



Consequences of weaning and separation for feed intake and milking characteristics of dairy cows in a cow-calf contact system

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ABSTRACT

In cow-calf contact (CCC) systems breaking the maternal bond may induce stress for the cow, thereby affecting feed intake, milk yield, milk flow rate, and milk electrical conductivity. This study aimed to determine the consequences of weaning and separation strategies in CCC systems for feed intake and milking characteristics of the cow. In 2 experiments, Swedish Holstein and Swedish Red cows either had (experiment 1) whole-day CCC (CCC1, $n = 12$) for 8.5 ± 1.2 wk (mean \pm SD) followed by 12 h of daytime CCC for 8 wk, before abrupt weaning and separation at 16.4 ± 1.2 wk, or (experiment 2) whole-day CCC for 16 ± 1.0 wk; thereafter half of the calves were weaned via nose flaps for 2 wk (NF, $n = 10$) before physical separation and half via nose flaps for 1 wk and fence-line contact for 1 wk (NFFL, $n = 9$). Cows were compared with conventionally managed cows (CONV1 or CONV2 in experiment 1 or 2) separated from their calves within 12 h postpartum. In experiment 1, the study period included the week before and after the system switch from whole-day to daytime CCC, and the week before and after separation. In experiment 2, the study period included the week before the start of weaning, during weaning, and 1 week after separation. All cows were milked in the same automatic milking unit. In experiment 1, feed intake of CCC1 cows at separation tended to be lower than CONV1 cows. In experiment 2, roughage intake of NF, NFFL, and CONV2 cows did not differ, but the concentrate intake of NF cows was lower than that of CONV2 cows. In experiment 1, the system switch did not affect milking characteristics. However, after separation, machine milk yield and milk electrical conductivity of CCC1 cows increased, remaining lower than CONV1 cows. In experiment 2, machine milk yield

of NF and NFFL cows increased when calves were fitted with nose flaps, but remained lower than CONV2 cows. In the week after separation, milk yield of NFFL cows was similar to that of CONV2 cows, and the NF cows remained lower. In the week before weaning, milk flow rates of NF cows were lower than those of CONV2 cows, and the NFFL cows did not differ. Before weaning, milk electrical conductivity of NF and NFFL cows was lower than that of CONV2 cows, but not thereafter. In conclusion, machine milk yield of CCC cows remained lower either until the week of separation, for NFFL cows, or until 3 or 11 wk after weaning and separation for CCC1 and NF cows of experiments 1 and 2, respectively. Cow-calf contact reduced milk electrical conductivity, and milk and peak milk flow rates increased the week after separation of cow and calf. Not for experiment 2, but for experiment 1, cow roughage and concentrate intake decreased at separation and recovered within a week, indicating that abrupt separation exerted a greater impact on the cow than separation after nose flap weaning or fence-line contact. Future studies should compare both weaning strategies within the same experimental setup, also focusing on the consequences for calves.

Key words: dam rearing, weaning stress, milk yield, milk flow, electrical conductivity

INTRODUCTION

In dairy production systems, the newborn dairy calf is generally separated from the dam within a few hours after birth and the cows return to the milking herd soon after parturition. This early separation is applied to control the amount and quality of colostrum and milk consumed by the calf, and to limit disease transmission between cows and calves (Çetinkaya et al., 1997; Weary and Chua, 2000; Fröberg et al., 2008). In such conventional dairy production systems, calves are given whole milk or milk replacer and are often weaned at around 2 to 3 mo of age, without being able to form a bond with the

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

dam (Sirovnik et al., 2020). To allow for more maternal contact and suckling, the calves can instead be housed together with their dams for the first months after calving (as reviewed by Johnsen et al., 2016). In these cow-calf contact (CCC) systems, calves are able to suckle and form a bond with their dam or a foster cow, while also being in contact with other calves and cows.

Milk intake is generally higher in CCC calves that can suckle freely or for large part of the day, compared with conventionally reared calves (as reviewed by Johnsen et al., 2016), who often are provided with limited amounts of milk or milk replacer. As a result, less saleable milk is available, which is one of the main reasons CCC systems are not implemented more commonly (Neave et al., 2022).

Furthermore, milk flow rate (MFR) in the milking unit (MU) was lower for cows in a whole-day CCC system (Zipp et al., 2018) or in a restricted-suckling CCC system with suckling allowed for 30 min after each milking (Mendoza et al., 2010), than in nonsuckled cows. The reduction in MFR is possibly because of reduced udder filling at milking, due to suckling being allowed in CCC systems (as reviewed by Johnsen et al., 2016; Zipp et al., 2018). In conventionally managed dairy cows, MFR and the peak milk flow rate (PMFR; highest MFR measured per milking) have been used as an indication of milking efficiency (Sandrucci et al., 2007), and has been positively correlated with milk yield (Weiss et al., 2004). Milk electrical conductivity (MEC) has been used as indicator for mastitis on dairy farms (Viguier et al., 2009; Bonestroo et al., 2022), because the increased concentrations of calcium, magnesium, potassium, chloride, and sodium in milk in inflamed udder quarters result in increased MEC (as reviewed by Norberg, 2005). A lower MEC may therefore indicate better udder health. Cows with prolonged CCC had a decreased risk of mastitis during the suckling period (Walsh, 1974; González-Sedano et al., 2010) as also reported in a recent review article by Beaver et al. (2019).

One concern of the later separation in CCC systems is that the weaning and separation process might be stressful for both cow and calf after the pair has bonded during the contact period (Flower and Weary, 2001, 2003; Stěhulová et al., 2008). Strategies for reducing weaning and separation stress after prolonged CCC have been to wean and separate calves gradually, thus either gradually reducing the suckling opportunities and daily contact time over a set period (Wenker et al., 2022a) or to apply a 2-step weaning and separation protocol (Loberg et al., 2008). One possible 2-step weaning and separation protocol is to fit calves with nose flaps that prevent suckling (Vogt et al., 2024). Calves are then abruptly weaned off milk, but are still housed with their dams. Another 2-step solution is to place calves behind a fence

line, allowing visual, tactile, and auditory CCC, with or without the opportunity to suckle for restricted times (Alvez et al., 2016; Wenker et al., 2022a; Bertelsen and Jensen, 2023). The stress experienced at separation at a later calf age, for example at 2 wk (Flower and Weary, 2001) or 10 wk of age (Veissier et al., 2013), compared with conventional separation within 24 h after birth, has mainly been indicated by increased behavioral responses in cows and calves, such as restlessness, movements, and vocalizations (Stěhulová et al., 2008; Meagher et al., 2019). Apart from behavioral responses, it can be hypothesized that weaning and separation for cows in CCC systems can also affect feed intake, milk production, and udder health. To our knowledge, studies evaluating consequences of weaning and separation in CCC systems on feed intake and milk yield of the cows are currently lacking.

The aim of this paper was to determine the consequences of different approaches to weaning and separation after prolonged CCC for feed intake and milking characteristics of the cow. Feed intake was expected to temporarily decrease as a behavioral response to weaning and separation, and the milking characteristics, including the machine milk yield, MFR, PMFR, and MEC of cows were expected to increase at weaning and separation because no further suckling was possible. In 2 experiments (Exp.), cows that had 16 wk of CCC followed by complete physical separation were compared with conventionally managed cows, where cow and calf were separated within 12 h after birth.

MATERIALS AND METHODS

This study was conducted at the Swedish Livestock Research Centre of the Swedish University of Agricultural Sciences in Uppsala, Sweden. The current study included 2 experiments: Exp. 1 was performed between August and December 2019, and Exp. 2 between September 2020 and February 2021. The study was approved by Uppsala Ethics Committee for Animal Research, Uppsala, Sweden (ID-number: 5.8.18–18138/2019).

Animals and Experimental Design

In both experiments, cows were followed from calving until 40 weeks in milk (WIM). Calves were not monitored in the current study. Inclusion criteria in both experiments were that the expected calving date was within 1.5 mo from start of the experiment, providing that cows did not test positive for *Staphylococcus aureus* mastitis, were not severely lame (based on the gait scoring of Flower and Weary, 2009) during the dry period or at calving, did not show aggression or fear to barn staff, and did not show adverse behavior toward the calves.

Experiment 1. Twenty-four cows were included in the experiment, with calving dates ranging from August 14 until September 24, 2019. The first 12 cows that calved were allocated to the cow-calf contact (CCC1) treatment group, where prolonged CCC was allowed. The subsequent 12 cows calved on average 20 d later and were conventionally managed according to standard herd practice (CONV1). This group allocation approach was done to reduce within-treatment group variation in calf age. The CCC1 cows calved on pasture in a mobile shed with 4 individual calving pens of 3 × 4 m each (Mobilt Vindskydd, Playmek, Röke, Sweden). Cows were kept in the calving pen together with their calf for 2 to 3 d postpartum, but were milked in the indoor automatic MU (DeLaval VMS, DeLaval, Tumba, Sweden) twice per day, before being introduced to the group. As part of a concurrent study, 6 of the cow-calf pairs remained on pasture until mid-October, and 6 pairs were moved to the indoor experimental unit directly from the calving pen. During the 6.7 (±1.1) wk period on pasture, whole-day CCC between cows and calves of the outdoor group was allowed. The indoor and outdoor CCC groups were balanced for parity and breed. The indoor CCC group consisted of 3 primiparous cows (1 Swedish Holstein and 2 Swedish Red breed) and 3 multiparous cows (1 Swedish Holstein and 2 Swedish Red breed), calving between

14 and 28 August. The outdoor CCC group consisted of 2 primiparous cows (1 Swedish Holstein and 1 Swedish Red breed) and 4 multiparous cows (2 Swedish Holstein and 2 Swedish Red breed), calving between 20 August and 10 September. The indoor CCC cows were allowed pasture access between 2200 and 0600 h, starting 1.9 ± 0.8 wk after calving. The CONV1 cows calved in individual indoor calving pens of 3 × 4 m; thereafter their calves were removed within 12 h of parturition. The CONV1 cows were moved to the same indoor experimental unit as the CCC1 cows 1 to 2 d after parturition and had pasture access during the same hours as the indoor CCC cows, but without physical access to calves of CCC1 cows.

Cow-calf pairs that were kept on pasture during early lactation were moved indoors to the same pen as other experimental animals 8 d before switching to 12-h daytime CCC and did not have further pasture access. During the period of daytime CCC, calves were placed in a closed calf creep during the night, preventing suckling from CCC1 cows, but allowing nose-nose contact. After an average of 8.5 ± 1.2 wk (mean ± SD) of full CCC throughout the whole day, followed by a system switch to daytime CCC for 8 more wk, calves of CCC1 cows were abruptly weaned and separated from the dams at 16.4 ± 1.2 wk of age (Figure 1A). Variation in duration was due to the differences in calving dates of the cows. Two

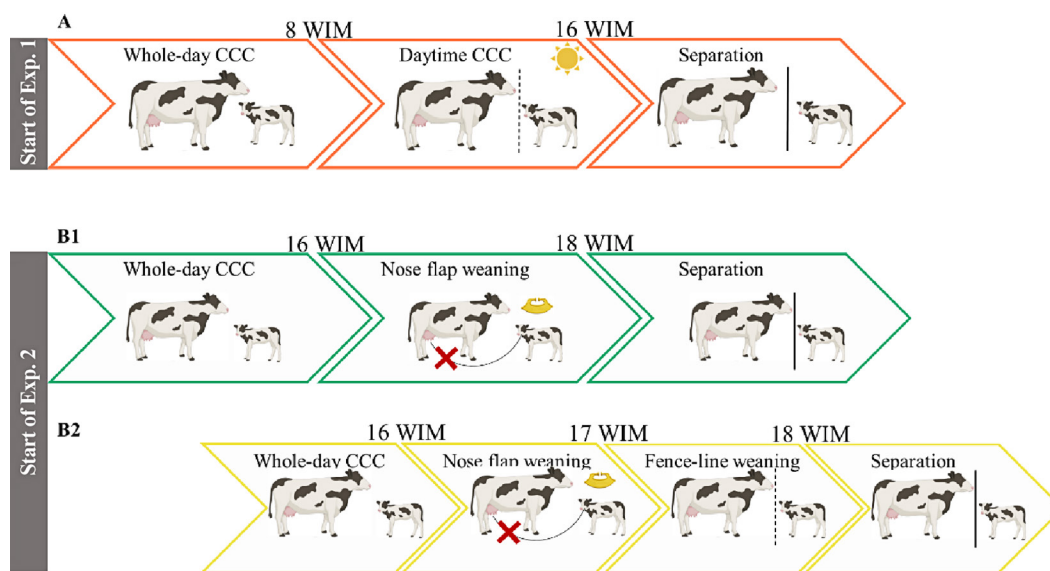


Figure 1. Cow-calf contact (CCC) during different periods in experiment (Exp.) 1 (A) and Experiment 2 (B). Experiments were performed separately, roughly 1 year apart. In Exp. 1, CCC1 cows ($n = 11$) were allowed full CCC throughout the whole day until 8 wk in milk (WIM). Thereafter, a system switch to a daytime CCC system was implemented, where CCC was allowed between 0700 and 1900 h for the following 8 wk. Calves of CCC1 cows were then abruptly weaned and permanently separated from the dams at 16 wk of age. In Exp. 2, (B1) full CCC throughout the whole day until ~16 wk WIM was allowed; thereafter calves were abruptly weaned by fitting them with a nose flap preventing suckling for 14 d (NF group, $n = 10$). (B2) Full CCC throughout the whole day until ~16 WIM was allowed; thereafter calves were abruptly weaned by fitting them with a nose flap for 7 d, followed by fence-line contact for 7 d (NFFL group, $n = 9$). The NFFL calves were fitted with nose flaps 3 wk after NF calves. The fence line prevented the calves from suckling, but allowed some tactile, visual, olfactory, and auditory contact with the cows. Cows in Exp. 2 were permanently separated from calves at ~18 WIM.

CCC1 bull calves were separated earlier, at an average of 11.3 ± 0.4 wk of age due to mounting behaviors, and a CCC1 heifer calf was separated at 16.1 wk because her dam was diagnosed with *Staphylococcus aureus* mastitis. Preliminary analyses indicated that the treatment effects on the milk yield and other milking characteristics did not differ if the cows of these calves were removed from the statistical analyses. We therefore decided to retain these data and adjusted their pre- and post-separation periods accordingly.

For Exp. 1, the 2 periods focused on were from 1 wk before until 1 wk after the initiation of daytime CCC (system switch) and complete physical separation, respectively. One CCC1 cow was euthanized due to trauma before the system switch and was not included in this study. The distribution of animals around the period of the system switch was 12 CONV1 cows, of which 5 were primiparous (Swedish Red breed) and 7 multiparous (4 Swedish Holstein and 3 Swedish Red breed), and 11 CCC1 cows, of which 5 were primiparous (1 Swedish Holstein and 4 Swedish Red breed) and 6 multiparous (2 Swedish Holstein and 4 Swedish Red breed). One CONV1 cow (Swedish Red breed, multiparous) was culled at 10 WIM; thus, 11 CONV1 and 11 CCC1 cows were included for analyses around separation.

Experiment 2. Forty cows were included in the experiment with calving dates ranging from September 1 until October 14, 2020. Cows were blocked on calf sex and alternately assigned to the cow-calf contact (CCC2) treatment group, where prolonged CCC was allowed, or to the conventionally managed treatment group (CONV2). The order of assignment was switched around on 6 occasions to balance treatment groups for breed and parity of the dams. All cows calved in individual indoor calving pens of 3×4 m. Cow-calf pairs in the CCC2 group remained in the calving pen for 2 to 3 d before being moved to the same indoor experimental unit used in Exp. 1. Calves of CONV2 cows were removed within 12 h of parturition and CONV2 cows were moved to the same experimental unit as CCC2 cows 2 to 3 d after parturition.

Separation and weaning of calves were done for 10 CCC2 pairs at a time, to reduce the variation in DIM and calf age when this was done. Therefore, the CCC2 group was divided into 2 groups based on the weaning strategies (Figure 1B). The 10 calves that were born first were aimed to be abruptly weaned (on January 4, 2021) by fitting them with a nose flap (QuietWean, JDA Livestock Innovations Ltd., Saskatoon, Canada) for 2 wk (NF group) after an average of 16 ± 1.0 wk of whole-day CCC. The remaining 9 cows' calves were abruptly weaned, 3 wk after weaning of NF calves (on January 25, 2021), after having 16 ± 0.9 wk of whole-day CCC by fitting them with a nose flap for 1 wk, followed by fence-line weaning for 1 wk (NFFL group). The fence line was a

fence between cows and calves that prevented the calves from suckling, but allowed some tactile, visual, olfactory, and auditory contact with the cows. Calves were separated from the cows after the weaning period of 2 wk, at an average age of 18 ± 1.0 wk.

For Exp. 2, the periods focused on were from 1 wk before the start of weaning, the 2 wk during weaning, and 1 wk after separation. One CONV2 cow was removed from the experiment at 10 DIM due to mastitis, and an NFFL cow was removed after her calf was euthanized due to trauma at 1 mo old. The distribution of animals during the period of weaning and separation was 18 CONV2 cows, of which 9 were primiparous (4 Swedish Holstein and 5 Swedish Red breed) and 9 multiparous (2 Swedish Holstein and 7 Swedish Red breed); 10 NF cows, of which 5 were primiparous (3 Swedish Holstein and 2 Swedish Red breed) and 5 multiparous (2 Swedish Holstein and 3 Swedish Red breed); and 9 NFFL cows, of which 6 were primiparous (1 Swedish Holstein and 5 Swedish Red breed) and 3 multiparous (1 Swedish Holstein and 2 Swedish Red breed).

Housing and Management

Experiment 1 was performed ~1 yr before Exp. 2 (Figure 2).

Experiment 1. Cows were housed on grooved concrete floors, apart from rubber flooring in the feed alley and concrete slatted floors in the waiting area of the MU. Alleyways were equipped with mechanical manure scrapers. Lying cubicles were lined with rubber mattresses (M40R, DeLaval International AB, Tumba, Sweden) and automatically topped with sawdust (JHminiStrø COW, MAFA i Ängelholm AB, Ängelholm, Sweden). The experimental unit consisted of a feed alley with 20 roughage bins equipped with scales (CRFI, BioControl A/S, Rakkestad, Norway), recording individual feed intake per feeding bout. Access to the roughage bins was unrestricted. Automatic water cups and cow brushes (DeLaval swinging cow brush, DeLaval International AB, Tumba, Sweden) were also present in the feed alley. Cow traffic was a semicontrolled feed-first system (DeLaval International AB, Tumba, Sweden). From the feed alley, cows passed by a 3-way selection gate (DeLaval Smart Selection Gate SSG, DeLaval International AB, Tumba, Sweden), where they were guided to the automatic MU (DeLaval VMS Classic, DeLaval International AB, Tumba, Sweden) if they had milking permission. Milking permission was granted after 6 h; CCC1 cows could be fetched for milking after 14 h and CONV1 cows after 12 h. Cows exited the MU into the feed alley. If not permitted to the MU, CONV1 cows were directed to the general lying area, while CCC1 cows were directed to the CCC area, where contact with the calves was allowed. The CCC area com-

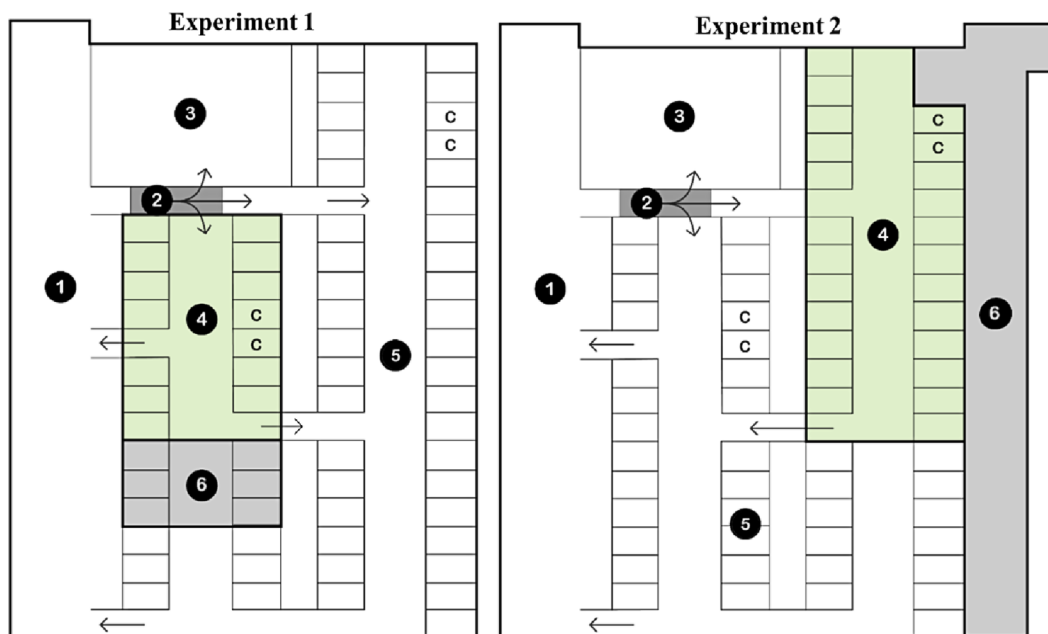


Figure 2. Layout of experimental units in Exp. 1 and Exp. 2. Cows had access to the feed alley (1), equipped with roughage intake control bins, water troughs, and cow brushes. From the feed alley, cows passed a 3-way selection gate (2) and were either directed to the slatted-floored waiting area to be milked in the automatic milking unit (3), or if not granted milking permission, cows with cow-calf contact (CCC) could enter the CCC area (4), and conventionally managed cows without physical contact with the calves could enter the general lying area (5). The calf creep was adjacent to the CCC area (6) and only calves were able to enter. Cows with CCC could exit the cow-calf area through 1-way gates, leading to either the feed alley or general lying area. Automatic concentrate feeders (C in the figure) were present in the CCC area as well as the general lying area. Traffic to the feed alley from the CCC area and general lying area was controlled through 1-way gates (indicated by arrows in the direction of traffic). Illustration created by C. S. Wegner, Swedish University of Agricultural Sciences (used with permission).

prised 12 cubicles. Both the general lying area and CCC area were equipped with 2 automatic concentrate feeding stations (DeLaval FSC400, DeLaval International AB, Tumba, Sweden). The CCC1 cows were also allowed to enter the general lying area at free will, passing through a spring loaded 1-way gate (FeedSelect, GEA Farm Technologies GmbH, Bönen, Germany) from the CCC area. A calf creep of 35 m² was present adjacent to the CCC area, allowing only calves to enter and rest. In the creep, calves had access to ad libitum water, hay, silage, and concentrates. Because the calf creep was adjacent to the general lying area, cows in the cubicles could have auditory, visual, and olfactory contact with the calves. Calves of CONV1 cows were housed in a separate area of the barn and had no contact with cows.

At the end of the 12 h daytime CCC period, at ~16 wk of age, calves of CCC1 cows were abruptly weaned and separated by moving all calves to the youngstock area in a separate part of the building, removing physical, visual, and olfactory contact between the CCC animals. The CONV1 and CCC1 cows were also moved to another automatic MU of the barn and all animals were subsequently managed according to standard herd procedures. The different unit where cows were housed was also equipped with an automatic MU (DeLaval VMS V300, DeLaval

International AB, Tumba, Sweden). The vacuum level, pulsation rate, pulsation ratio, and milk flow at detachment level remained the same in the new MU.

Cows received an ad libitum mixture of corn and grass-clover silage, including a maximum of 3% wheat straw, in the roughage bins according to standard barn routines, that is, at 5 occasions per day between 0500 and 2030 h. Feed was provided via an automated feed wagon (DeLaval FS1600, DeLaval International AB, Tumba, Sweden). Cows received commercial, pelleted concentrates (Lantmännen Lantbruk & Maskin, Malmö, Sweden) from automatic feeding stations, according to individual milk yield for conventionally managed cows and expected milk yield according to herd average for breed and parity for cows with CCC. Feed was provided according to the NorFor evaluation diet calculation system (Volden, 2011).

Experiment 2. Cows of Exp. 2 were housed in the same experimental unit used in Exp. 1. Although the general layout and equipment was the same as in Exp. 1, milking permission was again granted after 6 h, and NF and NFFL cows could be fetched for milking after 18 h and CONV2 cows after 12 h. Again, cows exited the MU into the feed alley. If not permitted to the MU, CONV2 cows were directed to the general lying area, and NF and

NFFL cows were directed to the CCC area, where contact with the calves was allowed. The CCC area comprised 24 cubicles, of which 2 were blocked off for calf use. As in Exp. 1, NF and NFFL cows were also allowed to enter the general lying area at free will. A larger calf creep of 73.2 m² was present adjacent to the CCC area. Calves of CONV2 cows were housed in a separate area of the barn and had no contact with cows.

The separation protocol in Exp. 2 meant that although the NF calves were prevented from suckling their dams during the first (NF) weaning period, NFFL calves were also present in the CCC area and could suckle all cows present in the contact area. After weaning, NF calves were moved to another side of the barn, without contact with the cows. During the second (NFFL) weaning period, fence-line contact between NFFL cows and calves was achieved by closing the calf creep with lightweight gates, whereby suckling was prevented on all cows. After the fence-line period, no more contact of any form was allowed between cows and calves, because calves were moved to another area of the barn and managed according to herd procedures. All cows (CONV2, NF, and NFFL) remained in the same unit for at least 3 wk after separation of NFFL calves.

Diet characteristics and feed provision in Exp. 2 were the same as in Exp. 1.

Data Collection and Handling

Roughage intake per feeding bout was individually measured for all cows in Exp. 1 and Exp. 2 in the roughage bins (CRFI, BioControl A/S, Rakkestad, Norway) and recorded throughout lactation. Concentrate intake per feeding bout was individually measured for all cows in Exp. 1 and Exp. 2 in the automatic concentrate feeding stations (DeLaval FSC400, DeLaval International AB, Tumba, Sweden) and recorded in the management software, DelPro (DeLaval International AB, Tumba, Sweden). Roughage and concentrate intake were then summed per cow per day (24 h), and expressed as kilograms per day. This was done for the focus periods of Exp. 1 (1 wk before 1 wk after the system switch and 1 wk before 1 wk after separation) and Exp. 2 (1 wk before the start of weaning, the 2 wk of weaning, and the week after separation).

Quarter-level milk yield (kg), MFR (kg/min), PMFR (kg/min), and MEC (mS/cm) were measured per cow per milking in the automatic MU and recorded in the management software. Preliminary analyses showed that calves did not prefer suckling a specific udder quarter, therefore milk yield, MFR, PMFR, and MEC were averaged over udder quarters to obtain one value per milking per cow for each variable. Milking characteristics were averaged per day (24 h) to obtain an average value per cow per day

(MFR and PMFR [kg/min], and MEC [mS/cm]) during the focus periods in the respective experiments. When milk yield for a specific quarter was 0 kg, MFR and PMFR were regarded as missing values. Machine milk yield throughout the first 40 WIM was summed per day (24 h) for each cow and expressed as kilograms per day.

In Exp. 1, preliminary analyses showed no difference in milk yield when CCC1 cows with or without full pasture access were moved indoors, and all CCC1 cows were therefore regarded as 1 treatment group in the analyses.

Statistical Analyses

Statistical analyses were performed in SAS (version 9.4, SAS Institute, Cary, NC). Visual inspection as well as the PROC UNIVARIATE procedure of all model variables and residuals was performed to check for normality. The individual cow was treated as the experimental unit for all statistical models. In all models, the first degree autoregressive covariance structure, AR(1), was used to account for within-cow variation. Interactions between fixed effects and their 2-way interactions were initially included in all models for roughage and concentrate intake, as well as milking characteristics. Interactions were retained in all models for feed intake when $P < 0.10$ for at least one model, as was the case for models for milking characteristics. The interaction between treatment group and focus period or WIM was forced into the model because this was the focus of the analyses. Differences were regarded as significant when $P < 0.05$. Values are presented as LSM \pm SEM, unless stated otherwise. Models were fitted separately for Exp. 1 and Exp. 2, but following the same strategy.

Experiment 1. Complete physical separation of cows and calves was achieved by moving both cows and calves to new areas: the cows to a nearby voluntary milking system and the calves to the youngstock stable. The CONV1 cows were moved together with the CCC1 cows, and experienced regrouping on the same date. In our comparisons, we chose to align the data from the CONV1 and CCC1 treatment groups by DIM, because milk yield changes as lactation progresses. As such, the CONV1 cows had already been housed in the new environment for an average of 20 d when reaching the same DIM as the CCC1 cows had at separation. One CCC1 cow was moved, without her calf, to the sick pen 3 d before separation until 5 d thereafter; data of those days were not analyzed.

For models analyzing data around the system switch from whole-day to daytime CCC and the separation of cow and calf, the “pre-system switch” period included the 7 d before the system switch and the “post-system switch” period the day of and 7 d thereafter. Similarly, the “preseparation” period included the 7 d before sepa-

ration of cow and calf and the “post-separation” period the day of and 7 d thereafter.

Roughage and Concentrate Intake: Roughage and concentrate intake were analyzed using repeated measurements models (PROC MIXED).

The final models for roughage and concentrate intake around the system switch from whole-day to daytime CCC and around separation in Exp. 1 was as follows:

$$y_{ijkl} = \mu + G_i + B_j + PC_k + P_l + (G \times P)_{il} + \epsilon_{ijkl},$$

where y_{ijkl} is the dependent variable (roughage intake [kg/d] or concentrate intake [kg/d]), μ is the mean, G_i is the treatment group ($i = \text{CONV1}$ or CCC1), B_j is the breed of cow ($j = \text{Swedish Holstein}$ or Swedish Red), PC_k is the parity class ($k = \text{primiparous}$ or multiparous), P_l is the period (either pre- or post-system switch or pre- or post-separation), $(G \times P)_{il}$ is the interaction between treatment group and period relative to the system switch or separation, and ϵ_{ijkl} represents the random residual term from a normal distribution. Day relative to the system switch or separation was regarded as the repeated variable, and the individual cow as repeated subject.

Concentrate intake data of CCC1 cows on d 4 post-separation were missing and thus not analyzed.

Milking Characteristics: Milking characteristics were analyzed using repeated measurements models (PROC MIXED).

The final models for milking characteristics around the system switch from whole-day to daytime CCC and around separation in Exp. 1 was as follows:

$$y_{ijkl} = \mu + G_i + B_j + PC_k + P_l + (G \times PC)_{ik} + (G \times P)_{il} + (B \times PC)_{jk} + \epsilon_{ijkl},$$

where y_{ijkl} is the dependent variable (milk yield [kg/d], MFR [kg/min], PMFR [kg/min], or MEC [mS/cm]), G_i is the treatment group ($i = \text{CONV1}$ or CCC1), $(G \times PC)_{ik}$ is the interaction between treatment group and parity class, $(B \times PC)_{jk}$ is the interaction between breed and parity class, and all other model parameters are as in the first model. Day relative to the system switch or to separation was regarded as the repeated variable, and the individual cow as repeated subject.

The final model for daily milk yield throughout the first 40 WIM in Exp. 1 was as follows:

$$y_{ijkl} = \mu + G_i + B_j + PC_k + WIM_l + (G \times PC)_{ik} + (G \times WIM)_{il} + (PC \times WIM)_{kl} + \epsilon_{ijkl},$$

where y_{ijkl} is the dependent variable (milk yield [kg/d]), G_i is the treatment group ($i = \text{CONV1}$ or CCC1), WIM_l

is the week in milk (1 through 40), $(G \times WIM)_{il}$ is the interaction between treatment group and week in milk, $(PC \times WIM)_{kl}$ is the interaction between parity class and week in milk, and all other model parameters are as previously described. Days in milk was regarded as the repeated variable, and the individual cow as repeated subject.

Experiment 2. For models analyzing data around the weaning and separation of cow and calf, the “preweaning” period included the 7 d before the start of weaning, the “during-weaning” period included the starting day of weaning and 13 d thereafter, and the “post-separation” period included the day of separation and 7 d thereafter. The interaction between treatment group and breed was not included in any model due to insufficient animal numbers. The Bonferroni adjustment was used for adjusting all P -values of pair-wise comparisons between CONV2, NF, and NFFL groups.

Roughage and Concentrate Intake: Roughage and concentrate intake were analyzed using a repeated measurements model (PROC MIXED).

The final models for roughage and concentrate intake around weaning and around separation in Exp. 2 was as follows:

$$y_{ijkl} = \mu + G_i + B_j + PC_k + P_l + (G \times P)_{il} + (B \times PC)_{jk} + \epsilon_{ijkl},$$

where y_{ijkl} is the dependent variable (roughage intake [kg/d] or concentrate intake [kg/d]), μ is the mean, G_i is the treatment group ($i = \text{CONV2}$, NF , or NFFL), B_j is the breed of cow ($j = \text{Swedish Holstein}$ or Swedish Red), PC_k is the parity class ($k = \text{primiparous}$ or multiparous), P_l is the period (either preweaning, during weaning, or post-separation), $(G \times P)_{il}$ is the interaction between treatment group and period relative to weaning and separation, $(B \times PC)_{jk}$ is the interaction between breed and parity class, and ϵ_{ijkl} represents the random residual term from a normal distribution. Day relative to the start of weaning was regarded as the repeated variable, and the individual cow as repeated subject.

Milking Characteristics: Milking characteristics were analyzed using a repeated measurements model (PROC MIXED).

The final model for milking characteristics around weaning and around separation in Exp. 2 was as follows:

$$y_{ijkl} = \mu + G_i + B_j + PC_k + P_l + (G \times PC)_{ik} + (G \times P)_{il} + (B \times PC)_{jk} + \epsilon_{ijkl},$$

where G_i is the treatment group ($i = \text{CONV2}$, NF or NFFL), P_l is the period around weaning and separation

(preweaning, during weaning, or postweaning), and all other model parameters were as previously described.

The final model for daily milk yield throughout the first 40 WIM in Exp. 2 was as follows:

$$y_{ijkl} = \mu + G_i + B_j + PC_k + WIM_l + (G \times PC)_{ik} + (B \times PC)_{jk} + (G \times WIM)_{il} + (PC \times WIM)_{kl} + \epsilon_{ijkl}$$

where y_{ijkl} is the dependent variable (milk yield [kg/d]), G_i is the treatment group ($i = \text{CONV2, NF, or NFFL}$), WIM_l is the week in milk (1 through 40), $(G \times WIM)_{il}$ is the interaction between treatment group and week in milk, $(PC \times WIM)_{kl}$ is the interaction between parity class and week in milk, and all other model parameters are as previously described. Days in milk was regarded as the repeated variable, and the individual cow as repeated subject.

RESULTS

Results of Exp. 1 and 2 are described separately.

Experiment 1

Figures of milking characteristics around separation of cow and calf are presented in Supplemental Figure S1 (see Notes).

Feed Intake Around System Switch and Separation.

From 1 wk before to 1 wk after the system switch, roughage intake of CCC1 and CONV1 cows did not differ and remained constant at an average of 39.4 ± 1.04 kg/d, but concentrate intake of CCC1 cows tended to be greater ($P = 0.06$) than that of CONV1 cows (14.7 ± 0.13 vs. 14.4 ± 0.13 kg/d, respectively). Roughage and concentrate intake of multiparous cows was greater ($P < 0.01$) than that of primiparous cows (42.9 ± 1.0 and 15.6 ± 0.1 kg/d for multiparous vs. 35.9 ± 1.24 and 13.4 ± 0.15 kg/d for primiparous cows, respectively). Swedish Holstein cows had a greater ($P = 0.05$) roughage intake than Swedish Red cows (41.1 ± 1.3 vs. 37.7 ± 0.9 kg/d), but no effect of breed was present for concentrate intake.

From 1 wk before to 1 wk after separation of cow and calf, roughage intake of CCC1 cows tended to be lower than that of CONV1 cows (pre: 41.8 ± 1.57 vs. 43.1 ± 1.57 kg/d; post: 36.9 ± 1.55 vs. 42.2 ± 1.51 kg/d, respectively; $P = 0.6$). From Figure 3A, roughage intake of CCC1 cows decreased the day after separation; thereafter it normalized again within a week, leading to a tendency for a lower intake across the week than CONV1 cows. From Figure 3B, concentrate intake of CCC1 cows also decreased after separation (from 14.0 ± 0.26 to 12.9 ± 0.26 kg/d; $P = 0.01$) and was consequently lower than

CONV1 cows (13.9 ± 0.27 kg/d; $P = 0.01$). Concentrate intake of multiparous cows was greater than that of primiparous cows (14.65 ± 0.19 kg/d vs. 12.80 ± 0.21 kg/d; $P < 0.01$). Swedish Red cows tended to have a greater concentrate intake than Swedish Holstein cows (13.99 ± 0.16 kg/d vs. 13.47 ± 0.25 kg/d; $P = 0.09$). Parity and breed did not affect roughage intake.

Milking Characteristics Around System Switch and Separation.

Daily Milk Yield: From 1 wk before to 1 wk after the system switch, no interaction between treatment group and period (i.e., pre- vs. post-system switch) was present, and milk yield of CCC1 cows was lower than that of cCONV1 cows ($P < 0.01$; Table 1). Machine milk yield was greater post-system switch independent of treatment group ($P = 0.02$) and also greater in multiparous cows than in primiparous cows independent of treatment group and period (24.14 ± 0.88 kg/d vs. 29.99 ± 0.56 kg/d; $P = 0.01$). No effect of breed was present for milk yield around the system switch.

From 1 wk before to 1 wk after separation of cow and calf, an interaction between treatment group and period (i.e., pre- vs. post-separation) was present for daily milk yield ($P < 0.01$). Daily machine milk yield of CCC1 cows increased by 40% from the pre- to the post-separation period ($P < 0.01$), but not for CONV1 cows. From 1 wk before to 1 wk after the separation, an interaction between treatment group and parity was present for daily milk yield ($P = 0.01$). Machine milk yield of CCC1 cows was lower than that of CONV1 cows, and this effect was greater in multiparous than primiparous cows: 38.98 ± 0.91 kg/d versus 25.06 ± 1.02 kg/d for multiparous CONV1 and CCC1 cows, and 30.25 ± 1.05 kg/d versus 22.52 ± 1.02 kg/d for primiparous CONV1 and CCC1 cows, respectively. An interaction between breed and parity class was present for machine milk yield ($P = 0.04$). Milk yield of primiparous Swedish Holstein cows was lower than that of primiparous Swedish Red cows (23.92 ± 0.82 kg/d vs. 28.85 ± 0.82 kg/d), but no effect of breed was present for multiparous cows (32.30 ± 1.10 kg/d vs. 31.74 ± 0.85 kg/d, respectively).

Milk Flow Rate and Peak Milk Flow Rate: From 1 wk before to 1 wk after the system switch, the MFR and PMFR of multiparous CCC1 cows was lower ($P = 0.04$ and $P = 0.05$, respectively) than those of multiparous CONV1 cows and remained constant (0.69 ± 0.06 vs. 0.97 ± 0.05 kg/min and 1.10 ± 0.06 vs. 1.40 ± 0.06 kg/min, respectively), but not for primiparous cows (Table 1). An interaction between breed and parity class was present for MFR ($P = 0.01$) and PMFR ($P < 0.01$), but post hoc comparisons did not differ for MFR. Within the Swedish Holstein breed, multiparous cows had a greater MFR and PMFR than primiparous cows (MFR: 0.92 ± 0.06 vs. 0.59 ± 0.11 kg/min; PMFR: 1.37 ± 0.07 vs. 0.97

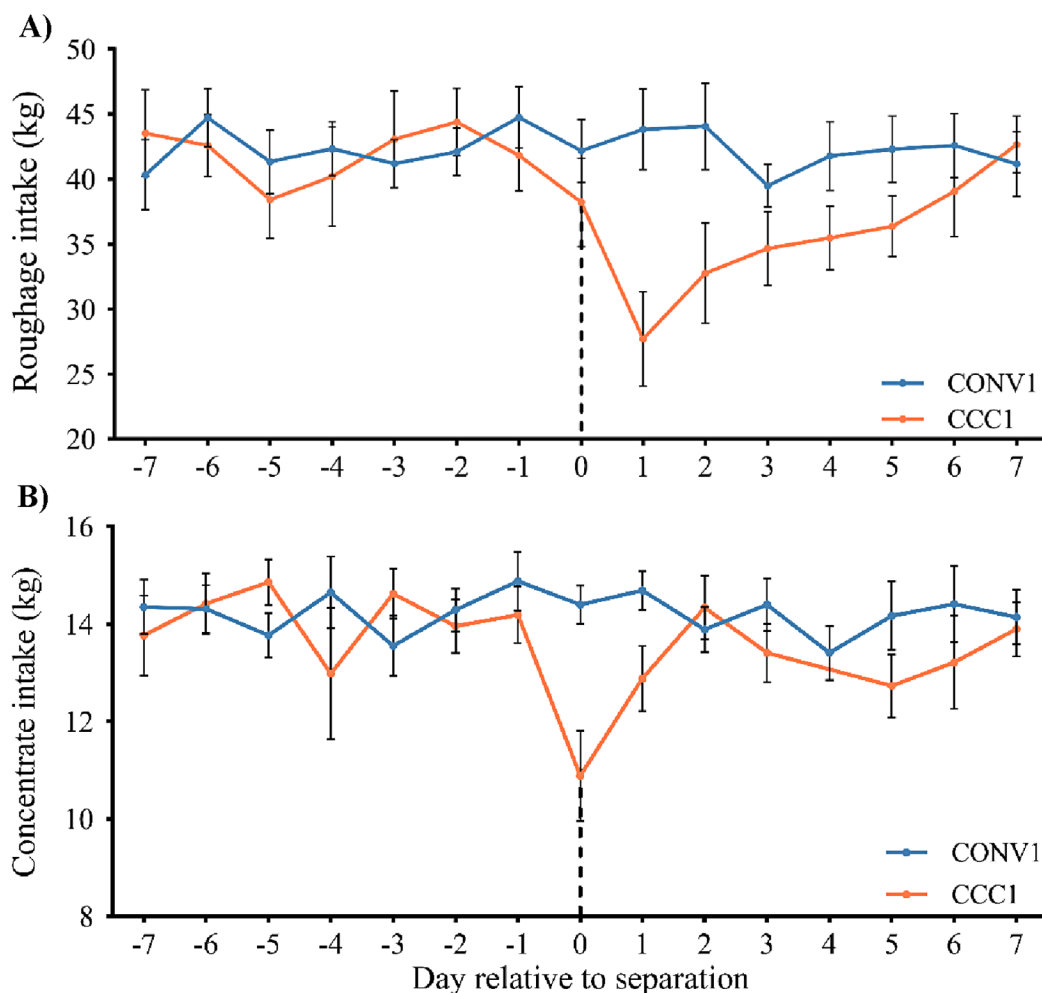


Figure 3. Individual roughage (A) and concentrate (B) intake (kg) per day from 7 d before until 7 d after separation of cow and calf (–7 to 7 d, where d 0 is the day of separation) in Experiment 1. CONV1 cows (n = 12) were conventionally housed cows, separated from their calves within 12 h postpartum, and CCC1 cows (n = 11) had whole-day cow-calf contact for ~8 wk, followed by a system switch to daytime cow-calf contact until abrupt weaning and separation at 16 wk. Dashed lines indicate day of separation (d 0) of cow and calf. Concentrate intake data of CCC1 cows on d 4 post-separation are missing and thus not presented. Values represent means \pm SEM.

± 0.11 kg/min for PMFR, respectively), and no parity effect was present with the Swedish Red breed (0.75 ± 0.05 vs. 0.84 ± 0.05 kg/min for MFR and 1.13 ± 0.06 vs. 1.29 ± 0.05 kg/min for PMFR, respectively).

From 1 wk before to 1 wk after separation of cow and calf, no interaction between treatment group and period (i.e., pre- vs. post-separation) was present for MFR and PMFR, but an interaction between treatment group and parity class was present ($P = 0.02$ and $P < 0.01$, respectively). Multiparous CCC1 cows had a lower MFR and PMFR than multiparous CONV1 cows (MFR: 0.75 ± 0.07 vs. 1.14 ± 0.07 kg/min; PMFR: 1.17 ± 0.08 vs. 1.55 ± 0.08 kg/min, respectively). An interaction between breed and parity class was also present for both MFR ($P = 0.04$) and PMFR ($P < 0.01$). Milk flow rate and PMFR were greater in multiparous Swedish Holstein cows than

multiparous Swedish Red cows (MFR: 1.07 ± 0.07 vs. 0.83 ± 0.07 kg/min; PMFR: 1.51 ± 0.09 vs. 1.20 ± 0.07 kg/min, respectively), but not for primiparous cows.

Milk Electrical Conductivity: From 1 wk before to 1 wk after the system switch, MEC of CCC1 cows remained constant and was lower ($P = 0.01$) than that of CONV1 cows (Table 1). Milk electrical conductivity of Swedish Red cows was greater than that of Swedish Holstein cows (4.54 ± 0.05 vs. 4.15 ± 0.08 mS/cm; $P < 0.01$).

From 1 wk before to 1 wk after separation of cow and calf, an interaction between treatment group and period was present for MEC ($P = 0.04$). Milk electrical conductivity of CCC1 cows increased by 2% from the pre- to post-separation ($P = 0.01$), but remained lower than the MEC of CONV1 cows ($P < 0.01$). Milk electrical conductivity of Swedish Red cows was greater than that of

Table 1. Daily milk yield (kg/d), quarter-level milk flow rate (kg/min), peak milk flow rate (kg/min), and milk electrical conductivity (mS/cm), measured per milking in the milking unit, of CONV1 (n = 12) and CCC1 (n = 11) cows of Exp.1 from 1 wk before to 1 wk after the system switch from a whole-day to daytime CCC system, as well as 1 wk before to 1 wk after separation of cow and calf¹

Measured item per focus period ³	Pre			Post			<i>P</i> -value ²						
	CONV1	CCC1	SEM	CONV1	CCC1	SEM	G	P	B	PC	G × P	G × PC	B × PC
System switch from whole-day to daytime CCC													
Milk yield (kg/d)	35.60 ^a	17.43 ^{b,y}	1.02	35.94 ^a	20.42 ^{b,x}	1.00	<0.01	0.02	0.18	<0.01	0.43	0.06	0.39
Milk flow rate (kg/min)	0.84 ^a	0.67 ^b	0.06	0.86 ^a	0.73 ^b	0.06	0.04	0.13	0.59	0.12	0.41	0.05	0.01
Peak milk flow rate (kg/min)	1.25 ^a	1.09 ^b	0.06	1.28 ^a	1.15 ^b	0.06	0.05	0.21	0.63	0.14	0.56	0.04	<0.01
Milk electrical conductivity (mS/cm)	4.47 ^a	4.15 ^b	0.08	4.47 ^a	4.29 ^b	0.08	0.01	0.21	<0.01	0.17	0.23	0.99	0.48
Separation of cow and calf													
Milk yield (kg/d)	35.05 ^a	19.74 ^{b,y}	1.11	34.18 ^a	27.84 ^{b,x}	1.05	<0.01	<0.01	0.10	<0.01	<0.01	0.01	0.04
Milk flow rate (kg/min)	1.01	0.82 ^y	0.08	1.01	0.89 ^x	0.08	0.14	0.14	0.98	0.80	0.14	0.02	0.04
Peak milk flow rate (kg/min)	1.35	1.26	0.08	1.35	1.30	0.08	0.48	0.41	0.67	0.38	0.38	<0.01	<0.01
Milk electrical conductivity (mS/cm)	4.46 ^a	4.25 ^{b,y}	0.06	4.63 ^a	4.44 ^{b,x}	0.05	<0.01	0.08	<0.01	0.87	0.04	0.77	0.34

^{a,b}Different superscripts indicate difference among LSM of treatment group within fixed effect.

^{x,y}Different superscripts indicate difference among LSM of milking characteristics within group between periods.

¹Values represent LSM and maximum SEM per period. Treatment groups included conventionally housed cows (CONV1) separated from their calves within 12 h postpartum, and cows with whole-day cow-calf contact (CCC1) for 8 wk, followed by a system switch to daytime cow-calf contact until 16 wk.

²*P*-value was considered significant when *P* < 0.05. G = treatment group; P = period relative to system switch from whole-day to 12 h daytime CCC or separation of cow and calf; B = breed, Swedish Holstein or Swedish Red breed; PC = parity class, primiparous or multiparous; G × P = interaction between treatment group and period relative to system switch or separation; G × PC = interaction between treatment group and parity class; and B × PC = interaction between breed and parity class.

³Period (P) relative to system switch from whole-day to 12 h of daytime CCC or separation of cow and calf, where d -7 to -1 relative to the day of the system switch or separation were included as the preweaning period and the day of the system switch or separation until 1 wk later were included as the postweaning period (d 0-7).

Swedish Holstein cows (4.65 ± 0.03 vs. 4.32 ± 0.05 mS/cm; *P* < 0.01).

Milk Yield over 40 WIM. During the first 40 WIM, an interaction between treatment group and WIM was present for machine milk yield (*P* < 0.01; Figure 4). The CCC1 cows had a lower daily machine milk yield than CONV1 cows until the 19th WIM (*P* < 0.01). Daily machine milk yield of CCC1 cows reached a peak in wk 20, with an average milk yield of 31.33 ± 1.01 kg/d; the peak milk yield of CONV1 cows was in wk 14 (36.47 ± 1.01 kg/d). In wk 20 and 21, daily milk yield of CCC1 and CONV1 cows did not differ. Thereafter, milk yield of CCC1 cows was again lower than that of CONV1 cows until 36 WIM, but did not differ from wk 37 to 40. An interaction between treatment group and parity class was present for daily milk yield (*P* < 0.01). Multiparous cows had a greater effect of treatment group on milk yield than primiparous cows: 25.04 ± 0.23 versus 34.25 ± 0.22 kg/d for multiparous CCC1 and CONV1 cows, and 22.81 ± 0.25 versus 29.51 ± 0.29 kg/d for primiparous CCC1 and CONV1 cows, respectively. An interaction between parity class and WIM was present (*P* < 0.01). Machine milk yield of multiparous cows was greater than for primiparous cows until 20 WIM. Thereafter, milk yield did not differ among the parity classes, except for 21 and 28 WIM. Throughout lactation, cows of the Swedish Hol-

stein breed had a greater daily milk yield than cows of the Swedish Red breed (28.64 ± 0.22 vs. 26.90 ± 0.15 kg/d, *P* < 0.01), independent of treatment group.

Experiment 2

Figures of milking characteristics around weaning and separation of cow and calf are presented in the Supplemental Figure S2 (see Notes).

Feed Intake Around Weaning and Separation. From 1 wk before weaning, during the 2 weaning weeks, until 1 wk after separation of cow and calf, roughage intake did not differ among treatment groups and was 42.2 ± 0.77 kg/d on average. During the same period, concentrate intake of NF cows was lower than that of CONV2 cows (13.4 ± 0.24 vs. 14.2 ± 0.19 kg/d, respectively; *P* = 0.03), but did not differ from NFFL cows (13.8 ± 0.27 kg/d; Figure 5A and B). For both roughage and concentrate intake, an effect of period was present (*P* < 0.01 and *P* = 0.04, respectively), but post hoc comparisons did not differ. An interaction between breed and parity class was present for roughage intake (*P* < 0.01). For primiparous cows, Swedish Red cows had a greater roughage intake than Swedish Holstein cows (48.1 ± 1.08 vs. 38.5 ± 0.88 kg/d, respectively), but this did not differ for multiparous cows of different breeds. Multiparous cows had a greater

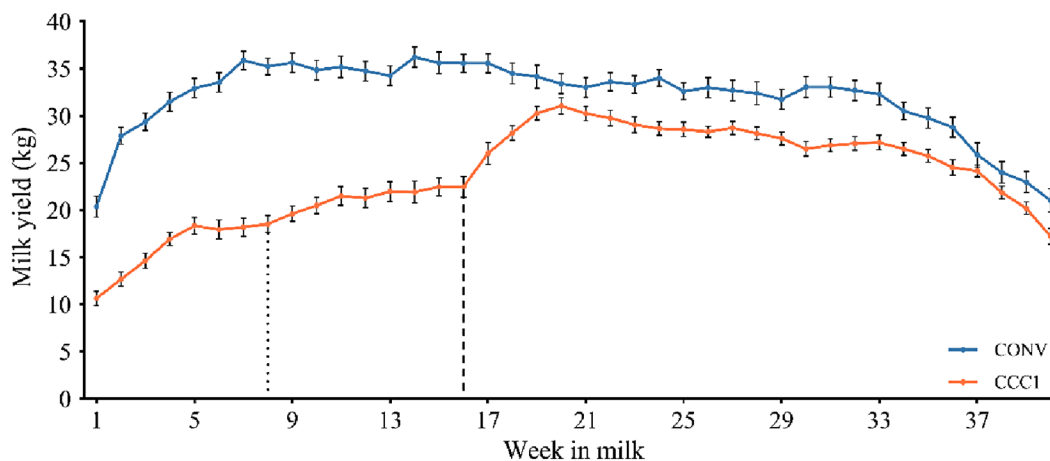


Figure 4. Machine milk yield (kg) per day of CONV1 and CCC1 cows of Experiment 1 from wk 1 until wk 40 in milk. CONV1 cows ($n = 12$) were conventionally housed cows, separated from their calves within 12 h postpartum, and CCC1 ($n = 11$) cows had whole-day cow-calf contact for ~8 wk, followed by a system switch to daytime cow-calf contact until abrupt weaning and separation at 16 wk. The short dashed line indicates the day of the system switch (at 8 wk), and the long dashed line indicates the day of separation of cow and calf (at 16 wk). Values represent means \pm SEM.

concentrate intake ($P < 0.01$) than primiparous cows from 1 wk before weaning, during the 2 weaning weeks, until 1 wk after: 14.94 ± 0.21 versus 12.62 ± 0.18 kg/d, respectively.

Milking Characteristics Around Weaning and Separation. Daily Milk Yield: From 1 wk before weaning, during the 2 weaning weeks, until 1 wk after separation of cow and calf, an interaction between treatment group and period (i.e., pre- vs. during vs. postweaning and separation) was present for daily milk yield ($P < 0.01$; Table 2). In the preweaning period, daily milk yield of NF and NFFL cows was lower than that of CONV2 cows ($P < 0.01$), with the milk yield of NF cows also being lower than that of NFFL cows ($P < 0.01$). Daily milk yield of NF and NFFL cows increased by 47% and 29%, respectively, from the preweaning to the during-weaning period; thereafter it remained constant. During weaning, daily milk yield of NF and NFFL cows was lower than that of CONV2 cows ($P < 0.01$), with the milk yield of NF cows being lower than NFFL cows as well ($P < 0.01$). Post-separation, milk yield of NFFL and CONV2 cows did not differ and was greater than that of NF cows ($P < 0.01$). An interaction between treatment group and parity class was present for daily milk yield around weaning and separation (i.e., preweaning vs. during weaning vs. post-separation). Milk yield of multiparous CONV2 cows was greater than that of the primiparous cows (41.34 ± 0.71 vs. 32.48 ± 0.65 kg/d, $P < 0.01$), and no parity was effect was present in NF and NFFL cows. An interaction between breed and parity class was present for milk yield ($P < 0.01$). For primiparous cows, Swedish Red cows had a greater machine milk yield than Swedish Holstein cows (34.10 ± 0.87 vs. 26.40 ± 0.71 kg/d, respectively), but this did not differ for multiparous cows of different breeds.

Milk Flow Rate and Peak Milk Flow Rate: From 1 wk before weaning, during the 2 weaning weeks, until 1 wk after separation of cow and calf, an interaction between treatment group and period was present for MFR (Table 2; $P = 0.04$). In the preweaning period, the MFR of NF cows was lower than that of CONV2 cows ($P < 0.01$), but did not differ from that of NFFL cows. Milk flow rate of NF cows increased by 14% from the preweaning period to during weaning, and that of CONV2 and NFFL cows remained constant. The MFR of NF and NFFL cows did not differ in any period around weaning and separation and were similar to that of CONV2 cows from the start of weaning onward. For the period around weaning and separation, thus 1 wk before weaning, throughout weaning, until 1 wk after separation, the PMFR of NF and NFFL cows was lower than that of CONV2 cows ($P < 0.01$), and post hoc comparisons indicated that PMFR of NFFL and CONV2 cows did not differ, and that of NF cows was lower only during preweaning and weaning, but not post-separation. An interaction between treatment group and parity class was present for MFR ($P = 0.04$), because the MFR of primiparous, but not multiparous, CONV2 cows was greater than that of primiparous NF cows. No effect of breed was present for MFR or PMFR.

Milk Electrical Conductivity: From 1 wk before weaning, during the 2 weaning weeks, until 1 wk after separation of cow and calf, an interaction between treatment group and period was present for MEC (Table 2; $P < 0.01$). Milk electrical conductivity of NF and NFFL cows did not differ and was lower than that of CONV2 cows in the preweaning period ($P < 0.01$). Milk electrical conductivity of NF cows increased significantly ($P < 0.01$), by 9%, from preweaning to during weaning, and a nonsignificant numerical increase of 4% was observed

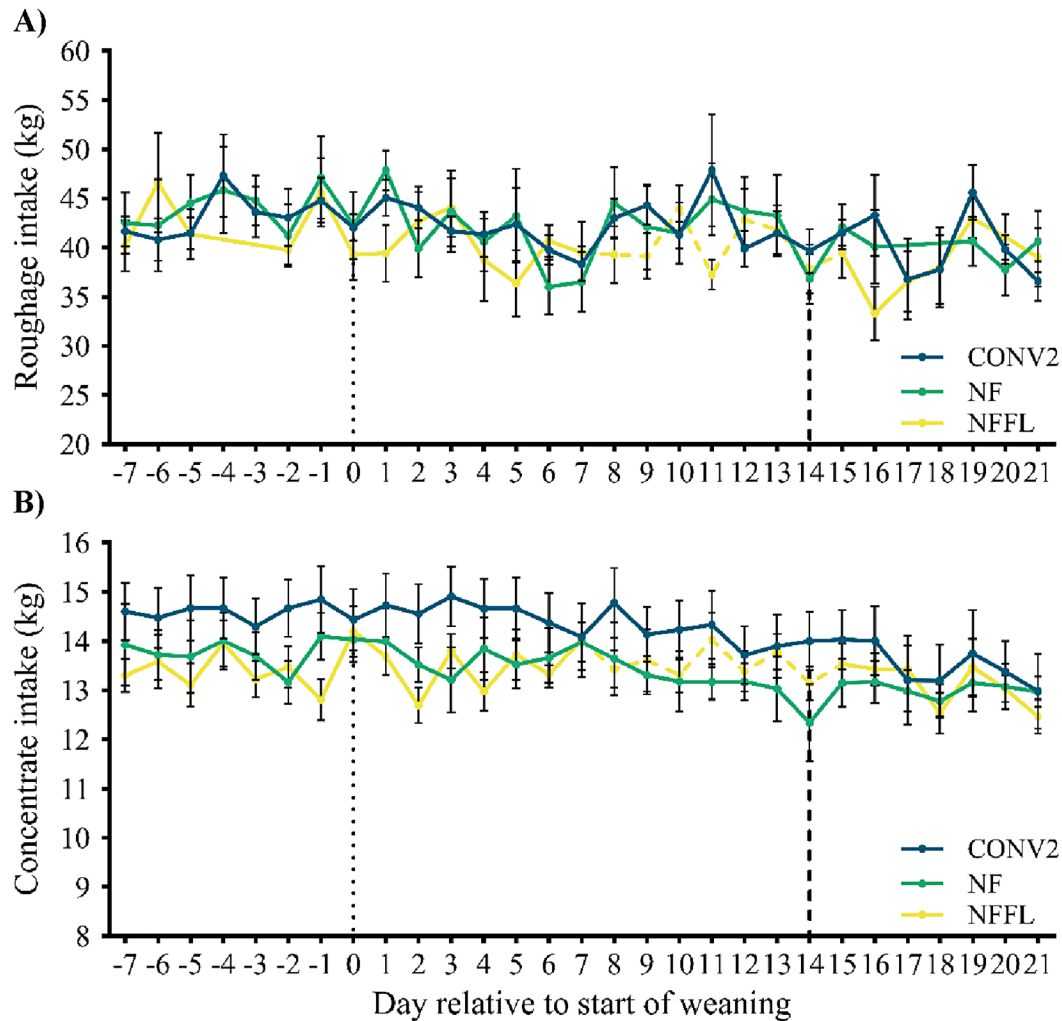


Figure 5. Individual roughage (A) and concentrate (B) intake (kg) per day of all Experiment 2 conventionally housed cows (CONV2, $n = 18$) separated from their calves within 12 h postpartum, and cows with whole-day cow-calf contact for 16 wk, after which calves were weaned via nose flaps for 14 d (NF, $n = 10$) or nose flaps for 7 d followed by fence-line contact with the cows for 7 d (NFFL, $n = 9$). The NFFL calves were fitted with nose flaps at the same age as NF calves, but 3 wk later due to the experimental setup. Day 0 was when nose flaps were fitted on the calves, and on d 7 NFFL cows had only fence-line contact with the calves for 7 d (until d 14). On d 14, thus 14 d after the start of the weaning process, all cows and calves were separated. Dashed horizontal line represents the period where fence-line contact between NFFL cows and calves was allowed. Dashed vertical lines indicate the start of weaning (d 0) and day of separation (d 14) of cows and calves. Values represent means \pm SEM.

for NFFL cows. No difference in MEC was present among CONV2, NF, and NFFL cows during weaning and onward. An interaction between breed and parity class was present for MEC ($P < 0.01$). For multiparous cows, Swedish Holstein cows had a greater MEC than Swedish Red cows (4.32 ± 0.04 vs. 4.51 ± 0.04 mS/cm, respectively), but this did not differ for primiparous cows of different breeds.

Milk Yield over 40 WIM. During the first 40 WIM, an interaction between treatment group and WIM was present for machine milk yield ($P < 0.01$; Figure 6). The CONV2 cows had a greater daily machine milk yield than NFFL cows until the week of separation (wk 18; $P < 0.04$), but not thereafter. Daily milk yield of CONV2

was greater than NF cows until 29 WIM, thus 11 wk post-separation, except for during wk 21 ($P < 0.01$). Milk yield of NFFL cows was greater than that of NF cows between wk 12 and 18, ($P < 0.05$). An interaction between treatment group and parity class was present for milk yield throughout the first 40 WIM ($P < 0.01$). Within CONV2 and NF treatment groups, multiparous cows had a greater milk yield than primiparous cows (37.25 ± 0.21 and 24.47 ± 0.29 kg/d vs. 30.33 ± 0.21 and 19.00 ± 0.28 kg/d, respectively), while no difference between parity classes was found for NFFL cows (25.80 ± 0.28 vs. 25.37 ± 0.38 kg/d). An interaction between parity class and WIM was present ($P < 0.01$). Machine milk yield of multiparous cows was greater than that of primiparous cows until

Table 2. Daily milk yield (kg/d), quarter-level milk flow rate (kg/min), peak milk flow rate (kg/min) and milk electrical conductivity (mS/cm) measured in the milking unit, of CONV2 (n = 18), NF (n = 10) and NFFL (n = 9) cows of Experiment 2 from 1 wk before weaning, during 2 wk of weaning and 1 wk after separation of cows and calves after prolonged cow-calf contact¹

Measured item per focus period ³	Pre						During						Post						P-value ²												
	CONV2		NF		NFFL		SEM		CONV2		NF		NFFL		SEM		G			P		B		PC		G × P		G × PC		B × PC	
Milk yield (kg/d)	37.94 ^a	15.13 ^{xy}	24.14 ^{by}	1.29	37.31 ^a	21.76 ^{cx}	30.68 ^{bx}	0.95	35.48 ^a	25.92 ^{bx}	32.30 ^{ax}	1.21	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Milk flow rate (kg/min)	0.98 ^a	0.63 ^{by}	0.90 ^{ab}	0.07	1.00	0.76 ^x	0.95	0.07	0.98	0.80 ^x	1.00	0.07	<0.01	0.23	<0.01	0.23	<0.01	0.40	0.03	0.04	0.04	0.13	0.13	0.04	0.04	0.04	0.04	0.04	0.04	0.11	0.11
Peak milk flow rate (kg/min)	1.40 ^a	1.02 ^b	1.32 ^{ab}	0.08	1.42 ^a	1.15 ^b	1.36 ^{ab}	0.07	1.39	1.18	1.41	0.08	<0.01	0.02	0.21	0.20	0.14	0.20	0.14	0.04	0.04	0.11	0.11	0.04	0.04	0.04	0.04	0.04	0.11	0.11	0.11
Milk electrical conductivity (mS/cm)	4.46 ^a	3.90 ^{by}	4.14 ^b	0.08	4.45	4.27 ^x	4.31	0.06	4.41	4.42 ^x	4.23	0.07	<0.01	<0.01	<0.01	<0.01	<0.01	0.84	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

^{a-c}Different superscripts indicate difference among LSM of treatment group within fixed effect.

^{xy} Different superscripts indicate difference among LSM of milking characteristics within treatment group between periods.

¹Values represent LSM and maximum SEM per period. Treatment groups include conventionally housed cows (CONV2) separated from their calves within 12 h postpartum, and cows with whole-day CCC for 16 wk, after which calves were weaned via nose flaps for 14 d (NF) or nose flaps for 7 d followed by fence-line contact with the cows for 7 d (NFFL). The NFFL calves were fitted with nose flaps at the same age as NF calves, but 3 wk later due to the experimental setup.

²P-value was considered significant when $P < 0.05$. G = group; P = period relative to the start of weaning and separation of cow and calf after prolonged contact; B = breed, either Swedish Holstein or Swedish Red; PC = parity class, primiparous or multiparous; G × P = interaction between treatment group and period relative to weaning and separation; G × PC = interaction between breed and parity class; and B × PC = interaction between breed and parity class.

³Period relative to the start of weaning and separation of cow and calf after prolonged contact, where days -7 to -1 relative to the start of weaning were included as the preweaning period, the start of weaning until the day before separation (d 0 to 13) were included as the during-weaning period, and the day of separation until 1 wk later (d 14 to 21) were included as the postweaning period.

17 WIM; thereafter it did not differ. An interaction between breed and parity class was present for milk yield: primiparous Swedish Red cows had a greater ($P < 0.01$) milk yield than multiparous Swedish Red cows (31.28 ± 0.26 vs. 27.02 ± 0.36 kg/d, respectively), but no parity effect was present for Swedish Holstein cows.

DISCUSSION

This study aimed to determine the consequences for the cow when weaning and separating dairy calves via different methods after 16 wk of CCC. Feed intake and milking characteristics, including machine milk yield, MFR, and MEC, of cows in a CCC system were compared with cows managed according to a conventional dairy system where cow and calf were separated within 12 h after calving. The choices for weaning approaches in the current study were based on gradual weaning methods commonly used on European farms (Eriksson et al., 2022) and what was practically possible at the specific research farm.

Feed Intake During Suckling, Weaning and at Separation

Roughage and concentrate intake of cows with prolonged CCC did not differ from conventionally managed cows during the suckling period. During the suckling period, cows maintained their feed intake while also allocating time to performing maternal behaviors, by increasing feeding rate (Johansson et al., 2024; who reported time budgets of the cows in Exp. 2 of the current study). The system switch from whole-day to daytime CCC did not affect feed intake of cows in Exp. 1, and neither did weaning and separation affect roughage intake of the cows in Exp. 2. In Exp. 2, calves of NF and NFFL cows were already fully weaned, thus not nutritionally dependent, when separated from the cows, possibly reducing the behavioral response of the cows to separation (Johnsen et al., 2018). In the week after separation, however, roughage intake of CCC1 cows decreased, a behavioral response to the probable stressful abrupt separation from their calves that were still dependent on them. The decreased feed intake of CCC1 cows could also be due to them being moved to another MU in the barn. Cows would have had to get used to the new pen, leading to a decrease in feed intake. However, in the new pen, the same roughage bins were still used as before. Although only anecdotally, CCC1 cows were seen standing in the part of the new pen closest to where they were housed with their calves, potentially searching for their calves. For statistical analyses of the current study, the data of CONV1 and CCC1 treatment groups were aligned by DIM, because milk yield changes as lactation progresses.

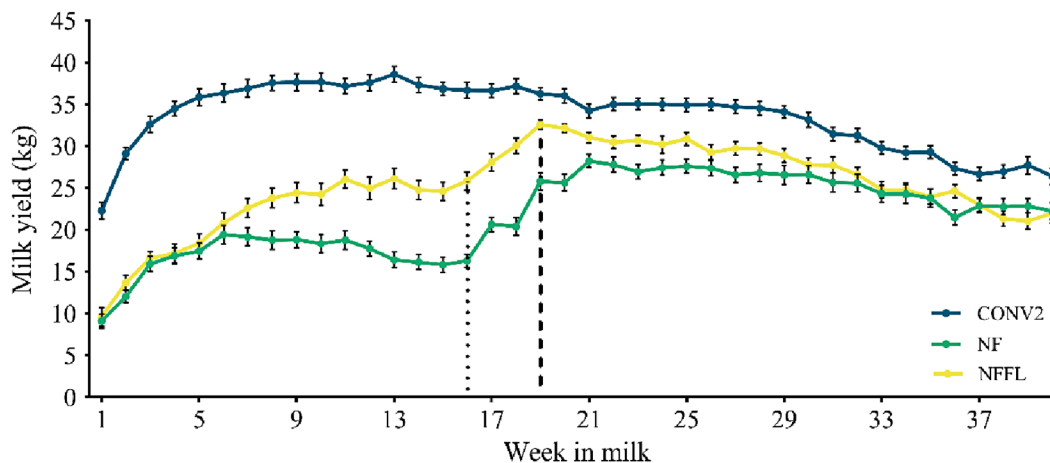


Figure 6. Machine milk yield (kg) per day from wk 1 until wk 40 in milk of all Experiment 2 conventionally housed cows (CONV2, $n = 18$) separated from their calves within 12 h postpartum, and cows with whole-day cow-calf contact for 16 wk, after which calves were weaned via nose flaps for 14 d (NF, $n = 10$) or nose flaps for 7 d followed by fence-line contact with the cows for 7 d (NFFL, $n = 9$). The NFFL calves were fitted with nose flaps at the same age as NF calves, but 3 wk later due to the experimental setup. The short dashed line indicates the start of weaning (at 16 wk), and the long dashed line indicates the day of separation of cow and calf (at 18 wk). Values represent means \pm SEM.

The actual dates of the days relative to separation of cow and calf are thus not the same across treatment groups. When using the actual calendar dates instead of DIM, still roughage intake of only CCC1 cows, not CONV1 cows, decreased on the day of separation and moving to the new pen (Supplemental Figure S3; see Notes). Roughage intake of CCC1 cows remained lower than that of CONV1 cows until 2 d after separation, providing more evidence for a stressful separation experience.

The strategy of reducing contact between cow and calf either via a system switch from whole-day to a 12 h daytime CCC system or by fitting calves with nose flaps did not affect the feed intake of the cow. However, the abrupt separation in Exp. 1 did result in a reduction in cow feed intake as effect. Not only feed intake, but also the daily feeding patterns of the cows with prolonged CCC could be further evaluated for a more accurate understanding of the weaning and separation response.

Milk Yield During Unrestricted Suckling

Machine milk yield of cows with prolonged CCC was, lower during the suckling period compared with conventionally managed cows. In Exp. 1, the difference in daily machine milk yield during the suckling period between cows with whole-day CCC (CCC1) and CONV1 cows (17.3 kg/d) was in line with the estimated up to 16 L/d that a beef breed calf can consume between 3 and 6 mo of age (Scholz et al., 2001). For dairy calves, daily milk consumption at 9 wk of age was 12.5 kg when calves were allowed to suckle their dams for only 2 h twice daily after each milking (De Passillé et al., 2008). This is still well above the milk allowance of 10% of the calf's

BW, as commonly used in conventional systems (Jasper and Weary, 2002). In Exp. 2, the difference in daily machine milk yield during the suckling period of NFFL and CONV2 cows was around 13 kg. This is in accordance with Wenker et al. (2022b), who found that saleable milk yield in the first 7 WIM was ~ 12 kg/d lower in cows with whole-day CCC than in conventionally managed cows. In the current study, the difference in daily machine milk yield during the suckling period of NF and CONV2 cows was around 22 kg. Similarly, in the study of Barth et al. (2009), cows with whole-day CCC or CCC for 15 min before each milking, twice per day, had an approximate 20 kg difference in daily milk yield at 3 mo in milk compared with conventionally managed cows. Even more so, daily milk yield of cows with whole-day CCC was ~ 25 L/d lower at 4 to 6 WIM than cows without CCC (12 vs. 37 L/d; Neave et al., 2024). It seems rather unlikely that this difference in milk yield was due to calf milk intake alone. The greater machine milk yield of NFFL and NF cows from 12 WIM until separation was not expected, because NF and NFFL cows did not differ in management or housing conditions until the start of the respective weaning periods. This might have been partially caused by an uneven distribution of cross suckling, a well-known phenomenon in CCC systems (Bertelsen and Jensen, 2023), between the NF and NFFL cows. Cow parity was imbalanced in Exp. 2; NFFL cows were either first or second parity, but parity of NF cows ranged from first to fourth. Milk yield of multiparous cows is generally greater than primiparous cows, which was also the case for the conventionally managed cows of the current study, but not for cows with prolonged CCC. It might thus be that NFFL calves generally preferred suckling the

older, multiparous NF cows from an early age on because they are able to consume more milk, or because multiparous cows expressed less adverse maternal behaviors, such as kicking and butting the calf, than primiparous cows (Edwards and Broom, 1982).

The difference in daily machine milk yield between cows with and without CCC was greater than expected, possibly pointing to additional influencing factors apart from calf milk intake. As also suggested by Jensen et al. (2024), understanding cross suckling behavior of calves in CCC needs more attention, whereby preferences among calves for factors such as parity of alien cow might become clear.

Milk Yield, MFR, and MEC During Restricted Suckling, Weaning, and at Separation

Machine milk yield of cows with prolonged CCC increased when suckling was restricted and when cows were separated from their calves. In Exp. 1, milk yield of CCC1 cows increased by ~3 kg/d (16%) after the system switch from whole-day to daytime CCC. This was less than expected, but still corresponding to the difference in milk yield between organically managed cows with whole-day CCC or only nighttime CCC for 90 d (Barth, 2020). The marginal difference between the end of the 24 h contact period and the beginning of the 12 h contact period indicates that the calves at this age were able to compensate for the reduced suckling opportunities by consuming similar amounts of milk also during the shorter daily contact period. As observed by Jensen et al. (2024), time spent suckling the dam was similar in calves with whole-day CCC and those with 10 h of daytime CCC followed by a gradual reduction to 2 h per day. Calves might thus be able to suckle more efficiently when their opportunity is limited (Jensen and Budde, 2006). In Exp. 2, machine milk yield of NF and NFFL cows increased at the start of weaning when the calves were fitted with nose flaps. Average daily machine milk yield of NF cows increased by ~7 kg/d (47%) at the start of weaning of NF calves. Because calves of NF cows were weaned 3 wk before calves of NFFL cows, although at the same average age, NF cows may still have been cross-suckled by NFFL calves during the 2-wk weaning period of NF calves. Average daily machine milk yield of NFFL cows also increased by ~7 kg/d (only 29%) at the start of weaning of NFFL calves. By this time, NF calves have already been completely separated, indicating that NFFL calves might have managed to still suckle during the weaning period, especially while fitted with nose flaps.

In the week after separation of cow and calf, average daily milk yield of CCC1 cows increased by 40%, as expected, and that of NF and NFFL cows in Exp. 2 was greater than at the start of weaning but remained

the same as during the weaning period. The daily milk yield of NF cows remained low in the week after separation. Because NF cows were still directed to the CCC area after their calves have been separated, NF cows may still have been suckled by NFFL calves also during the post-separation period, leading to a lower machine milk yield during the first weeks following separation from their own calves. Milk yield of NFFL cows recovered to the same level as the conventionally managed cows when calves were separated. The greater initial increase in machine milk yield of NFFL cows after weaning and separation might indicate that the partial CCC during the week of fence-line contact helped alleviate the stress at permanent separation of cow and calf. In line with that, calves of NF cows that were separated more abruptly might have led to greater separation stress for cows (and calves) and therefore resulted in a lower milk yield. It might also be a consequence of the speculated less suckling that occurred at NFFL cows due to cross suckling, whereby residual milk of the cows was lower, leading to less negative feedback on milk synthesis, as discussed later in this section.

The effects of restricting suckling and the separation of cow and calf on MFR and PMFR differed among treatment groups of both experiments. Before restricting suckling, the MFR and PMFR of CCC1 and NF cows, but not NFFL cows, were lower than those of the conventionally managed cows. Furthermore, in Exp. 1, MFR and PMFR were not affected by restricting suckling via the system switch from a whole-day to daytime CCC system. We expected both MFR and PMFR to increase with only daytime CCC, because the degree of udder fill and milk ejection was expected to increase with less suckling opportunities allowed (Sørby et al., 2024b). In an earlier study, the average MFR of cows in a short-time CCC, where 30 min of suckling was allowed 2 h after each milking for 6 wk, followed by suckling only after morning milking until 8 wk of age, was consistently lower during the CCC period than in conventionally housed cows (Mendoza et al., 2010). In that study, the average MFR of cows with CCC did not differ from conventional cows after the suckling period, which was not always the case in the current study. Even after calves were prevented from suckling by fitting calves with nose flaps, the PMFR of NF cows was lower than that of the conventionally managed cows. This might be due to carryover effects of the mechanism between suckling, oxytocin, and milk yield (De Passillé et al., 2008). In cows, suckling induces a greater oxytocin response in plasma than machine milking does (Bar-Peled et al., 1995; Lupoli et al., 2001), possibly leading to milk ejection problems when previously suckled cows are machine milked (Tančin and Bruckmaier, 2001). When not being suckled anymore, it might thus be that the oxytocin threshold for milk ejection is

not always reached during machine milking, whereby cows have to adapt to a lower oxytocin release, leading to a gradual increase in machine milk yield across days after separation. In line with the study of Sørby et al. (2024b), the MFR of NF cows was higher from the start of weaning onward than preweaning. In that study, MFR increased when calves were prevented from accessing cows compared with having short-time CCC. A greater MFR after separation can be explained by its positive correlation with machine milk yield (Weiss et al., 2004). In turn, this could explain the similarities in MFR and PMFR between NFFL and CONV2 cows, or it could be a consequence of the speculated less suckling that occurred at NFFL cows due to cross suckling.

Milk electrical conductivity of cows with prolonged CCC was lower during the suckling period compared with conventionally managed cows. Fröberg et al. (2008), for example, indicated that suckling might improve the udder health of the cow. Milk electrical conductivity can be used for indirect evaluation of udder health because it is used as indicator for mastitis (Bonestroo et al., 2022). A lower MEC might indicate better udder health, thereby indicating a possible advantageous effect of suckling for udder health. This was also the conclusion of the review of Krohn (2001). However, these results should be interpreted with caution because the effectiveness of MEC as an indicator for mastitis in automatic milking systems is questioned by some authors (Bruckmaier et al., 2004; Hovinen et al., 2006; Lehmann et al., 2015). As with somatic cell count, MEC can also be affected by the milk yield, but correcting for milk yield in the current study did not influence MEC outcome, apart from no difference between MEC of CCC1 and CONV1 cows shortly after the system switch to a daytime CCC system. The difference in MEC of conventionally housed cows and cows with CCC can thus only partially be explained by the milk yield of the cows. Besides the explanations related to udder health or milk yield level, lower MEC during the suckling period could be the result of an interplay between the high frequency of milk removal from the udder, blood prolactin concentration, and the permeability of the mammary epithelium tight junctions. The more frequent milk removal when being suckled could lead to increased prolactin secretion in the cow (Lupoli et al., 2001). In turn, the permeability of mammary epithelium tight junctions could decrease, when prolactin concentrations increase, leading to reduced ion leakage to the milk and thus a lower MEC (Stelwagen et al., 1999).

The strategy of reducing contact between cow and calf via a system switch from whole-day to 12 h daytime CCC system slightly increased machine milk yield, but not the other milking characteristics, including MFR, PMFR, and MEC, and also not as much as fitting calves with nose flaps. A gradual 2-step weaning and separation

protocol, as in NF and NFFL cows of this study, alleviates the stress response at time of separation (Johnsen et al., 2015; Wenker et al., 2022a). It seems that the week of fence-line contact, as in NFFL cows of Exp. 2, might have been more successful in doing so. As suggested by Vogt et al. (2024), a more gradual reduction in daily CCC allowed, for example 1 wk of 7 h daytime contact before another week of 3.5 h morning contact, followed by a week of fence-line contact can lower weaning and separation distress, possibly also with more pronounced effects for milking characteristics.

Milk Yield Beyond Separation

Later in lactation, beyond separation of cows and calves, machine milk yield of NFFL cows did not differ significantly from CONV2 cows after 18 WIM. This corresponds to the study of Wenker et al. (2022a), where cows with whole-day CCC for 8 wk had a nonsignificant lower milk yield of 6 kg/d after weaning compared with cows that had no CCC. Also in the prospective cohort study of Sørby et al. (2024a), average lactational machine milk yield, including the contact period and time beyond separation, did not differ significantly between cows that had CCC for longer than a month (17.6 kg/d), less than month (26.5 kg/d), or without CCC (27.9 kg/d). However, machine milk yield of CCC1 and NF cows in Exp. 1 and Exp. 2, respectively, did not fully recover to a similar level as conventionally managed cows. Similarly, post-separation milk yield of cows that had whole-day CCC or CCC for 15 min before each milking for 3 mo also did not recover to a similar level as cows without CCC (Barth, 2020). Milk yield of NF cows increased as soon as their calves were weaned and reached the same level of NFFL cows when their calves were separated at 19 WIM. Apart from the NFFL calves possibly cross suckling the NF cows and reducing their machine milk yield, the more gradual post-separation increase in milk yield of CCC1 and NF cows could indicate an adaptation to only being machine milked. This could be a consequence of the mechanism between suckling, oxytocin, and milk yield as discussed earlier. As thoroughly discussed in the Sørby et al. (2024a) study, a lower post-separation milk yield in cows that had CCC might reduce the secretory potential of the mammary cells, leading to reduced milk production in later lactation. The lower total milk production, and thus greater saleable milk losses, in cows with CCC, as calculated by Churakov et al. (2023), could indicate impaired milk ejection and thereby incomplete milk removal during machine milking in the CCC cows. Incomplete milk removal might have led to a negative feedback in early lactation, resulting in lower peak yields and lower milk production later in lactation for the CCC cows. Milk ejection during milking of cows with CCC

remains a hurdle and should be further investigated with the aim to improve milk let-down in the MU, while ensuring that the calf continues to receive sufficient milk.

The effects of prolonged CCC on the lactational milk yield of cows are thus contradictory between the experiments of the current study, and between other studies, and could depend on the contact duration and extent as well as weaning and separation strategies. In addition, further research is needed to determine the energy balance of the cow, as well as the metabolic effects of prolonged CCC for both the cow and calf, especially around the possibly more stressful weaning and separation periods.

Limitations and Future Studies

To our knowledge, this is one of the first studies comparing feed intake of cows in a CCC system with cows in a conventional dairy system. Like in many system comparisons, not only the contact between cow and calf varied between treatment groups, but also aspects related to animal management and housing differed. In Exp. 1, CCC1 cows were moved to another automatic MU, which might have caused additional stress due to the new environment, possibly reducing machine milk yield. Because of this, we were unable to differentiate between stress from separation from the calves and stress from the new environment. In addition, allocating cows that calved first to the CCC1 treatment group in Exp. 1 or NF treatment group in Exp 2, instead of blocking for cow characteristics, might have introduced bias in the experiments. This allocation protocol might lead to treatment groups experiencing different conditions, such as weather, management, or ration composition. A possible advantage of this allocation protocol is the low variation in calf age within the treatment groups. To our knowledge, however, studies are lacking comparing different distributions in calf age within a CCC group. Balancing the groups for calf age would have meant a more variable weaning age for the calves, with some calves being weaned at a younger age than intended. Moreover, future studies could avoid the cross suckling in the weaned NFFL calves with the NF cows by preventing contact between cows whose calves have been weaned or separated and calves that are yet to be weaned.

Clearly, in the current study the consequences of the CCC systems and different weaning and separation strategies on feed intake and milk yield of the cows are not unequivocal. Various types of weaning approaches and separation strategies should still be explored further. Although more gradual weaning approaches are advantageous, the duration as well as the extent thereof is still not certain. Finally, abrupt separation as in Exp. 1, although following repeated temporary separation, does not seem optimal in terms of cow and calf welfare, whereas a more

gradual separation strategy could alleviate the behavioral response at separation. Gradual separation strategies, for example, where cow and calf are partially separated and restricted suckling is still allowed, could be promising for both cow and calf welfare and should be further worked studied (Neave et al., 2024).

CONCLUSIONS

Machine milk yield of CCC cows remained lower either until the week of separation, for NFFL cows, or until 3 or 11 wk after weaning and separation for CCC1 and NF cows of experiments 1 and 2, respectively. Cow-calf contact reduced MEC, and although lower during the suckling period, MFR increased the week after separation of cow and calf. Not for experiment 2, but for experiment 1, roughage and concentrate intake decreased at separation and recovered within a week. Based on the temporary reduction in cow feed intake at separation in Exp. 1, reducing CCC to daytime contact followed by abrupt separation had a greater effect on the cow than weaning calves with nose flaps or via a fence line followed by separation, as in Exp. 2. This conclusion should be confirmed in future studies where both weaning strategies are compared within the same experimental setup, including focus on consequences of the weaning strategies for the calves.

NOTES

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Nonstandard abbreviations used: B = breed; CCC = cow-calf contact; CCC1 = cows with cow-calf contact in

experiment 1; CCC2 = cows with cow-calf contact in experiment 2; CONV1 = conventionally managed cows in experiment 1; CONV2 = conventionally managed cows in experiment 2; Exp. = experiment (1 or 2); G = treatment group; MEC = milk electrical conductivity; MFR = milk flow rate; MU = milking unit; NF = nose flap group in Exp. 2; NFFL = nose flap fence-line group in Exp. 2; P = period; PC = parity class; PMFR = peak milk flow rate; WIM = weeks in milk.

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