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Behavioural responses of dairy cows and calves to fenceline weaning after 4 or 6 months of full cow-calf contact

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A B S T R A C T

Cow-calf contact (**CCC**) systems, where cows and calves are housed together during all or part of the milk-feeding period, foster strong social bonds within dam-calf pairs. However, calves are still generally weaned and separated at younger ages than have been observed for semi-feral cattle. This study aimed to evaluate behavioural responses of dairy cows and calves to fenceline weaning after 4 or 6 months of full CCC. Additionally, the proportion of time spent by dam-calf pairs in close proximity (< 4 m indoors or < 8 m outdoors) prior to weaning was tested for its effect on behavioural responses. Dairy cows (n = 25) and their calves (n = 26) were housed in a freestall pen with free access to pasture for either 4 (**4MO**) or 6 months (**6MO**), after which calves were weaned outdoors via fenceline separation. Daily activity (lying time and step count) was recorded for all animals using accelerometers for 6 days before and for 11 days after weaning, while vocalisations and feed-seeking behaviour were collected for calves postweaning through direct observations. Scan sampling on 3 days during the end of the contact period was used to estimate proximity within each dam-calf pair, and calves were weighed regularly throughout the study. Calf feed-seeking behaviour and differences in lying time or step count (calculated as changes from a preweaning baseline value) for cows and calves were fitted with polynomial regression models. Directly after weaning, calves responded by decreasing their lying time, increasing their step count and vocalisations, and spending little time on feed-seeking; these responses were greater for 4MO calves. The calves, especially those weaned at 4 months, had reduced growth rates for several weeks postweaning, suggesting a lack of nutritional independence prior to weaning. Cow activity responses were similar but with no clear treatment differences in the first 3 days and with faster recovery times than for calves. Dam-calf proximity varied greatly between pairs but did not influence any of the modelled responses. Our results suggest that fenceline weaning causes behavioural responses indicative of distress in both calves and (to a lesser extent) cows, even when calves are weaned at a higher age.

Implications

Cow-calf contact systems allow dairy calves to form strong bonds with their mothers, but this can lead to stress when they are inevitably separated and the calves weaned from milk. In this study, we aimed to evaluate behavioural responses to weaning calves via fenceline separation at 4 or 6 months of age. Weaning at either age caused similar behavioural response patterns for both calves and cows, although younger calves' responses were stronger in the first few days. Methods for gradual weaning are needed to reduce weaning-related stress and encourage social and nutritional independence prior to weaning, especially in younger calves.

Introduction

Dairy production systems that allow some form of contact between cows and calves beyond the colostrum period – commonly known as cow-calf contact (**CCC**; Sirovnik et al., 2020) systems – are gaining recognition amongst consumers (Sirovica et al., 2022) and producers for being more natural and promoting good animal health and welfare (Eriksson et al., 2022; Neave et al., 2022). These systems provide cows and calves with increased opportunities to perform important behaviours (e.g., suckling) than if they are separated at calving, and facilitate the formation of

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social relationships, such as that between a dam and her calf. The dam-calf bond is a preferential mutual and emotional attachment, characterised by affiliative behaviours (e.g., allogrooming, maintaining proximity) and demonstrated in cattle to survive short periods of separation (Newberry and Swanson, 2008). This bond is established within the first few days after parturition through the dam's engagement in maternal behaviour (see review by von Keyserlingk and Weary, 2007), although quite quickly afterwards the dynamic begins to shift, to where the majority of interactions within the dam-calf pair are initiated by the calf (Jensen, 2011).

Spatial proximity (i.e., physical distance), a suggested measure of attachment between dam and calf (Claramunt et al., 2020), has been reported to be influenced by factors such as calf sex (Kour et al., 2021) or number of offspring (Price et al., 1985). To date, studies exploring spatial proximity in the context of dam-calf attachment have been largely limited to free-ranging beef cattle (Claramunt et al., 2020), Maremma cattle (Vitale et al., 1986) and the first 5 weeks postpartum in dairy cattle (Wenker et al., 2021). Time spent in close proximity provides opportunities for the calf to receive maternal care independent of suckling bouts (Johnsen et al., 2015; Kour et al., 2021). Thus, dam-calf pairs that spend more time at further distances may be said to exhibit a greater degree of social independence from one another. It could therefore be expected that pairs that spend more time in close proximity react more strongly to weaning.

Under extensive management conditions, weaning occurs gradually as calves gain nutritional and social independence, and may coincide with attempts by the dam to reject suckling by the calf (reviewed by Enríquez et al., 2011). Observations of semi-feral *Bos indicus* cattle have shown this process to occur between 7 and 14 months of age, with marked differences between calf sexes (Reinhardt and Reinhardt, 1981). Calves in CCC systems are instead often weaned by human intervention at 12–17 weeks (Eriksson et al., 2022), which is earlier than the weaning age observed in *Bos indicus* but later than the European average of 9 weeks for artificially-reared calves (Marcé et al., 2010). Near or concurrent to the time of weaning, calves in CCC systems are also separated from their dams, resulting in the cessation of both social contact and suckling, and therefore causing distress for the bonded individuals. Behavioural responses to early weaning and separation have been well-documented for cows and calves and often include increased vocalisations and locomotion for several days, paired with a temporary reduction in time spent lying and – for calves – feeding (see review by Lynch et al., 2019). Work on beef cattle suggests that weaning and separation at a higher calf age may reduce (but not eliminate) observed stress responses in calves (Lambertz et al., 2015b; de Souza Teixeira et al., 2021).

The question of when to wean and separate bonded dam-calf pairs in order to minimise the stress experienced by the animals still remains unanswered. To tackle this question, our study aimed to evaluate behavioural responses of dairy cows and calves to weaning via fenceline separation at two different calf ages (4 and 6 months), the older of which was chosen to be closer to the weaning ages observed for free-ranging *Bos indicus* cattle. The null hypotheses were that there would be no significant differences regarding time spent feed-seeking (calves), step count and lying time (cows and calves) after fenceline weaning at 4 or 6 months. Additionally, the study aimed to determine if dam-calf proximity prior to weaning influenced the responses postweaning, with the prediction that dam-calf pairs that were more spatially dependent (i.e., spent more time in close proximity) would show greater behavioural responses. To further understand the effects of fenceline weaning calves at these two ages, vocalisations and average daily gain in BW were explored descriptively.

Material and methods

Animals and treatments

This study enrolled 24 dam-calf pairs and one dam-calf triad with twin heifers and was conducted at the Swedish University of Agricultural Sciences' Swedish Livestock Research Centre in Uppsala, Sweden from 2 February 2022 to 3 March 2023. The study was an experimental trial with a parallel group design, with the total sample size selected based on the number of animals that could realistically be housed in the experimental pen. Cows were enrolled into a dam-calf contact system over a 6-week period and consisted of two breeds: Swedish Holstein (**SH**; primiparous: n = 5, multiparous: n = 4) and Swedish Red (**SR**; primiparous: n = 6, multiparous: n = 10). Enrolment was on the basis of birthing a heifer calf and containing no history of *S. aureus* mastitis prior to calving. Dam-calf pairs were kept for an average (± SD) of 4 ± 1.0 days in individual calving pens before being moved to the experimental pen and introduced to the herd. One SR dam-calf pair (dam parity: > 1) was removed 37 days after the enrolment period had ended due to the cow contracting and succumbing to *E. coli* mastitis. Another SH dam-calf pair (dam parity: 1) was removed due to the treatment and eventual euthanasia of a calf (age: 87 days) with congenital impaired digestive functioning. Both pairs were removed before the onset of data collection for this study.

Dam-calf pairs were blocked by parity of the dam (primiparous or multiparous) and breed (resulting in four blocks in total) and randomly assigned to one of two treatments: weaning at 4 (**4MO**) or 6 (**6MO**) months of age. Randomisation was achieved by listing pairs within each block by calf birth date and switching every third and fourth pair, then using an online, randomised coin flipper to determine to which treatment group the first listed pair in each block should be assigned to. After this, every other pair within a block was assigned to that group, with the remaining animals assigned to the other treatment. An online coin flipper was further used to elect which treatment group would receive an additional pair of animals for blocks with uneven numbers. During treatment allocation, the dam-calf triad containing heifer twins was treated as a single unit. Following weaning through physical separation (hereafter referred to as fenceline weaning) at 123 ± 12.8 (4MO group) or 182 ± 9.6 (6MO group) days of age, calves remained on fenceline contact for 4 weeks. After this, they were moved to a separate area of the farm and therefore fully separated from any form of contact (auditory, visual or olfactory) with dams. One SR 6MO cow (parity: >1) exited the trial on the day her calf was weaned due to a diagnosis of *S. aureus* mastitis in two quarters. The final number of animals available for statistical analysis was 11 4MO calves, 11 4MO cows, 12 6MO calves and 11 6MO cows (Fig. 1). After the final separation, the 4MO calves were kept as a group on a remote pasture for 37 days, where they were provided access to the same resources (i.e., feed, dry lying area) as the 6MO calves. The 4MO calves thereafter joined the general population of young stock, as did the 6MO calves immediately following their fenceline contact phase.

Housing and management

Indoor area

All animals were housed in an insulated barn with free cow traffic (VMS™, DeLaval International, Tumba, Sweden), freestalls and automatic milking. The experimental pen (Fig. 2) operated with full (i.e., whole-day, unrestricted contact; see Sirovnik et al., 2020 for definition) CCC and was calf-driven, so that calves had the primary initiative in choosing contact with cows. All resources – with the exception of a milking robot (DeLaval VMS™ V300, DeLaval AB

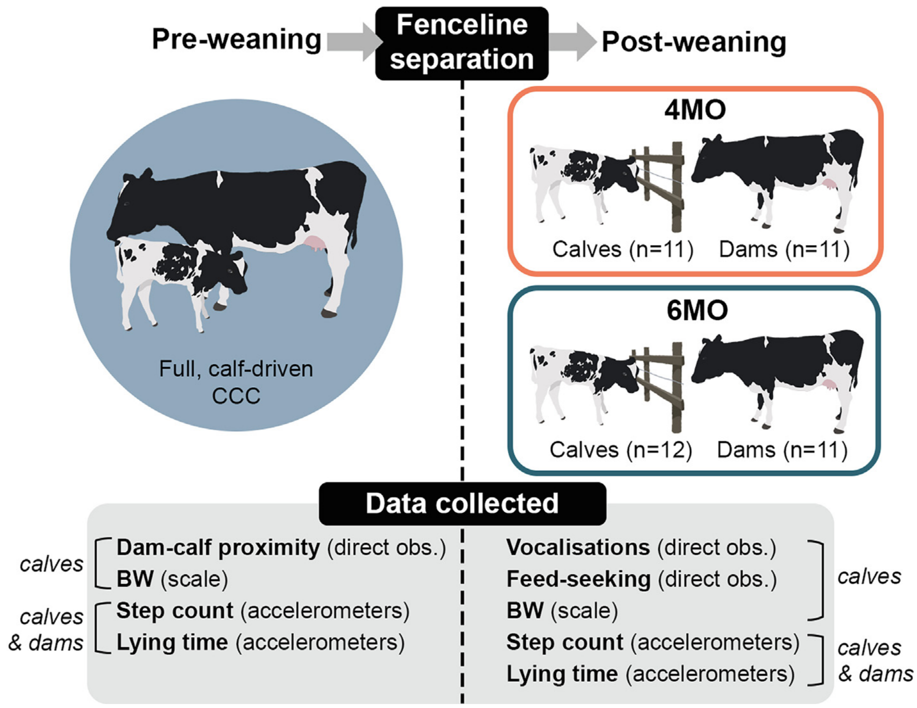

Fig. 1. Graphical summary of study design and data collection. Swedish Red and Swedish Holstein dams and calves were housed together, and calves were weaned via fenceline separation after either 4 (4MO) or 6 (6MO) months of cow-calf contact (CCC). Abbreviations: obs. = observations.

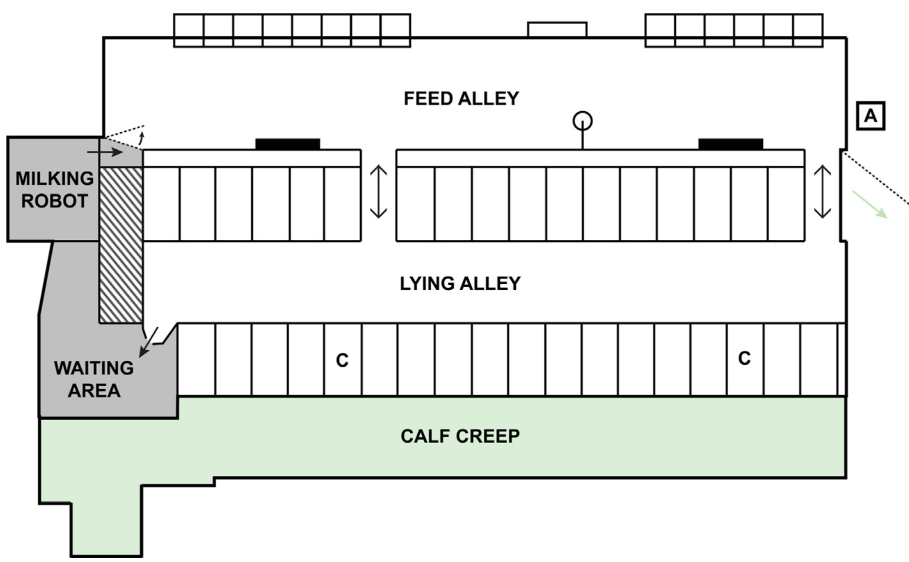

Fig. 2. Schematic illustration of the indoor areas available to dairy cows (n = 25) and calves (n = 26). Calves had access to these areas until weaning via fenceline separation at 4 (4MO) or 6 (6MO) months. All cows retained access until 4 weeks following weaning of the 6MO calves. Areas in grey are only accessible to cows, while the green area is exclusive to calves; all remaining areas are shared by both cows and calves. Arrows indicate the direction(s) of cow traffic. Observer location is depicted with an 'A' and a green arrow shows outdoor access from the pen (only available during summer). Cow concentrate feeding stations are indicated by a 'C', although they remained inactive for the duration of this study.

International, Tumba, Sweden), waiting area and calf creep – were shared between cows and calves. Access to the waiting area was restricted to only cows by use of a spring-loaded one-way gate (FeedSelect, GEA Farm Technologies GmbH, Bönen, Germany). A metal self-closing gate, modified with large plastic flaps, prevented calves from entering the robot in reverse.

The pen contained 33 freestalls bedded with rubber mattresses and a layer of sawdust, which was topped up two to four times per day using a rail-suspended bedding dispenser (JH miniStrø COW, MAFA i Ängelholm AB, Ängelholm, Sweden). Each stall was manually scraped several times per day to remove soiled bedding. There were also two concentrate feeding stations (DeLaval feed station FSC400, DeLaval International AB, Tumba, Sweden) located in the lying area, but these were turned off to encourage cow traffic through the milking robot; the robot was instead programmed to act doubly as a milking unit and concentrate feeding station.

In the feed alley, cows and calves had *ad libitum* access to one swinging brush (DeLaval SCB, DeLaval International AB, Tumba, Sweden), two self-filling water troughs (175 × 34.5 cm), salt blocks and feed. A grass-clover silage-based partial mixed ration (**PMR**) (grass-clover silage with 2% straw inclusion and 5–7 kg concentrate) was available for the cows via 14 individual feed bins (CRFI, BioControl AS, Rakkestad, Norway) and a feeding table containing eight headlock spaces. A 1.9 m long portion of the feeding table was modified to allow access for calves; in this area, headlocks were removed and replaced with horizontal bars that prevented calves from escaping, and a trough was used to bring feed within reach for calves. PMR was delivered to all feeding areas 5 times per day via a rail-suspended distribution wagon (DeLaval FS1600, DeLaval International AB, Tumba, Sweden). Flooring through the lying area and feed alley was rubber, while the milking and waiting areas contained slatted rubber flooring. Initially, automatic scrapers were run manually in both alleys to prevent calf injury; once the calves were deemed old enough by barn staff, scrapers were set to run automatically once per hour.

The calf creep was an 80 m^2 area that ran adjacent to the lying area, spanning the length of the pen, and was accessible through the fronts of lying stalls. As such, the head and neck rails on two stalls were eventually adjusted to prevent the growing calves from developing skin lesions on their backs when moving between the creep and lying area. Within the creep, calves had free access to hay and water, as well as controlled access to two concentrate feeding stations (DeLaval concentrate station calves, DeLaval International AB, Tumba, Sweden). Bedding in the calf creep was deep-bedded wood shavings, which was topped up as needed to maintain a dry lying surface. Additional wooden boards were placed between the creep and waiting area for milking; this was done to discourage cows from seeking contact with calves in this area and potentially disrupting cow traffic through the robot.

Outdoor areas

From 17 May onwards, cows and calves were granted free access to a shared outdoor pasture (2.4 ha), located 227 m from the barn and accessible via a walkway (Supplementary Fig. S1). Fencing surrounding the pasture and walkway was both wooden and electric, and water was available *ad libitum* via two water troughs. When each group of calves was weaned, they were moved to a separate calf pasture (1.1 ha), located between the shared pasture and the barn. The calf pasture contained one water trough and a shelter (48 m^2; PLAYMEK mobilt vindskydd, PLAYMEK, Röke, Sweden) that encompassed a deep-bedded straw area. Fresh feed (identical to that available in the barn) and minerals were freely available via a separate roofed feed wagon. A 5.4 m long portion of fencing was modified to allow for limited CCC (Supplementary Fig. S2); at this area, cows and calves could physically touch one another through a 0.4 x 5.4 m opening. The bottom of the opening was located at a height of 0.9 m from the ground, and suckling was prevented via closely placed horizontal beams below.

Data collection

To facilitate the identification of individuals, all cows were marked with a unique symbol on their sides and back using an animal-safe marking spray (blue, white or yellow). Markings were refreshed 2–3 times per week throughout the preweaning and postweaning observation periods, and all calves were equipped with coloured collars to aid in differentiation. Binoculars were used to identify animals at greater distances. A visual overview of the data collected pre- and postweaning is presented in Fig. 1.

Preweaning

In the week prior to fenceline weaning for each respective group, direct observations were conducted on 3 consecutive days by two trained individuals per day to determine the spatial proximity within dam-calf pairs. One observer was positioned indoors, standing on a 1.7 m tall step ladder, while a second observer was outdoors, sitting on a 3.3 m high hunting tower (Frisport AB, Malung, Sweden) with a clear view of the pasture and walkway. There were a total of four 2-hour observation periods each day, spanning over an 11-hour period each day (0700–1800 h). Each observation period was preceded by an additional 10-min period, to allow both cows and calves to acclimate to the observer's presence. During each 2-hour observation period, scan sampling was conducted at 10-min intervals, where each dam-calf pair was scored based on whether or not they were in "close" proximity. Close proximity was defined as a dam and calf being within 4 or 8 m of one another in the indoor or outdoor areas, respectively. Instances where the dam was located within the milking robot or milking waiting area were automatically scored as not being in close proximity. If the distance between a pair could not be determined (e.g., dam or calf were out of sight), this was additionally noted. Finally, whether or not a calf was engaged in suckling a cow was recorded during each interval using one-zero sampling.

Postweaning

Direct observations were conducted on days 1, 2, 3, 5, 8 and 11 postweaning, with the day of fenceline weaning considered as day 0. Observations were carried out by one of three trained observers from a hunting tower with a view over all experimental pastures (Supplementary Fig. S1). Similar to the preweaning observations, there were four 2-h observation periods daily, each with a 10-min acclimation period prior and spanning over 11 h (0800–1900 h). During each observation period, calves were scanned at 10-min intervals and noted for a presence or absence of feed-seeking behaviours, as well as if they were out of sight. Feed-seeking was defined as "actively picking at or consuming grass, hay, silage or minerals"; the calf could be still or moving. Additionally, one-zero sampling in 5-min intervals was used to detect vocalisations on a per-calf basis. No distinction was made between types of vocalisations (i.e., high-pitched vs low-pitched), but a calf was only recorded as having vocalised if the observer witnessed that particular individual emit a noise. As such, recorded vocalisations were primarily open-mouthed.

Activity and calf BW

All cows and calves were equipped with leg-mounted tri-axial accelerometers (IceQube, IceRobotics, Edinburgh, UK) which were used to record the time spent lying and number of steps taken each day, as reported in 15-min time periods. IceQubes were attached to one of the hind legs and scanned once weekly using an IceReader device (IceRobotics, Edinburgh, UK) and computer containing the IceManager software (IceRobotics, Edinburgh, UK) to download the raw data. IceQubes have been previously validated for recording daily lying time in dairy cows (Borchers et al., 2016) and calves (Finney et al., 2018). Step count has been validated against video observations for IceTag (IceRobotics, Edinburgh, UK) devices (Nielsen et al., 2010), which are similar to IceQubes but sample at a rate of 16 (compared to 4) Hz. All calves were weighed at birth and thereafter on a monthly basis. Animal weights were always recorded on the first Thursday of each month, so calf age at the first monthly weighing could vary between a few days and almost a month. Additionally, calves were weighed weekly from the day of fenceline weaning to 3 weeks after, resulting in four consecutive weeks of weight records for each respective treatment group. Weight data included all recorded weights from the first monthly weighing after the enrolment period ended (calf age: 42 ± 11.4 days) to the first monthly weighing after the experimental period ended (calf age: 372 ± 11.4 days).

Statistical analysis

All data handling and statistical analysis procedures were conducted using R version 4.4.2 (tidyverse, Wickham et al., 2019; R

Core Team, 2024). Significant differences were accepted at $P < 0.05$. The experimental unit for all analyses was the individual cow or calf. For the triad, one twin was randomly selected using an online coin flipper, and her data were used for all analysed variables. Data pertaining to the second twin heifer were removed. All analyses pertaining to calves were run both with and without the single twin heifer, and as this did not substantially affect the results, it was ultimately decided to retain this individual in the final dataset.

Dam-calf proximity

Fifty-five of 3 456 observations were initially removed due to noted external disturbances during the 10-min scan periods. Additionally, all observed instances when either the cow or calf could not be seen were considered missing values and thus removed (n = 99). An additional 18 observations were missing due to observer errors. From the remaining 3 284 observations, the number of scans recorded in close proximity was first summed per calf-day, then averaged across the 3 days to create a single mean value for each dam-calf pair. This value thus represents the average daily number of observations recorded in close proximity, and was thereafter used to calculate the mean percentage of observed time that each dam-calf pair spent in close proximity.

Calf growth performance

Calf weight measurements were examined visually and two erroneous weight records were removed, as they were 100–200 kg higher than those recorded for the same individuals both 1 week prior and later. Average daily gain (**ADG**) was calculated for four separate periods: from birth to weaning, and weekly from weaning until 3 weeks postweaning. ADG was calculated by taking the difference in BW over the observed time period (e.g., weaning weight subtracted from the weight 1–week postweaning) and dividing by the number of days. Calf BWs and ADG are reported descriptively due to a lack of *a priori* predictions.

Activity data

Raw IceQube data were converted to.csv files using the IceManager software and thereafter imported into R, where step count and lying time were handled separately. To correct for invalid recordings of step count due to leg movement whilst lying, all 15-min periods where the recorded time spent standing was 0 min were removed. Step count and lying time were then summed per cow- or calf-day, and each daily summary was assigned a day number relative to the day of fenceline weaning (Day 0). Afterwards, the dataset was filtered to include only data from Day −6 to Day 11 per treatment group. Overall, of the 374 possible cow-days and 391 calf-days (not including Day 0), 62 cow- and 48 calf-days were removed (see Supplementary Table S1 for details). Per day, at least 22 animals were available for analysis, with an average of 13.6 ± 0.80 and 15.5 ± 0.95 observation days for 4MO and 6MO animals, respectively. Data from Day 0 were not analysed due to data sampling of the animals on this day (e.g., calf weighing, accelerometer data download), which likely affected their behaviour. Finally, a preweaning baseline value was created per individual for both daily step count (steps/day) and daily lying time (min/day) by averaging the available data between Day −6 and Day −1. The two response variables used in the statistical analyses corresponded to the difference in steps or lying time for each day postweaning compared to the baseline value for either activity.

The effect of day after weaning was fitted as a polynomial function for both lying time and step count in linear mixed models. The mixed-effects polynomial regression analyses were performed using the lme function from the nlme package (Pinheiro et al., 2023). Fixed effects included treatment (4MO or 6MO), time (Day 1–11 as a numeric time-series), treatment × time, dam-calf proximity (percentage of observed time spent “close”) and parity (primiparous or multiparous; in cow models only), while cow or calf ID was specified as a random intercept. A first-order autoregressive correlation structure was specified to account for temporal autocorrelations between days. The difference in step count for cows and calves was transformed to fulfil the assumption of normal distribution of residuals. Second-order polynomial models were used for the fourth-root-transformed difference in step count, as well as the untransformed difference in lying time for both cows and calves. Likelihood ratio tests were performed to check model fit using the lrtest function in the lmtest package (Zeileis and Hothorn, 2002), wherein each final model was tested against a lower-order polynomial model. Likelihood ratio tests were also used to determine whether or not the treatment × time interaction had a significant effect on the response variables. Only interaction effects with $P < 0.05$ were included in the final models; as such, treatment × time interactions were removed from the cow model for differences in step count ($P = 0.590$). Results are reported as back-transformed values.

Vocalisations and feed-seeking behaviour

Data from two 10-min observation periods were removed due to cows blocking the observer’s view of the calf area, making it impossible to identify which calves were vocalising and if they were feed-seeking. Additionally, due to visual observations of oestrus behaviour in some calves, three calf-day observations were excluded from further analyses of feeding behaviour and vocalisations. Individual observations were further removed or missing due to external disturbances, such as barn staff entering the calf enclosure (n = 22), the calf being out of sight (n = 19), the observer not being in place (n = 13) or calves having escaped the enclosure (n = 26). At most, four of a total possible 48 daily observation periods were excluded from a single day; eight of the 12 postweaning observation days had no missing observation periods. Observations of vocalisation occurrence and feed-seeking behaviour were summed per hour and day to create an hourly average per calf per day. Due to a lack of independence between calves (vocalisations generally occurred clustered), vocalisations are reported descriptively as mean and SD per treatment group and observation day. A second-order polynomial model was used to analyse the time spent feed-seeking (min/h) after weaning, with treatment, time, treatment × time and dam-calf proximity included as fixed effects. Calf ID was included as a random intercept, and the covariance structure was specified as first-order autoregressive. Likelihood ratio tests were performed to test both model fit and the significance of interaction terms; treatment × time was ultimately removed from the final model ($P = 0.207$).

Results

Dam-calf proximity

When allowed full, free access to one another, 4MO dam-calf pairs spent an average 34% (range: 20–65%) of observed time within 4 and 8 m from one another in the inside and outdoor area, respectively. Descriptively, this was similar to 6MO pairs, who spent approximately 41% (range: 30–59%) of their time in close proximity to one another. Average dam-calf proximity did not have a significant effect on any of the changes in daily step count or lying time observed for dams and calves following fenceline weaning, nor on the feed-seeking behaviour of calves (Table 1).

Calves

Growth performance and vocalisations

Average calf BWs from the age of 6–53 weeks are displayed in Fig. 3. From birth to weaning, ADG appeared similar between treat-

Table 1
Regression estimates and SE for the daily difference in step count and lying time of dairy cows and their calves in the 11 days after weaning, measured as the difference from a baseline value (mean daily value in the 6 days prior to weaning) for either behaviour. Model estimates and SE for feed-seeking behaviour of calves on Days 1, 2, 3, 5, 8 and 11 are also reported. Dam-calf pairs were housed together with full cow-calf contact for 4 (4MO; n = 11) or 6 (6MO; calves, n = 12; cows, n = 11) months, after which the calves were weaned via fenceline separation. *P*-values are based on ANOVA output of main effects unless noted otherwise.

	Cows			Calves		
Model	Estimate	SE	*P*-value	Estimate	SE	*P*-value
Difference in steps (steps/day)[1]						
Treatment$_{(6MO)}$	−0.21	0.235	0.342	−0.77	0.514	0.482
Time	−0.77	0.102	<0.001	−1.16	0.128	<0.001
Time[2]	0.05	0.008	<0.001	0.07	0.011	<0.001
Treatment × time	–	–	–	0.09	0.185	0.048[3]
Treatment × time[2]	–	–	–	0.001	0.015	0.048[3]
Proximity	−0.003	0.010	0.777	0.01	0.010	0.374
Parity$_{(primiparous)}$	−0.08	0.222	0.732	–	–	–
ICC[4]	0.10			0.16		
Difference in lying time (min/day)						
Treatment$_{(6MO)}$	6.21	48.290	0.537	183.64	57.790	0.334
Time	46.74	13.285	<0.001	105.09	14.887	<0.001
Time[2]	−2.75	1.141	<0.001	−7.52	1.258	<0.001
Treatment × time	−3.31	18.768	0.015[3]	−71.06	21.054	<0.001[3]
Treatment × time[2]	−0.08	1.566	0.015[3]	5.98	1.734	<0.001[3]
Proximity	0.84	0.748	0.181	−0.95	1.061	0.372
Parity$_{(primiparous)}$	−36.92	17.109	0.046	–	–	–
ICC[4]	0.05			0.12		
Feed-seeking (min/h)						
Treatment$_{(6MO)}$	–	–	–	1.23	0.936	0.242
Time	–	–	–	4.32	0.450	<0.001
Time[2]	–	–	–	−0.24	0.037	<0.001
Proximity	–	–	–	−0.06	0.039	0.117
ICC[4]	–			0.10		

Abbreviations: ICC = Intra-class correlation coefficient.
[1] Variable was square-root-transformed in order to maintain normal distribution of model residuals.
[2] Referring to the numeric variable time raised to the power of two.
[3] Likelihood ratio test comparing models both with and without the interaction term was used to determine *P*-values.
[4] ICC was calculated using the formula $\sigma_i^2 / (\sigma_i^2 + \sigma_\varepsilon^2)$, where σ_i^2 is the variance of random effects and σ_ε^2 is the residual variance.

ments at (mean ± SD) 1.3 ± 0.14 kg/day for 4MO calves and 1.4 ± 0.13 kg/day for 6MO calves. ADG in the first week postweaning was on average negative for both groups (4MO: −0.4 ± 0.50 kg/day; 6MO: −0.4 ± 0.43 kg/day) but by the second week, the calves were again on average gaining weight (4MO: 0.2 ± 0.35 kg/day; 6MO: 1.1 ± 0.76 kg/day). In the third week following weaning, 4MO calves increased their ADG to preweaning rates of 1.4 ± 0.29 kg/day while calves weaned at 6 months once again reduced their ADG on average to 0.04 ± 0.68 kg/day. At the end of the study, when calves were around 12 months (372 ± 11.4 days) old, the 4MO and 6MO groups weighed 422 ± 24.4 kg and 451 ± 37.6 kg, respectively. Vocalisations across the 11 days immediately after fenceline weaning decreased numerically for both treatment groups, although 4MO calves were observed to vocalise in a higher proportion of sampling intervals than 6MO calves on Day 2 (Table 2).

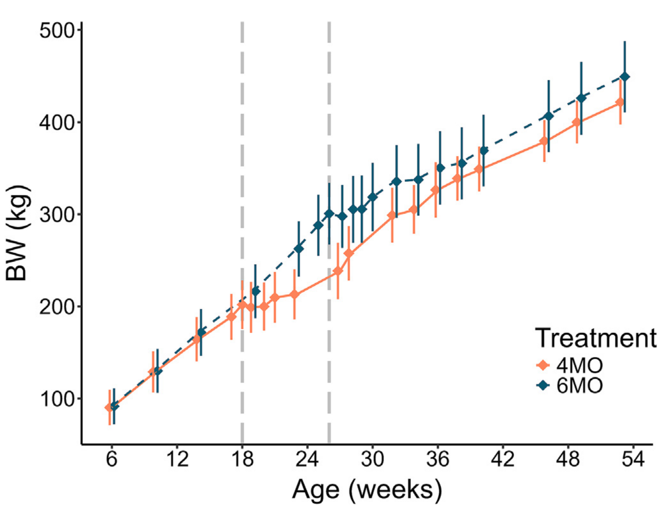

Fig. 3. Average BW (kg) for dairy calves of two treatment groups – weaning via fenceline separation after either 4 (4MO; n = 11) or 6 months (6MO; n = 12) of full contact with their dams. Vertical dashed lines represent weaning events. Error bars represent SD, while weeks refer to the average calf age.

Changes in activity and feed-seeking behaviour

Prior to weaning, 4MO and 6MO calves spent 15.6 ± 0.79 and 14.6 ± 0.94 h/day lying down, respectively. There was a significant effect of treatment × time for the difference in lying time (χ^2_2 = 19.34, *P* < 0.001; Fig. 4A) after fenceline weaning, with 4MO calves showing stronger behavioural responses in the first few days.

Table 2
Average percentage (mean ± SD) of 5-min sampling intervals per hour during which dairy calves of two treatments – weaning via fenceline separation at 4 (4MO) or 6 (6MO) months – vocalised at least once. Sampling was performed 1, 2, 3, 5, 8 and 11 days after weaning of each treatment group, with a total of 96 sampling periods per day.

	Treatment	
Day	4MO (n = 11)	6MO (n = 12)
1	40.2 ± 10.26	43.0 ± 11.16
2	30.2 ± 13.04	12.2 ± 5.89
3	15.5 ± 5.83	12.9 ± 12.75
5	4.3 ± 2.49	1.4 ± 1.68
8	4.3 ± 3.48	0.9 ± 1.24
11	3.3 ± 3.62	1.1 ± 1.29

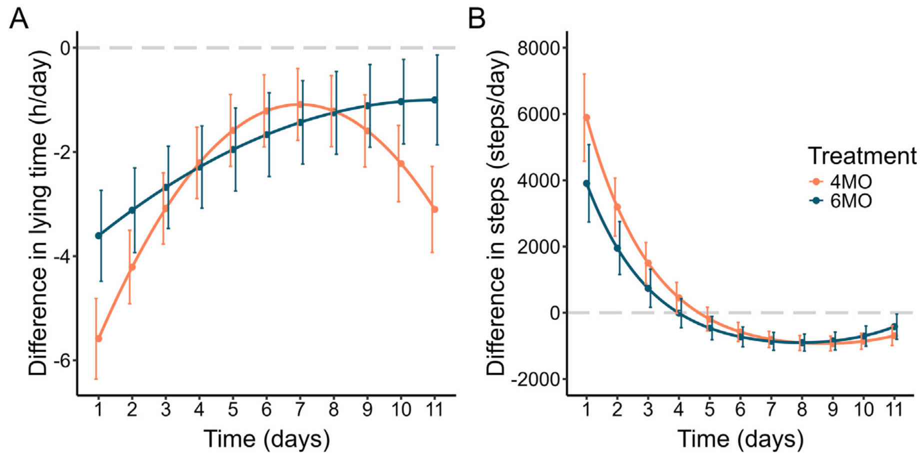

Fig. 4. Estimated quadratic regression lines for differences in lying time (h/day; A) and step count (steps/day; B) of dairy calves in the 11 days after weaning via fenceline separation at 4 (4MO; n = 11) or 6 months (6MO; calves, n = 12). Behaviours are displayed as the difference from baseline (indicated by the grey dotted line), which was calculated as the mean daily lying time or step count in the 6 days prior to weaning. Error bars show the estimated SE. Estimates for step count are back-transformed.

Across all calves, average daily step count increased from 2 451 ± 519.9 steps/day before weaning to 10 898 ± 3 298.5 steps/day on Day 1. The difference in step count differed between treatments depending on the day (χ^2_2 = 6.08, P = 0.048; Fig. 4B); 4MO calves had a higher step count on Day 1 and 2 postweaning.

While preweaning feed-seeking behaviour was not recorded, all calves were noted to engage in suckling on at least one occasion during the 3 preweaning observation days (median: 6, range: 1–12). From Day 1 after weaning, instances of feed-seeking behaviour increased in a quadratic manner but with no differences between 4MO and 6MO calves (Table 1). Across the days, calves increased their hourly time spent feed-seeking from 8.6 ± 3.32 mi n/h on Day 1 to a peak average of 24.8 ± 3.67 min/h on Day 8, after which there was a slight decrease to 21.7 ± 4.73 min/h on Day 11.

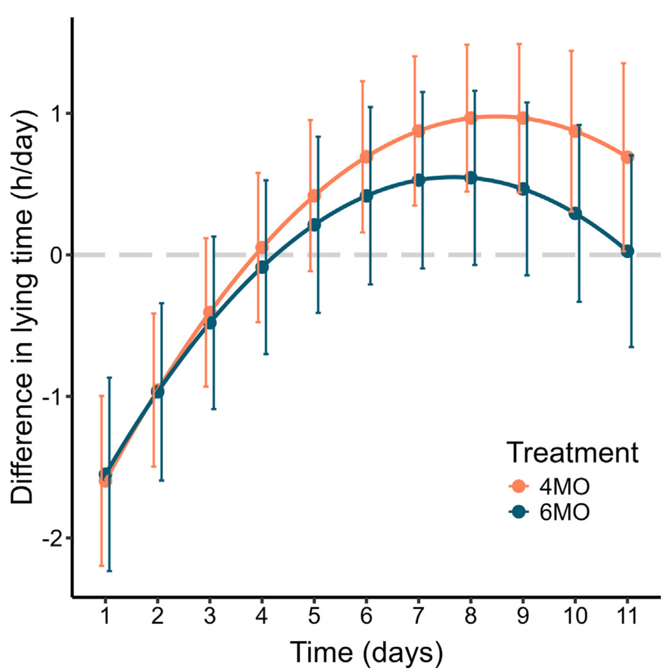

Fig. 5. Estimated quadratic regression lines for differences in lying time (h/day) of dairy cows in the 11 days after weaning of their calves via fenceline separation at 4 (4MO; n = 11) or 6 months (6MO; n = 11). Behaviours are displayed as the difference from baseline (indicated by the grey dotted line), which was calculated as the mean daily lying time in the 6 days prior to weaning. Error bars show the estimated SE.

Cows

Across both treatments, cows spent an average of 11.4 ± 1.16 h/day lying down and performed approximately 2 397 ± 753.8 steps/day in the week prior to fenceline weaning. Following the weaning of calves, there was a significant treatment × time interaction effect on the response in cows' lying time (χ^2_2 = 8.46, P = 0.015; Fig. 5), with both treatments initially reducing lying time to a similar degree but cows in the 4MO group increasing their lying time more than 6MO cows over the following days. However, this significant interaction was likely the result of a low number of 6MO individuals having extremely low values on Days 4 and 11 only (see Supplementary Fig. S3). Cows also responded to the weaning by increasing their step count but returned to preweaning levels by Day 2 and Day 3 for 4MO and 6MO cows, respectively, with no significant interaction between treatment and time (Fig. 6). Parity did not influence postweaning step count, but primiparous cows reduced their lying time to a greater extent than multiparous cows ($F_{1,18}$ = 4.62, P = 0.046; Table 1).

Discussion

This is the first study to compare the effects of fenceline weaning in dairy cows and heifer calves after 4 or 6 months of full CCC. We expected that delaying weaning until the calves had gained a higher status of social and nutritional independence as an effect of being older would reduce behavioural indications of weaning distress. However, both groups of calves initially responded to fenceline weaning by spending less time lying down, increasing their step count and vocalisations, and spending very little time engaging in feed-seeking behaviour relative to 1 week later. As is in line with previous work in dairy (Fröberg et al., 2011; Wenker et al., 2022; Bertelsen and Jensen, 2023) and beef (Price et al., 2003; Lambertz et al., 2015a) calves, these behavioural responses were strongest the first days of fenceline. The responses were evident regardless of the age at which calves were weaned, although the 4MO calves' responses were slightly stronger. While this difference in initial response is potentially, in part, due to the difference in calf age upon weaning, only a handful of studies have explored age-related differences in weaning response and with mixed findings. Lambertz et al. (2015b) found response patterns similar to our own for beef calves abruptly weaned at 6 and 8 months, with the younger calves having spent more time walking and less time lying down on the second day after weaning compared to calves weaned at an older age. Another study observed that beef calves weaned at

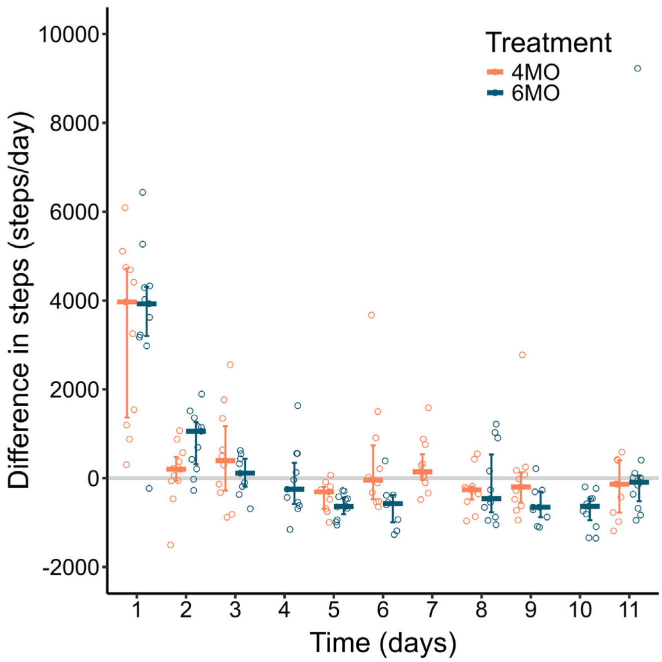

Fig. 6. Median values for differences in step count (steps/day) of dairy cows after weaning of calves via fenceline separation at 4 (4MO; n = 11) or 6 months (6MO; n = 11). Step count is displayed as the difference from baseline (indicated by the grey solid line), which was calculated as the mean daily step count in the 6 days prior to weaning. Presented data are raw, untransformed values. Error bars represent interquartile range, and daily individual cow observations are plotted as individual points.

30 or 75 days of age spent more time walking on the day of weaning than calves weaned at 6 months (de Souza Teixeira et al., 2021). Contrary to these findings, other work has reported a tendency for locomotor activity in beef calves to increase with weaning age (range: 5–8 months), although these results were based on only 6 h of postweaning observations (Stěhulová et al., 2017). Additionally, two of these studies reported the youngest calves to perform more frequent vocalisations than those weaned at later ages (Lambertz et al., 2015b; de Souza Teixeira et al., 2021), while Stěhulová et al. (2017) found no effect of weaning age on vocalisation frequency. In our study, vocalisations were measured with one-zero sampling, preventing us from evaluating the frequency of vocalisation. Nonetheless, on Day 2, younger calves were observed to vocalise in a visually greater proportion of sampling intervals than older calves.

Similar to calves, cows responded to the fenceline weaning by decreasing their lying time and increasing their locomotor activity. In the days immediately after weaning, the behavioural responses of cows did not differ between treatment groups. Contrary to our findings, other work on beef cattle has reported a potential effect of calf age, with dams of younger calves spending more time moving and vocalising in response to weaning (calf age range: 5–8 months; Stěhulová et al., 2017). Nevertheless, short-term behavioural changes are regularly observed for cows, irrespective of whether their calves are weaned at 8–10 weeks (Veissier et al., 2013; Ungerfeld et al., 2016; Neave et al., 2024) or 7 months (Lambertz et al., 2015a). Additionally, primiparous cows reduced their lying time to a greater degree in response to weaning compared to older cows. While previous work has generally reported dam age not to influence behavioural responses to weaning (Flower and Weary, 2001; Neave et al., 2024), it should be noted that some of the cows in our study had reared calves in a previous lactation. Meanwhile, the experience of being separated from a bonded calf was new for all of the primiparous cows. While our data did not allow us to investigate the potential carry-over effects of dam-rearing on cow responses – either across lactations or due to being reared with dam contact – this remains an important area for future research.

Calves of both treatment groups experienced clear depressions in ADG upon cessation of suckling, likely indicating a lack of nutritional independence prior to weaning. Comparatively, beef calves that were fenceline-weaned at 7 months maintained similar ADGs in the 2 weeks postweaning compared to non-weaned controls (Price et al., 2003). Even at 6 months of age, our dairy calves likely had nearly unlimited access to whole milk, while similarly-aged beef calves may be forced to start experimenting with solid feeds due to the more limited milk supply of their dams (3 kg/day at 6 months lactation; Rodrigues et al., 2014). The effect of dam milk yield on nutritional independence in calves is additionally reinforced by findings from Ungerfeld et al. (2009), who reported that beef calves reared by cows with low milk yields spent more time grazing and returned to baseline activity levels faster after weaning at 6 months than those reared by high-yielding beef cattle. Although we do not have detailed data on the feeding behaviour of our calves prior to weaning, all calves were confirmed to still engage in suckling bouts, regardless of weaning age. Moreover, previous research has reported that dairy calves with unrestricted access to CCC consume very little solid feed during suckling periods of 2–3 months (Roth et al., 2009; Fröberg et al., 2011).

In our study, the growth check after weaning (i.e., number of weeks before the slope in Fig. 3 stabilises) was longer in duration for 4MO than that of 6MO calves, suggesting that they may have been more nutritionally dependent on their dams upon weaning. Nutritional independence plays a large role in how calves respond to weaning, as demonstrated by Johnsen et al. (2018) in a study where calves were granted half-day access to their dams – either with or without the opportunity to suckle. Calves with dam access but prevented from suckling instead obtained milk from an automatic feeder; they were thus considered to be nutritionally independent and produced significantly fewer vocalisations upon fenceline weaning at 6 weeks. Other work has attributed vocalisations at weaning to gut fill; abruptly-weaned calves vocalised more compared to calves with continued access to a milk feeder with warm water substituted for milk (Budzynska and Weary, 2008). Considering the descriptively higher proportion of vocalisations for 4MO calves on Day 2, it is plausible that the younger calves were simply hungrier after weaning due to a lack of nutritional independence. Furthermore, the differences in growth check severity were still visible at 1 year of age, with 4MO calves weighing approximately 50 kg less than 6MO calves. However, it should be noted that calves of both treatments were, at 12 months of age, still considerably larger (4MO: 422 ± 24.4 kg; 6MO: 451 ± 37.6 kg) than the recommended minimum BW for 15-month-old Swedish Holstein and Swedish Red heifers, which is 380 kg and 350 kg, respectively (Greppa Näringen, n.d.).

The behavioural responses and growth checks observed in response to weaning in our study suggest that producers should avoid ending suckling abruptly, especially for younger calves. Fenceline weaning – while preferable to other two-stage methods such as the use of nose-flaps, which have been reported to cause nasal abrasions (Lambertz et al., 2015a; Valente et al., 2022; Wenker et al., 2022) – still involves an abrupt cessation of milk. Instead, we encourage future research to focus on developing and evaluating weaning methods that implement a gradual decrease in milk allowance, thereby fostering nutritional independence in calves prior to weaning.

While we did not differentiate between low-pitched (close-mouthed) and high-pitched (open-mouthed) vocalisations (Johnsen et al., 2015) during behavioural observations, our recorded observations included primarily the latter due to the relative ease with which open-mouthed vocalisations can be associated to a specific individual. Vocalisations – particularly those that are high-pitched – are thought to be a behavioural mechanism intended to locate and, when paired with locomotion, eventually reunite bonded pairs (Watts, 2000; Newberry and Swanson, 2001; Johnsen et al., 2015). The vocalisations observed in our calves immediately postweaning were paired with a simultaneous increase in locomotor activity, and from our own anecdotal evidence were generally noted to cease upon the reunion of dam-calf pairs across the fence. These behaviours were thus, at least in part, serving in an effort to join calves with their respective dams, regardless of their ability to suckle afterwards. This theory is further supported by work on free-ranging beef (Price et al., 1985; Padilla de la Torre et al., 2015) and non-suckling dairy (Johnsen et al., 2018) cattle, where vocalisations have been noted to occur during reunions of dam-calf pairs following short bouts of separation. Vocalisations in our study appeared in the highest proportion of sampling intervals on Day 1 for both groups. On Day 2, 4MO calves vocalised more than 6MO calves, while the values were similar for both groups for the remaining study period. From Day 5 postweaning and onwards, vocalisation occurrence was minimal, with the observers noting that many vocalisations could be attributed to events likely not related to weaning distress (e.g., feed delivery).

Changes in locomotor activity and movement have been greatly detailed as a weaning response for both dam-reared dairy (range of weaning age: 1 day to 9 weeks; Stěhulová et al., 2008; Fröberg et al., 2011; Bertelsen and Jensen, 2023) and beef (range of weaning age: 6–7.5 months; Price et al., 2003; Haley et al., 2005; Lambertz et al., 2015a) calves, with a return to preweaning levels usually reported by 5 days after weaning. In our study, all calves increased their step count considerably the day after fenceline separation but returned to baseline levels around Day 4, aligning with the findings of others. In contrast, the substantial decrease in daily lying time compared to baseline, observed on the first day of fenceline, had still not normalised on the last day of the observation period (Day 11). It is difficult to compare the relative extent to which lying behaviour changed between studies due to differences in behavioural recording protocols. Nevertheless, in contrast to our own findings, other studies report a stabilisation of lying time by 2–3 days after weaning, regardless of weaning strategy (Enríquez et al., 2010; Lambertz et al., 2015b). Part of the initial decrease in lying time we observed may be linked to the high levels of locomotion performed by the calves, as they may have exchanged some lying time for time spent standing and walking. However, even as vocalisations lessened and step count returned to baseline levels, daily lying time remained lower, implying that factors other than weaning distress were at play. Adult cows housed on pasture are known to have lower lying times compared to those in freestall systems (see review by Tucker et al., 2021). One explanation provided is that on pasture, cows need to spend more time consuming feed (i.e., grazing) than indoor-housed cows. After weaning, our calves increased their time spent on feed-seeking activities to a peak average of 25 min/h on Day 8, so it is possible that calves exchanged some lying time during this period for time spent seeking food.

While we are unable to evaluate changes in feed-seeking behaviour due to our lack of preweaning observations, the postweaning responses of our calves follow a similar increasing pattern as is reported for 6-month-old beef calves (Hötzel et al., 2010; de Souza Teixeira et al., 2021). As a result of our study design, we cannot determine if calves initially decreased their feeding-related activities as a stress response to weaning, or instead were simply seeking out very little solid feed to begin with and increased this following the cessation of milk.

Cows also responded to weaning events by decreasing their daily lying time, although this behaviour was affected to a lesser extent than for calves. Compared to preweaning lying times of 11 h/day, cows only reduced their lying by an estimated 2 h immediately after weaning. Moreover, cows returned to baseline levels by Day 5 (4MO) and 6 (6MO) after weaning, whereas calves failed to recover their lying time within the observation period. This is a slightly longer recovery period than the 2 days reported for dairy cows whose calves were weaned – either abruptly or gradually through a reduction in contact time – after 10 weeks of whole-day CCC (Neave et al., 2024). Our own findings therefore do not indicate that weaning at a higher calf age reduces stress responses in dairy cows, at least in terms of changes in lying time. In further contrast, findings from beef cattle suggest even shorter-lived reductions in lying time (weaning age: 2 months; Ungerfeld et al., 2016) or no changes whatsoever (weaning age: 7 months; Boland et al., 2008) for dams following weaning. The reductions in lying time seen in our cows, at least on Day 1, are likely due to the simultaneous increase in locomotor activity.

In terms of locomotor activity, the changes seen on Day 1 were nearly identical between treatments, with cows increasing their activity by median values of nearly 4 000 steps/day. By Day 2 and 3, 4MO and 6MO cows had returned to preweaning activity levels and remained near or slightly below baseline for the remainder of the observation period. Postweaning changes in movement have previously only been reported for individually-housed dairy cattle after short periods (i.e., up to 14 days) of CCC (Flower and Weary, 2001; Stěhulová et al., 2008). Nonetheless, the results of these studies mirror our findings, with increases in general cow movement reported for the first day following separation only. Limited findings are also available for beef cows; Ungerfeld et al. (2016) saw an increase in the percentage of observed time spent on locomotor activity following the abrupt weaning and separation of their 7-month-old beef calves, with a return to preweaning levels within 5 days.

To our knowledge, dam-calf proximity has not previously been explored as a predictor when modelling behavioural responses to weaning. We initially hypothesised that dam-calf pairs that were more spatially dependent would demonstrate stronger responses to fenceline weaning. In general, there was a large inter-pair variation in terms of time spent in close proximity, with some pairs spending up to 65% of their daily observed time near one another while others spent as little as 20%. Wenker et al. (2021) made a similar reflection regarding individual variation when recording how much time free-stalled-housed dairy cows spent standing within 2 m of their calves. Yet, contrary to our hypothesis, we ultimately found no effect of dam-calf proximity prior to weaning on any of the postweaning behaviours analysed. It is possible that for some individuals, spatial proximity alone is simply not a good measure of dam-calf attachment. Personality assessments of growing heifers have demonstrated clear differences between individuals (e.g., sociable vs pessimistic: Lecorps et al., 2019), making it plausible that calves may perceive spatial proximity in different ways. For example, some calves may feel socially "secure" at farther dam-calf distances, while others require a close physical proximity to meet the same social needs. In this way, two calves with similar levels of dam dependence may differ in how much time they spend within a close distance. Furthermore, as it is impossible to separate the calf's drive to maintain proximity from that of the dam in a free-ranging system, it is possible that socially and/or nutritionally independent calves were recorded as being in close proximity due to strong maternal behaviour on part of the cow – or vice versa. However, with so little existing work exploring spatial proximity in the context of dairy dam-calf attachment, it is difficult to determine what factors ultimately influenced our measures of dam-calf spatial proximity.

Our findings suggest that fenceline weaning was stressful for dairy calves regardless of weaning age, as seen by the decreased growth rate, short-term changes in behavioural patterns and prevalence of vocalisations. It is possible that the greater changes seen in calves for both daily lying time and step count compared to cows were not solely the result of milk removal and partial restriction in dam contact, but also as a result of being introduced to a new environment. Based solely on the differences in initial postweaning stress responses and in BW at 1 year, the 6MO calves appear to have been slightly better equipped to handle the fenceline weaning. Nevertheless, we recommend a further exploration of gradual methods that encourage the development of social and nutritional independence in calves prior to weaning.

Conclusions

Overall, dairy calves demonstrated clear behavioural responses to fenceline weaning at both 4 and 6 months, as shown by increases in step count and vocalisations, decreases in lying time, and little time spent feed-seeking during the days immediately after weaning. The calves, particularly those weaned at 4 months, had a reduced growth rate for a number of weeks postweaning, suggesting that they were not nutritionally independent from the dams at weaning. Cows similarly increased their step count and reduced daily lying time in the first few days postweaning, but to a lesser extent than calves and with no clear differences between treatments. Furthermore, we did not find dam-calf proximity during the contact time to be a predictor of behavioural responses to fenceline weaning, but we encourage further exploration in this area and on dam-calf relationships as a whole.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101525) can be found at the foot of the online page, in the Appendix section.

Ethics approval

All animal handling was approved by the Animal Experiments Ethics Board in Uppsala, Sweden (ID-No: 5.8.18–18138/2019).

Data and model availability statement

The analysed dataset and final models were deposited in an official repository and are publicly available for download at https://doi.org/10.5281/zenodo.11092911. Information can be made available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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