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Can a gradual weaning and separation process reduce weaning distress in dam-reared dairy calves? A comparison with the 2-step method

Anina Vogt,[1]* Kerstin Barth,[2] Susanne Waiblinger,[3]† and Uta König von Borstel[1]
[1]Division of Animal Husbandry, Behaviour and Welfare, Justus-Liebig-University of Giessen, 35392 Giessen, Germany
[2]Institute of Organic Farming, Johann Heinrich von Thünen Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries, 23847 Westerau, Germany
[3]Institute of Animal Welfare Science, University of Veterinary Medicine, 1210 Vienna, Austria

ABSTRACT

The weaning and separation phase remains one of the biggest challenges for cow-calf contact systems, but a gradual process that better mimics the naturally occurring reduction in milk intake has not yet been scientifically investigated. Therefore, the aim of our study was to compare behavioral and physiological indicators of distress in 3-mo-old dam-reared dairy calves (with previous full-time cow-calf contact) weaned and separated either via gradual reduction of contact time with the dam (GR; 1 wk of half-day contact, 1 wk of morning contact, and 1 wk of fence-line contact before complete separation, n = 18) or via 2-step weaning using a nose flap (NF, 2 wk of access to the dam with a nose flap, 1 wk of fence-line contact before complete separation, n = 18). Behavior was recorded 1 wk before (or for lying 3 wk before) weaning start and during the 3 wk weaning and separation period with direct observations on 4 d/wk or via accelerometers (locomotor play, lying behavior). Blood and fecal samples were taken twice per week from weaning start until 3 wk after weaning start. Calves were weighed weekly. Statistical analysis was conducted using (generalized) linear mixed models. Over the whole weaning and separation phase, NF calves showed a stronger decrease in the number of lying bouts, amount of locomotor play, and ADG, as well as a higher increase in TMR feeding time compared with GR calves, whereas GR calves vocalized more often and showed more searching behavior than NF calves. Also, the neutrophil: lymphocyte ratio of NF calves was elevated on d 3 after insertion of the nose flaps compared with baseline, but showed no change for GR calves on any sampling day. Overall, results point toward a favorable effect of a gradual weaning strategy on reduction of weaning and separation distress in dam-reared dairy calves, but the method requires further improvement from the protocol used in our study.

Key words: cow-calf contact systems, weaning, separation, stress

INTRODUCTION

Weaning and separation distress is one of the biggest challenges in cow-calf contact systems as reported by 87% of farmers practicing cow-calf contact across Europe (Eriksson et al., 2022). For the calf, the weaning and separation process is not only associated with loss of milk as main nutritional source and loss of contact with the dam, but often also with the loss of familiar peers, mixing with new conspecifics, and changes in physical environment (Weary et al., 2008; Lynch et al., 2019). These multifactorial stressors can result, among others, in reduced weight gains (Haley et al., 2005; Sweeney et al., 2010), a strong increase in vocalizations (Haley et al., 2005; Loberg et al., 2008), increased pacing and seeking behavior (Enríquez et al., 2010), reduced play behavior (Enríquez et al., 2010), and reduced lying times (Haley et al., 2005; Budzynska and Weary, 2008), as well as neutrophilia (O'Loughlin et al., 2011, 2014) and an increase in cortisol levels (Loberg et al., 2008; O'Loughlin et al., 2014) in abruptly weaned calves. Because separation from the dam further induces a pessimistic judgment bias in calves (Daros et al., 2014), indicative of a negative affective state, the aforementioned behavioral and physiological responses to weaning thus reflect that calves experience distress, in the sense of stress that adversely affects an animal's welfare (Moberg, 2000), during the process.

To reduce the weaning distress, different methods have been investigated in the past that separate the loss of the milk from the loss of social contact to the dam. These include for example weaning via 2-step separation, which means that suckling is prevented with so-called "nose

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*Corresponding author: anina.vogt@agrar.uni-giessen.de
†Current address: Centre for Animal Nutrition and Welfare, University of Veterinary Medicine, 1210 Vienna, Austria

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

flaps" before permanent total separation (Sirovnik et al., 2020), as well as fence-line weaning, which allows partial physical contact to the dam without suckling (Sirovnik et al., 2020). Beef and dairy calves that were weaned with nose flaps showed reduced behavioral reactions (Haley et al., 2005; Loberg et al., 2008; Enríquez et al., 2010) as well as reduced physiological reactions at the time of separation (Loberg et al., 2008) compared with abrupt weaning. Thus, nose flaps have been proposed as a low stress weaning method for beef calves (Haley et al., 2005) and are also considered as an alternative for dairy cow-calf contact systems (Sirovnik et al., 2020; Schneider and Ivemeyer, 2021; Barth et al., 2022). The major advantage of 2-step weaning with nose flaps is the high practicability for farms because, in contrast to other weaning and separation methods, it requires no modifications of the barn (Barth et al., 2022) or adjustments of on-farm routines for animals or personnel. Additionally, farmers value the lower number of vocalizations by cows and calves during weaning with nose flaps not only as a sign of reduced distress, but also because frequent vocalizations during weaning are emotionally challenging for them and lead to additional worries that others may mistake these as an indication for mistreatment of the animals (Waiblinger and Hebesberger, 2023; S. Waiblinger, University of Veterinary Medicine, Vienna, Austria, personal communication). However, the use of nose flaps results in diminished weight gains compared with abrupt or fence-line weaning (Boland et al., 2008; Enríquez et al., 2010; Wenker et al., 2022) and can lead to severe nasal abrasions and open wounds with or without bleeding and secretion (Lambertz et al., 2015; Valente et al., 2022), which potentially even initiate pituitary abscesses (Fernandes et al., 2000), and leave nose flaps questionable with regard to animal welfare. Thus, there is a need for further improvement or implementation of new weaning strategies.

One possible approach is a gradual weaning and separation method, in which the cow-calf contact time before permanent separation is gradually reduced. To date, this weaning method has not yet been scientifically investigated; however, a gradual reduction of contact time could also be a promising weaning and separation method for cow-calf contact systems for several reasons. First, it resembles more the naturally occurring reduction of milk intake during natural weaning, which is typically a gradual process in which the number of suckling bouts declines significantly with age of the calf over the course of several months (Reinhardt and Reinhardt, 1981). Second, Weary et al. (2008) suggested that during the increased times that cow and calf spend apart before weaning, the calves are potentially able to habituate to the periods of separation and increase their intake of solid feed, which eases the transition at weaning. Third, gradual weaning is commonly used as a standard procedure for artificially reared dairy calves, either by diluting the milk or milk replacer with water or by feeding the calves less often or with smaller amounts of milk during the end of the milk-feeding period. These practices showed that gradual weaning leads to reduced vocalizations (Jasper et al., 2008; Bittar et al., 2020), increased lying times (Scoley et al., 2019), as well as increased starter intake preweaning, and can alleviate part of the compromised postweaning weight gains compared with abrupt weaning (Khan et al., 2007; Sweeney et al., 2010; Omidi-Mirzaei et al., 2015). Therefore, a gradual reduction of the daily cow-calf contact time might cause less distress than weaning with a nose flap.

Accordingly, the aim of our study was to compare the behavioral and physiological distress responses of 3-mo-old dam-reared dairy calves during weaning and separation either via gradual reduction of contact time between dam and calf or via 2-step weaning using a nose flap. We hypothesized that a gradual process would ease the psychological and physiological adaption of calves to being separated from the mother and thus predicted a lower weight loss and fewer distress-related behaviors, that is, less vocalizations and searching behavior, but higher lying times, reduced number of lying bouts and more play behavior in gradually weaned calves compared with using a nose flap. Also, we expected that gradually weaned calves will react with less pronounced changes in hematological parameters and lower cortisol levels to the weaning and separation process compared with calves weaned with a nose flap.

MATERIALS AND METHODS

Animals, Housing, and Feeding

All experiments were performed in accordance with the German Animal Welfare Act (Federal Republic of Germany, 2020; animal experiment number V244–51520/2019, MELUND Schleswig-Holstein). The study was conducted from November 2019 till March 2020 with 36 dam-reared calves at the Thünen Institute of Organic Farming in Germany. All calves were of the German Holstein breed, but belonged to 2 different herds (19 horned: 8 female/11 male calves, 17 genetically polled: 8 female/9 male calves), that were kept in identically mirrored parts of an open-sided freestall barn (see Wagner et al., 2012 for a scheme of the barn). Calves stayed about 5 d with their mothers in an individual calving pen and were afterward kept in a calf section with full-time access to the cow herd, including their mothers, until the weaning and separation process started. For calves that were born during the grazing period (from August until mid-November 2019, applies for all but 2 calves) this included also pasture access with the cows during the preweaning phase. After the grazing period ended, the calves were kept completely indoors in a separate calf section of 96.5 m^2, which was directly connected to the cow section via an automatic transponder-controlled selection gate. Calves were free to enter the cow section and suckle their mothers the whole day except for the milking hours (0515–0830 h and 1530–1845 h) and during times when cows were feeding, because calves had no access to the milking parlor and the cows' feeding area. Consequently, the cow-calf contact was always initiated by the calf.

In the calf section the calves had ad libitum access to water, hay, and a TMR composed of about 63.6% grass silage, 30.0% corn silage, 6.2% concentrate feed in the form of coarse grain, and 0.2% mineral feed, which was provided freshly once a day in the afternoon. Additionally, calves had access to a concentrate feeder (Förster Technik GmbH, Germany) with an allowance of 1.5 kg concentrate per animal and day distributed in portions of 50 g. No additional milk was fed from buckets or an automatic feeder to the calves besides the milk that could be suckled from the mothers or other cows. The calf section was equipped with an automatic calf brush (Schurr Gerätebau GmbH, Germany) in the walking area (rubber-coated and concrete floor, 71.9 m^2) as well as 2 straw-bedded lying areas (12.6 m^2 and 12.0 m^2). In addition, the walking alleys and cubicles of the cows (490.0 m^2) could be entered by the calves as well. Group size of the calves was dynamic and varied from 15 to 23 calves (horned herd) and 14 to 20 calves (polled herd), due to new calvings or regrouping of fully weaned calves into the youngstock barn. In the calf sections of both herds, the number of study animals that were simultaneously at any stage of the weaning and separation process ranged from 2 to 7 calves. Thus, in addition to the study animals there were always other younger calves present. Group size of the cow herd varied from 45 to 49 cows (horned herd) and 43 to 48 cows (polled herd) throughout the experiment.

Experimental Design and Treatments

All calves were about 3 mo old when the weaning and separation process started. The calves were randomly allocated balanced for sex (male/female) and herd affiliation (horned/polled) to one of the following 2 treatments: The nose-flap calves (**NF**, n = 18 calves, 8 female, 10 male) were weaned and separated with a 2-step method using a nose flap (Quiet wean, JDA Livestock Innovations, Canada). Calves were equipped with the nose flap for 2 wk, allowing them whole-day contact with their dam without suckling. After these 2 wk, the NF was removed, and the calves were kept in the calf section for an additional week, that is, they were not allowed to access the cow barn but had fence-line contact to their mother via the selection gate and the pen boundaries. This still allowed visual, auditory, olfactory, and limited tactile contact with their mothers without suckling. After this 3-wk period the weaning and separation process was completed by moving the calves to the youngstock herd in a different barn (Figure 1). In the gradual reduction treatment (**GR**, n = 18 calves, 8 female, 10 male) the weaning and separation took place by gradually reducing the daily cow-calf contact time. For this purpose, access to the cow section was controlled automatically via transponders on the calves' collars. When the weaning and separation process started, the cow-calf contact time was reduced from full-time contact preweaning to half-day contact between milkings during the day (~7 h, about 0830 h to 1530 h). During the evening and night the calves had no access to the cow herd. After 1 wk of half-day contact, the cow-calf contact time was further reduced to ~3.5 h from the end of morning milking until noon. Following this week of morning contact, the GR calves had one more week of fence-line contact with their dam, like the NF calves, before they were moved to the youngstock barn (Figure 1).

The weaning and separation process was always initiated on the same day of the week (Tuesday) and started for half of the calves in both treatments at 12 wk of age (exact age of calves: NF_{12w}: range, mean ± SD; 83–89, 87.8 ± 2.0 d, GR_{12w}: 83–89, 85.0 ± 2.2 d at weaning start) and for the other half of the calves at 14 wk of age (NF_{14w}: 98–102 d, 100.0 ± 1.3 d, GR_{14w}: 97–103 d, 99.2 ± 1.9 d old at weaning start, n = 9 each). This was done to counterbalance the fact that, due to the nature of the treatments, the complete milk loss happened at treatment d 0 for calves in the NF treatment, but not until introduction of fence-line separation from the dam on d 14 in the GR treatment, thus with a 2-wk delay (Figure 1). By staggering the starting age by 2 wk in each treatment, calves with similar age at weaning start, at milk loss (NF_{14w}: 98–102 d, 100.0 ± 1.3 d old at milk loss; GR_{12w}: 97–103 d, 99.0 ± 2.2 d old at milk loss), and at introduction of fence-line separation from the dam were included in both treatments (details are included in Supplemental Table S1, see Notes). Consequently, age of all calves in the NF treatment ranged from 83 to 102 d (mean ± SD 93.9 ± 6.5 d) and age of all GR calves ranged from 83 to 103 d (92.1 ± 7.6 d at weaning start), and this age was later included in the statistical analysis to best reflect the existing variation.

Data Collection

For assessment of the calves' distress responses to the weaning and separation process a combination of physiological and behavioral indicators was used. All sampling types and time points are given in Figure 1.

Physiological Assessment

ADG. Calves were weighed once per week between d −21 and +21 relative to weaning start with a commercial cattle weighing scale (Patura Wiegekäfig, Wiegeset S1; accuracy ± 1%). All calves were weighed routinely every week starting from birth and were thus accustomed to being moved onto the weighing scale.

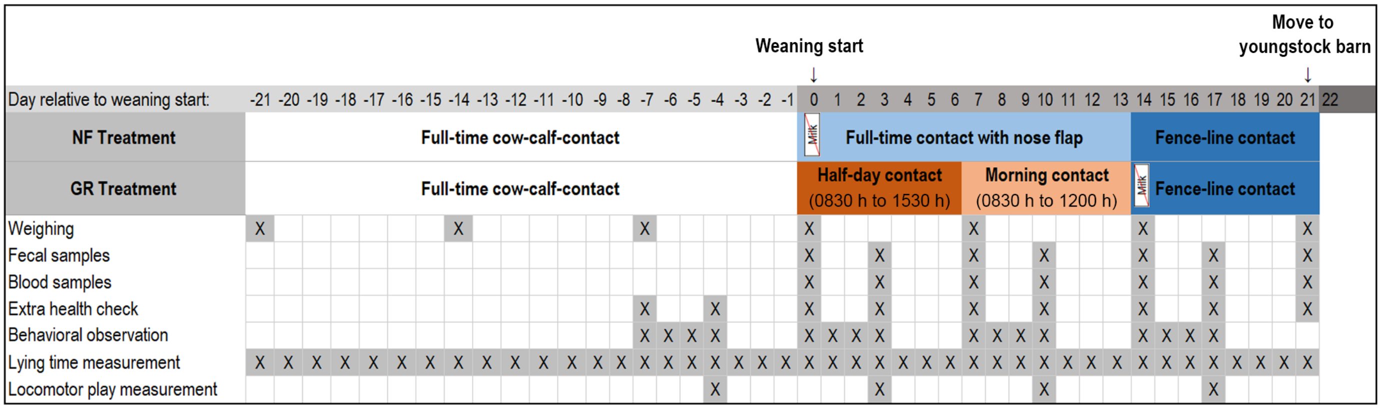
Fecal Cortisol Metabolites. Between d 0 and +21 relative to weaning start, fecal samples were collected twice per week in the morning at about 0730 h (±60 min). A minimum of 5 g of feces was collected by rectal stimulation, homogenized, and transferred into a commercial fecal sampling tube. Sampling tubes were immediately transferred into a cooling box and placed within 30 min into a freezer at −20°C, where they were kept until further analysis. Analysis of fecal samples in the laboratory followed the established protocol (Palme et al., 2013) with an 11-oxoetiocholanolone enzyme immunoassay (**EIA**) because this EIA was shown to be superior for fecal cortisol metabolites (**FGCM**) analysis in calves (Vogt et al., 2023).

Hematological Variables. Because O'Loughlin et al. (2014) emphasized neutrophil number as a robust biomarker of weaning distress in beef calves, blood samples of calves were taken twice per week between d 0 and 21 relative to weaning start. Two milliliters of blood was collected via jugular venipuncture always by the same qualified experimenter into EDTA (K EDTA)-coated sampling tubes (S-Monovette by Sarstedt) and kept refrigerated at 8°C until daily collection by a courier. The hemogram was determined on the same day by an external medical diagnostics provider with laboratory services for veterinary medicine (SYNLAB.vet GmbH, Hamburg).

Behavioral Assessment

Direct Observation of Behavior. Direct observation of calves' behavior was done always by the same observer on 4 consecutive days per week between d −7 and +21 relative to weaning start. All areas of the cow and calf section were visible to the observer at all times, except for the calves' lying and feeding area, which were observed via video recordings. Recorded behaviors are described in Table 1, although locomotor, social, and object play behavior were combined into "total play behavior" afterward. Observation times included: (a) 2 h following morning milking, (b) 1 h before the start of evening milking, and (c) 2 h following evening milking. These times were chosen because cows and calves were accustomed to being separated and reunited around milking and thus showed the most activity during those periods. Observations after the end of milking (a and c) started in the exact moment the cows and calves were allowed to reunite. Becuase there were 2 herds for observation, the observer switched between herds every 30 min, thus totaling 2.5 h daily observation time per calf. Observation start was alternated between the horned herd in even and the polled herd in odd calendar weeks and was also swapped each day within a week.

Video Analysis of Behavior. Analysis of TMR feeding time and brush use (Table 1) was done via video



																						Weaning start ↓																					Move to youngstock barn ↓	
Day relative to weaning start:	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
NF Treatment	**Full-time cow-calf-contact**																					~~Milk~~ **Full-time contact with nose flap**														**Fence-line contact**								
GR Treatment	**Full-time cow-calf-contact**																					**Half-day contact** (0830 h to 1530 h)							**Morning contact** (0830 h to 1200 h)							~~Milk~~ **Fence-line contact**								
Weighing	X							X							X							X							X							X							X	
Fecal samples																						X			X				X			X				X			X				X	
Blood samples																						X			X				X			X				X			X				X	
Extra health check															X			X				X			X				X			X				X			X				X	
Behavioral observation															X	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X					
Lying time measurement	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Locomotor play measurement																		X							X							X							X					

Figure 1. Overview of the weaning and separation process in the 2 treatments, sampling types, and sampling time points. NF = calves weaned with a 2-step weaning process using the nose flap (n = 18); GR = calves weaned by gradual reduction of cow-calf contact time (n = 18); half-day contact = cow-calf contact from 0830 h to 1530 h; morning contact = cow-calf contact from 0830 h to 1200 h; fence-line contact = partial separation from the mother by pen boundaries; weaning start (d 0) = weaning of calves started at either 12 or 14 wk of age as depicted in Supplemental Table S1 (see Notes); behavioral observation = sampling time points of video and direct observation; extra health check = detailed health check done by the experimenter in addition to the routine health check that was done daily by the barn personnel. The crossed-out "milk" label marks the day of complete milk loss for the calves.

Table 1. Ethogram of behaviors accessed by direct or video observation

Behavior	Definition	Recording rule[1]	Mode
Vocalization	Calf produces a clearly audible sound through the mouth (Loberg et al., 2008).	Continuous (freq.)	Direct
Searching behavior	Calf is moving parallel to, within 1 m of, the pen partition up and down, or standing with its head through the selection gate or standing beside the pen partition (radius 2.5 m) with head elevated with eyes and ears focused in the direction of the cow's section and scanning (adapted from Loberg et al., 2008; Enríquez et al., 2010).	Interval	Direct
Suckling attempt	Calves attempt to nuzzle the udder but did not obtain milk (because of nose flap or cow rejection; Enríquez et al., 2010).	Interval	Direct
Locomotor play	Calf is galloping, jumping, leaping, bucking, or buck-kicking (after Jensen et al., 1998).	Continuous (dur.)	Direct
Social play	Two calves are standing front to front, pushing, rubbing, or butting head against head/neck without force, often including rotating head movements (adapted from Jensen et al., 1998).	Continuous (dur.)	Direct
Object play	Calf is butting water bowl, hayrack, or bars in the pen, standing up. Or calf is butting straw or rubbing head, throat, or neck in straw, kneeling down on the two forelegs (Jensen et al., 1998).	Continuous (dur.)	Direct
Sociopositive behavior	Calf is sniffing, licking, or rubbing against another calf (Loberg et al., 2008).	One-zero	Direct
Rumination	Chewing after regurgitating boluses of feed (Enríquez et al., 2010).	Interval	Direct
Mounting	Calf lifts its front legs from the ground, supporting itself on another calf; the act of an animal raising the anterior part of its body generally onto the posterior part of another animal (Solano et al., 2005).	Continuous (freq.)	Direct
Cross-sucking	Calf sucking any part of another calf (Hepola et al., 2006).	One-zero	Direct
TMR feeding time	Calves' head is placed through the feeding rack (marker: ears in front of the rack).	Continuous	Video
Brush use	Calf having physical contact with the brush with any body part while being (automatically) brushed or scratching itself against the brush (adapted from Horvath and Miller-Cushon, 2019).	Continuous	Video

[1]Continuous = continuous behavior sampling; interval = scan sampling every 3 min, one-zero = one-zero sampling every 3 min, with 1 denoting the presence of a behavior in that period and 0 denoting the absence of the behavior in that period; freq. = frequency, dur. = duration.

observation on the same 4 consecutive days when direct observation took place, using Jovision Infrared Network Cameras with Jovision NVR System Software (Version 2.0.1.49). Cameras automatically switched to infrared mode when light conditions were not sufficient for color mode anymore. For better identification of calves during the infrared mode, photos of the calves' individual coat patterns were used. Analysis was conducted by 4 different observers for TMR feeding time (Cohen's kappa, $\kappa_{TMR} \geq 0.91$) and 3 different observers for brush use ($\kappa_{Brush\ use} \geq 0.88$; one observer—with part of data for 4 and all data for 3 calves—had to be excluded due to too low interobserver reliability for brush use) with the software BORIS (Friard and Gamba, 2016, Version 7.9.22). All behaviors were recorded continuously in intervals of 6 h (night: 0000–0600 h, morning: 0600–1200 h, afternoon: 1200–1800 h, and evening: 1800–0000 h) and specific intervals on days of treatment change were subsequently deleted from the dataset (i.e., the night interval of d 0 for NF calves because the nose flap was not inserted until the morning).

Automatic Assessment of Behavior

Concentrate Feed Intake. Output from the concentrate feeder (Förster Technik GmbH) was automatically recorded with Förster CalfCloud Software (Förster Technik GmbH). Daily allowance per calf was 1.5 kg concentrate; however, in the course of the experiment it was frequently observed that calves other than the intended recipient ate (parts of) the portion. Therefore, a precise allocation of the concentrate intake per calf was not possible and concentrate intake was not further considered for analysis.

Lying Behavior. For assessment of lying times and number of lying bouts 28 of the 36 calves (14 per treatment) were fitted with an accelerometer (Hobo Pendant G data logger, Onset Computer Corporation). The 3-axis accelerometers were placed directly above the metacarpal joint on the medial side of the left hind leg and tilted by 90° to allow for a better fit to the calves' legs, such that the y-axis was parallel to the ground and the x-axis was perpendicular to the ground pointing downward. Data loggers recorded g-force and degree of tilt of the x-axis at 60 s intervals from d −28 to +21 relative to weaning start, with d −28 to −22 used as the habituation period. The accelerometers were replaced every 14 d due to limited storage capacities. Data were downloaded using HOBOware software (Onset Computer Corporation, Version 3.7.17). Recordings at the time of fitting or replacement of loggers, that is, between 0700 and 1000 h on respective days, were omitted due to their potential nuisance effect on the data. The degree of vertical tilt (x-axis) was used to determine the lying position of the calf, such that readings ≤120° indicated the calf lying down, whereas readings >120° indicated the calf standing up (adjusted from Ito et al., 2009 for tilted logger position).

Afterward recordings were edited with an event filter, which converted single outlier readings (e.g., a single lying event that was preceded and followed by a standing event, or vice versa) to the behavior that preceded it, as these were potentially erroneous readings of lying or standing events (Ledgerwood et al., 2010). From these data, total lying time in percent per day and the number of lying bouts per day were calculated.

Locomotor Play. For automatic assessment of locomotor play, 28 of the 36 calves (all but 4 identical to those calves used for assessment of lying behavior, NF = 13 and GR = 15 calves) were fitted with a second accelerometer for 9 h (0800 h to 1700 h) on the medial side of the right hind leg on days −4, +3, +10, and +17 relative to weaning start. Loggers were set to measure g-force of the vertical axis (x-axis, as loggers were tilted by 90° again) at a rate of 1 Hz as previously validated by Luu et al. (2013). Readings were exported with HOBOware and analyzed using the peak acceleration method (Größbacher et al., 2020), that is, counts of acceleration peaks from the data logger determine the counts of play events of the calf. For validation, 30 min video sections (from continuous video recordings in the calf section) were selected in which the calves showed locomotor play (running, bucking, or single kick) during the times the "play accelerometer" was worn (available for 16 of the 28 calves). Then a Pearson correlation was calculated for different upper and lower threshold values of peak acceleration between counts of observed play events from video and counts of peak acceleration measurements from the data loggers. Our data showed the best results for peak threshold values of $\geq +3.0\ g$ and $\leq -2.0\ g$ ($r = 0.92$), which was used for further analysis. Thus, our data are reported in counts of peak acceleration measurements (**CPA**) from HOBO-loggers per 9 h sampling period for peak threshold values of $\geq +3.0\ g$ and $\leq -2.0\ g$.

Statistical Analysis

Exclusion Criteria. Data points from collection days at which calves showed clinical signs of ill health or gained access to the cow area during periods in which they were supposed to be separated from their dam (named thereafter "illegitimate access"), for example, GR calves during the night in the half-day phase, were excluded from analysis as described in the following paragraph.

Illegitimate Cow-Calf Contact. Video monitoring of the selection gate where calves entered the cow section was used to examine if any weaner calves obtained illegitimate access to their dams, for example, by forcing themselves through the gate along with younger calves. The percentage of time a calf entered the cow section from the total time it was not permitted in the cow section anymore was calculated per 6 h interval (morning, afternoon, evening, night) to account for the different permission times in the course of the GR treatment. An error score of 0 was given when a calf had 0–2% of time of illegitimate access with its dam and an error score of 1 was given when a calf had >2% illegitimate access to its dam per interval. If an error score of 1 was given for an interval, the calf automatically obtained this score for the subsequent interval as well, because we expected calves that had just suckled their dams to have a lower motivation (or vice versa less distress) to enter the cow section and were thus not representative for weaning distress examination during that period. For physiological indicators (except ADG) and automatically measured behaviors, the highest error score of the 4 intervals from that day was used for correction of data. For weight gains, which were measured only once per week, the 2% threshold was used as well, that is, calves that entered the cow section for >2% of time illegitimately within a week were not included in ADG analysis for that week. Only observations with an error score 0 were used in all analyses.

Twenty-one of the 36 study calves entered the cow section illegitimately (6 NF/ 15 GR calves). On average (±SD) these 21 calves illegitimately entered the cow section for 18.66% ± 18.12% (median: 13.5%, range 0.28%–86.4%) of time per 6 h observation; however, this was mainly due to 5 prominent calves (all from the GR treatment group). Within the GR treatment group the majority of observations with illegitimate access was recorded in the third week (55.61% of occurrences, 13 calves), followed by the first week (23.98%, 13 calves, 11 calves identical to wk 3) of the weaning and separation process. NF calves could only enter the cow section illegitimately during the third week (100% of occurrences of illegitimate access, 6 calves).

Health Monitoring. The health status of the calves was monitored in detail twice per week before collection of blood samples by the experimenter. Assessment included general condition, nasal and ocular discharge, coughing, ear position, navel health, lesions, cleanliness of hindquarters, fecal consistency, and measurement of rectal temperature (adapted from Roth et al., 2009). A calf was considered to have fever if its rectal temperature was above 39.1°C. Health problems were scored with a 0, 1, 2 system, with 0 equaling no health problems and a 2 equaling a severe symptom (see Roth et al., 2009), for example, fever, liquid feces or all conditions that made medications or veterinary care necessary. A 1 equaled a minor symptom, such as sporadic coughing or superficial lesions. The total health score for the day equaled the highest score the calf got on one of the single parameters on the specific day. If an animal was scored a 2 on its total health score (i.e., it scored a 2 in any of the health measurements) on a specific day, it was completely excluded from analysis for that day as well as the preceding and subsequent day. Only data for weight gain were based on a dataset that included calves with scours (fecal consistency scored 2) on single days. This was the case because the weight measurements were taken only once every week and single days with scours in between would be masked by the other days. Furthermore, deleting calves with nutritional scours due to high milk consumption could have led to an underestimation of the ADG. For FGCM analysis the health and error score data from the day before data collection were used, because in calves FGCM peak about 8 to 10 h after the occurrence of a stressor (Vogt et al., 2023), and thus our samples from 0730 h reflect the calves' FGCM concentrations from the previous evening (~2100 h until 0000 h).

Remaining data points after correction for health problems and illegitimate cow-calf contact are shown in Supplemental Figures S1 and S2 (see Notes).

Data Analysis

Statistical analysis was performed with SAS Version 9.4 (SAS Institute, Cary, NC). An a priori reduction of explanatory variables was done by checking for correlations between variables. For all behavioral parameters observed at several intervals per day (TMR feeding times, rumination, vocalizations, searching behavior, total play behavior, and brush use) the means per week for each individual calf were calculated before analysis. Data were analyzed according to their respective distribution (Gaussian: ADG (kg/d), rumination (% of scans), FGCM (ng/g), neutrophils (% of total leukocytes), lymphocytes (% of total leukocytes), vocalizations (frequency per 30 min), searching behavior (% of scans), total play behavior (seconds per 30 min), brush use (min per 6 h), lying times (% of 24 h) and suckling attempts (% of scans); β distribution (logit-link): neutrophil:lymphocyte ratio; Poisson distribution (log-link): locomotor play (CPA per 9 h) and lying bouts (number per 24 h); geometric distribution (log-link): TMR feeding time (min per 6 h) with a linear or generalized linear mixed-effects model with repeated measures. FGCM data were log-transformed before analysis and back-transformed using the omega method. Vocalizations, searching behavior, total play behavior, and suckling attempts were square root-transformed before analysis and back-transformed using the delta method. The (generalized) linear mixed-effects model included treatment (GR/NF), phase (baseline phase/weaning and separation phase), and the interaction between treatment and phase, as well as sex of the calf (male/female), as fixed effects. Age of the calf at start of the weaning procedure (in days) was included as continuous variable. The calf ID nested within herd (horned/polled) was included as a random effect. Additionally, the calendar week merged with herd was included as cross-classified random effect to account for the weekly changing group composition in the calf section of each herd throughout the study (Cafri et al., 2015). Correction for multiple testing of several parameters (in total 14) was calculated according to the Benjamini-Hochberg false discovery rate correction (Glickman et al., 2014; Supplemental Table S2; see Notes).

In case that the treatment × phase interaction showed significance ($P \leq 0.05$) or a tendency ($P \leq 0.1$) after correction for multiple testing, an extended model was run that included the exact week (baseline, week 1, week 2, week 3 of the weaning and separation phase) instead of the whole weaning and separation phase as the sampling time point. For all models, model requirements (normal distribution and homoscedasticity of residuals) were checked graphically. Pairwise differences of different treatment × phase interactions (or for extended analysis treatment × week interactions) were calculated using a Tukey-Kramer post hoc test. All results are presented as (back-transformed) LSM ± SE. Extended model results for variables not reported here are included in Supplemental Table S3 (see Notes), and confidence intervals for the estimates are presented in Supplemental Tables S4 and S5 (see Notes).

Exceptions to this procedure were FGCM concentrations, hematological variables, and suckling attempts. For FGCM data, only the extended model with the specific weeks was run, because a comparison of the whole weaning and separation phase was not valid due to the different nutritional bases in the course of the treatments, which was shown to heavily influence FGCM levels (Vogt et al., 2023). For hematological variables, the exact day of treatment was used as the sampling time point instead of the week in the extended analysis, because literature showed that changes in hematological parameters are transient and can return to baseline levels within just 3 d after an abrupt weaning stressor (O'Loughlin et al., 2012, 2014). For suckling attempts of the NF calves, we used the same mixed model as above, except that it contained only treatment day, age at weaning start, and sex of calf as fixed factors.

Analysis of sociopositive behavior, mounting behavior, and cross-sucking was not possible due to a too low occurrence of these variables during our selected observation times. Cross-sucking was observed once in 4 calves (2 NF, 2 GR) during the whole behavioral observation.

RESULTS

ADG

Weight gain (kg/d) differed between treatments depending on the phase (interaction treatment × phase,

Table 2. Model output of the main model for the effect of weaning and separation of calves with either 2-step weaning using a nose flap (NF) or gradual reduction of contact time to the dam (GR) on behavioral and physiological indicators of distress[1]

			Phase		P-values (F-value$_{\text{Num DF,Den DF}}$)		
Variable	Treatment	No. of calves	Baseline	Treatment	Treatment	Phase	Treatment × phase
ADG (kg/d)	NF	18	1.50 ± 0.07^{a}	0.26 ± 0.07^{bx}	0.003	<0.001	**0.028**
	GR	18	1.58 ± 0.07^{a}	0.68 ± 0.09^{by}	$(F_{1,28} = 10.6)$	$(F_{1,72} = 198.7)$	$(F_{1,70} = 5.1)$
TMR feeding time (min/6 h)	NF	18	6.81 ± 0.74^{a}	29.25 ± 2.56^{bx}	<0.001	<0.001	**<0.001**
	GR	18	6.51 ± 0.71^{a}	13.04 ± 1.15^{by}	$(F_{1,31} = 14.1)$	$(F_{1,93} = 186.6)$	$(F_{1,58} = 26.9)$
Rumination (% of scans)	NF	18	9.95 ± 2.25	16.31 ± 1.76	0.74	0.001	0.50
	GR	18	11.72 ± 2.27	16.05 ± 1.73	$(F_{1,29} = 0.1)$	$(F_{1,89} = 10.9)$	$(F_{1,72} = 0.5)$
Vocalizations^{2} (frequency per 30 min)	NF	18	0.50 ± 0.15	0.61 ± 0.10^{x}	0.12	0.003	**0.007**
	GR	18	0.16 ± 0.05^{a}	3.21 ± 0.47^{by}	$(F_{1,87} = 2.5)$	$(F_{1,128} = 9.2)$	$(F_{1,128} = 7.4)$
Searching behavior^{2} (% of scans)	NF	18	0.32 ± 0.09	0.50 ± 0.08^{x}	0.09	<0.001	**<0.001**
	GR	18	0.01 ± 0.00^{a}	3.62 ± 0.53^{by}	$(F_{1,90} = 3.0)$	$(F_{1,127} = 15.7)$	$(F_{1,114} = 12.2)$
Total play behavior^{2} (s/30 min)	NF	18	1.54 ± 0.26	0.19 ± 0.02	0.01	<0.001	0.71
	GR	18	2.59 ± 0.43	0.80 ± 0.09	$(F_{1,44} = 7.7)$	$(F_{1,90} = 36.5)$	$(F_{1,67} = 0.14)$
Locomotor play (CPA/9 h)	NF	13	34.77 ± 7.40^{a}	12.17 ± 2.14^{bx}	0.11	<0.001	**0.007**
	GR	15	31.38 ± 6.10	26.10 ± 4.45^{y}	$(F_{1,19} = 2.9)$	$(F_{1,43} = 14.0)$	$(F_{1,38} = 8.1)$
Brush use (min/6 h)	NF	16	3.57 ± 0.68	3.77 ± 0.54	0.73	0.67	0.25
	GR	17	4.18 ± 0.61	3.62 ± 0.49	$(F_{1,11} = 0.13)$	$(F_{1,51} = 0.2)$	$(F_{1,38} = 1.4)$
Lying times (% of 24 h)	NF	15	66.69 ± 0.71	62.11 ± 0.73	0.47	<0.001	***0.047***
	GR	14	66.64 ± 0.75	63.53 ± 0.83	$(F_{1,25} = 0.6)$	$(F_{1,248} = 86.9)$	$(F_{1,279} = 4.0)$
No. of lying bouts (per 24 h)	NF	15	18.52 ± 0.75^{a}	11.50 ± 0.50^{bx}	0.31	<0.001	**<0.001**
	GR	14	17.28 ± 0.74^{a}	13.89 ± 0.66^{by}	$(F_{1,25} = 1.1)$	$(F_{1,201} = 255.3)$	$(F_{1,211} = 37.4)$
Neutrophils (%)	NF	18	19.84 ± 2.43	25.05 ± 1.53	0.12	0.46	**0.016**$^{\#}$
	GR	17	27.49 ± 2.60	24.74 ± 1.75	$(F_{1,39} = 2.6)$	$(F_{1,97} = 0.6)$	$(F_{1,89} = 5.9)$
Lymphocytes (%)	NF	18	71.85 ± 2.57^{a}	65.76 ± 1.60^{b}	0.12	0.32	**0.012**
	GR	17	63.64 ± 2.74	66.25 ± 1.83	$(F_{1,41} = 2.5)$	$(F_{1,95} = 1.0)$	$(F_{1,85} = 6.5)$
Neutrophil:lymphocyte ratio	NF	18	0.30 ± 0.06	0.41 ± 0.04	0.21	0.62	***0.045***$^{\#}$
	GR	17	0.46 ± 0.07	0.40 ± 0.04	$(F_{1,43} = 1.6)$	$(F_{1,102} = 0.2)$	$(F_{1,87} = 4.2)$

[a,b] Indicate a difference between baseline and the weaning/separation phase within a treatment.

[x,y] Indicate a difference between the NF and GR treatment within a phase with $P \leq 0.05$.

[#] Individual pairwise comparisons were no longer significant or only tended to be significant after Tukey-Kramer adjustment.

[1] Values are expressed as LSM ± SE. A Benjamini-Hochberg false discovery rate calculation for the treatment × phase interaction confirmed the P-values in bold for rejection of the null-hypothesis with an $\alpha \leq 0.05$, while P-values in bold and italics correspond to an $\alpha \leq 0.1$. NF = nose-flap weaning; GR = gradual separation; DF = degrees of freedom; baseline = last 3 wk before weaning start for ADG, lying times, and lying bouts, 1 wk before weaning start for hematological responses, and all other behaviors; treatment phase = the 3-wk weaning and separation phase; CPA = counts of peak acceleration measurements.

[2] LSM and SE back-transformed from square-root-transformation.

Table 2). GR calves gained more weight than NF calves during the 3-week weaning and separation phase (pairwise post hoc test; t-statistic$_{\text{degrees of freedom}}$: $t_{77} = 3.6$, $P = 0.004$, Table 2). However, both treatments showed lower ADG during the weaning and separation phase compared with their individual baseline before start of the treatment (GR: $t_{81} = 7.8$, $P < 0.001$; NF: $t_{60} = 12.9$, $P < 0.001$, Table 2).

The extended model revealed the lowest gains for NF calves compared with baseline in the first week immediately after insertion of the nose flap ($t_{137} = -9.3$, $P < 0.001$), but ADG remained at a relatively low level for the second and third week as well (Table 3). In contrast, GR calves showed increasing weight gains over the weaning and separation period with the highest ADG in the third week, which also did not differ from the GR baseline anymore ($t_{164} = -2.4$, $P = 0.28$, Table 3, Supplemental Figure S1A).

TMR Feeding

Duration of feeding on TMR differed between treatments depending on phase (interaction treatment × phase, Table 2). Throughout the weaning and separation phase, NF calves spent more time feeding on TMR than GR calves ($t_{31} = -6.7$, $P < 0.001$). Both treatments showed an increase in TMR feeding duration during the weaning and separation phase compared with their individual baseline before the weaning procedure started (GR: $t_{81} = -6.5$, $P < 0.001$; NF: $t_{74} = -13.5$, $P < 0.001$), but this increase was stronger for NF calves (Table 2).

The extended model revealed that NF and GR calves each showed a significant increase in the duration of TMR feeding with each week of the weaning and separation procedure (Table 3), except for NF calves from wk 2 to wk 3 ($t_{63} = -2.3$, $P = 0.33$). In general, NF calves showed a noticeably stronger increase in TMR feeding duration

Table 3. Output of the extended model for the effect of weaning and separation of calves over 3 wk with either 2-step weaning using a nose flap (NF) or gradual reduction of contact time to the dam (GR) on behavioral and physiological indicators of distress[1]

			Week				P-values (F-value$_{\text{Num DF,Den DF}}$)		
Variable	Treatment	No. of calves	Baseline	Week 1	Week 2	Week 3	Treatment	Week	Treatment × week
ADG (kg/d)	NF	18	1.51 ± 0.07^{a}	0.07 ± 0.13^{c}	0.41 ± 0.13^{bc}	0.27 ± 0.14^{c}	<0.001	<0.001	0.03
	GR	18	1.58 ± 0.07^{a}	0.64 ± 0.18^{bc}	0.51 ± 0.16^{bc}	1.07 ± 0.21^{ab}	($F_{1,52} = 17.3$)	($F_{3,112} = 67.2$)	($F_{3,113} = 3.2$)
TMR feeding time (min/6 h)	NF	18	6.05 ± 0.63^{ab}	21.52 ± 2.30^{d}	30.56 ± 3.08^{e}	37.61 ± 3.84^{e}	<0.001	<0.001	<0.001
	GR	18	5.64 ± 0.58^{a}	9.09 ± 0.95^{b}	13.21 ± 1.39^{c}	18.06 ± 1.89^{d}	($F_{1,29} = 32.0$)	($F_{3,66} = 116.3$)	($F_{3,49} = 13.8$)
Vocalizations[2] (frequency/30 min)	NF	18	0.25 ± 0.05^{a}	3.68 ± 0.80^{b}	0.12 ± 0.24^{a}	0.04 ± 0.01^{a}	<0.001	<0.001	<0.001
	GR	18	0.30 ± 0.06^{a}	1.20 ± 0.24^{ab}	0.85 ± 0.17^{a}	13.78 ± 2.91^{c}	($F_{1,30} = 31.9$)	($F_{3,71} = 19.8$)	($F_{3,68} = 45.6$)
Searching behavior[2] (% of scans)	NF	18	0.14 ± 0.03^{ab}	1.61 ± 0.40^{bc}	0.02 ± 0.01^{a}	0.90 ± 0.22^{ac}	<0.001	<0.001	<0.001
	GR	18	0.05 ± 0.01^{a}	2.30 ± 0.53^{c}	0.70 ± 0.16^{ac}	15.40 ± 3.73^{d}	($F_{1,26} = 38.7$)	($F_{3,71} = 31.2$)	($F_{3,65} = 21.5$)
Locomotor play (CPA/9 h)	NF	13	35.66 ± 7.25^{a}	11.71 ± 3.49^{b}	11.12 ± 2.78^{b}	15.68 ± 4.08^{ab}	0.36	0.001	0.004
	GR	15	29.25 ± 5.51^{ab}	25.45 ± 6.17^{ab}	31.52 ± 6.34^{a}	7.42 ± 3.90^{ab}	($F_{1,23} = 0.9$)	($F_{3,34} = 6.7$)	($F_{3,31} = 5.4$)
Lying times (% of 24 h)	NF	15	66.69 ± 0.71	62.68 ± 0.86	62.89 ± 0.85	60.90 ± 0.86	0.49	<0.001	0.19
	GR	14	66.66 ± 0.74	64.19 ± 0.97	64.48 ± 0.99	60.49 ± 1.20	($F_{1,30} = 0.5$)	($F_{3,346} = 35.5$)	($F_{3,328} = 1.6$)
No. of lying bouts (per 24 h)	NF	15	18.54 ± 0.73^{a}	12.02 ± 0.62^{bc}	11.26 ± 0.57^{c}	11.25 ± 0.58^{c}	0.11	<0.001	<0.001
	GR	14	17.35 ± 0.72^{a}	14.69 ± 0.78^{b}	14.07 ± 0.78^{bc}	11.57 ± 0.86^{c}	($F_{1,31} = 2.7$)	($F_{3,285} = 91.9$)	($F_{3,276} = 12.6$)
FGCM[3] (ng/g)	NF	18	234.76 ± 38.72^{ac}	255.02 ± 28.07^{a}	146.50 ± 17.05^{c}	151.06 ± 17.29^{bc}	0.11	0.006	0.045
	GR	17	276.62 ± 48.22^{ab}	221.57 ± 27.03^{ac}	214.47 ± 28.69^{ac}	211.47 ± 33.99^{ac}	($F_{1,25} = 2.7$)	($F_{3,80} = 4.4$)	($F_{3,72} = 2.8$)

[a–e] Indicate statistically significant differences at $P \leq 0.05$ (all post hoc pairwise comparisons between treatment × week combinations for a variable considered to allow also for individual relevant comparisons like, for example, the week of milk loss in both treatments).

[1] Values are expressed as LSM ± SE. The baseline comprised the last 3 wk before weaning start for the variables ADG and lying times; for FGCM and all other behaviors it comprised only 1 wk before the weaning start. Week = sampling time point (week) and treatment × week = interaction of treatment × week. NF = nose-flap weaning; GR = gradual separation; DF = degrees of freedom; CPA = counts of peak acceleration measurements; FGCM = fecal cortisol metabolites.

[2] LSM and SE back-transformed from square-root-transformation.

[3] LSM and SE back-transformed from log-transformation. Note that for FGCM levels only weeks with an identical feeding base are reasonably comparable between treatments and within a treatment.

compared with GR calves, because they fed significantly longer from the TMR in each weekly comparison (Table 3, Supplemental Figure S1B).

Rumination Behavior

There was no treatment × phase interaction for the percentage of scans in which calves showed rumination behavior, but a main effect of the treatment phase (Table 2). Rumination behavior increased in all calves during the weaning and separation phase as compared with the baseline phase (Table 2).

Vocalizations

The frequency of vocalizations differed between treatments depending on phase (interaction treatment × phase, Table 2). Gradually weaned calves showed an increase in frequency of vocalizations from baseline to the weaning and separation phase ($t_{128} = -4.1$, $P = 0.001$), which was not the case for the NF calves ($t_{128} = -0.2$, $P = 0.99$, Table 2). Consequently, GR calves showed more vocalizations during the weaning and separation phase compared with NF calves ($t_{48} = 4.7$, $P < 0.001$, Table 2).

Additionally, the extended model revealed a difference in temporal distribution of vocalizations; GR calves vocalized most frequently during the third week of the treatment with fence-line contact and NF calves most frequently during the first week, which differed significantly from one another ($t_{122} = 5.9$, $P < 0.001$, Table 3, Supplemental Figure S2A, see Notes). This represented more vocalizations compared with the respective baseline value for both of the treatments (GR: $t_{95} = 11.4$, $P < 0.001$; NF: $t_{68} = 4.6$, $P < 0.001$, Table 3) and was for both treatments the time point when milk loss happened. Introduction of half-day (wk 1) and morning contact (wk 2) did not significantly increase vocalizations compared with the baseline in GR calves (Table 3, Supplemental Figure S2A).

Searching Behavior

The percentage of scans in which calves showed searching behavior differed between treatments depending on phase (interaction treatment × phase, Table 2). During the 3-wk weaning and separation phase, GR calves showed more searching behavior than NF calves ($t_{52} = 6.2$, $P < 0.001$, Table 2). The highest increase in scans with searching behavior compared with baseline was seen in GR calves during the first and especially the third week of the weaning and separation process, whereas NF calves showed no significant increase in searching behavior compared with their baseline in any treatment week (Table 3, Supplemental Figure S2B). Generally, GR calves showed significantly more searching behavior during the third week with fence-line contact than during any other week of the weaning and separation process (Table 3). Equally, the amount of searching behavior of GR calves in the third week was also higher than that of NF calves in any week of the weaning and separation period, including the first week in which the milk loss happened for NF calves (Table 3, Supplemental Figure S2B).

Total Play Behavior (Direct Observation)

There was no interaction of treatment × phase for the duration of total play behavior of the calves, that is, locomotor, social, and object play behavior combined, but a main effect of phase (Table 2). During the weaning and separation phase all calves played less in comparison with the baseline phase (Table 2).

Locomotor Play (Automatic Assessment)

Counts of peak acceleration (per 9 h), indicative of locomotor play, differed between treatments depending on the phase (interaction treatment × phase, Table 2). During the 3-wk weaning and separation phase, NF calves showed a decrease in locomotor play compared with their baseline phase ($t_{37} = 4.5$, $P < 0.001$), which was however not the case for GR calves ($t_{47} = 0.9$, $P = 0.82$, Table 2). Consequently, GR calves showed more locomotor play during the weaning and separation phase than NF calves ($t_{19} = 3.5$, $P = 0.007$, Table 2).

The extended model revealed that compared with baseline, the locomotor play behavior of NF calves was mainly reduced in the first and second week while wearing the nose flap (wk 1: $t_{33} = -3.5$, $P = 0.03$; wk 2: $t_{35} = -4.1$, $P = 0.007$), but not during the third week with fence-line contact ($t_{39} = -2.8$, $P = 0.13$, Table 3, Supplemental Figure S2C, see Notes). In contrast, no significant decline in locomotor play was observed for GR calves from baseline to any treatment week (Table 3, Supplemental Figure S2C). Nonetheless, GR calves showed a numerical reduction in play behavior compared with the baseline after introduction of fence-line contact to the dam in the third week (Table 3; note however that this was based on data of only 3 calves, denoted by the diamonds in Supplemental Figure S2C).

Brush Use

There was no interaction of treatment × phase for the duration of brush use of the calves and no main effect either (Table 2).

Table 4. Number of unsuccessful suckling attempts with the nose flap of calves weaned via 2-step weaning[1]

	Treatment day								*P*-value (*F*-value$_{\text{Num DF,Den DF}}$)
Variable	0	1	2	3	7	8	9	10	Sampling time point (day)
Suckling attempts (% of scans)[2]	9.31 ±3.34[a]	6.73 ±2.42[a]	0.77 ±0.24	2.84 ±0.89[a]	1.39 ±0.48	0.12 ±0.04	0.34 ±0.09	0.16 ±0.44	<0.001 ($F_{8,81}$ = 9.2)

[a] Indicates a statistically significant difference from zero behavioral observations with suckling attempts at $P \leq 0.05$. D = sampling time point (treatment day). DF = degrees of freedom.

[1] Data are presented as percentage of scans (LSM ± SE) in which suckling attempts occurred during direct observation with scan sampling every 3 min. Treatment d 0 represents the day of insertion of the nose flap.

[2] LSM and SE back-transformed from square-root-transformation.

Lying Times and Number of Lying Bouts per Day

Daily lying times of calves tended to differ after correction for multiple testing between treatments depending on the phase (interaction treatment × phase, Table 2). Numerically though, there was no difference in the percentage of total lying time per day between treatments over the whole 3-wk weaning and separation phase (Table 2) and the extended model revealed no difference between treatments in any weekly comparison either (Table 3). However, there was a main effect of the treatment phase on lying times (Table 2), because lying times of both treatments decreased during the 3-wk weaning and separation phase compared with the individual 3-wk baseline before treatment start (Table 2).

The number of lying bouts per day differed between treatments depending on phase (interaction treatment × phase, Table 2). Compared with baseline values, the number of lying bouts declined in both treatments, but this decline was stronger in NF calves (~40% fewer bouts) than in GR calves (~20% fewer bouts, Table 2). Thus, GR calves showed more lying bouts than NF calves over the whole 3-wk weaning and separation phase ($t_{38} = 3.0$, $P = 0.02$, Table 2). The extended model revealed no difference in the number of lying bouts between treatments in any weekly comparison (Table 3).

Suckling Attempts and Nasal Injuries of NF Calves

It is noteworthy reporting that calves in the NF treatment group, which wore the nose flap over a duration of 2 wk, all showed some tissue alterations caused by the nose flaps. Out of 18 calves in the NF treatment, 12 calves had pressure marks at the nasal septum from the nose flap, the other 6 had injuries, with 2 calves showing tissue bleeding, 3 calves having a purulent inflammation, and one calf having both bleeding and a purulent inflammation at the tissue of the nasal septum at the contact site with the nose flap.

Suckling attempts of NF calves decreased considerably over the 2-wk period while wearing a nose flap (Table 4). The percentage of scans in which suckling attempts were observed showed no significant difference from zero suckling attempts beyond the second treatment day and all days of the second treatment week (Table 4).

Hematological Responses

Neutrophil and lymphocyte percentage differed between treatments depending on phase (interaction treatment × phase) and tended to do so after correction for multiple testing for the neutrophil:lymphocyte ratio (Table 2). Nose flap calves showed a tendency for an increased neutrophil percentage ($t_{87} = -2.4$, $P = 0.09$) and an accompanying decreased lymphocyte percentage in blood ($t_{84} = 2.6$, $P = 0.048$) during the weaning and separation phase compared with the NF baseline phase (Table 2). This was also reflected in a numerically increased neutrophil:lymphocyte ratio of NF calves ($t_{88} = -1.9$, $P = 0.25$, Table 2). In contrast, no significant changes in neutrophil percentage, lymphocyte percentage, or neutrophil:lymphocyte ratio from baseline to the weaning and separation phase were observed for GR calves (Table 2).

Extended models with individual treatment days showed a numerical increase in neutrophil percentage and a numerical decrease in lymphocyte percentage in NF calves from d 0 to d 3, which was the first sampling point after insertion of the nose flap. This resulted in an elevated neutrophil:lymphocyte ratio for NF calves on d 3 compared with the NF baseline on d 0 ($t_{64} = -3.9$, $P = 0.02$, Table 5). For none of the other treatment days did significant changes compared with baseline become evident in either of the 2 treatments (Table 5).

Fecal Cortisol Metabolites

There were no significant differences in FGCM concentrations (ng/g) between the 2 treatments during the baseline phase or the third week with fence-line contact (Table 3). Within treatments, NF calves showed the highest increase in FGCM concentrations in reaction to inser-

Table 5. Effect of the weaning and separation procedure on total neutrophil and lymphocyte percentage and the neutrophil:lymphocyte (N:L) ratio of calves weaned with 2-step weaning using a nose flap (NF) or gradual reduction of contact time to the dam (GR)[1]

		Treatment day							*P*-values (*F*-value$_{\text{Num DF,Den DF}}$)		
Variable and treatment	No. of calves	0	3	7	10	14	17	21	Treatment	Sampling time point (d)	Treatment × day
Neutrophils, %											
NF	18	21.37 ± 2.48	28.54 ± 2.22	23.36 ± 2.26	24.57 ± 2.06	24.62 ± 2.71	25.11 ± 2.17	21.81 ± 2.54	0.58	0.49	0.17
GR	17	27.52 ± 2.66	24.75 ± 2.45	25.67 ± 2.56	24.81 ± 2.42	28.52 ± 3.36	24.31 ± 3.48	21.94 ± 3.21	($F_{1,26} = 0.3$)	($F_{6,76} = 0.9$)	($F_{6,76} = 0.9$)
Lymphocytes, %											
NF	18	70.52 ± 2.57	62.15 ± 2.30	69.58 ± 2.35	66.90 ± 2.13	65.93 ± 2.86	65.47 ± 2.24	66.55 ± 2.62	0.67	0.36	0.10
GR	17	63.34 ± 2.75	65.31 ± 2.53	65.37 ± 2.65	66.06 ± 2.51	63.66 ± 3.49	66.59 ± 3.61	70.28 ± 3.34	($F_{1,26} = 0.2$)	($F_{6,72} = 1.1$)	($F_{6,65} = 1.9$)
N:L ratio											
NF	18	0.30^a ± 0.05	0.48^b ± 0.05	0.34 ± 0.04	0.37 ± 0.04	0.36 ± 0.05	0.36 ± 0.04	0.34 ± 0.05	0.56	0.19	0.05
GR	17	0.46 ± 0.06	0.40 ± 0.05	0.36 ± 0.05	0.40 ± 0.05	0.38 ± 0.07	0.40 ± 0.07	0.34 ± 0.07	($F_{1,14} = 0.4$)	($F_{6,59} = 1.5$)	($F_{6,63} = 2.2$)

[a,b]Means with different superscript letters differ between sampling days within a row with $P \leq 0.05$. No further comparisons were significant.

[1]Values are expressed as LSM ± SE. DF = degrees of freedom.

tion of the nose flap in wk 1 compared with the other 2 treatment weeks (wk 2: $t_{83} = 4.3$, $P = 0.001$, wk 3: $t_{39} = 4.0$, $P = 0.004$, Table 3). For GR calves, treatment weeks were not reasonably comparable due to a different nutritional basis (Vogt et al., 2023).

DISCUSSION

The present study compared weaning and separation of calves through either 2-step weaning with nose flaps or a gradual reduction of contact time between cow and calf. Results showed that the weaning and separation procedure induced a considerable distress response in calves regardless of the weaning method, but revealed differences between the 2 methods in behavioral as well as physiological indicators (Table 2). Considering the whole 3-wk weaning and separation phase, NF calves showed a stronger decrease in the number of lying bouts, locomotor play, and ADG, as well as a higher increase in TMR feeding time compared with baseline than GR calves, whereas GR calves vocalized more often and showed more searching behavior than NF calves. Also, a tendency for an increased neutrophil percentage and a significantly decreased lymphocyte percentage compared with baseline could be found for NF, but not for GR, calves during the weaning and separation phase. Overall, in line with our hypotheses, results point toward a favorable effect of the GR method on reduction of weaning and separation distress in calves, which will be discussed in the following sections.

Physiological Adaption to the New Diet

Calves of both treatments showed only a modest roughage intake during the baseline phase, when milk was still available ad libitum, and hence reacted with a decrease in ADG once the weaning process started. This is in line with results of beef suckler calves weaned with nose flaps (Haley et al., 2005; Boland et al., 2008; Enríquez et al., 2010) as well as of artificially reared dairy calves in which the increased starter intake could not compensate for the reduced milk intake during gradual weaning (Sweeney et al., 2010). The lower ADG of the NF calves compared with our GR treatment over the whole weaning and separation phase was expected because GR calves were still able to suckle milk from their dams for 2 wk of the weaning phase, whereas the NF calves abruptly lost the milk and had to meet their nutritional requirements solely from solid feed. Accordingly, the TMR feeding time of NF calves over the whole weaning and separation phase was about twice as high as that for GR calves. Additionally, these high TMR feeding times could result from an altered, potentially more cautious, feeding behavior of calves with the nose flap, leading to a lowered feeding rate at the feeding rack. Thus, even if the feeding duration differed between our treatments, the actual feed intake might have been the same, which is also supported by the similar rumination times of calves in both treatments.

Importantly however, NF calves lacked an increase in ADG during the third week with fence-line contact, despite the highest TMR feeding times at that point and without impairment through the nose flap. In contrast, GR calves increased their TMR feeding and weight gains during the weaning and separation process and showed the highest ADG in the third week, which did not differ from their baseline at that time. This might be explained by an improved adaption of the gastrointestinal tract (**GIT**) to the changed diet in the 2 treatments, including a potentially smoother transition of the gut microbiome from the pre- to postweaning state (Li et al., 2012; Rey et al., 2014; Meale et al., 2016, 2017) with the gradual weaning method, as compared with the abrupt change in NF calves. In this regard Steele et al. (2017) discussed that increased starter intake in gradually weaned calves can potentially accumulate in the rumen as substrate for microbial fermentation, creating a greater nutrient pool to support growth. Accordingly, studies in artificially fed dairy calves showed that postweaning weight gains of gradually weaned calves are usually higher than those of abruptly weaned calves and prevent the typical depression in postweaning weight gains (e.g., Khan et al., 2007; Sweeney et al., 2010; Omidi-Mirzaei et al., 2015). In contrast, the abrupt loss of milk through the nose flap likely had not allowed enough time for a sufficient transition of the gut microbiome for an effective maintenance of body weight from solid feed after abrupt cessation of milk feeding. This is in line with the results of van Niekerk et al. (2021), who reported a delay in rumen structural development, inadequate short-chain fatty acid absorption, and reduced ruminal pH (acidosis) for up to 2 wk postweaning in calves that were weaned in one step from a high milk replacer diet. In addition, it has been shown that hay consumption from hay racks is restricted by the nose flap (Barth et al., 2015), which might have additionally reduced ruminal pH and delayed adaption of the GIT in our NF calves, because hay intake during weaning stimulates rumen development with a positive effect on ruminal pH (Khan et al., 2011; Castells et al., 2013; Pazoki et al., 2017) and ruminal bacterial diversity and abundance (Kim et al., 2016) and thus affects the establishment of its fermentative functions. An impeded adaption of the GIT in our NF calves was further supported by the results of our hematological variables. There was a tendency for an increased neutrophil percentage and a significantly decreased lymphocyte percentage during the whole weaning and separation phase in NF calves, whereas we found no significant change in relation to baseline values in GR calves at any sampling day. Such an increased neutrophil percentage and decreased lymphocyte percentage, resulting in an increased neutrophil: lymphocyte ratio, was equally reported for beef calves in response to abrupt weaning (e.g., Hickey et al., 2003; de Souza Teixeira et al., 2021), but no neutrophilia and lymphopenia were found in gradually weaned dairy calves fed at an automatic milk feeder (**AMF**; Johnston et al., 2016), which is in line with our findings. This increase in inflammatory blood markers can result from different weaning stressors leading to elevated glucocorticoid levels in the bloodstream (discussed in Burton et al., 2005; de Souza Teixeira et al., 2021), but might in this regard be especially likely to reflect a temporary hindgut acidosis of the calves during the weaning process as hypothesized by Steele et al. (2016). Thus, our hematological markers may mainly reflect an inflammation of the GIT in consequence of the abrupt change in diet in NF calves or the better nutritional adaption through the GR treatment. This is further supported by the reduced lying bout frequency and play behavior of NF calves compared with our GR calves, which might also be related to the different energy levels of the calves in consequence of a different speed of adaption of the GIT. Generally, calves of both treatments showed reduced lying times compared with baseline values during the weaning and separation process, which is in line with the literature; increased activity levels and reduced lying times have been frequently reported for beef (Budzynska and Weary, 2008; Hötzel et al., 2010) and dairy calves (Jasper et al., 2008; Eckert et al., 2015) in response to weaning. In our study, this was likely caused by conversely increased standing times at the feeding rack, as well as increased time spent for searching behavior in the GR treatment. The reduced number of lying bouts in NF calves compared with our GR calves was, however, contrary to expectations because we predicted more but shorter lying bouts in hungrier calves due to enhanced restlessness. Nonetheless, our results are in line with a study by Black et al. (2017), which reported a reduced lying bout frequency in dairy calves following abrupt weaning. Potentially, this could be a consequence of the energy deficit of calves during weaning, because increased bout duration reduces the number of necessary raisings and conserves energy. This is partly supported by the fact that dairy cows show less but longer lying bouts during cold conditions compared with hot or thermoneutral climates (Lovarelli et al., 2020). Also, the reduced amount of locomotor play of NF calves compared with GR calves during the weaning and separation phase supports this assumption; past research has repeatedly shown that the amount of locomotor play in calves decreases with a low energy intake (Krachun et al., 2010; Duve et al., 2012; Rushen et al., 2016). The high levels of locomotor play of GR calves in the first 2 wk of the weaning process while still being able to suckle milk are in line with this.

Last, we found the highest increase in FGCM concentrations within the NF treatment in the first week compared with the other weeks of the weaning and separation process, which is in line with hematological responses and equally points toward a heightened distress response in NF calves directly after insertion of the nose flap. However, it has to be kept in mind here that the composition of the gut microbiome was probably profoundly different in the first week of wearing the nose flap compared with afterward, due to the progressive transition toward a solid diet with time. We have shown in a previous study that FGCM concentrations in the same calves will be higher when the calves are fed a primarily milk-based diet in comparison to a diet based on solids (Vogt et al., 2023), thus the high FGCM concentrations in NF calves in the first week without milk are especially noteworthy. Nevertheless, the change in diet brings nuisance to the FGCM results and complicates comparability not only between weaning methods but also between weaning stages. Therefore, we conclude that FGCM concentrations are of limited use as a marker for weaning distress, and this should be considered in the design of future studies.

Lack of Vocalizations Seems not Equivalent to Absence of Distress

Contrary to our hypothesis and also contrary to the aforementioned results, we found significantly more vocalizations and searching behavior in GR calves compared with the NF calves over the whole weaning and separation period. For the NF calves this is in line with the literature because a low frequency of vocalizations has been repeatedly reported in studies on beef (Haley et al., 2005; Enríquez et al., 2010) and dairy calves (Loberg et al., 2008) weaned with a nose flap. However, in contrast to the generally accepted view, we argue that this low frequency of vocalizations does not indicate that the NF calves experienced less distress during the weaning and separation procedure, because our remaining indicators do not support this assumption, but rather reflects the different situation regarding the necessity of vocal communication between cow and calf. In general, contact calls of cow-calf pairs seem to be linked to the desire to reunite and the need to obtain milk or have milk removed from the udder because calls are often followed by reunion and nursing events (Padilla de la Torre et al., 2015) and increasing time spans between nursing events increase the probability that one or both members of a cow-calf-pair vocalizes (Watts, 2001). Also, calf vocalizations are likely to be linked to hunger because bottle-fed calves call less frequently and at a lower fundamental frequency when fed higher amounts of milk (Thomas et al., 2001). This makes sense in the view of the honest signaling theory: the energy cost and risk of being caught by predators during vocalizing is high, and thus the frequency of calling must be balanced against the urgency of obtaining resources from the dam (dicussed in Weary and Fraser, 1995; Enríquez et al., 2011). Interestingly, however, De Paula Vieira et al. (2008) reported a lack of vocalizations in dairy calves fed at an AMF, even though their nutritional needs were verifiably not met. It might therefore be reasonable to assume that calves' vocalizations are not simply an expression of hunger, but more an attempt to regain contact with the dam or communicating with a caretaker that has been previously associated with milk provision (De Paula Vieira et al., 2008). Accordingly, there is less need for vocalizations by the calf when direct contact with the cow is already re-established, which was the case for our NF treatment. Similarly, searching behavior in this study covered only standing next to or walking up and down the pen barrier or standing with the head through the selection gate, for which NF calves had a reduced necessity due to access to the cow herd for most of the treatment. In line with this, beef calves weaned with a nose flap showed less fence-line pacing compared with abruptly or fence-line weaned calves (Enríquez et al., 2010), which might equally be explained by their possibility of direct contact with the dam. Altogether it might therefore be stated that calves that show searching behavior with or without vocalizations are often likely to be hungry or wish to reunite with the dam for other reasons, but that in turn it seems not reasonable to assume that calves that are not showing these behaviors are not stressed and that their nutritional needs are satisfied.

Frustration of Behavioral Needs During Weaning

Although the higher frequency of vocalizations and proportion of searching behavior in GR calves compared with NF calves over the whole weaning and separation period seem to reflect mainly the differing necessity for vocal communication with the dam over a distance, it is noteworthy that NF calves showed almost no searching behavior and vocalizations in response to fence-line separation from the dam in the third week, whereas GR calves reacted to this step with the highest increase in these behavioral responses, despite the presumably better nutritional adaption. This signals a high motivation of GR calves to reunite with the dam, which is supported by the comparatively high proportion of GR calves that gained illegitimate access to the cow area over the whole weaning period, but especially during the third week with fence-line contact. The main explanation here might be that suckling of the dam was still possible for GR calves until the fence-line separation commenced, which has been shown to lead to oxytocin release in calves (e.g., Lupoli et al., 2001; Uvnäs-Moberg et al., 2001) and does reinforce the strength of the cow-calf bond. Particularly, dairy calves that had only partial contact with their dam, without suckling, up from birth showed little distress in response to debonding (Wenker et al., 2022), and also cows showed a greater motivation for calf contact in the days following parturition when suckled compared with nonsuckled controls (Wenker et al., 2020). Thus, the prevention from suckling through the nose flap has probably loosened the cow-calf bond in the NF treatment, whereas the continued possibility of GR calves to suckle has presumably maintained the bond to the dam and consequently led to frustration when access to their dam was denied in the third week. This is in line with results in beef calves, for which fence-line separation has equally been shown to cause increased frustration levels (Enríquez et al., 2010). Given the comparably high weight gains of GR calves in the third week, the high increase in vocalizations and searching behavior is more likely a reaction to the loss of access to the dam per se, rather than to the loss of milk, which is supported by the fact that abrupt maternal separation leads to a pessimistic judgment bias in dairy calves, even when the calves are nutritionally independent (Daros et al., 2014). With regard to the NF calves, it can be assumed that the inability to obtain milk from the dam during the weeks with the nose flap had equally caused a state of frustration in the NF calves, because they were not able to suckle milk from the dam despite the perceived potential for access to the udder (Enríquez et al., 2010, 2011). In fact, exposure of fasted animals to situations in which they previously learned to expect food, but could unexpectedly not obtain it, has been used as an experimental technique to create a state of frustration in study animals in the past (Lewis, 1999; Mason and Burn, 2011). This assumption is supported by the observation that the NF calves in our study as well as beef calves weaned with a nose flap (Enríquez et al., 2010) showed a high number of unsuccessful suckling attempts for 2 to 4 d after the nose flap was inserted. Such repeated attempts to consume an inaccessible food have been described as a typical behavioral reaction in situations were strong motivations are frustrated (Mason and Burn, 2011). Furthermore, the severe drop in locomotor play behavior in the 2 wk while wearing the nose flap, which was found in our study and also in beef calves (Enríquez et al., 2010), equally points toward a possible negative affective state in these calves, but is likely confounded with reduced energy intake as discussed above. Generally, play behavior is discussed as an indicator of positive welfare in animals (Boissy et al., 2007; Held and Spinka, 2011) and a reduction of play behavior in calves indicates, among other things, impaired welfare such as pain (Mintline et al., 2013) or social deprivation (Jensen et al., 2015). Because the nose flap likely caused discomfort or potentially even pain in calves and has potentially impeded several behaviors such as self- or allogrooming as well as hay intake and milk consumption, it is probable that wearing the nose flap created a state of frustration in NF calves, which was reflected in the reduced play levels. In contrast, removal of the nose flap would have caused an improved affective state due to a relieflike effect, which could partly explain the enhanced play levels of NF calves in the third week despite the presumably low energy level as reflected in ADG. In line with this, studies on other ruminants showed that the release from different stressors (restraint, isolation, shearing) creates a positive judgment bias in animals (Doyle et al., 2010; Sanger et al., 2011). Differences in space allowance also strongly affects play levels in calves (Waiblinger et al., 2020) but provide no explanation for the changes in locomotor play within the NF treatment or the differences among the GR calves, because calves with the longest access to the additional space in the cow section (NF calves in wk 1 and 2) played the least. Last, the reduced lying bout frequency of NF calves compared with their baseline might additionally hint toward a negative affective state in NF calves because reduced activity levels have been discussed as being related to a depressionlike state in nonhuman animals as well (Fureix and Meagher, 2015). For this reason, it seems possible that the low levels of vocalizations and searching behavior of NF calves in response to fence-line separation from the dam could point not only toward a weakened cow-calf bond, but might additionally stem from the negative affect of frustration which further persisted in the third week and decreased their motivation to enter the cow section. Importantly however, NF calves showed no reduction in brush use, which has been reported in dairy cows in reaction to stressors like acute metritis, heat stress, regrouping, or cow and calf separation (Mandel et al., 2013, 2017; Lecorps et al., 2020, 2021) and has been discussed as indicating an anhedonia-like affective state in cows (Lecorps et al., 2020, 2021). The lack of reduction in brush use in response to weaning with the nose flap in our study is contrary to our other indicators and suggests that brush use might not be a similarly reliable indicator for stress in calves like it is for older cattle, at least not for weaning distress. One possible alternative could be that brush use during weaning in dam-reared calves could act as a partial substitution for the loss of maternal grooming as has been suggested for artificially reared calves by Zobel et al. (2017). Future studies are encouraged here to enhance our understanding of brush use in dairy calves during stressful events.

General Evaluation of the 2 Weaning Methods

On the whole, results of our study suggest that both types of weaning and separation methods caused a distress response in the calves. However, the abrupt change in diet during 2-step weaning with the nose flap apparently compromised adaption of the GIT and led to a greater energy deficit in NF calves compared with the gradual weaning method. The fact that the high TMR feeding times of NF calves were not reflected in respective high weight gains gives reason for concern because it indicates major impairment of feed intake by the nose flap. Next to the energy deficit, the severe reduction in lying bouts and locomotor play behavior in addition to the high number of suckling attempts with the nose flap, also hints toward a negative affective state in these calves. Because the nose flap also caused pressure marks and partly even injuries in the nasal septum of calves, 2-step weaning with nose flaps cannot be classified as a high-welfare weaning method. This is in line with recent results from Wenker et al. (2022), who compared different separation methods for cow-calf contact systems with varying types of contact and concluded that for dairy calves with full contact to the dam, the nose flap was the less effective method for reducing weaning distress compared with fence-line weaning. That said, it should be considered that classical fence-line weaning also leads to an abrupt termination of milk feeding and probably causes similar problems within the GIT, which merits further research.

Notwithstanding, our study demonstrated that the gradual weaning and separation method still provoked distress and frustration in the GR calves, especially in the third week with fence-line separation from the cow. Thus, even if the GR treatment seemed favorable compared with 2-step weaning with a nose flap, it requires further refinement from the protocol used in our study. In this regard, future studies should investigate the effect of enabling a closer contact between cow and calf during the last step of the gradual weaning and separation procedure, because separation by the selection gate used for the fence-line phase in our study provided the pairs with only very limited visual and tactile contact. Furthermore, a GR treatment with smaller steps will potentially be more successful, as suggested by a study in lambs that showed little response during definite weaning after a gradual increase in separation time from the dam from 2 to 23 h/d over a period of 2 mo (Orgeur et al., 1998). Nevertheless, many small reductions in cow-calf contact time might be problematic with regard to on-farm practicability because this may be associated with a considerable workload as long as there are no technical solutions such as automatic selection gates that are both affordable for farmers and reliably prevent unauthorized access of calves.

An additional factor to consider with the gradual weaning and separation method is that the results of the milk yields of the dams during evening milking (Barth et al., 2021) suggest that the calves in our GR treatment drank comparable amounts of milk during the daytime in the morning contact phase (wk 2) as during the daytime of the half-day contact (wk 1) and baseline phase. This implies that the calves partly compensated for the reduced contact time by drinking higher amounts of milk during the shortened access times during the day, but not enough to compensate for the lost suckling time during night. Consequently, the reduction in milk consumption of the GR calves only truly happened by prevention of suckling during the night and was therefore not as gradual as planned, but rather stepwise from baseline to wk 1. This is in line with results from Roadknight et al. (2022), who compared a full-time to a half-day cow-calf contact system and reported that half-day contact calves suckled twice as long around milking as calves with full-time access to their dams and that weight gains were similar between treatments. In general, it was a prominent result of our study that the highest changes in behavioral and physiological indicators of weaning distress of the calves occurred constantly at the time point of cessation of suckling and accompanying milk loss in both treatments (NF wk 1 and GR wk 3, Table 3). This was less clear for the GR calves because the separation from the dam happened at the same week and thus effects of milk loss and loss of the dam cannot be examined separately for this treatment. However, for the NF calves our indicators point toward a higher distress response after the removal of milk compared with separation from the dam and indicate that the former needs to be alleviated to achieve a comparably mild weaning and separation procedure. This is supported by studies on dam-reared dairy calves provided with additional access to an AMF, which were shown to spend more time playing (Rushen et al., 2016; Johnsen et al., 2018) but less time close to the separation barrier and to produce fewer vocalizations during or after separation from the dam than calves with no additional access to an AMF (Johnsen et al., 2018). Equally, Wenker et al. (2022) found that dairy calves that had partial contact with their dams without suckling, but were bucket fed, showed minimal signs of distress during the debonding process from the dam compared with calves with full cow-calf contact. Altogether, these results from the literature in conjunction with results from our NF calves underline that calves' weaning and separation distress is largely affected by the nutritional dependency from the dam. Therefore, the GR method will probably be more successful in alleviation of weaning and separation distress, if it can be ensured that calves really reduce suckling times and do not compensate for the reduced contact time by spending more time suckling and drinking larger amounts of milk in the remaining time.

Limitations

A limitation of our study was that no data collection took place after actual permanent total separation from the cow when calves were moved to a different barn, which is a main stressor during the weaning and separation process. However, data collection after movement to the youngstock barn would have been heavily influenced by introduction into a new herd, loss of known peers, new surroundings with different germs and different feed. These confounders would have left results not clearly attributable to the particular treatment and therefore, we limited our data collection to direct comparison of calves' distress responses in the third week in which cows and calves were kept separated in adjacent pens with fence-line contact through pen boundaries. This way, external factors were kept stable and we could ensure that calves' behavioral and physiological distress responses were caused by loss of direct contact to the dam, rather than, for example, an unfamiliar, dominant conspecific in the youngstock herd. Also, this way all direct behavioral observations were conducted by exactly the same observer. Future studies that are able to control such external stressors after permanent total separation from the dam are encouraged.

CONCLUSIONS

Results showed that both weaning and separation methods led to distress and likely caused frustration in calves. However, the gradual method was favorable compared to weaning with a nose flap in most indicators, foremost allowing a better adaptation to the dietary change. This included higher weight gains as well as a lower decrease in lying bouts and locomotor play levels in gradually weaned calves, indicating higher energy levels and a potentially less compromised affective state. Moreover, inflammatory blood markers increased in reaction to the NF but not to the GR weaning method. In conclusion, gradual weaning and separation is a promising method for dam-reared dairy calves, but it requires further improvement from the protocol used in our study. This recommendation needs however to be confirmed regarding effects on the dams, because a weaning and separation method must always consider both partners of a cow-calf-pair.

NOTES

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Nonstandard abbreviations used: AMF = automatic milk feeder; CPA = counts of peak acceleration; DF = degrees of freedom; dur. = duration; EIA = enzyme immunoassay; FGCM = fecal cortisol metabolites; freq. = frequency; GIT = gastrointestinal tract; GR = gradual reduction of contact time method; NF = 2-step nose-flap method.

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ORCIDS

Anina Vogt https://orcid.org/0000-0002-6896-2244
Kerstin Barth https://orcid.org/0000-0001-7070-6445
Susanne Waiblinger https://orcid.org/0000-0003-3565-4306
Uta König von Borstel https://orcid.org/0000-0002-7313-1197