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Comparing weaning methods in dairy calves with different dam-contact levels

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ABSTRACT

Allowing the dam to rear her calf is an alternative practice in the dairy industry where cow and calf may gain welfare benefits from performing natural and highly motivated behaviors. However, this system has been linked to an increased separation and weaning response. Reducing the daily dam-calf-contact time may be a way to prepare the calf for weaning and separation. The first aim of the present study was to investigate the effect of 8 weeks of half-day dam-calf-contact on calves' response to weaning and separation, compared with calves reared with whole-day dam-calf-contact and an artificially reared, group-housed control with unrestricted access to milk for 20 min twice daily. Weaning off milk and separation from the dam can be viewed as 2 independent stressors. By introducing each stressor separately, it may be possible to reduce the overall behavioral response. The second aim of the present study was to investigate the effect of one-week fence-line weaning before permanent separation. The study was conducted with a 3x2 factorial design with dam-contact treatments: "Whole-day," "Half-day" and "Control" and weaning treatments "Simultaneous" and "Stepwise." Whole-day calves were separated twice daily from their dams during milking while Half-day calves were separated daily from the afternoon milking and until next morning milking. Simultaneous weaning and separation were done in wk 9, while Stepwise weaning and separation started in wk 8 with calves being fence-line weaned before permanent separation in wk 9. Data were collected on 69 dairy calves in wk 8 and wk 9, and data were summarized over the 2 weeks for analysis. Stepwise weaning and separation reduced the number of high-pitched vocalizations and activity of dam-reared dairy calves, while having little impact on control calves. There was no difference between Whole-day and Half-day calves in their response to separation, but as expected, dam-reared calves reacted more strongly than the control group. This was also reflected in the average daily BW gain the week after weaning, with Control calves having a higher average daily gains than Whole-day, while Half-day calves were intermediate. However, the behavioral response did not fully wane within the observation period (0–48 h of interventions). In conclusion, one-week fence-line weaning reduced the summed weaning and separation response in dam-reared calves. However, no difference between half-day dam-calf contact and whole-day dam-contact was detected as regards the behavioral response to weaning and separation.

Key words: Maternal Behavior, Cow-Calf Contact, Weaning Performance, Half-day Contact

INTRODUCTION

Most modern dairy farms separate the calf from the dam within 24 h of birth and artificially rear the calves on either whole milk or milk replacer. Rearing dairy calves with their dam has potential to improve animal welfare through the opportunity to express natural and highly motivated behaviors by both the dam and the calf (Reviewed by Meagher et al., 2019; Newberry and Swanson, 2008). Rearing calves with the dam also aligns better with consumer expectations (Boaitey et al., 2022; Sirovica et al., 2022; Weary and von Keyserlingk, 2017), especially to organic farms, where natural and ethical considerations are expected to a higher degree (Harper and Makatouni, 2002).

One challenge of rearing calves with the dam is that whole-day dam-contact leads to reduced bulk tank milk (Barth, 2020) due to calves suckling a large amount of milk and issues with milk let-down (Tancin et al., 2001; Zipp et al., 2018). Further, compared with separation within 24 h of birth, later separation of the dam and her calf leads to an increased response to weaning, manifested as reinstatement behaviors such as vocalization, pacing and standing with the head out of the pen (Johnsen et al., 2015b; Nicolao et al., 2022; Weary and Chua, 2000; Wenker et al., 2022), as well as reduced calf weight gain (Fröberg et al., 2011; Johnsen et al., 2015a).

Natural weaning age in cattle is estimated to be earliest at 8 mo of age (Reinhardt and Reinhardt, 1981a),

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and natural weaning is a gradual process where the cow gradually reduces the calf's access to suckle and eventually rejects the calf. However, frequent interaction and close proximity between the 2 continues beyond the weaning (Reinhardt and Reinhardt, 1981b). Present dairy systems are usually based on weaning off milk at approx. Eight weeks of age (typically 12 weeks in organic production in Europe), and therefore a substantial behavioral response to weaning and separation is to be expected when cows and calves are bonded and the calf is drinking large [12–15L (Barth, 2020)] amounts of milk.

Due to these challenges, part-time dam-calf contact, where the calf and the dam are kept together for some, but not all of the day, has been suggested (Johnsen et al., 2016; Meagher et al., 2019). Examples of part-time systems are restricted suckling (e.g., 2 short suckling opportunities either pre or post milking) and half-day contact between 2 daily milkings, either during day hours or during night hours (e.g., Johnsen et al., 2015b; Nicolao et al., 2022; Roadknight et al., 2022). The practical aspects of half-day contact, compared with shorter or longer daily contact times, are that under conventional milking management, the caretaker is already handling the cows at the time of the daily separation and reunion of dam and calf due to milking (Bertelsen and Vaarst, 2023 [Unpublished data, manuscript submitted to J Dairy Sci]).

Half-day contact may prepare the calf for permanent weaning and separation by stimulating calves to eat more solid feed and calves may become accustomed to periods of separation (Newberry and Swanson, 2008). Indeed, in the companion paper based on the same experimental study (Bertelsen and Jensen, 2023), we found that Half-day calves spend twice as along eating solid feed as Whole-day calves, especially during the night, when Half-day calves did not have access to their dam. The first aim of the present study was to investigate the effect of half-day contact on dairy calves' response to weaning and separation.

Weaning and separation can be considered as 2 independent stressors, as they need not happen simultaneously (Loberg et al., 2008; Weary et al., 2008). In cow-calf-contact systems weaning off milk can happen before separation from the dam, e.g., if the calf is prevented from suckling the dam's udder. This may be achieved by fitting the dam with an udder net, by fitting the calf with a nose-flap, or by placing dam and calf on each side of a fence that allows relatively close contact, but prevents suckling (Wenker et al., 2022). Another option is weaning off milk after separation from the dam, by continuing milk feeding from another source (e.g., bucket or automatic milk feeder), and then wean off milk at a later age (Johnsen et al., 2015a). It has been shown that when calves are less dependent of the dam as a source of milk, they react less to separation from her (Johnsen et al., 2015a; Wenker et al., 2022). On the other hand, calves that were never allowed to suckle their dam, but had all other aspects of maternal contact, showed a negative bias upon separation (Daros et al., 2014), indicating that both weaning off milk and separation from the dam are stressful.

When milk feeding calves artificially, gradual weaning is recommended to reduce weaning stress. When calves are gradually weaned, they gradually receive less milk over an extended period, which stimulates them to increase their concentrate intake (e.g., Eckert et al., 2015). Gradual weaning has been recommended especially for calves receiving large amounts of milk, as their pre-weaning concentrate intakes are much lower than calves fed low milk allowances, and thus they risk substantial weaning stress (Reviewed by Khan et al., 2011).

Knowing that especially calves fed large amounts of milk should be gradually weaned, there is a challenge in dam-calf contact systems where calves are reported to consume high amounts of milk and to eat only little concentrate, compared with artificially reared calves (Fröberg et al., 2011). Gradually weaning the calf from a high-yielding cow would require preventing the calf from suckling ad libitum. The use of automatic gates to reduce the daily duration of dam-calf contact has been investigated (Johnsen et al., 2021) and may combine the benefits of a gradual weaning off milk and gradual separation from the dam. However, calves that had access to restricted suckling of the dam for 30 min twice daily managed to suckle approx. 10L/d in the study by Fröberg et al. (2008) which may suggest that gradual weaning calves off milk, while calves continue to have even short periods of full contact to the dam, may be a challenge. A simpler approach, which may be more feasible, is fence-line weaning. Fence-line weaning has been used and researched especially in beef cattle. Here, the calves are first weaned off milk, but remain in close contact to their dam, and then subsequently separated from the dam (Enríquez et al., 2011; Taylor et al., 2020). While this does not allow for a gradual weaning off milk, it separates the stressors of weaning and separation in time, which has been shown to reduce the vocalization response (Taylor et al., 2020; Wenker et al., 2022). The second aim of the present study was to investigate the effect of fence-line weaning before separation of dam-reared dairy calves on their response to weaning and separation.

Our hypotheses were, first, that calves on the half-day contact treatment would react less strongly to weaning and separation than calves with whole-day contact, while control calves were expected to react the least.

Second, we hypothesized that calves weaned and separated by use of a fence-line would react less overall, i.e., compiling the response from the first and the second step, compared with calves subjected to simultaneous weaning and separation.

METHODS AND MATERIALS

Animals, housing and management

The study was conducted in the experimental barn at the Danish Cattle Research Centre at Foulum, Aarhus University, Denmark, from September 2020 to May 2021, including purebred Danish Holstein (*Bos Taurus*) cows and their newborn calves. The experimental study was based on a 3x2 factorial design repeated in 6 blocks. Animals were enrolled in blocks of 12 cow-calf pairs and within block allocated to one of 3 dam-contact treatments on a rotation basis: whole-day contact except at milking [Whole-day], part-time contact between morning and afternoon milking [Half-day] and separation at birth and artificial rearing [Control], balancing for dam parity, calf sex and calf age. This amounted to a total of 72 calves, which were the observational units. The sample size was based on the availability of cows in the resident herd and supported by post hoc power calculations to detect significant differences (at 5% significance level) of high-pitch vocalizations between the cow-contact and weaning treatments, and with a power of at least 80%.

Due to illness 3 cow-calf pairs were excluded from the study. The experimental timeline began when the block was full and no further interventions, except weekly weighing and health scoring, were made until the time of weaning and separation. All references to "week" are the experimental week, while calf age varied due to calves being enrolled over a period: mean age (95% CI) at first weaning intervention, wk 8; Whole-day: 54.9 (53.1–56.6) days, Half-day: 59.3 (58.2–60.5) days, Control: 55.7 (54.4–56.9) days). Calves were further randomly allocated to one of 2 weaning and separation treatments using an online random generator (one simultaneous step [Simultaneous] or 2 separate steps [Stepwise]). No blinding of the observers to the treatments was possible.

All calves were born in individual calving pens and fed 4 L of their dams' colostrum within 6 h of birth using a teat bottle. From the 2nd day of life, all calves had access to ad libitum calf-starter concentrate, hay, water and cows' total mixed ration.

Dam-Contact treatments

Whole-day and Half-day (collectively: dam-reared). Dam-reared calves stayed in the calving pen with the dam for approx. Twenty-four h and were assisted in suckling if necessary. The cow-calf pair was housed in a deep-straw-bedded group pen from the second day after birth, together with 3 other cow-calf pairs on the same dam-contact treatment. The group pens were 9 m x 7.5 m in size and had 2 calf creeps where calves had access to hay, concentrate and water (1.5 m x 1.5 m and 3 m x 3 m, respectively) in the back corners. The creeps were made from tubular metal bars with narrow enough gaps to prevent calves from sticking their head through the fixture. Calves had access to the creeps along the wall on each side (see Figure 1). Calves received all of their milk from suckling.

Calves on the Whole-day treatment were with their dam throughout the day and night except during milking time (5:00 h to 5:30 h and 15:30 h to 16:00 h) while calves on the Half-day treatment were housed with the dam between the morning milking and the afternoon milking, but cows were housed in a separate barn from after the afternoon milking until after next morning milking (15:30 h to 5:30 h).

Control. Control calves were separated from the dam 12 h after birth and managed largely according to standard farm procedure. Upon separation from the dam, they were moved to individual straw-bedded pens (1.5 m x 3 m) with sides made from tubular metal bars allowing visual and tactile contact with neighboring calves of the same treatment. When the youngest calf reached an age of 7 d, the partitions between pens were removed resulting in 4 control calves of a block being housed together as a group. Control calves were fed

Figure 1. A picture showing the larger calf creep, which was used for fence-line weaning of 2 calves. The entrances along each wall were closed off during the weaning step using fixtures similar to the vertical, tubular partitions. Calves could not suckle through the bars.

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milk in 8 L teat buckets (one bucket per calf). During the first 7 d, Control calves were fed daily amounts of milk gradually increasing from 6 L to 8 L in 2 daily feedings. For the rest of the milk-feeding period they were fed to satiation, meaning the bucket was continuously topped up when half-full during 20 min, twice daily at 06:30 h and 17:00 h. Milk allotted and left-over milk was measured (mean daily intake per calf ± SD ranged from 7.9 L ± 0.93 in wk 2 to 11.08 L ± 1.7 by wk 8).

Weaning and separation treatment.

Simultaneous In wk 9, calves on the Simultaneous treatment were abruptly and simultaneously weaned off milk and moved from their home pen to a new environment (group pens for 4 calves of 3 m x 3 m) at 11:00h, in the other end of the barn, together with the calves from the same dam-contact treatment and block. This effectively also separated the dam-reared calves from their dams, who were moved away to a separate barn at the same time. Cows and calves were to some degree within auditory reach after separation, but sound was muffled. Although Control calves were already separated from their dam and thus only moved to a new environment, the described procedure will for simplicity be referred to as separation (or, being separated) throughout (see Figure 2).

Stepwise For calves on the Stepwise treatment, weaning off milk started in wk 8, at 11:00 h, where dam-reared calves were confined in the larger calf creep (2 calves, 9 m^2), abruptly weaning the calves off milk, while the other 2 calves remained with their dams (and the dams of the Stepwise calves) in the main pen, with no change. At the same time, the control pen was divided in 2 equally sized pens, each holding 2 calves. Control calves on the Stepwise weaning treatment were also abruptly weaned off milk, but stayed in the familiar environment. One week later, in wk 9 and at the same time and way as for calves on the Simultaneous treatment, calves were moved to a group pen with the calves from the same dam-contact treatment and block, and the dams were moved to a separate barn (see Figure 2).

Observations We carried out behavioral observations on all calves at each of the 2 weaning interventions (wk 8 and 9, see Figure 2). We used focal animal sampling and continuously counted high-pitched (communicate intent to reunite over long distances, Padilla et al., 2015) and low-pitched (communicate intent to suckle over short distances, Padilla et al., 2015) vocalizations and recorded the behaviors "activity" and "cross sucking" (see Table 1 for ethogram) at 1 min intervals using one-zero sampling [did the behavior occur 'yes' or 'no', (Bateson and Martin, 2021)]. Observations were made at 4 time points after the interventions had taken place: after 4 h (d 0, 15:00 h), 21 h (d +1, 08:00 h), 29 h (d +1, 16:30 h) and 45h (d +2, 8:00 h), respectively. At each observation time point, the 4 calves on each of the 3 dam-treatments were observed for 3 × 5 min at 10 min intervals within a 45 min observation period. Thus, observations amounted to 15 min of observation per calf per observation time point, and a total of 2 h observation per calf for wk 8 and 9 combined. For Simultaneous calves, we expected wk 8 to correspond to a baseline level, as they had not experience any weaning and separation yet.

Growth and health measures All calves underwent a weekly health scoring and weighing. The health score was based on the procedure suggested by McGuirk and Peek (2014) and involved scoring nasal discharge, ocular discharge, naval inflammation, coughing and fecal consistency on a scale from 0 (perfectly normal) to 3 (heavy clinical signs of illness). Lastly, the rectal temperature was measured. Calves were also weighed on a walk-on calf scale weekly.

Statistical Analysis

Statistical analysis was performed in R, using RStudio (R Core Team, 2022) and the package "glmmTMB" (Brooks et al., 2017) for generalized linear mixed models. The assumptions of distribution and homoscedasticity were checked by graphical inspection of the residuals. Statistical significance was decided at the $P < 0.05$ level. Multiple pairwise comparisons were corrected using the Tukey methods when there was one set of means and the Sidak method when there was more than one set of means. The number of experimental units were: Control-Stepwise: n = 12, Whole-day-Stepwise: n = 10, Half-day-Stepwise: n = 12, Control-Simultaneous: n = 12, Whole-day-Simultaneous: n = 11, Half-day-Simultaneous: n = 12.

Vocalizations To compare the aggregated stress response of both weaning and separation, vocalization counts (high- pitched and low-pitched, respectively) were summed across wk 8 and 9 within each observation time point (e.g., Obs 1 from wk 8 + Obs 1 from wk 9; see Figure 2). The summed vocalization counts were analyzed with a generalized linear mixed model with log link and a negative binomial (linear parameterization) distribution. The fixed effects were: weaning and separation treatment [Stepwise; Simultaneous], dam-contact treatment [Whole-day; Half-day; Control], observation time point relative to intervention [4 h, 21 h, 29 h, 45 h], and their 2- and 3-way interactions, as well as a random effect taking into account the repeated measures on each animal and the random effect of block and pen.

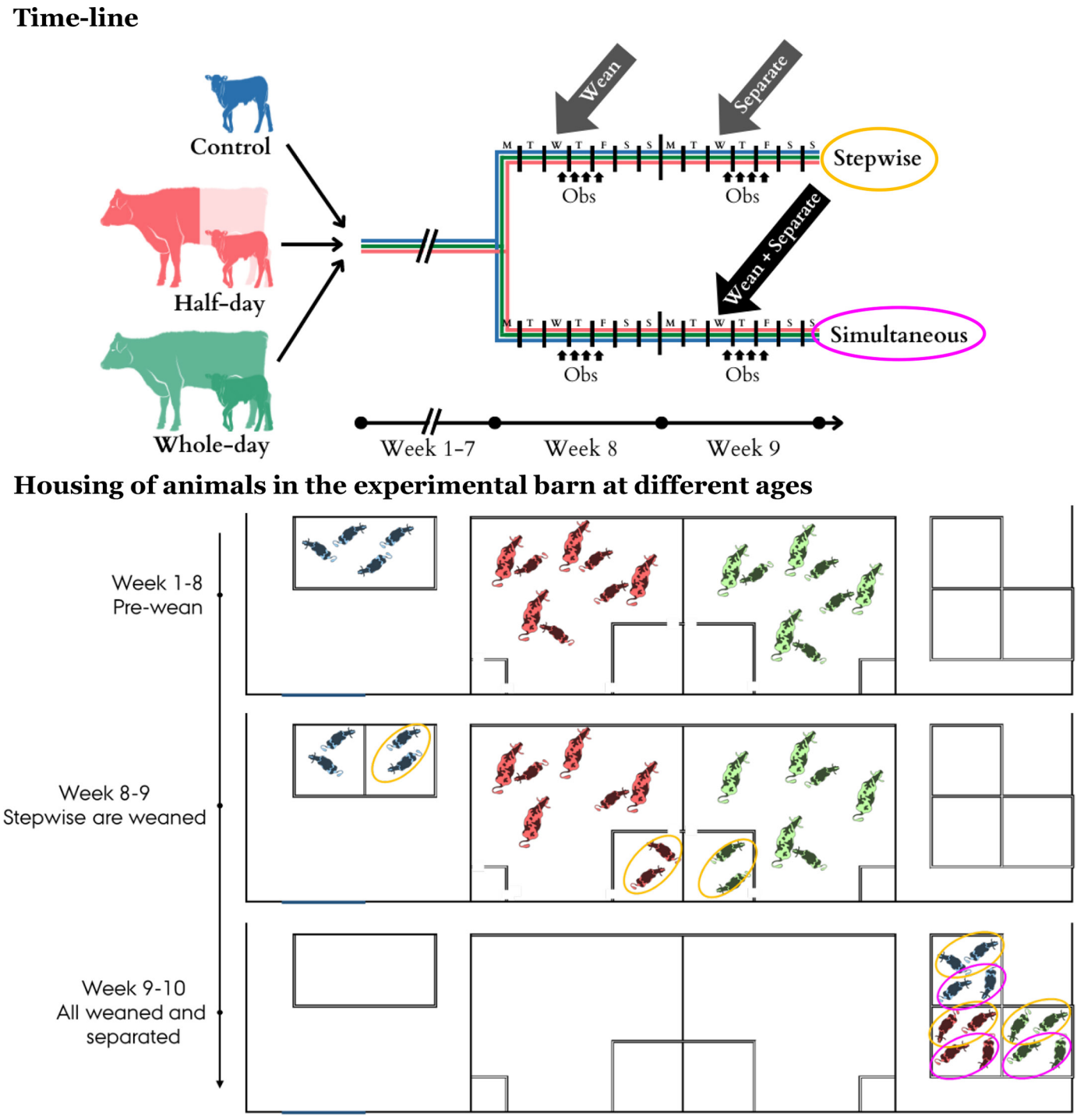

Figure 2. A graphical illustration of the experimental timeline and corresponding housing of animals. Calves were housed according to their allocated dam-contact treatment throughout the experimental period [Control (blue), Half-day (red) or Whole-day (green)]. From wk 8, half of the calves started a Stepwise weaning and separation (yellow circle) while the remaining calves were simultaneously weaned and separated in wk 9 (pink circle). Behavioral observations on 3 d post weaning and separation interventions are indicated by arrows on the time-line. Observations were carried out 4 h, 21 h, 29 h and 45 h after interventions. Simultaneously weaned and separated calves were also observed in wk 8, giving a baseline measure, before any intervention.

Activity The activity, recorded as the number of minutes where the calf moved (computed as success/ failures), were analyzed with a generalized linear mixed model with logit link and a binomial distribution.

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Table 1: The description of vocalizations and behavior of dairy calves upon weaning and separation (modified from Johnsen et al. (2015b))

Behavior	Description	Recording rule
High-pitched vocalizations	The calf gives a high-pitched (loud), open mouth sound. Taking a breath interrupts a vocalization.	Continuous recording
Low-pitched vocalizations	The calf gives a low-pitched (muffled), close mouth sound. Taking a breath interrupts a vocalization.	Continuous recording
Activity	The calf took more than two steps in any direction	One-Zero sampling [×]
Cross sucking	The calf is sucking on another calf's body parts e.g., ears, muzzle or scrotum.	One-Zero sampling [×]

[×] 1min intervals.

The fixed effects were: weaning and separation treatment [Stepwise; Simultaneous], dam-contact treatment [Whole-day; Half-day; Control], and observation time point relative to intervention [4 h, 21 h, 29 h, 45 h] and all their possible interactions, as well as a random effect taking into account the repeated measures on each animal and the random effect of block and pen.

Cross sucking A total of 58% of the calves were never observed to perform cross sucking during the weaning and separation observations and therefore this variable was transformed to a binary variable and the number of calves on each treatment combination that performed cross sucking at least once were analyzed using Fishers' Exact test. Among the calves that did perform cross sucking this behavior was recorded in on average 2.8 (min-max: 1 – 8) of the 1-min observations intervals.

Growth The last weighing before any weaning interventions was in wk 8. The average daily gain (ADG) was calculated as the difference in weight between 2 weighings divided by the number of days between. ADG was calculated for respectively: the period from birth to wk 8 (before weaning), for the week after weaning off milk (differing between weaning and separation treatments: wk 9 for Stepwise and wk 10 for Simultaneous) and (for calves on the Stepwise weaning and separation treatment only) for the week after separation from the dam, 2 weeks after weaning of milk. The BW in wk 8 and the measures of ADG were analyzed with a generalized linear mixed model with identity link and a Gaussian distribution. The fixed effects in the model were: weaning and separation treatment [Stepwise; Simultaneous], dam-contact treatment [Whole-day; Half-day; Control], dam parity [primiparous; multiparous], calf sex [heifer; bull], as well as 2-way dam-contact interactions (dam-contact treatment × weaning and separation treatment, dam-contact treatment × dam parity, dam-contact treatment × calf sex) and the random effect of block and pen in the model.

For ADG 2 weeks after weaning off milk we could only compare dam-contact treatments because we only had this measure for stepwise weaned and separated calves.

Health For health scores no statistical analysis was made, but group wise frequency distributions are reported. After initial graphical inspection, an interesting pattern in body temperature, seemingly related to the timing of the weaning interventions, was investigated post-hoc. Body temperature was analyzed with identity link and a Gaussian distribution with the fixed effects: weaning and separation treatment [Stepwise; Simultaneous], dam-contact treatment [Whole-day; Half-day; Control], week [1 to 9], and all their possible interactions, dam parity [primiparous; multiparous] as well as a random effect taking into account the repeated measures on each animal and the random effect of block and pen.

RESULTS

Vocalizations

For both high- and low-pitched vocalizations there was a 3-way interaction between dam-contact treatment, weaning and separation treatment and the observation time point (High-pitched vocalizations: $\chi^2 = 14.8$, $P < 0.01$, See Figure 3A, Low-pitched vocalizations: $\chi^2 = 22.1$, $P < 0.01$, See Figure 3B).

The effect of weaning and separation treatment

For Control calves, the number of high-pitched vocalizations on the 2 weaning treatments were similar (Figure 3A). For Whole-day calves, the number of high-pitched vocalizations were lower for Stepwise weaning and separation than for Simultaneous weaning and separation 21 h, 29 h, and 45 h after the intervention (Figure 3A). For Half-day calves, the number of high-pitched vocalizations were lower 21 h and 29 h after the intervention for Stepwise weaning and separation than for Simultaneous weaning and separation (Figure 3A). Regarding low-pitched vocalizations, there was no difference for any of the dam-contact treatments when comparing the Stepwise and Simultaneous weaning and separation within treatment (Figure 3B).

The effect of dam-contact treatment

Under the Simultaneous treatment, the number of high-pitched vocalizations was higher for both Whole-day and Half-day than for Control 21 h and 45 h after the intervention (Figure 3A). After 4 h only Half-day had a higher number of high-pitched vocalizations than Control while after 29 h only Whole-day did. There was no difference between the Whole-day and Half-day treatment at any of the time points. Under the

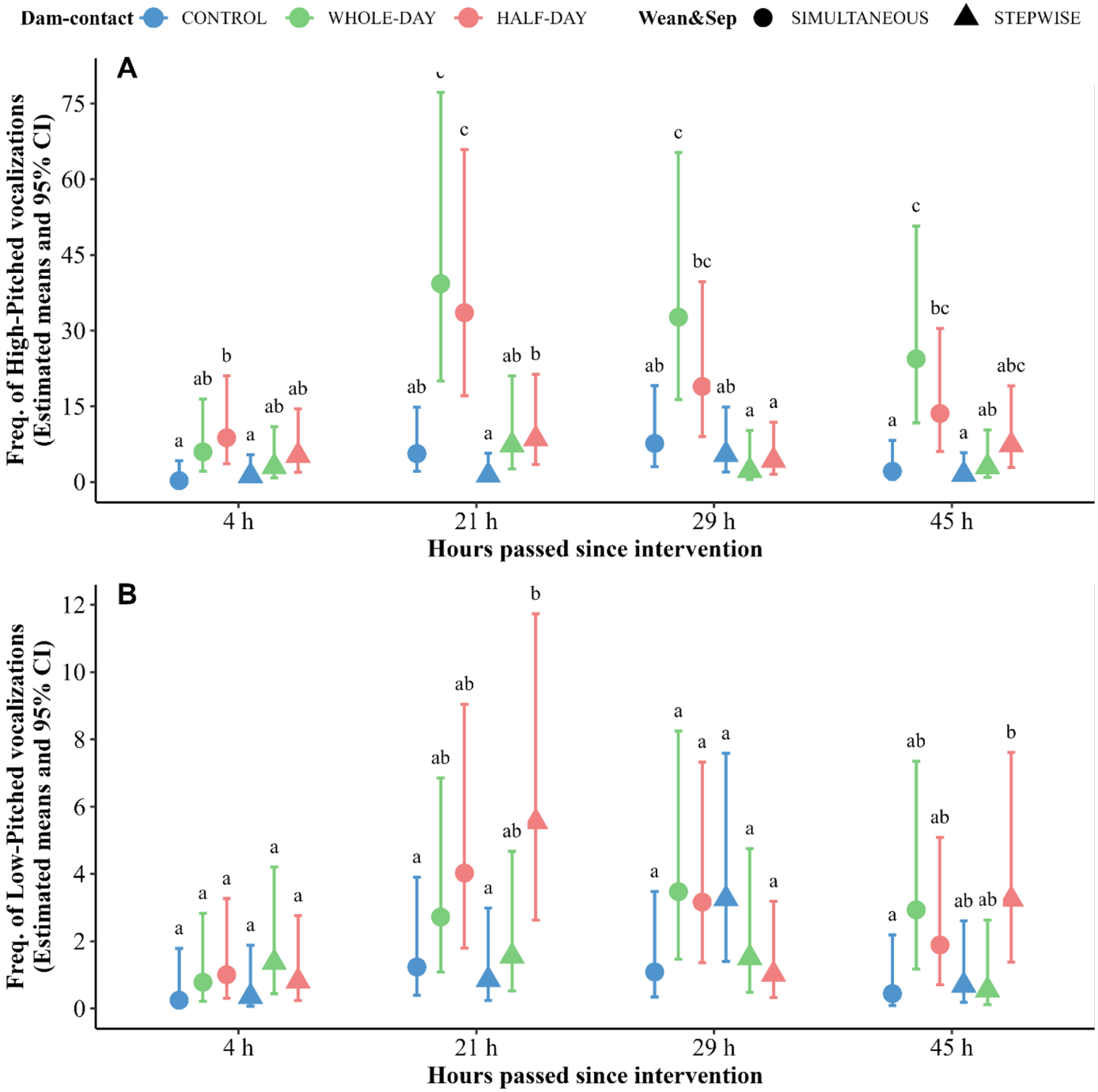

Figure 3. The estimated mean number and 95% confidence interval of the summed A) high-pitched vocalizations and B) low-pitched vocalizations of dairy calves after weaning and separation intervention, at each of 4 observation time points, each estimate is based on 30 min of observations. Statistical, pairwise comparisons were made between all 6 treatment combinations within each observation time point. Within time point, means that share the same superscript letter are not significantly different at the $P < 0.05$ level.

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Stepwise treatment, the only difference in number of high-pitched vocalizations was 21 h after separation where Half-day had higher numbers than Control (Figure 3A). Again, there was no difference between Whole-day and Half-day at any of the time points. For low-pitched vocalizations, there was no difference under the Simultaneous weaning and separation (Figure 3B). Under the Stepwise weaning and separation there was a difference 21 h after the intervention with Half-day calves emitting more low-pitched vocalizations than Control calves (Figure 3B).

Activity

There was a 3-way interaction between dam-contact treatment, weaning and separation treatment and the observation time point for the response variable "activity" ($\chi^2 = 22.6$, $P < 0.001$, See Figure 4). The activity response was higher under the Simultaneous weaning and separation than the Stepwise for Whole-day calves at 21 h, 29 h and 45 h. For Half-day calves, a higher activity response under the Simultaneous weaning and separation was seen at 21 h and 29 h and for Control only at 21 h.

For calves on the Simultaneous treatment, there were no dam-contact treatment differences in the activity response. However, for calves on the Stepwise treatment, Half-day calves showed a higher activity response after 21 h than Whole-day and Control, while Control calves showed higher activity response than Whole-day after 29 h (Figure 4)

The timing of the response. The above results are based on the summed behavioral reactions from wk 8 and 9. To get a sense of the timing of the behavioral response in relation to the weaning and separation interventions, the raw data for high- and low-pitched vocalizations are plotted in Figure 5A and 5B for each of the 2 weeks and each of the 2 weaning and separation treatments. For Simultaneous calves, no change happened in wk 8 and, expectedly, simultaneously weaned calves did virtually not vocalize in wk 8. For Stepwise calves, the response appeared to be distributed between the 2 weeks, but with numerically more vocalizations after the weaning step in wk 8 (Figure 5A).

Cross sucking A total of 42% of all calves performed cross sucking at least once during the weaning and separation observations. A Fishers' exact test revealed no difference in the number of calves observed performing cross sucking between any of the treatment combinations (see Table 2, $P = 0.874$).

Growth At 8 weeks of age, just before any weaning interventions, there was no difference between treatments in body weight (Estimated marginal means [95% CI]; Control: 89.6 [83.0–96.2] kg, Whole-day: 91.2 [84.2–98.1] kg, Half-day; 88.0 [81.0–94.9] kg). There was, however, an effect of both actual calf age ($\chi^2 = 30.1$, $P < 0.001$), calf sex ($\chi^2 = 6.4$, $P < 0.001$) and the dams' parity ($\chi^2 = 13.4$, $P < 0.05$) on body weight. Older calves were 1.7 (SE: 0.39) kg heavier for each extra day of age, calves of multiparous cows (95.2 [89.6–100.8] kg) were heavier than calves of primiparous cows (84.2 [77.3–91.1] kg), and bull calves (93.2 [87.2–99.1] kg) were heavier than heifer calves (86.1 [79.8–92.5] kg).

There was no treatment effects on ADG from birth to 8 weeks of age (estimated marginal means [95% CI]; Control: 930 [800–1060] g, Whole-day: 918 [783–1050] g, Half-day; 883 [752–1010] g), but calves of multiparous (993 [888–1099] g) cows had a higher ADG than calves of primiparous cows (827 [702–953] g), across dam-contact treatments.

When comparing the ADG during the first week after weaning off milk, (wk 9 for calves on the Stepwise treatment and wk 10 for calves on the simultaneous treatment) there was a main effect of the dam-contact treatment ($\chi^2 = 10.83$, $P < 0.01$) with Control calves (estimated marginal means [95% CI]; 230 [33 to 428] g) having a higher ADG than Whole-day calves (−230 [-447 to −14] g), while Half-day were intermediate (7 [-19 to 21] g). Pairwise comparisons showed that Control calves had higher ADG than Whole-day calves (t = 3.06, $P < 0.01$) but Half-day calves were not different from neither Control calves (t = 2.04, $P = 0.11$) nor Whole-day calves (t = 1.14, $P = 0.49$). There was also a tendency for an interaction between dam-contact treatment and weaning and separation treatment ($\chi^2 = 4.8$, $P < 0.09$) for the ADG after weaning, likely driven by Control calves (estimated marginal means [95% CI]; 402 [127 to 676] g) having higher ADG than Whole-day (−316 [-610 to −21] g) and Half-day (−86 [-37 to 192] g), under the Simultaneous weaning and separation but not under the Stepwise weaning and separation (estimated marginal means [95% CI]; Control: 59 [-222 to 341] g, Whole-day: −145 [-451 to 161] g and Half-day: 101 [-177 to 379] g).

The ADG for the second week after weaning for the Stepwise weaned and separated calves was also calculated. Here, there was an effect of dam-contact treatment ($\chi^2 = 12.0$, $P < 0.01$), with Control calves (estimated marginal means [95% CI]; 1136 [753–1519] g) having higher growth rates than both Whole-day (497 [11–888] g and Half-day (380 [1–759] g). In addition, bull calves (estimated marginal means [95% CI]; 894 [612–1176] g) had higher ADG at this point in time than heifers (448 [73–875] g, $\chi^2 = 5.2$, $P < 0.05$).

Health For the observations on health, the frequency distribution summed across the experimental period is shown in Figure 6. In general, the included calves were healthy and showed only mild symptoms of clinical

disease. Three cow-calf pairs (all from the whole-day treatment) were however removed from the data set as either the cow or the calf had severe clinical symptoms (2 calves with diarrhea and fever, and one cow with mastitis).

For the rectal temperature, there was an interaction between the experimental week and the dam-contact treatment ($\chi^2 = 32.6$, $P < 0.05$), as well as between the experimental week and weaning and separation treatment ($\chi^2 = 20.2$, $P < 0.05$). In wk 1 Control calves had a lower temperature (estimated marginal means [95% CI]; 38.4 [38.1–38.74] °C) than Whole-day (38.8 [38.5–39.2] °C) and Half-day calves (38.9 [38.6–39.2] °C) when averaging over weaning and separation treatment. In wk 9, there was a difference between Simultaneous (estimated marginal means [95% CI]; 38.8 [38.6–39.0] °C) and Stepwise (38.4 [38.1–38.7] °C), when averaging over dam-contact treatments, due to a drop in temperature for Stepwise. Post-hoc analysis revealed a similar drop in temperature for Simultaneous the week after weaning off milk. See Figure 7.

DISCUSSION

The present study compared the response to weaning and separation of dairy calves that were housed with either whole-day or half-day contact with their dam to a control group of artificially reared calves. Dam-calf contact calves were either weaned off milk and separated from the cow simultaneously at 9 weeks of age, or weaned off milk at 8 weeks of age and separated from the dam in a second step at 9 weeks of age. Control calves were also either weaned off milk in wk 8 or wk 9. This experimental design allows us to ask 2 main

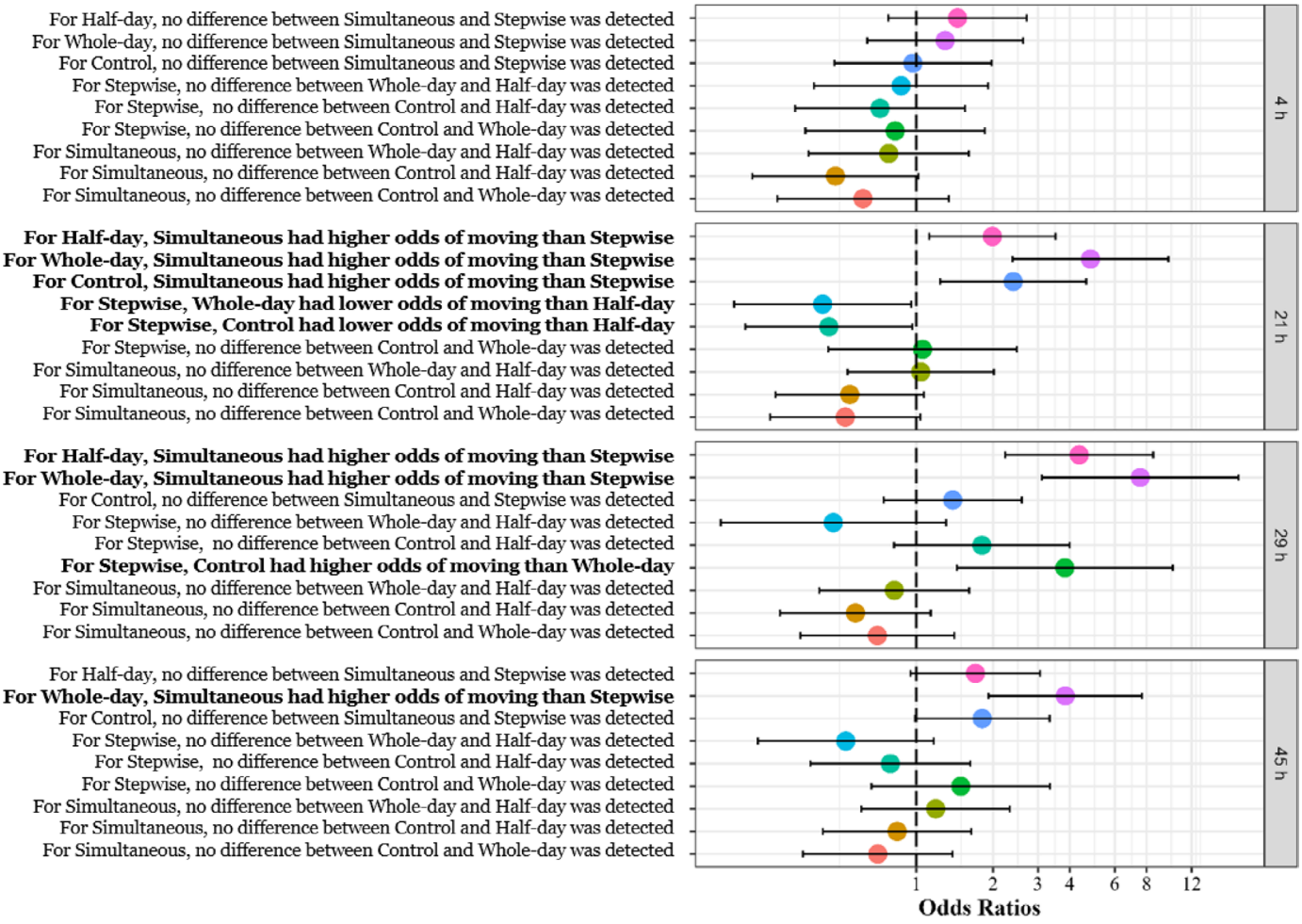

Figure 4. The odds ratios and the 95% confidence interval for dairy calves being active for each minute of a 30 min observation period, at each of 4 observation time points (4 h, 21 h, 29 h and 45 h), after a weaning and separation intervention. Each point represents the odds ratio between 2 treatment combinations. Odds ratios are shown on a log10 scale to ease visual interpretation (odds ratios of 0.5 and 2 shows as the same magnitude). Statistical, pairwise comparisons were made within each observation time point. Odds ratios between 2 treatment combinations whose confidence interval transects the vertical dashed line at "1" are not significantly different at the $\alpha = 0.05$ level.

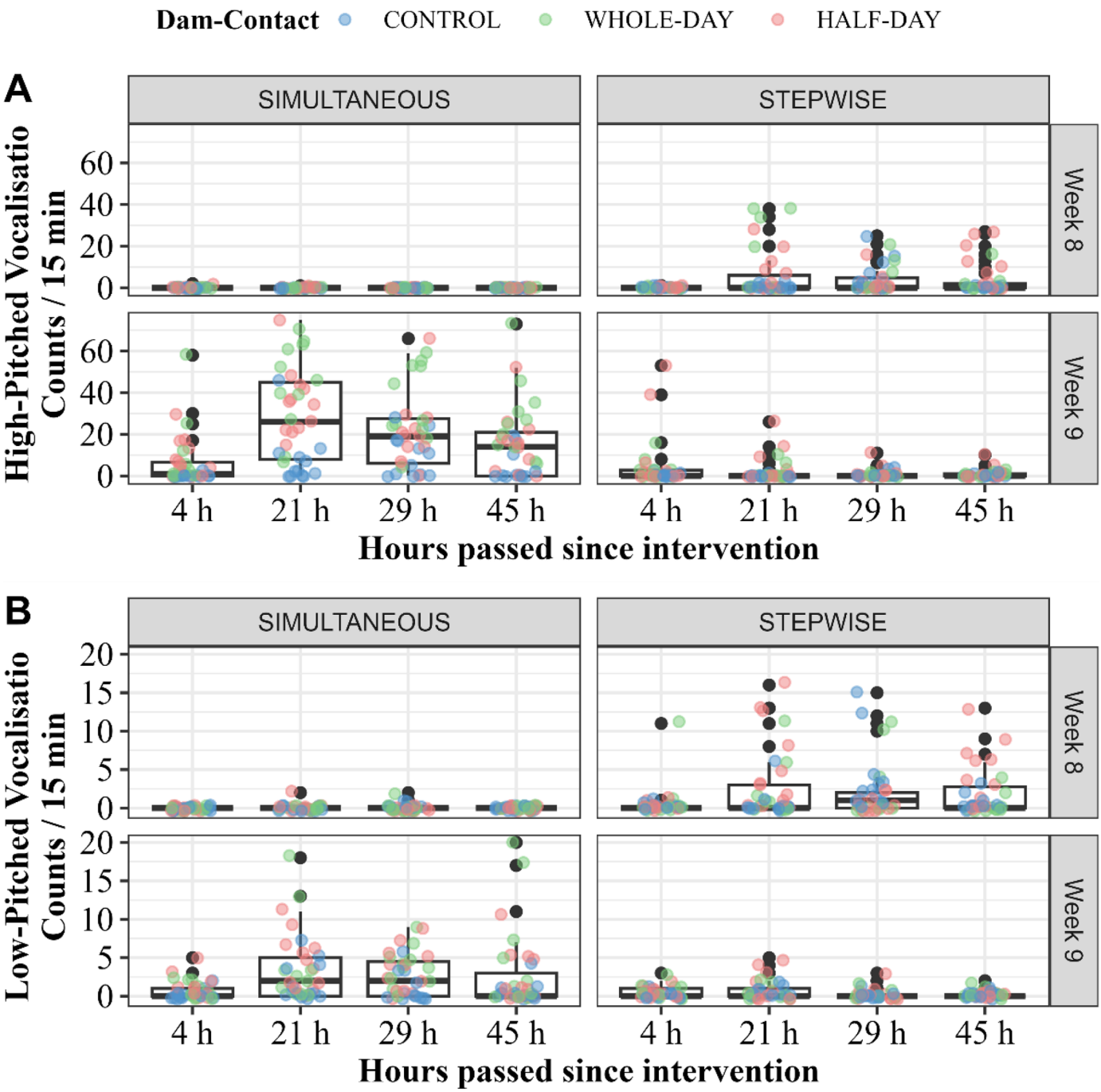

Figure 5. Descriptive plot of respectively the A) high- and B) low-pitched vocalizations in response to the weaning and separation interventions, illustrating the contribution from wk 8 and wk 9, respectively. Each calf's vocalization count is illustrated with a point of the color corresponding to treatment [Control (blue), Whole-day (green), and Half-day (red)]. Box plots illustrate the median (mid-line within box), 25th and 75th percentiles (outside edges of the boxes) and whiskers going out to the upper and lower adjacent values (the most extreme values within 1.5 of the interquartile ranges of the nearer percentile) and black dots are outside values >1.5 of the interquartile ranges.

questions: 1) Does half-day contact result in a lower behavioral response to weaning and separation compared with whole-day contact, and is this comparable to the behavioral response observed among artificially reared calves when they are weaned off milk? 2) Does a stepwise weaning and separation by the use of fence-line weaning reduce the summed behavioral response, compared with a simultaneous weaning and separation?

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Table 2: The number of calves on each treatment combination performing cross sucking at least once during 2 h of observations following weaning and separation, in dairy calves on three different dam-contact treatments and two different weaning and separation treatments

	Control	Whole-day	Half-day	Total
Simultaneous	8 (n = 12)	3 (n = 11)	3 (n = 12)	14 (n = 35)
Stepwise	9 (n = 12)	1 (n = 10)	5 (n = 12)	15 (n = 34)
Total	17 (n = 24)	4 (n = 21)	8 (n = 24)	

The effect of dam-contact treatment

Comparing the 3 dam-contact treatments, the general picture is that under the Simultaneous weaning and separation treatment, Control calves had a lower response to weaning and separation than dam-reared calves. This was evident from Control calves showing fewer high-pitched vocalizations after weaning and separation and a having higher ADG after weaning, which is in line with the literature (Fröberg et al., 2011; Johnsen et al., 2015a; Wenker et al., 2022). While we do not have data on the solid feed intake, a suggestion could be that Control calves, like in the studies of Fröberg et al. (2011) and Fröberg and Lidfors (2009) had higher solid feed intake before weaning (possibly due to longer daily periods without milk access) and/or were less affected by being weaned and moved to a new environment than dam-reared calves, who in addition were separated from their dam at this point. Alternatively, Control calves were vocalizing during periods without observations (though this was not noted by farm staff) or may not have the same communication strategy as dam-reared calves (Padilla de la Torre and McElligott, 2017), but this is an area which needs further research.

The differences were less clear under the Stepwise treatment, where response levels were generally lower across dam-contact treatments. On one hand, Control calves had fewer high- and low-pitched vocalizations than Half-day calves at 21 h, but on the other hand, they were more active than Whole-day calves at 29 h (See discussion on timing of observations below). It thus seems that the difference between treatments was less pronounced when calves were weaned and separated in a stepwise, fence-line manner. This is also supported by the measures of ADG: in wk 8, dam-reared calves on the Stepwise treatment were housed inside the group pen in the familiar calf creep and during this week had similar ADG as Control calves. Whether this is caused by dam-reared calves being in the known environment and with the dam present and thus allocating time to eating solids is unclear. However, one week later, 2 weeks after weaning off milk and one week after being moved to the weaning pens, permanently away from the cow, Control calves on the Stepwise treatment had regained pre-weaning ADG while dam-reared calves on the Stepwise treatment had not, indicating that dam-reared calves were more strongly affected by the separation step.

Contrary to expectations, there was no difference between Whole-day and Half-day under any of the 2 weaning and separation treatments, except that Half-day calves were more active than Whole-day calves on the Stepwise weaning and separation treatment, 21 h after interventions. Thus, we found no support for the hypothesis that half-day rearing reduces the behavioral response to weaning and separation as suggested by Veissier et al. (2013). We did find, that Half-day calves had an intermediate ADG between the significantly different Control and Whole-day group, but since the Half-day group was not significantly different from neither the Control nor the Whole-day group, no clear interferences can be made based on this data. Spending 14h apart every night for the first 2 mo of life did not appear to fully prepare Half-day calves better for weaning and separation, although we showed in the companion paper (Bertelsen and Jensen, 2023) that Half-day calves spend twice as long daily eating solid feed pre-weaning. This presumably was associated with a higher solid food intake, but this may not have been enough to reduce the sensation of hunger, though it may be the reason, that Half-day calves ADG was intermediate to Whole-day and Control calves. Further, in Bertelsen and Jensen (2023) we showed a large variation in daily eating time between animals, which may explain some of the variability in vocalization responses between calves at weaning and separation. We encourage future research to explore the effect of further reducing the daily dam-contact duration before separation or delaying weaning and separation to a later age, as a way to stimulate solid feed intake before weaning.

The effect of weaning and separation treatment

The summed behavioral response (as measured by vocalizations and activity) to the Stepwise weaning and separation was lower than the Simultaneous weaning and separation, which is in accordance with results from studies in beef cattle (Enríquez et al., 2011). However, this did depend on the dam-contact treatment. For dam-reared calves, the Stepwise treatment consistently resulted in a lower number of high-pitched vocalizations at the 21 h, 29 h (and for Whole-day also 45 h) observation time points compared with Simultaneous. The largest mean difference of the number of high-pitched vocalizations between weaning and

separation treatments was seen after 21h, which is in accordance with peak vocalization latency found in literature (Fröberg et al., 2011; Loberg et al., 2008). Under the Stepwise weaning and separation dam-reared calves did not differ from Control calves except at 21 h after the intervention. The found differences supports our hypothesis that Stepwise weaning and separation result in an over-all lower behavioral response, which is in accordance with studies on dairy (Johnsen et al., 2015a; Taylor et al., 2020) and beef cattle (Price et al., 2003). It thus seems that weaning and separation in the present study were not additive stressors. In contrast, Enríquez et al. (2010) and Solano et al. (2007) found that the summed response to fence-line weaning of beef calves was similar, or higher, than control, but extended over longer time. This will be further discussed in the section "Timing of observations."

Not surprisingly, it appeared that Control calves benefitted the least from the Stepwise treatment. Due to Control calves already being separated from their dam within 24 h of birth, there was no separation happening in wk 8 or 9 for Control calves. Control calves were however still weaned off milk in one of 2 ways, either weaned at wk 8, but staying one week further in the familiar environment, or being moved to an unfamiliar pen at the same time as being weaned off milk at wk 9. The only indication that being moved and weaned simultaneously, rather than separately in time, was more stressful for Control calves was more activity under the Simultaneous weaning and separation 21 h after intervention. In the present study all calves were weaned abruptly off milk, including the artificially reared Control calves, to better allow for comparison. Abrupt weaning is not recommendable practice, as it induces a strong weaning stress response (Khan et al.,

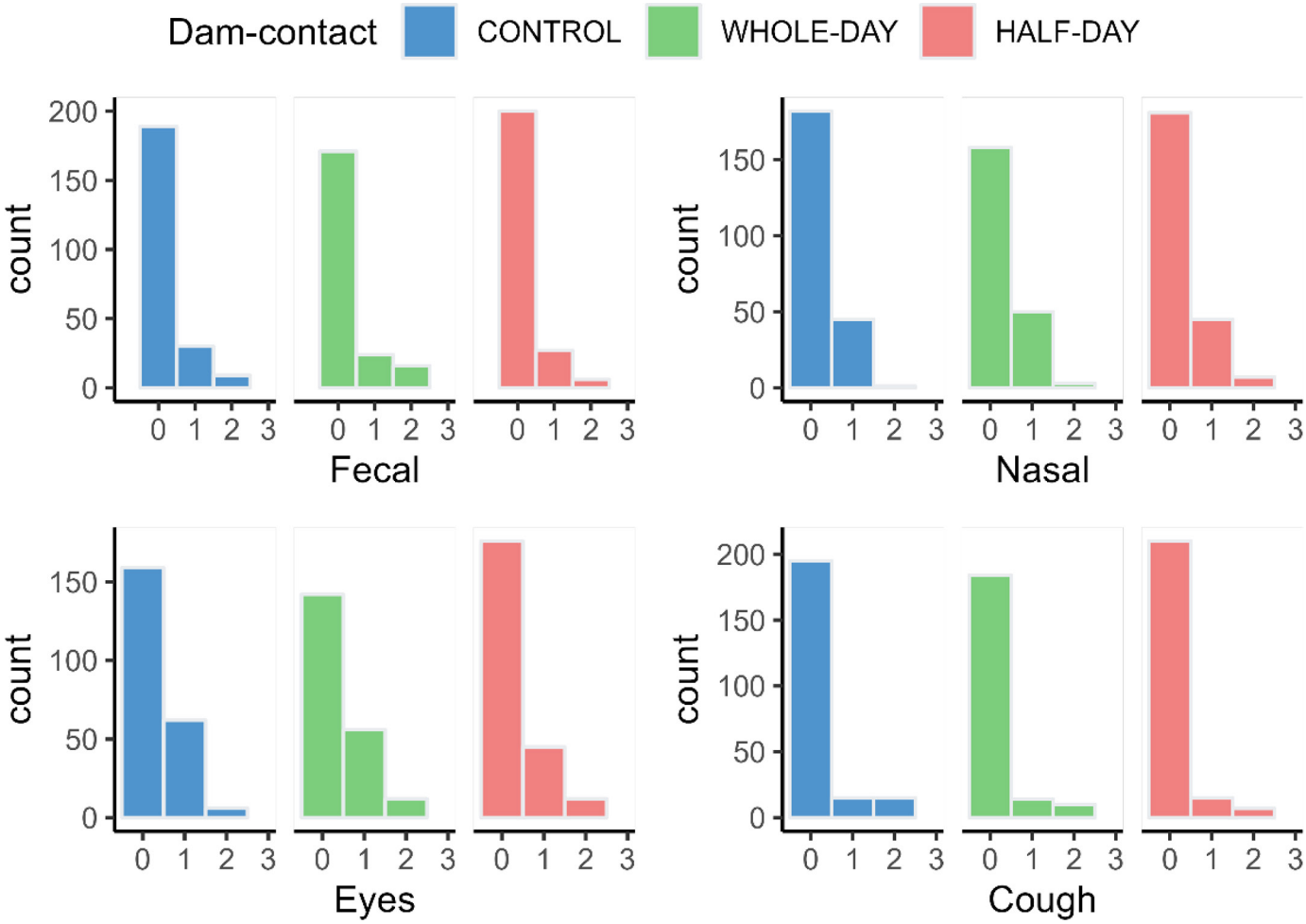

Figure 6. Frequency distribution of health scores related to fecal consistency, nasal discharge, ocular discharge and coughing summed across the 10 weekly health checks. A score of zero indicates no signs of disease and a score of 3 is the highest possible, indicating severe clinical symptoms of disease.

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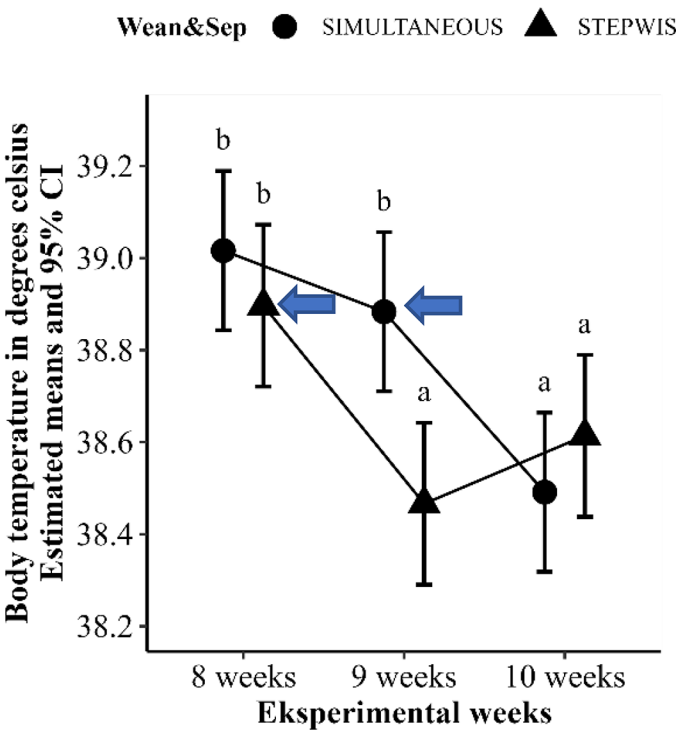

Figure 7. Post-hoc analysis of temperature drop around weaning. Estimated marginal mean temperature and 95% confidence interval for dairy calves weaned and separated either simultaneously (weaning in wk 9) or stepwisely (weaning in wk 8). Blue arrows indicate the time of weaning for each weaning and separation treatment. Statistical, pairwise comparisons were made between all treatments, combinations and experimental weeks. Means that does not share a letter are not significantly different at the $\alpha = 0.05$ level.

2016), and we encourage future research to investigate gradual weaning methods for dam-reared dairy calves .

Regarding low-pitched vocalizations, the effect of weaning and separation treatments are less clear, and these vocalizations appear to be much influenced by the time of day (see section: "Timing of observations").

Concerning the distribution of behavioral responses between wk 8 and 9, we recorded few vocalizations for Simultaneous calves in wk 8 (high-pitched: 2, low-pitched: 3), i.e., before they had experienced any weaning or separation, which was expected. We consider this level of vocalizations illustrative of a close-to baseline vocalization level for the calves in the study. However, these calves did experience a change, as the largest calf creep was closed off to confine the 2 calves undergoing stepwise weaning and separation during wk 8. The 2 calves undergoing stepwise weaning and separation were vocalizing, which may also have affected the Simultaneous calves in wk 8. For Stepwise calves, high-pitched vocalizations appeared similar in each of the 2 steps (wk 8 and wk 9, not statistically tested), which indicates that weaning off milk (while staying in fence-line contact to the dam) and being separated from the dam one week later constitute 2 individual stressors of similar strength. However, further studies that control for calf age are needed to investigate if they are indeed similar in strength.

There was a large variation in both high-pitched and low-pitched vocalization frequency for dam-reared calves and especially among calves on the Simultaneous treatment. Neither calf sex, calf age or dam parity could explain these. Future research is encouraged to investigate whether personality traits play a role in the vocalization response to weaning.

We were not able to detect any differences in the occurrence of cross sucking between the 6 treatment combinations with the Fishers' Exact test. On one hand, it could be expected that dam-reared calves would perform more cross sucking as this behavior has been linked to hunger and unsatisfied suckling need (Roth et al., 2008), but on the other hand dam-reared calves have been reported to show low levels of cross sucking (Margerison et al., 2003). Future studies are encouraged to look further into this area around the time of weaning, since the lack of difference in the current study may be attributed the relatively low number of animals in each treatment combination

Timing of observations

Padilla et al. (2015) suggested high-pitched vocalization to be aimed at long-distance communication to reinstate contact and opportunity to suckle, while low-pitched vocalizations are used for signaling intent to suckle when the dam is within visual contact. Indeed, as seen from Figure 3, among Half-day calves on the Stepwise treatment, low-pitched vocalizations peaked during those 2 observation time periods where the dam is present in the pen during wk 8 (dams had recently returned to the pen at 21 h and 45 h) and was at its lowest when Half-day calves were alone in the pen at night (29 h). Similarly, Stepwise Control calves' low vocalizations and activity peaked during milk-delivery to the calves in neighboring pens at 29 h. The fact that calves were influenced by either the time of day, the physical presence of the cows or milk feeding warrants a discussion of the best time to do observations in relation to weaning and separation interventions. Had we e.g., not included the afternoon time point where Control calves were normally fed, we would have observed very little response in Control calves. When comparing weaning and separation in calves with different housing, feeding and timing of management, future studies should carefully choose when to record and preferably have many repeated observation points, or an automatic recording of vocalizations throughout the 24 h

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of the day. In relation to the choice of observation time periods, the response should reach baseline levels at the time of the last observation. Based on previous studies, we expected the weaning response to have ceased by the last observation at 45 h (Johnsen et al., 2015b), but the estimated mean was 15 high-pitched vocalizations for Simultaneous and 4 high-pitched vocalisations for Stepwise during the 15min observation window 45 h after weaning off milk (and separation from the dam, for Simultaneous). Compared with our own "baseline" level (Simultaneous, wk 8, before any interventions, mean = 0.0), these are still elevated levels. We might thus have missed responses to weaning and separation between the last observation from wk 8 and the first in wk 9 and again after the last in wk 9. This is an important limitation of the present study. It also further illustrates the need to develop reliable, automatic detection of vocalizations when comparing responses that are expected to last over several days. Another consideration is how much calves influence each other, i.e., if each calf is indeed independent. In the present study we did see large differences between calves (Dam-reared vs. Control) observed at the same point in time, thus even though calves were able to react to each others calling. However, we do not know if the difference had been more pronounced if they had been out of auditory reach. In the present study, the statistical model took into consideration the effects of pen and block to best account of this. Lastly, the present study is also limited by the number of animals on each treatment combination (n = 10–12).

Health

Calf health was monitored to ensure that we would detect any severe ill health, and the data set is too small to make statistical interferences on dam-calf contact's effects on the health score. However, it was interesting to note that rectal temperature dropped approx. half a degree the week after weaning off milk, for both Stepwise and Simultaneous treatments, and that for Stepwise it started increasing again after 2 weeks, mirroring the ADG pattern. Indeed, Silva et al. (2021) also found an effect of larger colostrum intake on the immediate rectal temperature, which they attribute to increased metabolized energy. We also found lower body temperature for Control calves in wk 1, compared with the other treatments. It can be speculated whether this was caused by a lower daily milk intake than dam-reared calves, at this age. This preliminary result indicates that rectal temperature may be used as a proxy for energy uptake in dairy calves, and caution should be taken when interpreting temperature differences for calves on different milk allowances.

CONCLUSION

Dairy calves reared by the dam had a higher response to weaning (higher number of high-pitched vocalizations and higher level of activity) than artificially reared calves, when calves were weaned and separated simultaneously. The response of dam-reared calves that had been with the dam daily from morning to afternoon milking only (Half-day) was similar to the response of dam-reared calves that had been with the dam all day, except for milking twice daily (Whole-day). Thus, we found no support for the hypothesis that half-day rearing reduces the behavioral response to weaning and separation. Among dam-reared calves, the summed response to stepwise weaning and separation using a fence-line was lower than the response to simultaneous weaning and separation at 21 h, 29 h and 45 h after the intervention. Therefore, using a fence-line weaning method shows promise as a way to reduce the response to weaning and separation in dairy calves reared by the dam, but further studies on gradual weaning methods for dam-reared dairy calves are needed.

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