC are known to perform locomotor play in the alleys of the cow barn (Wagner et al., 2013; Waiblinger et al., 2020), so they may have been less motivated for locomotor play in the test arena compared to calves that had less space in their home pen (Jensen and Kyhn, 2000; Wagner et al., 2013). However, all PC-calves were housed in similar calf boxes as NC calves but did not show this rebound activity during the NOT. This finding suggests that merely maternal contact in the first weeks of life affects calves' activity but, given that we did not document play behaviour in the home pens, we recommend further studies to assess play behaviour in different CCC systems to further understand the role of CCC for calf development.

Exposing animals to stressors could also reduce animals' immune competence and increase susceptibility to diseases (Blecha, 2000; Griffin, 1989). Previous studies have compared two-step debonding strategies with abrupt weaning. These studies demonstrated lower plasma cortisol levels (Loberg et al., 2008), greater humoral antibody titer responses to a viral vaccination (Lippolis et al., 2016), and reduced morbidity (Boyles et al., 2007) indicating reduced stress responses and thus enhanced immune functioning when debonding occurs. Yet, we found no significant differences between two-step debonding strategies for calves' immune functioning, health status, plasma cortisol concentrations or hair cortisol levels, as inter-individual variability for the various parameters was large. Some individuals showed mild physiological stress responses during debonding, whereas others seemed to have experienced more severe stress. Given that individual animals may profoundly differ in stress responsiveness (e.g. Nogues et al., 2020; van Reenen et al., 2005), we encourage the development of tailored debonding strategies that can be adapted to individual cow-calf pairs. For instance, the use of computer-automated access gates that facilitate access to either the calf or the cow could gradually reduce contact for specific individuals over a longer period of time. One possible confounder in our work is the fact that the different debonding strategies differed in timing, intensity, and duration, which made it difficult to control the stressor severity (Sapolsky, 2015). For example, FC-FS calves may have experienced more social stress than the other groups due to the additional loss of contact with peers once they moved behind the fence-line, and gradual weaning was initiated at another moment in time in FC calves (week 7 and 8) compared to the handfed calves (from week 5 onwards). Moreover, the space-allowance in the different treatment groups varied, which may have affected the activity levels of the calves (Sutherland et al., 2014). Hence, future work should aim to standardize debonding strategies and this could be facilitated by increasing the size of relevant groups.

Interestingly, FC cows' machine-harvested milk yield seemed to recover to some extent once calves were weaned, as FC-FS and FC-NF cows no longer differed statistically significant in their daily milk yield two weeks after calves were weaned compared to PC-BW, PC-AW, NC cows. However, the non-significant numerical difference may still have economic consequences for farmers (de Andrade Ferrazza et al., 2020), and more research is needed on milk production effects of CCC systems over time with larger numbers of cows. The decreased volume of milk yielded in the parlor during the period when calves suckle freely is well reported, although there is no consistent evidence of reduced milk production beyond the suckling period (see review by Meagher et al., 2019). Recent work reported, however, that machine-harvested milk yields were negatively impacted throughout the whole lactation period, perhaps because the frequency of milk removal went down from several times per day to twice daily machine milking after the calves were weaned (Barth, 2020). Other studies found that cow performance recovers in full CCC systems once calves were weaned (de Passillé et al., 2008; Johnsen et al., 2015b).

In terms of calf performance, weaning negatively affected the weight gain of FC-NF calves compared to FC-FS, PC-BW, PC-AW, or NC calves. This finding is in line with Enríquez et al. (2010), who also reported a reduced growth in beef calves weaned with a nose-flap compared to fence-line separation. The small proportion of time spent ruminating in FC-FS and FC-NF calves before weaning accompanied by a relatively high prevalence of liquid manure between weeks 7–10 indicates a suboptimal adaptation to solid feed prior to weaning (De Passillé et al., 2011), which in combination with the abrupt cessation of milk supply after insertion of the nose-flap may have enhanced the weaning stress in FC-NF calves. Moreover, previous work reported heavy nasal abrasions 7 days after fitting the nose-flaps in beef calves (Lambertz et al., 2014), and, although not documented, we also observed injuries to the calves' nostrils that may have been caused by the pressure of the nose-flaps. We also suspect that the nose-flaps might have caused some pain or irritation that affected calves' activity levels.

A limitation of the current study is the small sample size, which limited the statistical power of the analysis and increased the possibility of Type II error. The precision of the study would have increased with a larger sample size, which would have allowed for the detection of smaller differences between treatment groups. Overall, we strongly recommend future research to include a higher number of animals and accelerometer data that provides a more comprehensive activity patterns (e.g. number of steps, frequency/duration of lying bouts) supported by video material to enhance our understanding of stress responses in dairy cattle to debonding after prolonged CCC.

Overall, it appears that partial CCC minimized calves' debonding distress. In dairy calves with full CCC, nose-flaps seem less effective at reducing weaning stress compared with fence-line separation. Given that stress responses may be affected by the duration of fence-line separation and the design of the fence, we strongly recommend to further explore methods that can gradually reduce contact prior to or after weaning for full CCC systems. Alternatively, we would argue that in full CCC systems a more gradual reduction of physical contact prior to weaning could be accompanied by delayed weaning age, which might result in less distress given that calves may then be even more socially and nutritionally independent from the dam.

5. Conclusion

Calves with partial cow-calf contact (CCC) showed minimal signs of distress during weaning and separation compared to calves with full CCC. Our results imply that debonding by reducing contact via nose-flaps was more stressful for full CCC calves compared to debonding via fence-line separation. Milk production of full CCC cows was only significantly negatively affected before weaning, and seemed to recover to some extent after calves no longer suckled. However, besides efforts that investigate cows' distress responses to debonding after prolonged contact, more strategies to mitigate stress responses in calves with full CCC need to be explored.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This study was financed by DairyNL (ZuivelNL; organization of the Dutch dairy supply chain, The Hague, the Netherlands) and the Dutch Ministry of Agriculture, Nature and Food Quality (LNV, The Hague, the Netherlands) as part of the research program 'One Health for Food' (1H4F, The Hague, the Netherlands). The contribution to this article by Dr. E.A.M. Bokkers was supported by the ERA-net CORE Organic Cofund Project GrazyDaiSy (ID 1871). We thank the staff of the Knowledge Transfer Centre (Zegveld, the Netherlands) for their help and assistance during the experiment. Thanks to Deborah Bomfim, Bianca Costa, Anne Hoogstra, Claudia Meeusen, Ilona Wardenaar, and Fokje Steenstra for their valuable help with the experiment and data acquisition. We are grateful to Lars Ravesloot from Wageningen Bio-Veterinary Research for the serum IgG analysis. Thanks to Agis Automatisering (Harmelen, the Netherlands) allowing us to use their technology in this study and for giving access to detailed CowManager sensor data. We thank Joop van der Werf from Wageningen Livestock Research for his assistance with processing the CowManager sensor data.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2022.105694.

References

de Andrade Ferrazza, R., Lopes, M.A., de Oliveira Prado, D.G., de Lima, R.R., Bruhn, F.R. P., 2020. Association between technical and economic performance indexes and dairy farm profitability. Rev. Bras. Zootec. 49. https://doi.org/10.37496/RBZ4920180116.

Barth, K., 2020. Effects of suckling on milk yield and milk composition of dairy cows in cow–calf contact systems. J. Dairy Res. 1–5. https://doi.org/10.1017/S0022029920000515.

Bikker, J.P., van Laar, H., Rump, P., Doorenbos, J., van Meurs, K., Griffioen, G.M., Dijkstra, J., 2014. Technical note: evaluation of an ear-attached movement sensor to

record cow feeding behavior and activity. J. Dairy Sci. 97, 2974–2979. https://doi.org/10.3168/jds.2013-7560.
Blecha, F., 2000. Immune system response to stress. In: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. CAB International.
Boissy, A., Bouissou, M.F., 1995. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Appl. Anim. Behav. Sci. 46, 17–31. https://doi.org/10.1016/0168-1591(95)00633-8.
Boyles, S.L., Loerch, S.C., Lowe, G.D., 2007. Effects of weaning management strategies on performance and health of calves during feedlot receiving. Prof. Anim. Sci. 23, 637–641. https://doi.org/10.15232/S1080-7446(15)31034-2.
Buchli, C., Raselli, A., Bruckmaier, R., Hillmann, E., 2017. Contact with cows during the young age increases social competence and lowers the cardiac stress reaction in dairy calves. Appl. Anim. Behav. Sci. 187, 1–7. https://doi.org/10.1016/j.applanim.2016.12.002.
Burnett, T.A., Madureira, A.M.L., Silper, B.F., Tahmasbi, A., Nadalin, A., Veira, D.M., Cerri, R.L.A., 2015. Relationship of concentrations of cortisol in hair with health, biomarkers in blood, and reproductive status in dairy cows. J. Dairy Sci. 98, 4414–4426. https://doi.org/10.3168/JDS.2014-8871.
Busch, G., Weary, D.M., Spiller, A., Von Keyserlingk, M.A.G., 2017. American and German attitudes towards cow-calf separation on dairy farms. PLoS One 12, 1–20. https://doi.org/10.1371/journal.pone.0174013.
Calderón-Amor, J., Beaver, A., von Keyserlingk, M.A.G., Gallo, C., 2020. Calf- and herd-level factors associated with dairy-calf reactivity. J. Dairy Sci. 103, 4606–4617. https://doi.org/10.3168/jds.2019-16878.
Cliquet, F., Aubert, M., Sagné, L., 1998. Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. J. Immunol. Methods 212, 79–87. https://doi.org/10.1016/S0022-1759(97)00212-3.
De Passillé, A.M., Marnet, P.G., Lapierre, H., Rushen, J., 2008. Effects of twice-daily nursing on milk ejection and milk yield during nursing and milking in dairy cows. J. Dairy Sci. 91, 1416–1422. https://doi.org/10.3168/jds.2007-0504.
De Passillé, A.M.M., Borderas, T.F.F., Rushen, J., 2011. Weaning age of calves fed a high milk allowance by automated feeders: effects on feed, water, and energy intake, behavioral signs of hunger, and weight gains. J. Dairy Sci. 94, 1401–1408. https://doi.org/10.3168/jds.2010-3441.
Duve, L.R., Weary, D.M., Halekoh, U., Jensen, M.B., 2012. The effects of social contact and milk allowance on responses to handling, play, and social behavior in young dairy calves. Journal of Dairy Science 95, 6571–6581. https://doi.org/10.3168/jds.2011-5170.
Enríquez, D., Hötzel, M.J., Ungerfeld, R., 2011. Minimising the stress of weaning of beef calves: a review. Acta Vet. Scand. 53, 28. https://doi.org/10.1186/1751-0147-53-28.
Enríquez, D.H., Ungerfeld, R., Quintans, G., Guidoni, A.L., Hötzel, M.J., 2010. The effects of alternative weaning methods on behaviour in beef calves. Livest. Sci. 128, 20–27. https://doi.org/10.1016/j.livsci.2009.10.007.
Flower, F.C., Weary, D.M., 2001. Effects of early separation on the dairy cow and calf: 2. Separation at 1 day and 2 weeks after birth. Appl. Anim. Behav. Sci. 70, 275–284. https://doi.org/10.1016/S0168-1591(00)00164-7.
Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E., Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92, 340–374. https://doi.org/10.1016/J.PHYSBEH.2007.03.016.
González-de-la-Vara, M., del, R., Valdez, R.A., Lemus-Ramirez, V., Vázquez-Chagoyán, J. C., Villa-Godoy, A., Romano, M.C., 2011. Effects of adrenocorticotropic hormone challenge and age on hair cortisol concentrations in dairy cattle. Can. J. Vet. Res. 75, 216.
Griffin, J.F.T., 1989. Stress and immunity: a unifying concept. Vet. Immunol. Immunopathol. 20, 263–312. https://doi.org/10.1016/0165-2427(89)90005-6.
Grignard, L., Boissy, A., Boivin, X., Garel, J.P., Le Neindre, P., 2000. The social environment influences the behavioural responses of beef cattle to handling. Appl. Anim. Behav. Sci. 68, 1–11. https://doi.org/10.1016/S0168-1591(00)00085-X.
Haley, D.B., Bailey, D.W., Stookey, J.M., 2005. The effects of weaning beef calves in two stages on their behavior and growth rate. J. Anim. Sci. 83, 2205–2214. https://doi.org/10.2527/2005.8392205x.
Heimbürge, S., Kanitz, E., Otten, W., 2019. The use of hair cortisol for the assessment of stress in animals. Gen. Comp. Endocrinol. https://doi.org/10.1016/j.ygcen.2018.09.016.
Hemsworth, P.H., Price, E.O., Borgwardt, R., 1996. Behavioural responses of domestic pigs and cattle to humans and novel stimuli. Appl. Anim. Behav. Sci. 50, 43–56. https://doi.org/10.1016/0168-1591(96)01067-2.
Hill, T.M.M., Suarez-Mena, F.X.X., Hu, W., Dennis, T.S.S., Schlotterbeck, R.L.L., Timms, L.L.L., Hulbert, L.E.E., 2017. Technical note: evaluation of an ear-attached movement sensor to record rumination, eating, and activity behaviors in 1-month-old calves. Prof. Anim. Sci. 33, 743–747. https://doi.org/10.15232/pas.2017-01623.
Jensen, M.B., 1999. Effects of confinement on rebounds of locomotor behaviour of calves and heifers, and the spatial preferences of calves. Appl. Anim. Behav. Sci. 62, 43–56. https://doi.org/10.1016/S0168-1591(98)00208-1.
Jensen, M.B., 2017. The role of social behavior in cattle welfare. In: Advances in Cattle Welfare. Woodhead Publishing, pp. 123–155. https://doi.org/10.1016/B978-0-08-100938-3.00006-1.
Jensen, M.B., Kyhn, R., 2000. Play behaviour in group-housed dairy calves, the effect of space allowance. Appl. Anim. Behav. Sci. 67, 35–46. https://doi.org/10.1016/S0168-1591(99)00113-6.
Johnsen, J.F., de Passille, A.M., Mejdell, C.M., Bøe, K.E., Grøndahl, A.M., Beaver, A., Rushen, J., Weary, D.M., 2015a. The effect of nursing on the cow-calf bond. Appl. Anim. Behav. Sci. 163, 50–57. https://doi.org/10.1016/j.applanim.2014.12.003.
Johnsen, J.F., Ellingsen, K., Grøndahl, A.M., Bøe, K.E., Lidfors, L., Mejdell, C.M., 2015b. The effect of physical contact between dairy cows and calves during separation on their post-separation behavioural response. Appl. Anim. Behav. Sci. 166, 11–19. https://doi.org/10.1016/j.applanim.2015.03.002.
Johnsen, J.F., Zipp, K.A., Kälber, T., Passillé, A.M. de, Knierim, U., Barth, K., Mejdell, C. M., 2016. Is rearing calves with the dam a feasible option for dairy farms?—Current and future research. Appl. Anim. Behav. Sci. 181, 1–11. https://doi.org/10.1016/j.applanim.2015.11.011.
Johnsen, J.F., Mejdell, C.M., Beaver, A., de Passillé, A.M., Rushen, J., Weary, D.M., 2018. Behavioural responses to cow-calf separation: the effect of nutritional dependence. Appl. Anim. Behav. Sci. 201, 1–6. https://doi.org/10.1016/j.applanim.2017.12.009.
Kenward, M.G., Roger, J.H., 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53, 983–997.
Khan, M.A., Weary, D.M., von Keyserlingk, M.A.G., 2011. Invited review: effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. J. Dairy Sci. 94, 1071–1081. https://doi.org/10.3168/jds.2010-3733.
Lambertz, C., Bowen, P.R., Erhardt, G., Gauly, M., Lambertz, C., Bowen, P.R., Erhardt, G., Gauly, M., 2014. Effects of weaning beef cattle in two stages or by abrupt separation on nasal abrasions, behaviour, and weight gain. Anim. Prod. Sci. 55, 786–792. https://doi.org/10.1071/AN14097.
Lambertz, C., Farke-Röver, A., Gauly, M., 2015. Effects of sex and age on behavior and weight gain in beef calves after abrupt weaning. Anim. Sci. J. 86, 345–350. https://doi.org/10.1111/ASJ.12285.
Lecorps, B., Weary, D.M., von Keyserlingk, M.A.G.G., 2018. Pessimism and fearfulness in dairy calves. Sci. Rep. 8, 1421. https://doi.org/10.1038/s41598-017-17214-3.
Lippolis, K.D., Ahola, J.K., Mayo, C.E., Fischer, M.C., Callan, R.J., 2016. Effects of two-stage weaning with nose flap devices applied to calves on cow body condition, calf performance, and calf humoral immune response. J. Anim. Sci. https://doi.org/10.2527/jas2015-9624.
Loberg, J.M., Hernandez, C.E., Thierfelder, T., Jensen, M.B., Berg, C., Lidfors, L., 2007. Reaction of foster cows to prevention of suckling from and separation from four calves simultaneously or in two steps1. J. Anim. Sci. 85, 1522–1529. https://doi.org/10.2527/jas.2006-813.
Loberg, J.M., Hernandez, C.E., Thierfelder, T., Jensen, M.B., Berg, C., Lidfors, L., 2008. Weaning and separation in two steps—A way to decrease stress in dairy calves suckled by foster cows. Appl. Anim. Behav. Sci. 111, 222–234. https://doi.org/10.1016/j.applanim.2007.06.011.
Marcé, C., Guatteo, R., Bareille, N., Fourichon, C., 2010. Dairy calf housing systems across Europe and risk for calf infectious diseases. Animal 4, 1588–1596. https://doi.org/10.1017/S1751731110000650.
McGuirk, S.M., 2008. Disease management of dairy calves and heifers. Vet. Clin. North Am. - Food Anim. Pract. 24, 139–153. https://doi.org/10.1016/j.cvfa.2007.10.003.
Meagher, R.K., Beaver, A., Weary, D.M., von Keyserlingk, M.A.G., 2019. Invited review: a systematic review of the effects of prolonged cow–calf contact on behavior, welfare, and productivity. J. Dairy Sci. 102, 5765–5783. https://doi.org/10.3168/jds.2018-16021.
Neave, H.W., Sumner, C.L., Henwood, R.J.T., Zobel, G., Saunders, K., Thoday, H., Watson, T., Webster, J.R., 2021. Dairy farmers' perspectives on providing cow-calf contact in the pasture-based systems of New Zealand. J. Dairy Sci. 105, 453–467. https://doi.org/10.3168/JDS.2021-21047.
Newberry, R.C., Swanson, J.C., 2008. Implications of breaking mother–young social bonds. Appl. Anim. Behav. Sci. 110, 3–23. https://doi.org/10.1016/j.applanim.2007.03.021.
Nogues, E., Lecorps, B., Weary, D.M., Keyserlingk, M.A.G. von, 2020. Individual Variability in Response to Social Stress in Dairy Heifers, 1440 10 Anim 2020 Vol. 10, 1440. https://doi.org/10.3390/ANI10081440.
Ochsenbein, A.F., 1999. Control of early viral and bacterial distribution and disease by natural antibodies. Sciences (80-.) 286, 2156–2159.
de Passillé, A.M., Rushen, J., Martin, F., 1995. Interpreting the behaviour of calves in an open-field test: a factor analysis. Appl. Anim. Behav. Sci. 45, 201–213. https://doi.org/10.1016/0168-1591(95)00622-Y.
Pereira, G.M., Heins, B.J., Endres, M.I., 2017. Technical note: validation of an ear-tag accelerometer sensor to determine rumination, eating, and activity behaviors of grazing dairy cattle. J. Dairy Sci. 101, 2492–2495. https://doi.org/10.3168/jds.2016-12534.
Price, E.O., Harris, J.E., Borgwardt, R.E., Sween, M.L., Connor, J.M., 2014. Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behavior and growth rate. J. Anim. Sci. 81, 116–121. https://doi.org/10.2527/2003.811116x.
Renaud, D.L., Duffield, T.F., LeBlanc, S.J., Haley, D.B., Kelton, D.F., 2017. Clinical and metabolic indicators associated with early mortality at a milk-fed veal facility: a prospective case-control study. Journal of Dairy Science 101, 2669–2678. https://doi.org/10.3168/jds.2017-14042.
Santo, N.K., Von Borstel, U.K., Sirovnik, J., 2020. The influence of maternal contact on activity, emotionality and social competence in young dairy calves. J. Dairy Res. 87, 138–143. https://doi.org/10.1017/S0022029920000527.
Sapolsky, R.M., 2015. Stress and the brain: individual variability and the inverted-U. Nat. Neurosci. 18, 1344–1346. https://doi.org/10.1038/nn.4109.
Schwinn, A.C., Knight, C.H., Bruckmaier, R.M., Gross, J.J., 2016. Suitability of saliva cortisol as a biomarker for hypothalamic-pituitary-adrenal axis activation assessment, effects of feeding actions, and immunostimulatory challenges in dairy cows. J. Anim. Sci. 94, 2357–2365. https://doi.org/10.2527/JAS.2015-0260.
Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166, 869–887. https://doi.org/10.1007/S00442-011-1943-Y.

Sirovnik, J., Barth, K., Oliveira, D., De, Ferneborg, S., Haskell, M.J., Hillmann, E., Jensen, M.B., Mejdell, C.M., Napolitano, F., Vaarst, M., Verwer, C.M., Waiblinger, S., Zipp, K.A., Johnsen, J.F., 2020. Methodological terminology and definitions for research and discussion of cow-calf contact systems. J. Dairy Res. 87, 108–114.

de Souza Teixeira, O., Kuczynski da Rocha, M., Mendes Paizano Alforma, A., Silva Fernandes, V., de Oliveira Feijó, J., Nunes Corrêa, M., Andrighetto Canozzi, M.E., McManus, C., Jardim Barcellos, J.O., 2021. Behavioural and physiological responses of male and female beef cattle to weaning at 30, 75 or 180 days of age. Appl. Anim. Behav. Sci. 240, 105339 https://doi.org/10.1016/J.APPLANIM.2021.105339.

Stěhulová, I., Valníčková, B., Šárová, R., Špinka, M., 2017. Weaning reactions in beef cattle are adaptively adjusted to the state of the cow and the calf. J. Anim. Sci. 95, 1023–1029. https://doi.org/10.2527/JAS.2016.1207.

Stygar, A.H., Gómez, Y., Berteselli, G.V., Dalla Costa, E., Canali, E., Niemi, J.K., Llonch, P., Pastell, M., 2021. A systematic review on commercially available and validated sensor technologies for welfare assessment of dairy cattle. Front. Vet. Sci. 8, 177. https://doi.org/10.3389/FVETS.2021.634338/BIBTEX.

Sutherland, M.A., Worth, G.M., Stewart, M., 2014. The effect of rearing substrate and space allowance on the behavior and physiology of dairy calves. J. Dairy Sci. 97, 4455–4463. https://doi.org/10.3168/JDS.2013-7822.

Ungerfeld, R., Quintans, G., Hötzel, M.J.J., Hotzel, M.J., Hötzel, M.J.J., 2016. Minimizing cows' stress when calves were early weaned using the two-step method with nose flaps. Animal 10, 1871–1876. https://doi.org/10.1017/S1751731116000793.

Vaarst, M., Hellec, F., Verwer, C.M., Johanssen, J.R.E., Sørheim, K., 2020. Cow-calf contact in dairy herds viewed from the perspectives of calves, cows, humans and the farming system. Farmers ' perceptions and experiences related to dam-rearing systems. J. Sustain. Org. Agric. Syst. 70, 49–57. https://doi.org/10.3220/LBF1596195636000.

Van Reenen, C.G., Engel, B., Ruis-Heutinck, L.F.M., Van Der Werf, J.T.N., Buist, W.G., Jones, R.B., Blokhuis, H.J., 2004. Behavioural reactivity of heifer calves in potentially alarming test situations: a multivariate and correlational analysis. Appl. Anim. Behav. Sci. 85, 11–30. https://doi.org/10.1016/j.applanim.2003.09.007.

Van Reenen, C.G., O'Connell, N.E., Van Der Werf, J.T.N., Korte, S.M., Hopster, H., Jones, R.B., Blokhuis, H.J., 2005. Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. Physiol. Behav. 85, 557–570. https://doi.org/10.1016/J.PHYSBEH.2005.06.015.

Van Reenen, C.G., Hopster, H., Van der Werf, J.T.N., Engel, B., Buist, W.G., Jones, R.B., Blokhuis, H.J., Korte, S.M., 2009. The benzodiazepine brotizolam reduces fear in calves exposed to a novel object test. Physiol. Behav. 96, 307–314. https://doi.org/10.1016/J.PHYSBEH.2008.10.016.

Veissier, I., Caré, S., Pomiès, D., 2013. Suckling, weaning, and the development of oral behaviours in dairy calves. Appl. Anim. Behav. Sci. 147, 11–18. https://doi.org/10.1016/j.applanim.2013.05.002.

Ventura, B.A., von Keyserlingk, M.A.G., Schuppli, C.A., Weary, D.M., 2013. Views on contentious practices in dairy farming: the case of early cow-calf separation. J. Dairy Sci. 96, 6105–6116. https://doi.org/10.3168/jds.2012-6040.

Wagner, K., Barth, K., Hillmann, E., Palme, R., Futschik, A., Waiblinger, S., 2013. Mother rearing of dairy calves: Reactions to isolation and to confrontation with an unfamiliar conspecific in a new environment. Appl. Anim. Behav. Sci. 147, 43–54. https://doi.org/10.1016/j.applanim.2013.04.010.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.V., Janczak, A.M., Visser, E.K., Jones, R.B., 2006. Assessing the human–animal relationship in farmed species: a critical review. Appl. Anim. Behav. Sci. 101, 185–242. https://doi.org/10.1016/J.APPLANIM.2006.02.001.

Waiblinger, S., Wagner, K., Hillmann, E., Barth, K., 2020. Play and social behaviour of calves with or without access to their dam and other cows. J. Dairy Res. 87, 144–147. https://doi.org/10.1017/S0022029920000540.

Weary, D.M., Jasper, J., Hötzel, M.J., 2008. Understanding weaning distress. Appl. Anim. Behav. Sci. 110, 24–41. https://doi.org/10.1016/j.applanim.2007.03.025.

Wenker, M.L., Bokkers, E.A.M., Lecorps, B., von Keyserlingk, M.A.G., van Reenen, C.G., Verwer, C.M., Weary, D.M., 2020. Effect of cow-calf contact on cow motivation to reunite with their calf. Sci. Rep. 10, 14233. https://doi.org/10.1038/s41598-020-70927-w.

Wenker, M.L., van Reenen, C.G., de Oliveira, D., McCrea, K., Verwer, C.M., Bokkers, E.A.M., 2021. Calf-directed affiliative behaviour of dairy cows in two types of cow-calf contact systems. Appl. Anim. Behav. Sci., 105461 https://doi.org/10.1016/J.APPLANIM.2021.105461.

Whalin, L., Weary, D.M., von Keyserlingk, M.A.G., 2021. Understanding behavioural development of calves in natural settings to inform calf management. Animals 11, 2446. https://doi.org/10.3390/ani11082446.