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# Dairy calves' time spent in the cow herd in a calf-driven cow-calf contact system during two-step separation with a nose flap

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ABSTRACT

Although originally developed for weaning of beef calves, nose flaps are also used on dairy farms nowadays to wean calves which are reared with contact to their dam or a foster cow. These calves are often weaned younger than beef calves and the time they wear nose flaps is highly variable, since it is unknown for how long younger calves need to wear the nose flap in order to induce an effective weaning. The aim of our study was therefore to track changes in dam-contact initiated by dairy calves after insertion of a nose flap in order to determine the minimum duration a nose flap needs to be worn to effectively reduce suckling motivation of calves. The study was conducted in two sequential experiments using 3-month-old dairy calves that were reared with full-time dam contact. Calves were weaned with a nose flap over either 14 (experiment I, 9 calves in herd A and 9 calves in herd B) or 7 days (experiment II, 11 calves in herd A and 12 calves in herd B) before they were fence-line separated from their dams. The total time a calf spent within the cow herd (TIC) per day was determined from continuous video recordings during 7 days before nose flap insertion (baseline), as well as during the 7 or 14 days in which calves had free access to the cow area while wearing the nose flap. Statistical analysis was conducted using linear mixed effects models. Results from both experiments showed that calves reduced their TIC at the fourth day of wearing the nose flap compared to their individual baseline ($p=0.03$ and $p=0.001$ respectively). In experiment I, calves showed a further numerical reduction in TIC from day 4 to day 5, while in experiment II, calves showed no further reduction in TIC after the 4 days of wearing the nose flap. After these 4 or 5 days respectively, TIC of calves in both experiments stayed at a constant low level of several hours per day. Taken together, these results indicate that in 3-month-old dairy calves it needs at least 4 days until the motivation to spend time with the cow decreases after insertion of a nose flap, while there seems to be no further decrease in motivation when using a nose flap longer than this time. However, results were subject to considerable inter-individual variability already during the baseline week and need replication in a pasture-based setting with less space-restrictions.

## 1. Introduction

Two-step weaning and separation of cow and calf entails that prior to permanent total separation of the pair, suckling by the calf is prevented with a so-called nose flap (defined by Sirovnik et al., 2020). This way the calves' stress of losing milk as their main nutritional source and losing contact to the dam is dispersed over two time points, which is why this method is regarded as an advantageous alternative to abrupt weaning and separation in beef calves (Haley et al., 2005). With the emergence of dairy cow-calf contact (CCC) systems, two-step weaning with nose flaps is also discussed as an option for dairy calves which were reared with contact to their dam or a foster cow (Barth et al., 2022; Schneider and Ivemeyer, 2021; Sirovnik et al., 2020) and could be especially valuable for weaning of such calves in pasture-based environments where options for alternative weaning strategies are more limited. For example, supplementary milk feeding with an automatic milk feeder during the hours of separation from the dam in combination with fence-line (Johnsen et al., 2018) and gradual (Johnsen et al., 2024) separation methods

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successfully reduced weaning distress in the past and will be more challenging in a pasture-based system, which equally applies to separation of pairs via a gradual reduction of cow-calf contact time with the assistance of automatic selection gates (Vogt et al., 2024). However, in the dairy CCC systems that practice two-step weaning with nose flaps so far, the calves are typically weaned at a much younger age than beef suckler calves for which the nose flaps were originally developed, and the time span calves wear the nose flaps is highly variable, ranging from a few days up to a month (Barth et al., 2022). In this context it is important to consider that nose flaps have been shown to cause reduced weight gains in beef calves (Boland et al., 2008; Enriquez et al., 2010; Valente et al., 2022), and for parts of the animals, lesions, bleeding and/or inflammation at the calves' nasal septum was evident after usage for 5–7 days (Lambertz et al., 2015; Taylor et al., 2020; Valente et al., 2022). Additionally, the calves' welfare might already be reduced even before lesion development, e.g. through discomfort or pain as well as interference with self-grooming or exploratory behavior, which has to our knowledge not been systematically measured in the aforementioned studies or elsewhere yet.

Regarding the duration of nose flap usage, Alvez et al. (2016) reported no difference in weight gains or behavioral indicators of distress in beef calves that wore nose flaps for 7 or 21 days prior to permanent separation from the dam. Equally, no differences in vocalizations after total separation from the dam were found in beef calves wearing the nose flap for 3 or 14 days beforehand (Haley et al., 2005). Given these results, nose flap usage for short time periods appears favorable and might potentially also be an option to prevent lesion development in the calves, but up until today it is unknown how long it actually takes for the suckling motivation to cease after insertion of a nose flap. Therefore, the aim of our study was to track changes in dam-contact initiated by the calves after insertion of a nose flap in order to determine the minimum time period the nose flap needs to be worn until the calves show reduced motivation to spend time within the cow herd as a proxy for suckling motivation. We hypothesized that calves would reduce their time spent with the cows around 5 days following insertion of the nose flap, given that Price et al. (2003) reported that fence contact to the dam declined significantly over 5 days following fence-line weaning in 7-month-old beef calves.

## 2. Material and methods

The study consisted of two sequential experiments, which were both carried out in the experimental barn of the Thünen Institute of Organic Farming in Germany. There, cows and calves are kept in two separate herds (A and B) in identically mirrored parts of the same barn (see Wagner et al., 2012 for a scheme of the barn). Experiment I was conducted from 11/2019–03/2020 as part of a larger study by Vogt et al. (2024), which compared calves' stress responses during different weaning and separation methods and a detailed description of the methods can be found there. Experiment II was conducted over the following two years (08/2020 – 04/2021 and 08/2021 – 06/2022) as part of the study by Schneider et al. (2024), which compared the effects of whole-day contact to half-day contact between cow-calf pairs. During the first year of experiment II, pairs from herd A had whole-day contact and pairs from herd B had half-day contact, which was reversed in the second year, so that pairs from herd B had whole-day contact and pairs from herd A had half-day contact. For the present study, we only included the pairs with whole-day contact at the weaning stage from the study by Schneider et al. (2024).

The required sample size for the study of Vogt et al. (2024) was calculated a priori using G*Power 3.1 (Faul et al., 2007) for an assumed large effect (f=0.5) with an acceptable α-error of 5 % and β-error of 10 %. For the study by Schneider et al. (2024), a post-hoc power analysis was conducted using R (Version 4.3.1) and setting $\alpha = 5$ %, which returned a high power ($\geq 0.98$).

### 2.1. Animals and housing

There were 18 calves included in experiment I (9 calves in herd A and 9 calves in herd B) and 23 calves in experiment II (11 calves in herd A and 12 calves in herd B, see Table 1). All calves and their dams were of the German Holstein breed. The animals were kept in two separate herds (A and B) in an open sided free-stall barn. Each part of the barn is comprised of a lying area separated into 51 straw-bedded cubicles (128.0 $m^2$ plus additional 64.0 $m^2$ headspace), a feeding area (237.5 $m^2$) and a rubber-coated walking area (298.0 $m^2$) for the cows.

Calves were housed in their own separate calf area, which was directly connected to the cow area of the respective herd via automatic selection gates. In the calf area, calves were provided with water, hay, a total mixed ration, concentrate feed, a calf brush (Schurr Gerätebau GmbH, Germany), a walking area (71.9 $m^2$) and a straw bedded lying area (12.6 $m^2$; and an additional 12.0 $m^2$ during the whole study period of experiment I and for part of the study period of experiment II in both herds whenever this was required due to a too high number of calves). All calves were reared with full-time contact to their dam up from birth, i.e. calves were free to enter the cow area through the selection gate during the whole day, except for the two daily milking times (starting at about 5 a.m. and 4 p.m.). In the cow area, calves had access to the lying and walking space of the cows, but could not enter the cows' feeding area. Group size of the cow herd in experiment I varied from 43 - 48 cows (herd A) and 45–49 cows (herd B) throughout the experiment. In experiment II, group size of the cow herd ranged from 32 - 47 cows (herd A) and 28–46 cows (herd B). This was due to inclusion of primiparous cows into the herd and/or selling and culling of older cows.

### 2.2. Experimental treatment

All calves were equipped with a nose flap (Quiet Wean, JDA Livestock Innovations, Canada) for initiation of the weaning process when they were about 3 months old (details see Table 1). During experiment I the nose flap remained in the nostrils for 14 days, while in experiment II, the calves were equipped with the nose flap only for 7 days. During the days when the calves wore the nose flap, they continued to have full-time access to the cow area including their mothers via the selection gate. Afterwards, the nose flap was removed and calves had one more week of fence-line contact to their dams through the pen boundaries between the cow and the calf area, before they were fully separated by moving the calves to the Youngstock barn. In both experiments the nose flap was inserted at around 7:30 a.m.

**Table 1**
Overview of attributes of calves included in the study.

| | Experiment I Herd A | Experiment I Herd B | Experiment II Herd A | Experiment II Herd B |
|---|---|---|---|---|
| Term of experiment | 11/2019–03/2020 | 11/2019–03/2020 | 08/2020–04/2021[#] | 08/2021–06/2022[#] |
| No. of calves | 9 calves | 9 calves | 11 calves | 12 calves |
| Sex of calves | 4 female/5 male | 4 female/5 male | 4 female/7 male | 7 female/5 male |
| Horn status | all polled | all horned | all polled | all horned |
| Age of calves at day of nose flap insertion (range, mean ± S.D.) | 83–102 days (92.7 ± 7.2 days) | 89–101 days (95.1 ± 5.9 days) | 90–96 days (93.5 ± 2.3 days) | 91–96 days (93.0 ± 2.0 days) |
| Number of days wearing the nose flap | 14 days | 14 days | 7 days | 7 days |

[#] weaning of the calves happened in the period between 12/2020-03/2021 (herd A) and 11/2021-05/2022 (herd B).

*2.3. Data collection and analysis*

As described above, calves were free to enter the cow area voluntarily during the pre-weaning phase as well as when wearing the nose flap. The selection gate which allowed calves to change sides from the calf area into the cow area was video monitored for 24 hours per day. From these videos, the total duration a calf spent within the cow herd (time in cow herd, **TIC**) was determined per day using BORIS software (Friard and Gamba, 2016, Version 7.9.22) during one week before the nose flap was inserted (baseline), as well as the one (experiment II) or two weeks (experiment I) while the calves wore the nose flap. A calf was considered to have entered or exited the cow area, if the calf's rear end, including the tail, was behind the top bar (videos were recorded from above) of the selection gate. Video analysis in experiment I was done by two different observers (Cohen's kappa $\kappa = 0.99$) and in experiment II by six different observers (Fleiss' kappa $\kappa = 0.92$).

For further analysis the mean TIC of a calf during the baseline week was calculated to define the individual baseline value per calf. This baseline value was then subtracted from the TIC of a given day of wearing the nose flap (hereafter called treatment day) to calculate the difference to baseline for each treatment day. Statistical analysis was done separately for both experiments using linear mixed effects models with repeated measures (Littell et al., 1998) in SAS Version 9.4, with differences to baseline as the dependent variable. As fixed effects, the models included treatment day (days 1–7 or days 1–14 for exp. II or I respectively), sex of the calf (male/female) and parity group of the cow (1st lactation/2nd-3rd lactation/4–7th lactation), as well as the continuous variables age when the nose flap was inserted (in days) and size of the cow herd (heads of animals in the herd at the observation day). The calf ID nested within herd was included as random effect and additionally, the calendar week of the year merged with herd was included as a cross-classified random effect to account for the dynamic group composition in the calf and cow area of each herd throughout the studies (Cafri et al., 2015). The covariance structure between repeated measures was set to autoregressive. In order to test for significant changes in TIC during the treatment days compared to baseline, it was tested whether the differences to baseline differed from zero. In order to test for differences between treatment days, all possible post-hoc pairwise comparisons between treatment days were calculated using a Tukey-Kramer post-hoc test (Rafter et al., 2002). Model requirements (normal distribution and homoscedasticity of residuals) were checked graphically. Confidence intervals for the estimates of TIC for each treatment day are presented in supplementary tables S1 and S2.

During the studies, calves' health status was routinely checked daily by the trained barn personnel of the research station according to a specific checklist. Calves which were scored as severely sick, i.e. had fever or needed medical and/or veterinary treatment, were excluded for the respective day as well as one day before and afterwards each before statistical analysis. Additionally, days with technical failures of the videos were excluded, e.g. when some hours of a day were missing on videos. In total 35 out of 378 data points were excluded in experiment I, and 26 out of 322 data points were excluded in experiment II.

## 3. Results

### *3.1. Experiment I*

The time that calves spent in the cow area during the baseline week ranged from 2.6 to 16.6 hrs/day (mean±S.D.: 5.4 ±3.9 hrs/day) in herd A and from 0.9 to 8.3 hrs/day (mean±S.D.: 1.9 ±1.8 hrs/day) in herd B (see Fig. 1). Within a calf, differences between the shortest and longest TIC per day during the baseline week rangend from 2.2 to 12.1 hrs/day (5.9±3.1 hrs/day) in herd A and from 1.2 to 6.1 hrs/day (2.4±1.5 hrs/day) in herd B, but exceeded $\geq$ 6 hours only for 5 of the 18 calves (4 calves from herd A and 1 calf from herd B, see Fig. 1).

The number of days the nose flap was worn influenced the time the calves spent in the cow herd ($F_{13,163}=16.8$, $p<0.0001$). During the first (least square means (LSM) ± S.E.:+0.98±0.8 hrs/day, $t_{24}=1.2$, p= 0.24) and second day (+1.71±0.8 hrs/day compared to the individual baseline, $t_{24}=2.1$, p=0.04) of wearing the nose flap, the calves increased

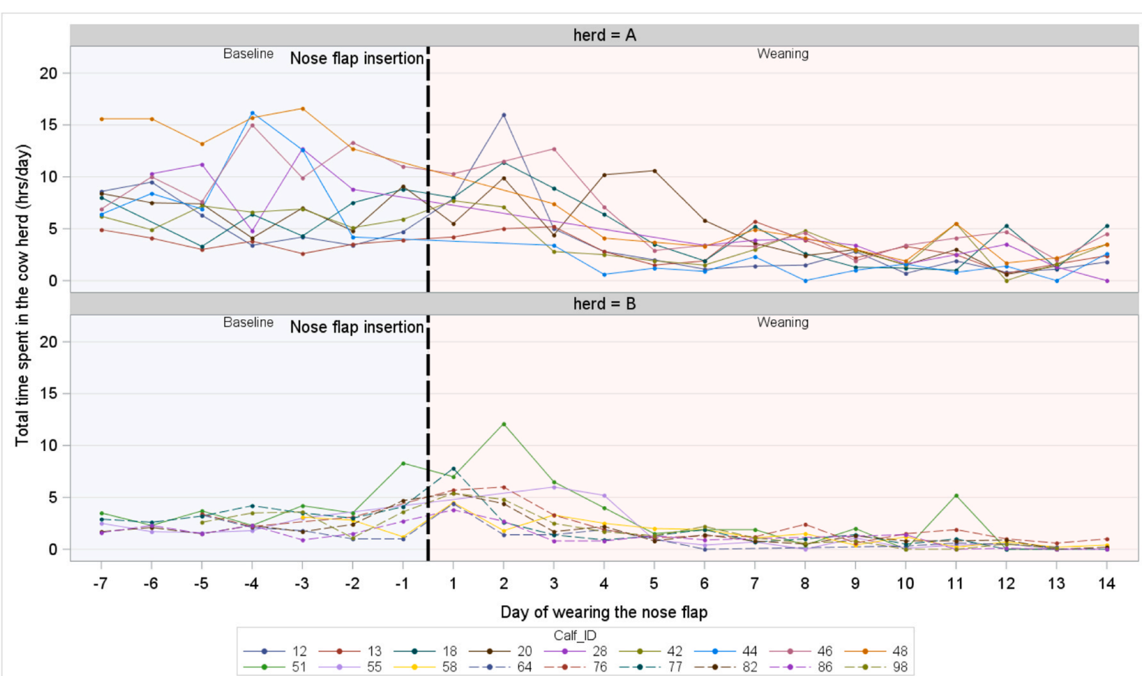


**Fig. 1.** Individual daily time spent within the cow herd (TIC) including the calves' mothers in hours per day of dam-reared dairy calves during one week before and two weeks while wearing a nose flap (experiment I). Data are displayed separately for the two herds in the barn (n=9 calves per herd). The nose flap was inserted at day 1 in the morning (dashed lined was offset to leave data points on day 1 visible). Missing data points for single days refer to days at which calves were classified as sick or contact times could not be fully retraced due to technical failure (n=35).

their TIC compared to baseline, however, this increase was only significant at the second day (Fig. 2). Up from day 3, TIC of calves decreased and the calves spent significantly less time within the cow herd compared to their individual baseline for the first time at the forth day of wearing the nose flap (-1.9±0.8 hrs/day, $t_{22}$=-2.4, p=0.03, Fig. 2). There was a further reduction in calves' TIC on the fifth day of wearing the nose flap (day 5: −3.0±0.8 hrs/day compared to individual baseline), that was significantly lower than on day 3 ($t_{176}$=4.2, p=0.004), but only numerically lower compared to day 4 ($t_{150}$=2.4, p=0.49, Fig. 2). Up from the 5th day of wearing the nose flap there was no further reduction in TIC until the end of the observation period (pairwise comparisons of days 5–14 all p > 0.05, Fig. 2). The mean time (mean ± S.D.) that calves still spent in the cow area at day 4 was 3.4±2.6 and at day 5 was 2.3±2.3 hrs/day.

Sex and weaning age of the calf, as well as parity of the cow or group size of the cow herd did not significantly influence TIC (all p>0.1). On the other hand, the random effect of calf ID nested in herd significantly influenced the measured time that calves spent in the cow herd (Z=2.3, p=0.01, see also Fig. 1).

### 3.2. Experiment II

Baseline TIC between calves ranged from 1.7 to 19.1 hrs/day (mean ±S.D.: 11.0 ±4.4 hrs/day) in herd A and from 0.8 to 16.9 hrs/day (mean ±S.D.: 4.5 ±3.7 hrs/day) in herd B (see Fig. 3). Within calves, differences between the shortest and longest TIC per day during the baseline week rangend from 2.6 to 12.3 hrs/day (6.1±3.0 hrs/day) in herd A and from 1.2 to 14.7 hrs/day (5.5±3.4 hrs/day) in herd B, but exceeded ≥ 6 hours only for 8 of the 23 calves (4 calves from herd A and 4 calves from herd B, see Fig. 3).

The number of days that calves wore the nose flap influenced the time the calves spent in the cow herd ($F_{6,80}$=10.5, p<0.0001). Compared to baseline, the calves numerically increased their TIC in the first two days after insertion of the nose flap (day 1: +0.23±0.8 hrs/day, $t_{44}$=0.3, p=0.76, day 2: +0.26±0.8 hrs/day compared to individual baseline, $t_{49}$=0.3, p=0.74, Fig. 4). Afterwards TIC of calves decreased and was significantly reduced compared to baseline up from the forth day of wearing the nose flap (-2.78±0.74 hrs/day, $t_{40}$= -3.8, p=0.001, Fig. 4). Calves showed no further reduction in TIC after the 4th day of wearing the nose flap (pairwise comparisons of days 4–7 all p > 0.05, Fig. 4). The mean time (mean ± S.D.) that calves still spent in the cow area at day 4 was 5.4±5.8 hrs/day.

Group size of the cow herd significantly influenced TIC of calves ($F_{1,21}$=5.8, p=0.03), since the time that calves spent in the cow area decreased (-0.41±0.17 hrs/day) per head of animals that the cow herd increased. Also, the measured TICs were significantly influenced by the random effect of calf ID nested in herd (Z=2.0, p=0.02, see also Fig. 2).

Sex and weaning age of the calf, as well as parity of the cow did not significantly influence TIC (all p>0.1).

## 4. Discussion

Results of this explorative study showed that insertion of a nose flap significantly altered the time that calves spent within the cow herd, which was measured as a proxy for suckling motivation. Whilst there was a considerable individual variability in TIC already during the baseline period between calves, we still found a general pattern in both experiments that calves increased their TIC, at least numerically, during the first two days after insertion of a nose flap, followed by a reduction in TIC that remained at a constant, reduced level starting from day 4–5 after insertion of the nose flap with no further decrease afterwards. In both experiments, calves spent significantly less time within the cow area after 4 days of wearing a nose flap compared to their individual baseline, which implies that the motivation for dam contact of these calves was reduced after this time without milk provision. Largely in line with this, fence-line separated beef calves show a significant decline in time spent within 3 m of the fence over 5 days following introduction of the fence-line (Price et al., 2003). Similarly, Enriquez et al. (2010) found a significant reduction in percentage of beef calves observed within 5 m of the fence over the first 3 days after start of fence-line weaning, with no further reduction at days 4 and 5. In the same study, beef calves that were not weaned via fence-line separation but with nose flaps, showed a slight increase in vocalizations after fitting of the nose flaps, but this vocal response had returned to zero at the 5th day after nose flap insertion as well (Enriquez et al., 2010). Furthermore, it is interesting to note that calves in experiment I showed a significant increase in TIC at the second day of wearing the nose flap, which is in agreement with results of fence-line separated beef calves which also showed the highest behavioral reaction 48 hours after separation through the fence (Pérez-Torres et al., 2016; Solano et al., 2007). In general, increased walking activity in calves for the first 1–2 days after fitting of the nose flap, which quickly decreases afterwards, was reported in several studies

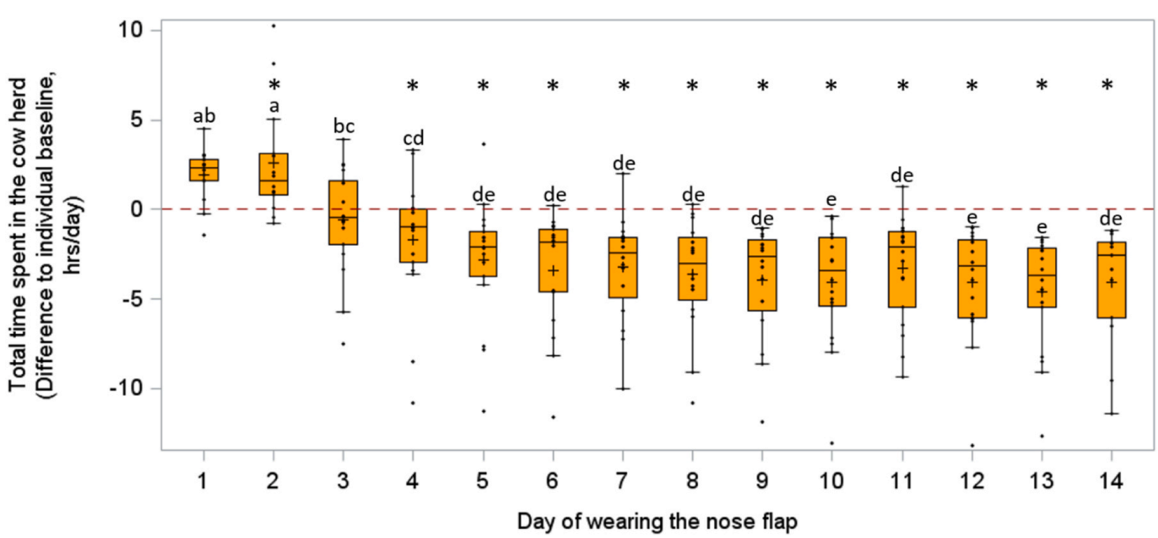


**Fig. 2.** Time spent within the cow herd (TIC) including the calves' mothers expressed as difference to the individual mean baseline in hours per day of dam-reared dairy calves (n=18) after insertion of a nose flap in the morning of day 1 (experiment I). Asterisks indicate differences of days of wearing a nose flap from baseline at p <0.05. Lowercase letters (a-e) indicate differences between days of wearing a nose flap at p <0.05. The plus symbol indicates the mean. Diamonds display the values of the individual calves at each day.

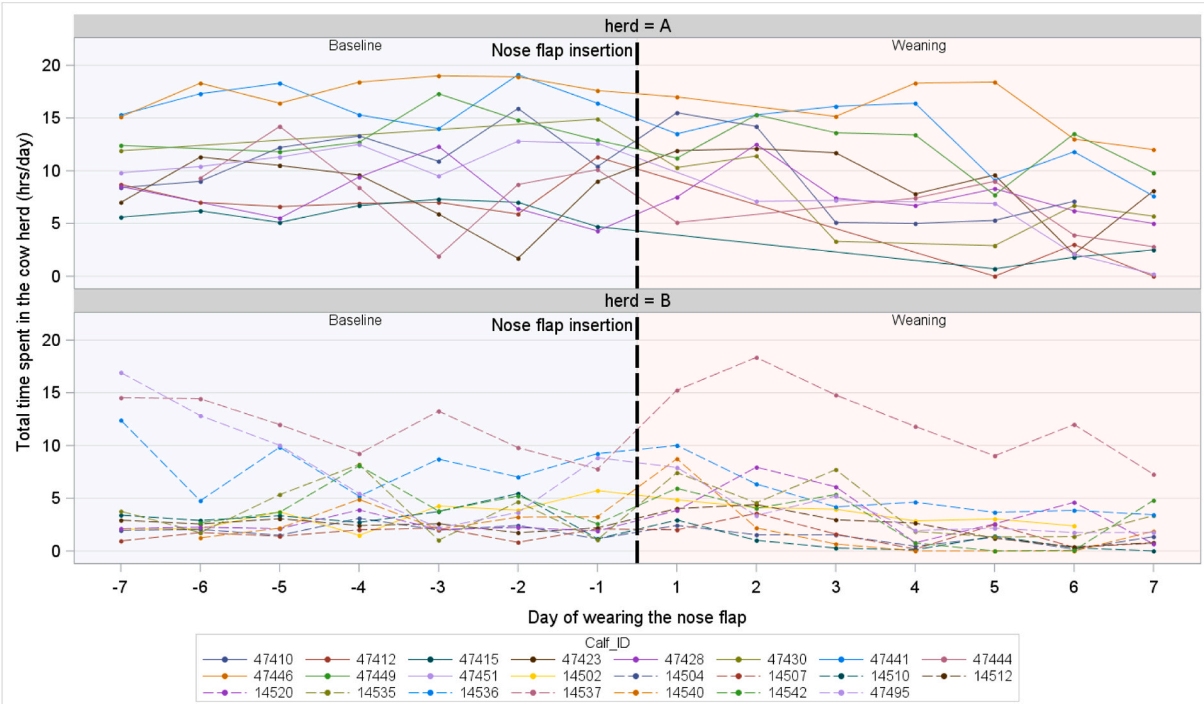


**Fig. 3.** Individual daily time spent within the cow herd (TIC) including the calves' mothers in hours per day of dam-reared dairy calves during one week before and one week after insertion of a nose flap (experiment II). Data are displayed separately for the two herds in the barn (herd A: n= 11 calves, herd B: n = 12 calves). The nose flap was inserted at day 1 in the morning (dashed lined was offset to leave data points on day 1 visible). Missing data points for single days refer to days at which calves were classified as sick or contact times could not be fully retraced due to technical failure (n=26).

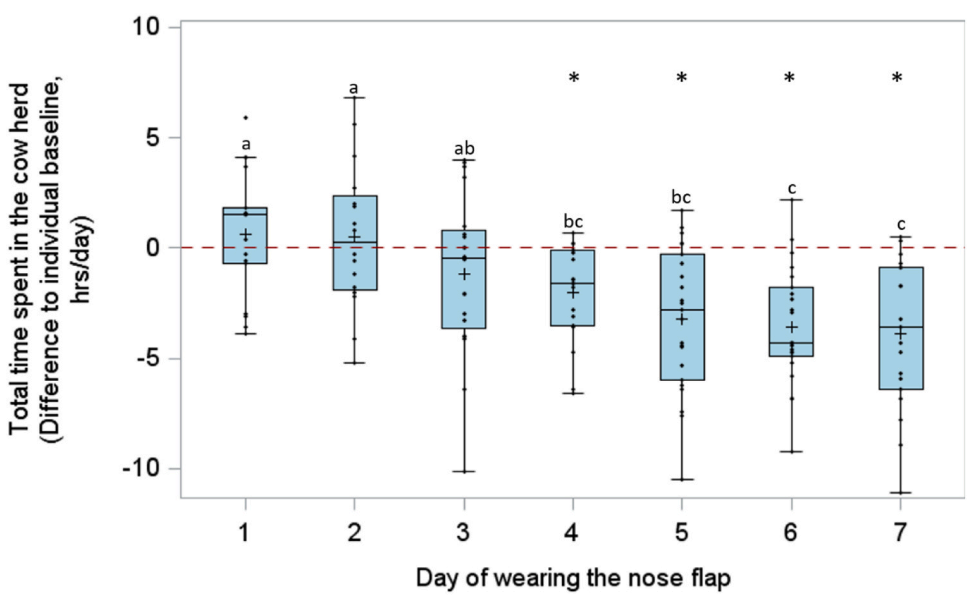


**Fig. 4.** Time calves spent within the cow herd (TIC) including the calves' mothers expressed as difference to the individual mean baseline in hours per day of dam-reared dairy calves (n=23) after insertion of a nose flap in the morning of day 1 (experiment II). Asterisks indicate differences of days of wearing a nose flap from baseline at p <0.05. Lowercase letters (a-c) indicate differences between days of wearing a nose flap at p <0.05. The plus symbol indicates the mean. Diamonds display the values of the individual calves at each day.

with dairy and beef calves (de la Cruz-Cruz et al., 2021; Hötzel et al., 2012; Lambertz et al., 2015; Loberg et al., 2008). Additionally, the same calves as used in experiment I (Vogt et al., 2024) as well as beef calves weaned with a nose flap (Enriquez et al., 2010) showed a high number of unsuccessful suckling attempts for 2–4 days after the nose flap was inserted. This indicates a persistently high suckling motivation of calves for the first days after nose flap insertion, which is likely caused by hunger due to insufficient nutrient supply from solid feed at this time point. Taken together, our results in conjunction with literature indicate that calves' behavioural responses to two step separation with a nose flap are predominantly present for about 3–5 days after fitting of the nose flap, after which the animals seem to adjust somewhat to the new situation.

### 4.1. Reasons for the reduction in TIC are likely multifactorial

The reduced need and/or motivation for contact to the dam after insertion of the nose flap is probably caused by an interplay of several factors. First, suckling of the dam's udder leads to the release of oxytocin in calves (e.g. Lupoli et al., 2001; Uvnäs-Moberg et al., 2001), which is known to be involved in reinforcement of the mother-young social attachment. Also, the receipt of milk has been reported to lead to the release of endogenous opioids in young animals of several species (reviewed in Nelson and Panksepp, 1998; Newberry and Swanson, 2008). In addition, Weary et al. (2008) already discussed that milk could potentially also act as a source of exogenous opioids to the calf, as specific tetra peptides in the cow's milk can also bind to opioid receptors (Yoshikawa et al., 1986). Thus, milk ingestion and the act of suckling itself both strengthen attachment to the dam, while prevention of this will lead to a diminished motivation for social contact to the dam, as part of the rewarding hormonal effect is missing.

At the same time as the attachment becomes weaker, calves will increase their nutritional independence from the dam by progressive adaptation of the gastrointestinal-tract (**GIT**) to a diet based on solids. This is supported by observations of significantly increased eating and rumination times by the fourth day, compared to first day, after fitting of nose flaps in 5-week-old dairy calves (Haley, 2006). Likewise, the calves from experiment I showed a significant increase in the duration of TMR feeding time compared to their baseline in both weeks while wearing the nose flap (Vogt et al., 2024). However, in the latter study, it remained uncertain, if this was due to an actual increase in roughage intake or simply due to an altered, slower, feeding behavior with the nose flap since the actual feed intake of calves was not measured and weight gains of calves remained low (Vogt et al., 2024).

Furthermore, not only the physical dependence, but also the calves' general motivation for dam contact might have become weaker after cessation of milk supply in our study. Generally, the number of suckling bouts per day (Reinhardt and Reinhardt, 1981a), as well as the time cow and calf spend within close distance from another (Lidfors and Jensen, 1988) decreases with age of the calf under extensive conditions. Up from the age of about 3 weeks, calves also form groups with other calves, so called créches, which are accompanied by only 1–2 guardian cows while the mothers are away to graze (Reinhardt and Reinhardt, 1981b). Thus, calves become socially more independent of the mother with increasing age. Since, the design of both experiments lacked a control group in which calves were dam-reared and not separated from their mothers, it is possible that the reduced TIC of calves might also have been partly a result of the fact that calves simply got older with each day of wearing the nose flap. However, the age range of almost 3 weeks (83–102 days of age at weaning start) of calves during experiment I showed no significant effect on TIC of calves. Thus, we infer that during the one or two week period that the nose flap was worn in the two experiments of this study, the age effect is not a dominant factor and that consequently, the individual baseline times from the week directly before nose flap insertion give a reasonable enough indication of TICs that could have been expected for the respective one or two following weeks in case calves would have remained without a nose flap. This assumption is further supported by the fact that the decline in suckling bouts naturally takes place over several months instead of days and that calves under semi-natural conditions are not weaned until an average age of 9–11 months (Reinhardt and Reinhardt, 1981a).

More likely than the age effect is that the outer conditions in the barn reduced the motivation of calves to enter the cow area and thus further enhanced the observed reduction in calves' TICs after insertion of the nose flaps. Since observations of TIC during both experiments took place in an artificial barn setup during the winter months, i.e. without pasture access, calves were limited to spend time with their dams in the cow area of the barn. Consequently, they had to share the restricted walking area and cubicles with the cow herd and might have been repeatedly displaced by cows other than their dam (Waiblinger et al., 2020), while on the other hand they had all necessary resources available in the calf area. This is especially likely for calves of cows with lower social rank in the herd and for the time periods with a high group size of the cow herd of 48 or 49 heads for the 51 available cubicles, which is supported by the result that during experiment II the TIC of calves was reduced by about 25 min/day per increase in herd size by one cow. As a result, motivation of calves to continue to reunite for other purposes than suckling after nose flap insertion might not have been as strong in our studied calves than it would have been under natural conditions. In any case however, TIC never decreased to zero in our study, but the calves still visited the cows after nose flap insertion for a decreased but consistent level of about 2.3–5.4 hours/day (experiment I and II respectively). This implies that our calves were nonetheless still motivated to reunite with their dams for purposes other than suckling, just in decreased amount. This is in line with observations of cattle under semi-natural conditions where weaning is never accompanied by complete separation, but dams form grazing and licking associations with their (eldest) offspring that remain constant for several years after weaning (Reinhardt and Reinhardt, 1981b).

In this context it might also be worth noting that the dams of the calves used in experiment I, showed no obvious decline in interest in their calves over the first 4 days after calves got the nose flap inserted, as the amount of strong vocal responses by cows recorded during direct observations was highest on days 3 and 4 after nose flap insertion compared to the first two days (see supplementary material of Vogt et al., under review). This points towards the fact, that the observed reduction in calves' TIC within the 4 days is probably not a counter-reaction to a reduced interest from the dams after their calves discontinued nursing, but seems more so to be due to a reduced motivation for dam contact by the calves themselves. However, this theory requires verification in future studies.

### 4.2. Individual variation

There was a considerable inter-animal variation in baseline TICs of the calves during both experiments, which likely contributed to the difference of about 2.3 vs. 5.4 hrs/day that calves continued to spent time in the cow area at the 4th or 5th day after cessation of the suckling opportunity in the two experiments. In this regard, descriptive analysis of TIC in both experiments showed that baseline TICs of calves from herd A were in general considerably higher than from herd B, while calves from herd B showed often quite constant, low TICs already during the baseline week of the study and thus no considerable further decrease in TIC after insertion of the nose flap (see Figs. 1 and 3). The most obvious difference between the herds was the horn status, as herd A included predominantly polled cows, whereas all cows in herd B were horned. As reviewed by Knierim et al. (2015), there is only limited knowledge on social behavior in horned compared to polled cattle herds, but it seems evident that horned cattle maintain greater inter-individual distances and show less frequent agonistic physical interactions compared to polled cattle, leading to generally more stable herds of horned cattle. It is thus possible, that due to the greater inter-individual distances kept in horned cattle, the calves from herd B were less likely to use the lying spaces of the cows if another cow was already lying close by and rather limited the time they spent in the cow herd to shorter activities like suckling. This needs however to be confirmed in future studies specifically monitoring cubicle use in both herds. Next to the horn status, there are several further factors which may have influence the individual (baseline) TIC of calves which we did not control for in our study, including for example personality of the calf (Finkemeier et al., 2018; Veissier and Boissy, 2007), milk yield and milk composition of the cow (Ungerfeld et al., 2009), the resulting weaning weight of the calf (Lidfors and Jensen, 1988) and in general the calves' establishment on solid feed and thus nutritional independence from the dam at the point of weaning start (Johnsen et al., 2018). Regarding the latter it has, for example, been reported that dairy calves which had access to a supplementary milk feeder and were thus nutritionally independent from their dams, spent less time close to the separation barrier which separated their pen from that of the dams during the day within the nursing phase (calves had night-time access to their dams) and also vocalized less during the separation phase compared to calves that were nursed by their dams (Johnsen et al., 2018). Hence, it seems likely that calves with a higher level of nutritional independence in our study might have also been the ones with lower TICs during the baseline week. With regard to personality of the calves, it can be suggested that individuals which are very sociable (a description of the sociability trait can be found in Lecorps et al., 2018b) are potentially the ones which expressed higher TICs during the baseline in our study, since more sociable calves are generally more motivated to remain in close proximity to conspecifics (Lecorps et al., 2018a). Also, there seems to be a link between personality of the calf and the level of nutritional independence at weaning, since a recent study reported that calves which are scored more 'explorative' and 'active' show higher willingness to try new feed and have higher solid feed intakes pre-weaning compared to less exploratory calves (Neave et al., 2018), which again might have influenced how motivated calves were for cow contact during the baseline.

It is additionally possible that the yield of the dams also influenced baseline variability in TIC of calves in a way that cow-calf pairs with high yielding dams had more contact to each other. At least in beef calves it has been reported that individuals which are heavier at weaning showed more vocalizations during the weaning process than lighter calves, which could be related to the fact that they lost access to higher yielding dams and thus a higher milk supply (Stěhulová et al., 2017). However, a possible influence of the milk yield of dams on motivation of calves for dam contact during the baseline was not verified during our study as parity of the cow had no significant influence on the measured TICs.

Lastly, maternal care of the dam towards the calf will affect the strength of the cow-calf bond and has been reported to be influenced by several factors such as personality and body condition of the dam (Stěhulová et al., 2013), age of the dam (Green, 1993; Stěhulová et al., 2017) as well as sex and weight of the calf (Stehulová et al., 2013). Hence, all of these factors could have played an additional role in the high variability in baseline TICs found between calves in our study.

But not only the inter-animal variation was considerable, also intra-animal variation between days within the baseline period was also quite large for single individuals. This might be explained by further factors not accessed in our study, such as e.g. high restlessness in the cow herd at single days due to cows in oestrus. For calves with a generally high intra-animal variation already during the baseline, it is a limitation of our study that we compared the TIC on single days during the weaning period to the average of the baseline week, as this average might not be a good representation of the actual time these calves would still have spent within the cow herd, if no nose flap had been worn. Nonetheless, we found quite consistent results in the general pattern of the calves' response to nose flap insertion over the two experiments included for this study, despite the comparably low sample size and considerable inter- and intra-animal variation. On the whole, the 4 days seem to give a reasonably good indication for the time point when calves' motivation to spend time within the cow herd, and thus likely also their suckling motivation, is decreased after insertion of a nose flap in an artificial barn setting after calves were kept with 3 months of full-time contact to their dams, but of course this can never apply to all individuals. More research is needed here to determine which factors mainly contribute to the found considerable differences between and within individuals as well as between herds in the present study.

### 4.3. Limitations and future directions

To the best of our knowledge this is the first study that attempted to track changes in dam-contact initiated by dairy calves after insertion of a nose flap so far. Hence the result from this explorative study in 3-month-old Holstein calves in a barn with a separate calf area needs verification in different husbandry systems as well as with calves of different weaning ages, different levels of nutritional dependence and also from dual-purpose breeds. Like discussed above, space-availability seemed to be a relevant confounder of TIC and thus results might differ in open pasture settings which have typically less space-restrictions, as well as in more spacious barn conditions. Also, the separate calf area, where calves had access to all essential resources but were undisturbed from the cows, likely influenced TIC in our study. Therefore, future studies that track cow-calf interactions initiated by the calf over the days after nose flap insertion in joint deep-bedded loose housing systems, as well as in pasture-based rearing systems with and without a calf creep area would help our understanding of this confounding factor.

Next to the low generalizability of our results, it is also a limitation that it was not possible to systematically score the integrity of the calves' nasal septum over the course of our two experiments. Since tissue alterations or even lesions occur frequently at the contact site where the nose flap rubs against the skin of the nasal septum, it would be essential to assess whether lesions in the calves' noses are also present after the suggested 4–5 days of nose flap usage. In this context, Valente et al. (2022) reported open wounds at the nasal septum of 8-month-old beef calves after 5 days of usage in all tested animals (n=41). Compared to that, we found partly only pressure marks (n=12) and partly lesions with bleeding and secretion (n=6) in the 3-month-old calves used in experiment I when the nose flap was removed after the longer period of 14 days (results presented in Vogt et al., 2024; in experiment II lesion scoring was unfortunately not possible). A daily scoring would have been desirable, but since pressure marks and lesions are covered up by the nose flap as long as it sits inside the nose and a frequent removal and re-insertion of the nose flap is highly stressful to the animals and might also cause lesions, an inspection of the nasal septum was only possible after the final removal of the flap after 14 days. Thus, it is unfortunately unknown, if the lesions found in the 6 calves from experiment I would already have been present after 4 days of nose flap usage as well. The current literature suggests in this regard however, that lesions occur consistently in dairy and beef calves regardless of age or duration of nose flap usage (Lambertz et al., 2015 (7.5 mo old calves, 7 days of usage); Freeman et al., 2021 (8 mo, 7 days usage); Valente et al., 2022 (8 mo, 5 days usage); Wenker et al., 2022 (2 mo, 14 days usage); Kirk and Tucker, 2023 (7 mo and different weights, 7 days usage); Vogt et al., 2024 (3 mo, 14 days usage)). Therefore, the result of our study that calves need to wear the nose flap for a minimum duration of 4 days in order to effectively reduce suckling motivation in conjunction with results by Valente et al. (2022) imply that a functional weaning of calves with nose flaps might adversely affect the physical integrity of some calves. However, systematic studies that specifically investigate nasal septum injuries through the nose flap with standardized scoring systems (e.g. as recently developed by Kirk and Tucker, 2023) in younger calves after a short-period of 4 days are still lacking and are encouraged here.

Lastly, other negative welfare consequences of 4–5 days of nose flap usage need to be examined as well. In this context, the two recent comprehensive studies (Vogt et al., 2024; Wenker et al., 2022) which assessed different types of alternative weaning methods for 2–3-month-old dairy calves in full-time cow-calf contact systems with regard to the calves' stress responses and performance are rather discouraging for the nose flaps. Compared to fence-line separation, two-step separation with nose flaps over 14 days led to lower weight gains during the weaning period and also to higher activity levels in the post-weaning period, so that the authors concluded that fence-line separation is the more effective method to reduce weaning and separation stress for dam-reared dairy calves (Wenker et al., 2022). Also, the results of the larger study where calves of experiment I were part of, suggested that the two-step separated calves had problems with adaptation of the GIT, resulting in greater energy deficits, compared to gradually weaned calves and also showed indicators of a more compromised affective state than gradually weaned calves (Vogt et al., 2024). Next to this and like already mentioned in the introduction, no comprehensive studies have to our knowledge assessed the potential discomfort and pain that could be caused by the nose flaps, as well as the impact of nose flaps on self-grooming or exploratory behavior so far. Anecdotal results of two 10-week-old calves that were weaned with a nose flap over 14 days, reported increased self-grooming frequency in the calves in the days after total separation from the cow (Verwer and Kok, 2012). The authors discussed that this might be an attempt to compensate for the loss of grooming through the mother after separation (Verwer and Kok, 2012), but likewise this could also be a re-bound behavior after the preceding prevention of self-grooming by the nose flap, since this behavior is also important for ensuring hygienic body maintenance (Kohari et al., 2009). Importantly, all of the aforementioned was assessed in calves weaned with nose flaps over 14 days, but at least part of these effects are likely also present in the first 4–5 days of nose flap usage, so that a thorough consideration of these negative welfare consequences is necessary before employing the nose flap method, even for shorter time periods. Nonetheless, results of our study suggest that there is little benefit of using nose flaps for longer periods than 4–5 days, which might give a valuable indication for those farms which currently still use the nose flap due to its high practicability and local restrictions for implementation of other weaning methods. Furthermore, a time period of 4–5 days per step could also be useful for the design of future alternative stepwise weaning protocols.

## 5. Conclusion

Results of our study indicate that in 3-month-old dairy calves that were raised with full-time contact to the dam, it needs at least 4 days until the motivation to spend time with the cow decreases after insertion of a nose flap. When using the nose flap for longer than 4-5 days there was no further reduction in contact times, and contact times remained at a relatively constant level of several hours per day despite the loss of the suckling opportunity. However, there was a considerable variability between individuals already during the baseline and thus, the result of this explorative study can only give an indication and needs replication, foremost in a pasture-based environment with less space-restrictions.

## Notes

This study received no external funding.

All experiments were performed in accordance with the German Animal Welfare Act (Federal Republic of Germany, 2020). Animal experiment number for experiment I: V244–51520/2019, institution of approval: MELUND Schleswig-Holstein.

For experiment II, the local Animal Welfare Committee decided that no ethical approval was required as calves were only monitored using commercially available sensors and video recordings while kept with their standard husbandry and living conditions in the barn. For weaning, nose flaps were used by default in the barn.

## CRediT authorship contribution statement

**Anina Vogt:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Marie Schneider:** Writing – review & editing, Data curation, Conceptualization. **Kerstin Barth:** Writing – review & editing, Supervision, Conceptualization. **Susanne Waiblinger:** Writing – review & editing, Supervision, Conceptualization. **Uta König von Borstel:** Writing – review & editing, Supervision, Conceptualization.

## Declaration of Competing Interest

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

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