Weaning and separation in two steps—A way to decrease stress in dairy calves suckled by foster cows

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Abstract

The aim of this study was to investigate whether the prevention of suckling and the separation from a foster cow in two steps lower the behavioural and physiological stress reaction in calves compared with if the two events occur simultaneously. Twelve groups, each consisting of one cow and four calves, were formed when the calves were 1 week old, and the calves were prevented from suckling at 10 weeks of age. In six of the groups, the calves were prevented from suckling by simultaneous separation from the cow (control). In the other six groups, the calves were fitted with a nose-flap, which prevented them from suckling while they were kept together with the cow for another 2 weeks before they were separated at 12 weeks of age (two-step). The behaviour and the heart rate of the calves were recorded, and saliva cortisol samples were taken after the calves were prevented from suckling (both treatments), as well as after the two-step separation. The two-step calves vocalised and walked significantly less when prevented from suckling than control calves (p < 0.05), although no effect of treatment on the heart rate or cortisol was found. Calves in the two-step treatment sniffed the interior of the pen significantly less (p < 0.05), and directed less social behaviours towards other calves in the group (p < 0.01) than calves in the control treatment. At the time of separation, calves in the two-step group vocalise and walked significantly less (p < 0.01), and also had lower heart rates (p < 0.001) than the control calves. The cortisol levels decreased in the two-step group, whereas it increased in the control group as compared with baseline values (p < 0.05). We conclude that

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two-step weaning, when compared with the simultaneous weaning and separation, reduces the behavioural reaction both at weaning and separation and the physiological reaction at separation when dairy calves are weaned from foster cows.

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Keywords: Behaviour; Cortisol; Foster calves; Heart rate; Stress; Weaning

1. Introduction

In order to reduce labour costs and to improve the opportunity of dairy calves to perform their natural behaviours, foster cows may be used to raise several alien calves during the milk period. In such a practice, the calves are held in small groups with one cow, and are allowed to suckle whole milk either freely or restrictedly. Weaning of calves from the foster cow is usually performed abruptly, where the calves are simultaneously separated from the cow and weaned from milk. At weaning, the calves are restless and vocalise (Jonasen and Krohn, 1991; Hansson, 2005), and there may also be negative effects on the weight gain after weaning (Jonasen and Krohn, 1991). Studies on weaning of beef cattle using fence-line contact (Stookey et al., 1997; Price et al., 2003) or a nose-flap to prevent suckling (Haley et al., 2001, 2005) have shown that both mother and calf showed less signs of stress when they were given the opportunity to maintain physical contact at weaning. With the nose-flap, the two events “stop suckling” and “separation from the mother” are separated in time, and this could reduce the stress experienced by the calves. In the studies mentioned, however, the calves were weaned at about 5–7 months of age (Haley et al., 2001, 2005; Price et al., 2003), which is closer to the natural weaning age of cattle (8–11 month, Bos indicus; Reinhardt and Reinhardt, 1981) than the age at weaning in dairy production (2–3 month). Behaviour may, however, not always tell us everything about the stress experienced by animals (Rutherford et al., 2006; Øverli et al., 2007). This is the reason why additional measures of heart rate and cortisol are important; in the case of lacking behavioural signals, they capture physiological signs of stress.

The aim of this study was to investigate if the prevention of suckling and the separation from a foster cow in two steps, at 10 and 12 weeks of age, reduces the behavioural and physiological stress reaction in calves compared with if the two events are simultaneous at 10 weeks of age.

2. Materials and methods

2.1. Animals and housing

The study was conducted on an ecological dairy farm (KRAV, 2006) in the southwest of Sweden, where the cows were kept in a cubicle-based loose housing system with a milking parlour containing 2 × 12 places (Fullwood, England). The calves were housed in an adjacent barn in large groups together with several tied foster cows during their milk period, and in smaller groups after weaning. The farm had 296 cows, whereof 40% were of the Swedish Red breed and 60% were of the Swedish Holstein breed. The experimental procedures used in this study were approved by the Research Animal Ethics Committee (Swedish Animal Welfare Agency).

During the experiment, the foster cow-calf groups were housed in the same barn as the calves. Each group of one cow and four calves was kept in a pen of approximately 20 m² (range 14.4–26 m²). The floor was concrete equipped rubber mats (used for 10 groups) and wood shavings. Twelve cows (5 Swedish Red breed, 7 Swedish Holstein breed) and 47 calves (15 Swedish Red breed, 32 Swedish Holstein breed) were
used. All the calves were kept with their own mother during the first week, after which they were moved together with three other calves to a pen with a foster cow. All calves within each group were born during the same week. The foster cow was tied for the first 12 h and the calves left to run free, where-after the cow was untied and the whole group was video recorded for 1 h. During this hour, a person observed the group to detect any aggressive behaviour of the cow towards the calves. However, none of the foster cows needed to be replaced due to aggression, nor for any other reason. All calves were allowed to suckle. The groups were kept together in the pen for 9 weeks prior to the application of treatments. They were fed a Total Mixed Ration (TMR) twice a day, and had ad libitum access to water in water bowls.

2.2. Prevention of suckling, and separation

In six of the groups, the calves were prevented from suckling by separation from the foster cow at 10 weeks of age (control). In the other six groups, the calves were prevented from suckling at 10 weeks of age, and separated 2 weeks later at 12 weeks of age (two-step). They were prevented from suckling by fitting a plastic device (QuietWean nose-flap, JDA Livestock Innovations, Saskatoon, Canada) in the nostrils that allowed them to eat and drink. In addition, in case any calf should lose its nose-flap, the cow was fitted with an udder net to prevent calves from suckling. Since it was not known how the nose-flap would affect the initial feed intake, and since one of the purposes was to compare the weight gain of calves in different treatments, calves in the control group were also fitted with a nose flap after separation from the cow. Some of the calves had difficulties learning how to press the paddle of the water bowl after being fitted with the nose-flap, but they had all learned this within 24 h. During the first 24 h the staff on the farm and the people making observations helped to fill the water bowls.

In 8 of the 12 groups, both breeds were represented among the calves; whereas in the remaining 4 groups, there were only Swedish Holstein calves. Groups from the two treatments were balanced among the differently sized pens. At the moment of separation, each group of calves was moved to an empty group pen in the same building, at a distance of 4–10 m from the original pen. The distance between the original pen and the calf pen was balanced across treatments. The foster cows stayed in the original pen until the observations had ended, which means that the calves were able to see and hear their foster cow after separation. When the last observation periods were finished, the cows were moved to the loose housing and the calves were kept in the initial group or mixed with other calves in a larger group.

2.3. Behavioural observations, cortisol sampling, heart rate measurements and weighing

The sampling schedule for the calves in both treatments is described in Table 1. During all behavioural observations, we observed a focal animal and recorded the frequency per minute of different behaviours by continuous recording. The four calves of the same group were observed for 1 min each, every fourth minute during the observation period. The order between calves was randomized for each observation period. Two different people made simultaneous observations of the cow and the calves in the same group. Before the start of the study, the three people involved in the observations practiced together to ensure equal definitions of the different behaviours (Table 2). Results from the observations of the foster cows have been published in a separate paper (Loberg et al., 2007).

At saliva sampling, each calf was gently pushed towards one side of the pen and held there by two people. The person holding the head placed a cotton swab (Salivette; SARSTEDT, Aktiengesellschaft & Co.), which was held with a metal surgical clamp, in the mouth of the calf until enough saliva was collected (approximately 1 mL). The saliva samples were centrifuged at 4500 × g for 15 min immediately after collection, and then frozen at −20 °C until analysis. The saliva cortisol concentration was measured using a solid phase RIA (Count-A-Count RIA kit, Diagnostic Products Corp., Los Angeles, CA, USA) following standard procedures. The intra-assay CV ranged from 4.0 to 6.6%, and the inter-assay CV was <10%. The detection limit of the assay was 0.02 µg/dL. The RIA was validated for saliva with a spiking recovery of 101.4% using pig saliva and an incubation time of 3 h in room temperature (Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala).
The heart rate was recorded on three of the calves in each group, since only four heart rate measuring equipments were available and one of them was placed on the foster cow. Heart rate was recorded in beats per minute (bpm) using the Polar Horse XTrainer (Polar Electro Oy, Finland) and the two electrodes were fitted on the calves with an elastic girth. To facilitate contact between the skin and the electrodes, a conductive gel (Lectro Derm, Swevet Piab, Sweden) was used. A watch receiver, which stored the measurements from the electrodes, was also attached to the girth on the calves back, and was set to calculate the heart rate at 15 s intervals. The Polar monitors calculate a value every 15 s derived from a moving average algorithm. Both the receiver and the electrodes were covered with a flexible bandage (usually used for horses, Horse Guard, Denmark) in order to prevent the animals from manipulating the equipment. The data from the receiver was transferred to a computer via a Polar Interface (Polar Electro Oy, Finland).

On Monday of week 10, the calves in the control treatment were separated from the foster cow and fitted with a nose-flap, while the calves in the two-step treatment remained with the cow and were also fitted with the nose-flap. The measures taken in week 9 and 10 for both treatments were repeated for the two-step calves after 2 weeks with the nose-flap (week 11 and 12) when the calves were separated from the foster cow.

The heart rate was recorded on three of the calves in each group, since only four heart rate measuring equipments were available and one of them was placed on the foster cow. Heart rate was recorded in beats per minute (bpm) using the Polar Horse XTrainer (Polar Electro Oy, Finland) and the two electrodes were fitted on the calves with an elastic girth. To facilitate contact between the skin and the electrodes, a conductive gel (Lectro Derm, Swevet Piab, Sweden) was used. A watch receiver, which stored the measurements from the electrodes, was also attached to the girth on the calves back, and was set to calculate the heart rate at 15 s intervals. The Polar monitors calculate a value every 15 s derived from a moving average algorithm. Both the receiver and the electrodes were covered with a flexible bandage (usually used for horses, Horse Guard, Denmark) in order to prevent the animals from manipulating the equipment. The data from the receiver was transferred to a computer via a Polar Interface (Polar Electro Oy, Finland).

Table 1
Schedule for all measurements performed on calves in both treatments

<table>
<thead>
<tr>
<th>Week</th>
<th>Day</th>
<th>Time</th>
<th>Type of measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>Weighing of calves when forming group</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>Weighing of calves</td>
</tr>
<tr>
<td>9</td>
<td>Sunday</td>
<td>12.00</td>
<td>Saliva sample (baseline)</td>
</tr>
<tr>
<td>10</td>
<td>Monday</td>
<td>09.15–12.15</td>
<td>Heart rate (period 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>09.50</td>
<td>Removal of calves/putting on nose-flap</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.00–12.00</td>
<td>Behavioural observation 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.00</td>
<td>Saliva sample 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.30</td>
<td>Weighing of calves</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.00–19.30</td>
<td>Heart rate (period 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.30–19.30</td>
<td>Behavioural observation 2</td>
</tr>
<tr>
<td></td>
<td>Tuesday</td>
<td>09.15–12.15</td>
<td>Heart rate (period 3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.00–12.00</td>
<td>Behavioural observation 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.00</td>
<td>Saliva sample 2</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td>09.15–12.15</td>
<td>Heart rate (period 4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.00</td>
<td>Saliva sample 3</td>
</tr>
<tr>
<td></td>
<td>Thursday</td>
<td>09.15–12.15</td>
<td>Heart rate (period 5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.00–12.00</td>
<td>Behavioural observation 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.00</td>
<td>Saliva sample 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.30</td>
<td>Weighing of calves</td>
</tr>
</tbody>
</table>

On Monday of week 10, the calves in the control treatment were separated from the foster cow and fitted with a nose-flap, while the calves in the two-step treatment remained with the cow and were also fitted with the nose-flap. The measures taken in week 9 and 10 for both treatments were repeated for the two-step calves after 2 weeks with the nose-flap (week 11 and 12) when the calves were separated from the foster cow.

The heart rate was recorded on three of the calves in each group, since only four heart rate measuring equipments were available and one of them was placed on the foster cow. Heart rate was recorded in beats per minute (bpm) using the Polar Horse XTrainer (Polar Electro Oy, Finland) and the two electrodes were fitted on the calves with an elastic girth. To facilitate contact between the skin and the electrodes, a conductive gel (Lectro Derm, Swevet Piab, Sweden) was used. A watch receiver, which stored the measurements from the electrodes, was also attached to the girth on the calves back, and was set to calculate the heart rate at 15 s intervals. The Polar monitors calculate a value every 15 s derived from a moving average algorithm. Both the receiver and the electrodes were covered with a flexible bandage (usually used for horses, Horse Guard, Denmark) in order to prevent the animals from manipulating the equipment. The data from the receiver was transferred to a computer via a Polar Interface (Polar Electro Oy, Finland).

Table 2
All behaviours included in the analysis together with their respective definitions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vocalising</td>
<td>Making an audible sound through the mouth</td>
</tr>
<tr>
<td>Walking</td>
<td>All four hooves are moved in a forward direction, in at least one sequence</td>
</tr>
<tr>
<td>Lying</td>
<td>Lying down in any resting position</td>
</tr>
<tr>
<td>Eating</td>
<td>Taking TMR in to the mouth followed by chewing and swallowing</td>
</tr>
<tr>
<td>Ruminating</td>
<td>Chewing after regurgitating</td>
</tr>
<tr>
<td>Sniffing interior</td>
<td>Appearing to inhale air through the nose with the nose being within 10 cm of interior of the pen or the floor</td>
</tr>
<tr>
<td>Social</td>
<td>Sniffing, licking or rubbing towards another calf</td>
</tr>
<tr>
<td>Head out</td>
<td>Standing with the head outside the pen with the eyes and ears focused in the same direction</td>
</tr>
</tbody>
</table>
The calves were weighed (EziWeig 2, Australia) on the day they were grouped at about 1 week of age, at 5 weeks of age and on Monday and Thursday at 10–12 weeks of age (Table 1).

2.4. Statistics

2.4.1. Behaviour

All relationships between behavioural responses and potential predictors were analyzed with log-linked Poisson regression models (PROC GENMOD, SAS 9.1, SAS Inst., Inc., Cary, NC, USA) since behaviours were observed as frequency counts on approximately Poisson distributed random variables. As individual calves within each group were being repeatedly observed over equidistant time, quasi likelihoods were used to estimate first-order autoregressive correlation structures within the repeated subjects (i.e. within group). The analyses were initiated by inferring the calves’ reactions to the prevention of suckling via the observations during week 10 for both treatments. The relationship between treatment (two-step, control), observation time (0–2, 8.5–9.5, 24–26 and 72–74 h after prevention of suckling and/or separation), and breed (Swedish Red, Swedish Holstein) together with the corresponding second degree interactions and the behaviours ‘walking’, ‘lying’, ‘eating’, ‘ruminating’, ‘sniffing interior’ and ‘social’, were inferred. For the behaviour ‘vocalising’ a simpler model with treatment, observation time, and breed was used, since there were no recordings in some of the combined categories of interaction. The observations during week 10 for control calves were also analysed together with week 12 for two-step calves, representing the calves’ reactions to separation. The Poisson model was again used for the behaviours ‘vocalising’, ‘walking’, ‘lying’, ‘eating’, ‘ruminating’, ‘sniffing interior’ and ‘social’. When applied to the behaviour ‘head out’ there were, again, no recordings in some of the combined categories of interaction. Therefore, a simpler model including treatment, observation time, and breed, together with the interaction between treatment and breed, as well as between observation time and breed, was used. With the chosen models being relatively well balanced, least square means should provide the best linear-unbiased estimates for the respective models designs (Milliken and Johnson, 1992). The mean values presented were calculated from the log-estimates produced by SAS. For the behaviours ‘vocalising’, ‘walking’, ‘eating’, ‘ruminating’, ‘sniffing interior’, ‘social’, and ‘head out’ the mean values are reported as the number of recordings per hour (rec/h), and for the behaviour ‘lying’ the mean values is reported as the percentage of observations during which the behaviour occurred (%obs). The accompanying prob-values are asymptotically $\chi^2$-distributed.

2.4.2. Heart rate

All heart rate data collected before the calves were separated or fitted with the nose-flap (between 9.15 and 9.50, Table 1) were removed from the data set. This time period was used as acclimatization for the calves to the equipment, which is the reason why these data were not used in the analysis. Errors in the data set were removed by inspection of histograms. With the moving average structure of the Polar monitor being unknown (it is confidential), inter-observational effects could not be considered. Instead, errors were considered as randomly occurring, whereby approximately 1.7% of the dataset was removed. With heart rates measured equidistantly every 15th second, strong auto-regression prevailed with auto-regressive orders varying from one calf to another. In order to correctly specify the significance of cross-correlative effects, auto-regression was filtered out with AR-filters (Box and Jenkins, 1976) using Statistica 7.1 (StatSoft, Inc. Tulsa, OK). Prior to AR-filtering, each heart rate series was characterized with mean, median, and inter-quartile range. The slope of the heart rate was calculated after the application of a 4253H-filter, whereas the heart rate standard deviation was determined after AR-filtering. The relationship between the resulting heart rate characteristics and treatment, observation time, and breed, together with the corresponding second degree interactions, was inferred with a general linear model (GLM, Statistica). As a post hoc test, planned comparisons of LS Means were used (Statistica).

2.4.3. Cortisol

The effect of treatment and day on cortisol concentration, increase in cortisol as compared with baseline, and difference in cortisol between two consecutive samples, was inferred using PROC GENMOD (SAS)
specified with normal distributed responses and identity link functions. The repeated statement (calf) group was included with an autoregressive correlation structure. The accompanying prob-values are asymptotically $\chi^2$-distributed. Results presented are average from four samples taken on four consecutive days.

2.4.4. Weight gain

The average daily gain (ADG) was calculated for the first 9 weeks when all calves suckled freely from a foster cow, and between the weeks 10 and 12 when the two different treatments were applied. The relationship between ADG and treatment, sex (female, male), and breed was inferred with a generalized linear model (PROC GENMOD, SAS) specified with normal distributed responses and identity link functions. Since all calves within each group had suckled the same foster cow and we did not know the exact amount of milk the cows produced, ‘group’ was used as a repeated factor with a symmetric correlation structure (exchangeable). Interactions were inferred and found not significant, and therefore excluded in the final model. Again, the resulting prob-values are asymptotically $\chi^2$-distibuted.

3. Results

3.1. After the prevention from suckling

3.1.1. Behaviour

Calves in the control group vocalised more (66.8 rec/h) after the simultaneous prevention from suckling and separation than the two-step calves (1.6 rec/h) that were only prevented from suckling with the nose-flap ($\chi^2 = 10.5, p < 0.01$). Calves vocalised more during the second (26.4 rec/h, $\chi^2 = 19.8, p < 0.001$) and third observation (25.6 rec/h, $\chi^2 = 13.7, p < 0.001$) than during the first (6.4 rec/h) and fourth observation (2.7 rec/h). When looking at the number of recorded vocalisations for the two treatments during the different times after prevention of suckling (Fig. 1), it appears that the control calves increased their vocalisation frequency during observations three and four. This interaction could not be tested due to the lack of recordings in some of the combined factor levels.

Calves in the control group also walked more (32.8 rec/h) than the two-step calves (11.1 rec/h) after the prevention of suckling ($\chi^2 = 6.6, p < 0.05$). The frequency of walking decreased with

![Fig. 1](image.png) Observed mean number of recordings per hour of vocalisations for calves during the four observation periods after the prevention of suckling either by separation (control, n = 6), or by a nose-flap (two-step, n = 6).
time in both treatments ($\chi^2 = 9.15, p < 0.05$). During the first two observations, there was no difference in the frequency of walking (29.8 rec/h and 31.5 rec/h), but the frequency decreased during the third observation (20.3 rec/h, $\chi^2 = 6.36, p < 0.05$) and even more during the fourth observation (7 rec/h, $\chi^2 = 25.4, p < 0.001$).

The number of recordings of eating did not differ between calves in the two treatments. In both groups, the calves had very few recordings of ‘eating’ during the first 2 h after the nose-flap was fitted (0.3 rec/min) and eating frequency peaked during the second observation about 9 h after fitting the nose-flap (14.8 rec/min, $\chi^2 = 87.6, p < 0.001$). The number of recordings of ‘eating’ decreased from the second to the third observation (9 rec/h, $\chi^2 = 8.9, p < 0.01$) but not from the third to the fourth (7.4 rec/h). Two-step calves were lying down more than control calves (54% obs and 34% obs, respectively, $\chi^2 = 5, p < 0.05$), and calves in both treatments were lying down more with time after prevention of suckling ($\chi^2 = 9.3, p < 0.05$). Two-step calves also ruminated more than control calves (17.4 rec/h and 10.2 rec/h, respectively, $\chi^2 = 9.5, p < 0.01$). Calves in the two-step treatment increased their frequency of rumination with observation time, while calves in the control treatment had a drop in rumination during the second observation (Fig. 2).

As expected, the calves in the control treatment that were separated and moved to a new pen, sniffed the interior and the floor of the pen much more frequently than calves in the two-step treatment that remained in the original pen (16 rec/h and 6.3 rec/h, respectively, $\chi^2 = 6.1, p < 0.05$). In both treatments the sniffing decreased with time ($\chi^2 = 9.9, p < 0.05$).

Calves in the control treatment performed a higher frequency of social behaviours (6.4 rec/h) than calves in the two-step treatment (2.5 rec/h, d.f. = 1, $\chi^2 = 6.8, p < 0.01$).

### 3.1.2. Heart rate

The mean heart rate was unchanged during the three first periods (108.4 bpm, 108.7 bpm and 108.8 bpm for 0–2, 8.5–9.5 and 24–26 h after prevention of suckling respectively), but decreased at period 4 and 5 (101.2 bpm and 99.3 bpm for 48–50 and 72–74 h after prevention of suckling respectively). There was an interaction between treatment and observation time ($F_{4.158} = 4.02, p < 0.01$). Calves in the two-step treatment had higher mean heart rate during the first observation period after the nose-flap was fitted (114.1 bpm) than the control calves that were

![Fig. 2. Estimated mean number of recordings per hour of ruminating for calves during the four observation periods after the prevention of suckling either by separation (control, n = 6), or by a nose-flap (two-step, n = 6). There was a difference between the two treatments at 0–2 ($\chi^2 = 5.4, p < 0.05$), at 8.5–9.5 ($\chi^2 = 34.4, p < 0.001$), at 24–26 ($\chi^2 = 10.8, p < 0.001$) and at 72–74 h ($\chi^2 = 4–39, p < 0.05$) after the prevention of suckling.](image-url)
separated from the cow (102.7 bpm). After that, however, the heart rate decreased for the two-step calves but increased for the control calves so that, at period 3, the two-step calves had a mean heart rate of 104.2 bpm and the control calves had a mean heart rate of 113.4 bpm.

3.1.3. Cortisol

The cortisol concentration in saliva was higher in the control calves (0.07 μg/dL) than in the two-step calves (0.06 μg/dL, χ² = 3.9, p < 0.05), but the increase in cortisol from baseline did not differ between treatments (0.01 μg/dL and 0.02 μg/dL for control and two-step respectively, χ² = 0.31, p = 0.58). When baseline samples were compared before any treatment was applied, it was found that control calves had initially higher cortisol concentration (0.06 μg/dL and 0.04 μg/dL for control and two-step respectively, χ² = 4.6, p < 0.05).

3.2. After the separation

3.2.1. Behaviour

When calves in the two-step group were separated, 2 weeks after the prevention of suckling, they vocalised less (1.8 rec/h) than when the control calves were prevented from suckling and separated simultaneously (68.4 rec/h, χ² = 7.53, p < 0.01). There was a strong tendency that the calves vocalised less with time since separation (χ² = 7.8, p = 0.051).

Calves in the two-step treatment walked less than calves in the control treatment (χ² = 6.9, p < 0.01), where the difference between treatments was greatest at the second and third observation (Fig. 3).

After separation, calves in the two-step group had more recordings of eating (9.6 rec/h) than in the control group (4.9 rec/h, χ² = 4.8, p < 0.05). The difference was most pronounced during the first observation period, where the control calves just had been fitted with the nose-flap, and where the nose-flap just had been removed from the two-step calves (Table 3). Calves in the control treatment decreased their lying and ruminating from the first to the second observation period, whereas the two-step calves increased their lying and ruminating during the same time (Table 3). After separation, calves decreased their frequency of sniffing with time in both treatments (χ² = 10.3, p < 0.05).

Fig. 3. Estimated mean number of recordings per hour of walking for calves during the four observation periods after separation from the foster cow, either simultaneous with the prevention of suckling (control, n = 6), or 2 weeks after prevention of suckling (two-step, n = 6). There was a difference between the two treatments at 8.5–9.5 (χ² = 16.2, p < 0.001) and at 24–26 h (χ² = 69.8, p < 0.001) after separation.
After separation, the calves social behaviour decreased with time in both treatments ($\chi^2 = 8.9$, $p < 0.05$). Swedish Red calves performed more social behaviours (7.3 rec/h) than Swedish Holstein calves (3.8 rec/h, $\chi^2 = 3.8$, $p = 0.05$). After separation from their foster cow, there was a tendency that the behaviour stand with the head outside the pen was more frequently observed in the control calves (4 rec/h) than in the two-step calves (0.5 rec/h, $\chi^2 = 3.4$, $p = 0.06$).

### 3.2.2. Heart rate

When all calves had been separated from the foster cows the control calves had higher mean heart rate (107.2 bpm) than the two-step calves (98.9 bpm, $F_{1,160} = 12.4$, $p < 0.001$). The two treatments differed during the second, third and fourth period (Fig. 4).

### 3.2.3. Cortisol

After separation, two-step calves had lower cortisol concentration in saliva (0.04 µg/dL) than control calves (0.07 µg/dL, $\chi^2 = 54.2$, $p < 0.001$), and there was also a difference between treatments in the cortisol change from baseline. During the 4 days following separation, the cortisol decreased from the baseline in two-step calves ($-0.02$ µg/dL) but increased in control...
calves (0.01 µg/dL, d.f. = 1, \( \chi^2 = 6.4, p < 0.05 \)). The baseline values did not differ between treatments before separation.

3.3. Weight gain

There was no effect of treatment, sex or breed on the ADG. The ADG for the first 10 weeks was 0.71 kg (±0.02 S.E.), and during the 2 weeks following the prevention of suckling the ADG was 0.93 kg (±0.04 S.E.).

4. Discussion

4.1. After the prevention from suckling

All behaviours indicate that calves that were prevented from suckling with the nose-flap and remained with the foster cow experienced less stress than calves that were prevented from suckling and separated simultaneously. Calves in the two-step group vocalised less, walked less and were lying and ruminating more, than the control calves. In studies of extensively held beef cattle, vocalising and walking was inversely related to the time it took for cows and calves to reunite after periods of short separation (Watts, 2001). This implies that vocal behaviour and walking is a response to separation in cattle. This response, however, was not immediate. Especially vocalising was most frequent at the second (8.5–9.5 h) and third periods of observation (24–26 h) after the prevention of suckling of the control group. This delayed reaction has been described previously (Weary and Chua, 2000; Flower and Weary, 2001), and Haley (2006) suggests that it might be an adaptation for hider species where the young is repeatedly separated from the mother when she is foraging. The low frequency of vocalisations in the two-step group, when calves were prevented from suckling indicates that they experienced a low level of stress, as previously suggested by Haley (2006). However, in this study, heart rate and saliva cortisol was also recorded in order to investigate the physiological response. The control calves that were moved to a new pen and fitted with the nose-flap, had a lower average heart rate during the first 2 h of observation than the two-step calves that were only fitted with the nose-flap. We also found the same increase in cortisol in both treatments. One possible explanation is that the nose-flap and the separation are both stressful procedures, but that they provoke an increase in heart rate of different duration. The reaction to separation from the foster cow, appear to last longer and even to increase during the first period of separation. An alternative explanation is that the behavioural reaction to separation is to vocalise (Watts, 2001), and that the vocalisations in the control treatment might have led to the lower heart rate recorded in this group. It has been shown that steers that vocalised when isolated had a greater reduction in heart rate than steers that were silent when isolated (Watts and Stookey, 2001). There are of course other measurements of stress that could have been used, such as ACTH challenge or a heterophil/lymphocyte ratio, and those might have given us more information of the stress experienced by the calves. However, in this study we chose to measure heart rate and cortisol in saliva in ways that minimised stress caused by the sampling. Thus, the behavioural parameters were not disturbed by the recordings of physiological measurements of stress.

4.2. After the separation

It has previously been reported that even if calves are not allowed to suckle, they stay attached to the cow (Veissier et al., 1990), and this attachment does not seem to change after weaning.
The attachment includes non aggressive contacts, seek and maintain proximity and synchronisation of behaviour. So, if an increase in vocalisation and walking is a response to the separation from the cow, then the calves in the two-step treatment would have been expected to increase their response at separation after the 2 weeks without suckling. This was, however, not the case. Two-step calves walked and vocalised less than control calves, even though they had the same number of recordings of walking during the first 2 h after separation. This is in accordance with previous studies on the effect of two-step weaning, where beef calves that had been weaned prior to separation vocalised and walked less than abruptly weaned and separated calves (Haley et al., 2005).

In this study, as in other studies on separation (Flower and Weary, 2001; Stehulová et al., 2008), the behaviour standing with the head outside the pen has been used to reflect the willingness to reunite between the cow and calf. Control calves tended to stand with their head outside the pen more often than two-step calves. Heart rate and cortisol levels were lower for the two-step calves than for control calves after separation, and the cortisol decreased in the two-step group and increased in the control group. In the foster cows, no increase in cortisol or heart rate was found (Loberg et al., 2007), which was ascribed to missing the peak in cortisol because the samples were taken 2 h after separation. However, calves might have experienced more stress at weaning and separation compared to cows. In horses, it has been shown that the increase in cortisol concentration after weaning lasts longer in the foals than in the mares (Malinowski et al., 1990).

The increase in cortisol in both groups when fitted with the nose-flap may indicate that the nose-flap adversely affects the calves. In the two-step group, a cortisol decrease was noted when the nose-flap was removed. Both the behavioural and the physiological responses indicate that separation 2 weeks after weaning is less stressful for calves than simultaneous weaning and separation, although the effect of the nose-flap needs further investigation. The reaction when weaning and separation occurs simultaneously is probably caused by the simultaneous loss of nutrition (milk) and the loss of social contact with the mother. When splitting these two events in time, as is done when applying the two-step weaning, Haley (2006) argues that the responses are not diluted over time, but that the total response over the weaning period is actually lowered.

Two-step calves had more recordings of eating, lying, ruminating, and sniffing the interior. This could be due to a lower stress response, but also to more time available to spend on such behaviours since they did not spend as much time on walking and vocalising after separation as the control calves. These results are also in accordance with Haley et al. (2005). It has to be noted that when comparing the two groups at the time of separation, the control calves were observed when they were 2 weeks younger than the two-step calves. This age difference may also have affected the results.

Swedish Red calves were more social with other calves than Swedish Holstein calves. A similar difference of social behaviour in foster cows has been found previously by Loberg and Lidfors (2001). In Sweden the selection criteria applied in the breeding program for both breeds has been focused at both production and health status (M. Häård, S. Avel, personal communication, 2006). However, the Swedish Holstein population is, to a large extent, based on high producing US imports (M. Häård, S. Avel, personal communication, 2006). These different targets might have resulted in differences in behaviour, which would be an interesting topic for further investigations.

4.3. Weight gain

One problem with abrupt weaning and separation is the reduction of weight gain (Price et al., 2003). It has been shown that the cow’s presence, although the calf is not suckling, may increase the
weight gain of the calf during the colostrum period (Krohn et al., 1999). In Haley et al. (2005), the abruptly weaned and separated calves had a higher average daily weight gain when observed during the whole study period, whereas the two-step weaned calves had a higher average daily weight gain some time after separation. In these studies, the control calves suckled the cow for a longer period than the two-step calves, probably resulting in an overall higher average daily weight gain. In the present study, the calves of both treatments suckled the foster cow for 9 weeks, where-after the average daily weight gain was studied with respect to differences between treatments during the 2 weeks after weaning when the two-step calves remained with the cow. Since there were no such differences observed, it seems that the presence of the cow during those 2 weeks did not affect the feed intake, or the nutrient uptake. Perhaps, the long period spent with the foster cow before the weaning and separation of the calves in the control group had made the calves eat enough solid food to compensate for the loss of milk at weaning. Also, the presence of the cow may only influence the weight gain of the calves when they are drinking milk and at a younger age.

5. Conclusion

We conclude that weaning in two steps by first preventing suckling and secondly separating the calf from the cow reduces the behavioural reaction during both procedures, as well as the physiological reaction at the time of separation, in dairy calves when weaned from their foster cows.

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