Effects of two gradual debonding strategies on machine milk yield, flow and composition in a cow-driven cow-calf contact system

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ABSTRACT

This controlled study compared the effects of 2 different gradual debonding strategies on machine milk yield, flow and composition in a cow-driven cow-calf contact (CCC) system with automatic milking. Cows had 24 h/d access to their calves during the first weeks of lactation. In the long debonding (LD) treatment (n = 16), a gradual reduction of cows’ access to their calves was initiated 4 weeks after calving over a total period of 28 d; first to 12 h/d (14 d), and then to 6 h/d (14 d). In the short debonding (SD) treatment (n = 14), gradual reduction was initiated 6.5 weeks after calving over a total period of 10 d; first to 12 h/d (5 d), and then to 6 h/d (5 d). From 6 h/d, access was finally reduced to 0 h/d for 7 d for both treatments. Machine milk yield, somatic cell count (SCC), peak and average milk flow were automatically registered at milking. During the 9 weeks study period, composite samples were analyzed for milk composition. Data were analyzed with linear mixed effect models. Results showed that machine milk yield during 24 h/d access varied between cows (range 1.2–49.9 kg/d, average ± SD 13.2 ± 7.82 kg/d). LD cows had a higher daily machine milk yield than SD cows at the end of and after access reduction was completed (+5.0 ± 1.63 and +5.1 ± 1.55 kg during the last 5 d of 6 h/d access, and 0 h/d access, respectively). SCC was on a healthy level, with no difference between treatments. Milk fat content increased with reduction in access, regardless of treatment. Short debonding cows tended to show higher milk protein content and lower milk lactose content than cows with a longer debonding. This study has shown that a longer debonding initiated earlier may give a higher milk yield in the short term. The variation in machine milk yield may indicate differences in milk ejection, suckling and visiting patterns and preferences among cows.

Key words: Dam rearing, separation, weaning, automatic milking system

INTRODUCTION

In modern dairy production, the cow has usually been separated from its calf straight after birth. This practice of separation is likely to be connected to tradition, economic considerations and for the best intentions regarding cow and calf health (Beaver et al., 2019; Neave et al., 2022). However, the practice has in recent years become a point of criticism in society’s discussion about dairy farming (Busch et al., 2017; Sirovica et al., 2022). Consumers, the industry and many farmers (Hansen et al., 2023; Neave et al., 2022) show increased interest in different management systems allowing cow-calf contact (CCC, Sirovnik et al., 2020) and this topic has become an important object of research for a sustainable dairy industry (Agenäs, 2020; Health et al., 2023). CCC systems vary considerably in terms of type and duration of contact between cow and calf (Johnsen et al., 2016). There can be contact either between a dam and her calf or between a foster cow and her foster calf, and the contact can be either full (allowing suckling) or partial (suckling is prevented, either whole-day or part-time) (Sirovnik et al., 2020). In such systems, cows and their calves can stay together for a prolonged period of time, allowing them to express their natural behaviors such as cow-calf bonding, suckling and care-taking which has the potential to enhance animal welfare in addition to live up to consumers expectations (Wagenaar & Langhout, 2007). However, beyond practical and economic concerns the major challenge in CCC systems is the moment of separation (Hansen et al., 2023; Neave et al., 2022). Both the cow and calf show increased signs of stress behavior compared with separation immediately after birth (Weary & Chua, 2000). Debonding, hereby defined as any process of gradually adapting cow and calf to separation, for example by reducing access to suckling, physical, olfactory, tactile...
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and visual contact through gradual or partial separation, may ease separation.

A cow-driven CCC implies that the cow visits the calf in a designated area either by free or restricted access (Sirovnik et al., 2020) and in such systems, debonding may be facilitated by gradually diminishing cow access to the calf. While gradual debonding is known to alleviate the stress-response of cow and calf compared with abrupt separation (Enríquez et al., 2011; Johnsen et al., 2015; Wenker et al., 2022), knowledge is needed on how gradual debonding may affect the cow’s milk production. Additionally, more research is needed on debonding strategies in cow-driven CCC systems, specifically length and initiation time.

Multiple studies report that cow’s machine milk yield (i.e., saleable milk) in CCC systems show great variation (Barth, 2020; Johnsen et al., 2021a; Mutua & Haskell, 2022), with a clear reduction of machine milk yield during (Barth, 2020; de Passillé et al., 2008; Wenker et al., 2022; Zipp, 2018), but not necessarily after, the suckling period (Barth, 2020; Meagher et al., 2019; Wenker et al., 2022). Reduced machine milk yield is an important barrier to adopt CCC (Hansen et al., 2023; Neave et al., 2022), thus more research is needed to explain the physiological mechanisms and find solutions that minimize the loss of saleable milk for the farmers. There are many factors behind the complexed system of lower milk production. An increased frequency of udder emptying by calves has the potential to increase milk production (Bar-Peled et al., 1995), but it is questionable whether calves empty the udder sufficiently at each suckling. Nursing cows show lower oxytocin secretion during machine milking and thereof an impaired milk ejection (de Passillé et al., 2008). Furthermore, the more frequent peak oxytocin levels associated with nursing might reduce mammary gland sensitivity to oxytocin in the same way as chronic exogenous oxytocin causes desensitization, affecting milk ejection negatively (Ferneborg et al., 2017; Macuhová et al., 2004).

Milk flow is closely related to milk ejection and is also affected by udder fill and anatomical aspects (Pfeilsticker et al., 1995; Tančín et al., 2006). Nursing cows have been found to have lower peak and average flow compared with non-nursing cows (Barth et al., 2010; Mendoza et al., 2010; Zipp, 2018), likely due to a combination of udder fill and milk ejection. Johnsen et al. (2021a) found that gradual debonding could lead to higher machine milk yields, possibly due to higher udder fills and improved milk ejection.

Similarly, milk composition may be affected by nursing and frequency of nursing (Barth, 2020; Cozma et al., 2013; Zipp, 2018). Several studies show that the milk fat content of machine milk from nursing cows is reduced (e.g., −0.66% (Zipp, 2018), −0.72% (Barth, 2020)), but reaches normal levels after separation (4.28% (Barth, 2020)). This is likely primarily linked to the difference in milk ejection between nursing and non-nursing cows, leading to evacuation of different fractions of milk (Ontsouka et al., 2003). Milk protein content is known to increase when milking frequency is increased (Boujenane, 2019; Campos et al., 1994; Klei et al., 1997). Therefore, increased machine milk protein may be expected among nursing cows, but available studies have shown inconclusive results (Barth, 2020; Mendoza et al., 2010; Zipp, 2018). There is a lack of research on milk lactose content from nursing cows as lactose variation is known to be small (Forsbäck et al., 2010), but lower levels have been reported (Zipp, 2018) since lactose is closely related to milk yield (Costa et al., 2019; Fox et al., 1998). Milk SCC among individual CCC cows can vary widely (Zipp, 2018). Margerison et al. (2002) reported that SCC was reduced in nursing cows, whereas most studies report no differences (Barth et al., 2007; Krohn, 2001; Zipp, 2018). More knowledge is needed on how milk composition is affected by gradual debonding in CCC systems.

The objectives of this study were to determine short-term effects of 2 gradual debonding strategies on machine milk yield, milk flow and milk composition in a cow-driven CCC system. We hypothesized that long debonding would lead to a higher machine milk yield and higher milk flow at the end of debonding and during no access. Milk fat was hypothesized to increase while milk protein was hypothesized to decrease with the reduction in cow access, whereas milk lactose and SCC were hypothesized not to be affected.

MATERIALS AND METHODS

The study was conducted at Norwegian University of Life Sciences’ Livestock Production Research Centre, Ås, Norway. The research center has 2 main calving periods throughout the year, this being from September to November and January to February. The study was conducted from October 2020 to March 2022, divided into 4 batches, each 9 weeks long. All procedures were in accordance with the regulations controlling experiments/procedures in live animals in Norway, and the study complied with the policies relating to animal ethics. Our study schedule was to our best knowledge a novel design.

Animals

In total, 32 cow-calf pairs were enrolled in the study, allocated into 4 batches based on calving date. For each of the 4 batches, it took 13, 17, 7 and 12d respectively
to collect all cow-calf pairs (n = 8). All cows and calves were of the Norwegian Red breed (a dual-purpose breed). Each batch was scheduled to coincide with peak number of calvings, to minimize within-batch calf age and DIM difference. The herd has approximately 160 calvings during calving season, with about 6–7 calvings each week. As it was necessary to prioritize short age and DIM differences, it was therefore not possible to balance treatments for parity in our study.

Healthy cow-calf pairs were included immediately after calving. Exclusion criteria were previous experiences with CCC, calving difficulties, calving outside of an individual calving pen, failure of establishment of suckling, ill health necessitating cow antibiotic treatment or aggression toward calf or personnel. Heifer calves were prioritized due to practical reasons and considerations for future research, but each batch could include up to 4 bulls. Five cow-calf pairs were excluded within 3 d after calving, due to aggressive behavior toward personnel (n = 1 pair), failure of suckling due to cow’s udder confirmation (n = 1 pair), or health events of the cow (interdigital dermatitis n = 1 pair, mastitis n = 2 pairs) requiring antibiotic treatments. New cow—calf pairs replaced these 5 pairs immediately. Two additional SD cows were excluded on d 21 and 45 respectively, due to interdigital dermatitis necessitating antibiotic treatment, resulting in a total of 30 cows in the data set. In total, the study included 9 primiparous, 9 2nd lactation, 7 3rd lactation and 5 4th lactation cows, with an average lactation number of 1.9 and 2.5 for LD and SD cows respectively.

Experimental design

Cow-calf pairs were managed in 4 batches, each of which included 8 pairs and ran over 9 weeks. Due to practical limitations, treatments could not be run in parallel. Each batch was therefore assigned to one of the 2 treatments, resulting in 2 separate batches per treatment. Treatments were long debonding (LD) and short debonding (SD). Potential seasonal differences were balanced for by conducting the treatments in the following order: LD (fall) – SD (winter) – SD (fall) – LD (winter). Before debonding, cows were given access to visit the calves in a meeting area 24 h/d (see details below). For cow-calf pairs with LD, cows’ access to the calf was gradually reduced from 4 weeks after calving over a total period of 28 d; first to 12 h/d (14 d), and then to 6 h/d (14 d). For cow-calf pairs with SD, gradual reduction was initiated 6.5 weeks after calving over a total period of 10 d; first to 12 h/d (5 d), and then to 6 h/d (5 d). From 6 h/d, access was finally reduced to 0 h/d for 7 d for both treatments, resulting in 4 phases (Figure 1). Reduction of cows’ access was executed simultaneously for all cows in a batch and the timing was based on median calving date in each batch. LD and SD cows were on average at 25.6 ± 4.2 and 44.5 ± 4.5 DIM (mean ± SD), respectively, when debonding started, and 55.6 ± 4.2 and 57.6 ± 4.3 DIM when the 0 h/d phase was initiated, respectively. LD cows spent in total 62.9 ± 3.4 d and SD cows spent 63.6 ± 4.3 d in the study.

Housing and management until debonding

At signs of imminent calving, cows were moved to an individual straw-bedded calving pen (2.9 × 3.2 m). After calving, all calves were offered the dam’s colostrum from a teat bottle within 1 h, assisted in suckling if needed and thereafter navel sprayed, ear tagged and weighed. Cows were bucket milked twice daily within the calving pen. Calves were not offered any supplemental milk in the calving pen, but suckling success was monitored. The cow-calf pair remained in the calving pen 3.4 ± 0.7 (mean ± SD) days, providing time to form a bond. Thereafter they were moved to a CCC-area consisting of a meeting area, cow area and a calf creep (Figure 2). During the 24 h/d access phase, cows had free access to the meeting area (36.7 m²) in which cows and calves could interact. The cow access was made possible by computer-controlled access gates (smart gates), in which cows are shown to be motivated to use for accessing their calves (Johnsen et al., 2021b). When the cow-calf pair was moved from the calving pen to the CCC area, a detailed protocol described training of the cows to use the smart gates. The cow was led in and out from the meeting area through open smart gates, then encouraged to try to self-open the smart gates by personnel retaining the calf in the calf creep. The cow was rewarded when she entered through the smart gate by the calf being released into the meeting area. The training ended once the cow voluntarily passed through the smart gates. In the cow area, cows had access to cubicles with rubber mats bedded with sawdust, ad libitum grass silage (DM 32 ± 2%, NEL20 6.1 ± 0.3 MJ/kg DM, CP 142 ± 9 g/kg DM and NDF 504 ± 37 g/kg DM), water, and concentrate (DM 87%, NEL20 7.3 MJ/kg DM, CP 211 g/kg DM and NDF 190 g/kg DM) in an automatic feeder as well as in the automatic milking system (AMS). Cows were following Norwegian standards of feeding concentrate; amount of daily concentrate provided for each cow was allotted according to a feeding template based on expected milk production. Cows were milked in an AMS (DeLaval VMS, 44 kPa, pulsation rate 60 cycles/min, pulsation ratio 65:35). Milking permission was set to 5.5 h and cows were fetched for milking to ensure that they were milked minimum twice daily. The 8 calves in a batch

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were housed in a calf creep (21.3 m²) with straw-bedded lying area (14.3 m²) and constant access to the meeting area. They had access to ad libitum water, hay and concentrate, but no access to supplementary milk. Cows were prevented from accessing the calf creep by a concrete wall with 2 small openings for calves to enter. Calves had no access to the cow area.

**Housing and management during debonding**

After the phase with 24 h/d access, cows’ access to the calves was gradually decreased by limiting their entry through the smart-gates, and thereby access to the meeting area, to the following time slots: 11:00 – 23:00 (12 h/d access), 11:00–17:00 (6 h/d access) and finally 0 h/d access. Cows were led out from the meeting area if they remained 1 h after the smart gates had closed (i.e., cows seen in the meeting area after 18:00 p.m.). From the point of first reduction in cow access, calves were provided ad libitum supplemental whole milk from an automatic feeder (DeLaval International AB, Tumba, Sweden). Calves were trained to use the automatic feeder by first being offered milk from a teat bottle before receiving individual assistance in the automatic feeder the 5 first days of 12 h/d access, then led to the automatic feeder as a reminder the first 2 d of 6 h/d and 0 h/d access. Independent of cow access through the smart gates, cows and calves always had the possibility for fence line contact.

**Data collection**

Individual cow machine milk yield, SCC, peak and average milk flow were automatically registered at every milking (Delpro software, DeLaval International AB, Tumba, Sweden) and downloaded weekly. Six composite milk samples were automatically collected by the milking robot individually from each cow, minimum once per access phase and sent for analysis of gross milk composition to TINE’s Laboratory in Heimdal, Norway, and analyzed using an FTS combi (Bentley Instruments).

**Statistical methods**

Basic data handling was performed in Excel (version Office 2016, Microsoft). All statistical analyses were performed using Stata (Stata SE/16, Stata Corp., College Station, TX, USA), and linear mixed models were used to analyze the data. Cows were treated as the experimental unit, and model selection followed a step-wise backward method, starting with all explanatory variables and possible interactions included. Thereafter, based on biological relevance and significance, variables and interactions were excluded from the model. The best fitted model was chosen after comparisons of Akaike information criterion (AIC). Regardless of statistical significance, treatment and phase effects were the main focus of the study. All models therefore included the following fixed effects: treatment (LD/SD), phase (24 h/d, 12+6 h/d, 0 h/d for milk composition, or last 5 d of 6 h/d access and 0 h/d access for machine...
milk yield, flow and SCC) and treatment*phase. Thus, these variables remained in the model even if no significant effect could be observed. The statistical models were specified to reflect the hierarchical structure of the data: batch > animal id. Within animal id (cow), data was collected repeatedly on different test days which was accounted for by the covariance structures (see below for details). For all models, different covariance structures were assessed to account for repeated measures. For all outcome variables except milk flow, autoregressive (AR) covariance structure was the best fitted covariance structure. Milk flow did not converge for AR, and therefore the covariance structure vce(robust) was used to estimate robust standard errors. The fit-

Figure 2. A cow-driven CCC system consisting of a meeting area (purple), cow area (blue) and a calf creep (yellow). The meeting area was for cow and calf interaction only, without any resources. Cows had all resources available in their cow area, while calves had access to forage, concentrate, water and an automatic milk feeder in their straw-bedded calf creep. Calves had free access to the meeting area, whereas cows’ access was controlled by smart gates.
ted residuals of the models were inspected graphically, and influential outliers not detected. We ended up with having one model for each outcome variable; machine milk yield, average and peak milk flow, SCC, milk fat, milk protein and milk lactose. All models included the fixed effects of treatment, phase (24 h/d, 12 h/d, 6 h/d and 0 h/d for milk fat, protein and lactose, while last 5 d of 6h/d access and 0 h/d access for milk yield, flow and SCC), treatment*phase interaction, days in milk and the random effect of animal id.

Significant differences were declared at \( P \leq 0.05 \), and trends discussed at 0.05 < \( P < 0.1 \). Data is presented as marginal means ± SE unless otherwise specified.

**Machine milk yield, average and peak milk flow and SCC.** A treatment effect on machine milk yield was evident already before any treatment was applied (24 h/d access phase), likely because of parity imbalance. The average for each outcome variable from treatment wk 1–3 was therefore used as a covariate in each model, as it would make the base level for treatment effects. Due to the differing lengths of phases in our study, comparing each phase between treatments would be incorrect due to differing DIM. Therefore, we chose to only include days in which cows were at similar stage of treatment and with similar DIM. Comparable days between treatments were the last 5 d of 6 h/d access and the 0 h/d access phase, d 53 until d 64 of treatment. The final model for machine milk yield, average and peak milk flow and SCC consisted of the fixed effects of treatment and phase as well as their interaction, days in milk, covariate from wk 1–3 and the repeated effect of cow.

Cow daily machine milk yield was calculated per 24 h period using moving averages over 3 d, due to high day-to-day variability. The final model included 12 observations per cow (d 53 - 64).

Data on cows’ average and peak milk flow included observations from each quarter at each milking. The model needed to take the repeated measures of teat into account; teat was nested within cow. The final model for milk flow included an average of 109 observations per cow (d 53 until d 64).

SCC was automatically registered by the AMS, but not at each milking. This resulted in 10.8 ± 1.5 (mean ± SD) observations per cow (d 53 until d 64), and to achieve normal distribution, data were log10-transformed.

**Milk fat, protein and lactose content.** Due to fewer observations for milk fat, protein and lactose content, we included all samplings for the analysis (d 1 until d 64). From the total of 24 sampling days for milk composition, one sampling was mistakenly not taken while 2 consignments went missing during transport or analysis, resulting in 5.3 ± 0.5 (mean ± SD) observations per cow. Samples were collected on treatment d 10 ± 0.24.5 ± 0.7, 30.6 ± 3.1, 52 ± 0, 61.5 ± 2.1 and 10.5 ± 6.4, 29.5 ± 0.7, 41 ± 1.4, 50.5 ± 0.7, 56 ± 0 and 58.5 ± 7.8 d for LD and SD respectively. The model for milk composition consisted of the fixed effects of treatment and phase as well as their interaction, days in milk and the repeated effect of cow.

**RESULTS AND DISCUSSION**

**Machine milk yield**

As previous CCC studies (Bar-Peled et al., 1995; Barth, 2020; Johnsen et al., 2021a; Mutua & Haskell, 2022), our study also showed great individual variation in machine milk yield from cows during the 24 h/d access phase, ranging from 1.2 to 49.9 kg/d, with an average ± SD of 13.2 ± 7.82 kg/d (Figure 3). This proves that relatively high machine milk yields in CCC systems are possible. The large variation persisted also after separation, with a range in yield from 8.6 to 50.1 kg/d and an average of 27.7 ± 7.51 kg/d (mean ± SD) during 0 h/d access. The large individual variation can be related to the differences in nursing frequency and intervals between suckling and milking during 24 h/d access, but also by the individual differences in alveolar milk ejection (Zipp, 2018). The average of 27.7 kg/d is lower than what is expected from cows at the research barn (28 kg/d and 37 kg/d for primiparous and multiparous, respectively.)

Descriptively, both LD and SD cows showed increasing machine milk yields with every reduction in access (Figure 3). This increase was expected based on the normal lactation curve, which also makes it difficult to distinguish the effect of phase from the effect of days in milk. The indication of increase with reduction in access was however in line with Zipp (2018) where a greater loss in machine milk yield was found for cows from whole-day contact than from cows with half-day contact compared with a control with no contact (−13 kg/d and −9.9 kg/d, respectively).

Comparing the last 5 d of 6 h/d access (5d6h) and the whole 0 h/d access phase between treatments, we found a significant effect of treatment on daily machine milk yield (Table 1). LD cows had 5.0 ± 1.63 kg higher daily machine milk yield than SD cows during 5d6h \( (P = 0.002) \), and 5.1 ± 1.55 kg higher yields than SD cows during 0 h/d access \( (P = 0.001) \). The different machine milk yield between the 2 treatments during reduced access and 0 h/d access may be due to a higher milk consumption by SD calves, but also due to differences in the degree of impaired alveolar milk ejection during milking. Insufficient milk ejection can be caused by inhibition of oxytocin release by the
central nervous system or by peripheral inhibition of oxytocin effects on the mammary gland due to receptor downregulation (Bruckmaier & Blum, 1998). Macuhová et al. (2004) stated that chronic oxytocin treatment reduced mammary gland sensitivity to oxytocin, and frequent oxytocin administration has been found to affect milk ejection negatively (Ferneborg et al., 2017). During nursing, oxytocin levels are greater than during machine milking (de Passillé et al., 2008; Lupoli et al., 2001), it may be possible that the repeated high levels of oxytocin during nursing can cause the same peripheral response as exogenous oxytocin injections. As SD cows had a longer phase of 24 h/d access than LD (6.5 weeks and 4 weeks respectively), the chronic effects of oxytocin might be more prominent resulting in a lower increase in machine milk yield during reduced access (5d6h) and after access was fully reduced (0 h/d). The lower machine milk yield of SD cows during 5d6h could also be related to the differing lengths of the 6 h/d access phases (14 d and 5 d for LD and SD respectively). Based on subjective observations, cows were perceived to show increased stress responses during the first days of a new phase, which may have affected machine milk yield negatively for SD cows.

We also found a significant effect of phase on machine milk yield, regardless of treatment (Table 1). With machine milk yield increasing from 5h6h to 0 h/d access phase. This was expected both from the normal lactation curve, but also from previous studies reporting lower machine milk yields from nursing cows (Barth, 2020; de Passillé et al., 2008; Wenker et al., 2022; Zipp, 2018) that increases after separation (Barth, 2020; Wenker et al., 2022).

**Milk flow**

There was a tendency of a treatment effect for both average and peak milk flow (Table 1), with LD cows having a higher flow during 5d6h ($P = 0.011$, $P = 0.015$, respectively), and a tendency of a higher flow during 0 h/d access ($P = 0.057$, $P = 0.056$, respectively). The higher flow from LD cows during 5d6h was as expected as we thought that a longer debonding could lead to higher udder fills and possibly improved milk ejection. Independent of treatment, phase affected average and
peak milk flow (Table 1), with a higher flow during the 0 h/d access phase compared with 5d6h. This was in line with previous studies (Barth, 2020; Mendoza et al., 2010; Zipp, 2018), and in close association with machine milk yield.

**Milk composition**

As hypothesized, there was no treatment effect on SCC in our study (Table 1), supporting previous findings (Barth, 2020; Krohn, 2001; Zipp, 2018). SCC remained on a healthy level (<100 000 cells/ml, (Nørstebø et al., 2019)) throughout the study for both treatments, with a range from 2 000 to 6 497 000 cells/ml. Four multiparous cows from LD seemed to be the major contributors to highest cell counts. Margerison et al. (2002) reported reduced SCC from suckled cows, and as the LD treatment consists of a shorter period of 24 h/d access this may be a reason for some LD cows having higher cell counts. However, those cows had cell counts > 100 000 cells/ml evenly distributed throughout the study, hence already before treatments were applied.

Collecting representative milk samples from cow-calf contact systems is very challenging, due to the variation in milk ejection and the influence of suckling. The samples collected in our study were representative for the delivered milk from each cow at that particular milking, but not necessarily for the actual produced milk of that cow, which is likely to have caused increased variation.

Overall, milk fat content in this study varied from 0.77% to 6.47% with an average of 3.37 ± 1.24% (mean ± SD). Milk fat content varies between breeds and that of Norwegian Red contains on average 4.29% fat (TINE, 2022) means that the average fat content found in the present study can be considered low. No effect of treatment was found ($P = 0.502$, Table 2), but phase significantly affected fat content ($P = 0.003$), with an increase from 3.20 ± 0.17% to 4.49 ± 0.30% between 12+6 h/d and 0 h/d access, respectively, supporting the study by Barth (2020). Due to the low specific gravity and adhesiveness of milk fat globules, milk fat content will increase during milking with a proper milk ejection. A decreased milk fat content can therefore be evidence of disturbed milk ejection (Onsouka et al., 2003). The fairly low fat content during phases of nursing and the increased fat content with decreased cow access may indicate disturbed milk ejection in nursing cows, which is in line with other studies (Carbonneau et al., 2012; Margerison et al., 2002; Zipp, 2018). In a CCC system, it can also be speculated that the calves may evacuate milk fat more efficiently than the AMS, resulting in a removal of some residual milk. The individually
higher fat levels are likely due to recent suckling, but it can also be evidence for proper milk ejection among individual CCC cows.

Milk protein content ranged from 2.69% to 4.84%, with an average (±SD) of 3.51 ± 0.29%. Protein content in milk from Norwegian Red breed cows is on average 3.56% (TINE, 2022), which is similar to our results. There was a tendency of a treatment effect \((P = 0.089)\) with a slightly higher milk protein content from SD cows. There was also an interaction effect between treatment and phase on milk protein content \((P = 0.010)\), with phase 12+6 h/d and 0 h/d access in SD treatment showing slightly higher protein content than the same phases in LD treatment, while just a tendency of a difference during 24 h/d access. The higher milk protein content from SD cows may be explained by the longer period with nursing before reduced access compared with LD cows (6.5 weeks and 4 weeks, respectively), as Barth (2020) reported significantly higher protein content in milk from CCC cows during the nursing period and several studies has reported increased protein content with increased milking frequency (Boujenane, 2019; Campos et al., 1994; Klei et al., 1997). However, protein content surprisingly also increased with every reduction in access regardless of treatment, with phase being significant \((P = 0.006, \text{Table 2})\). This may however be related to the expected increase in crude protein as well as several of the individual milk protein fractions starting at approximately 5 weeks in lactation (Franzoi et al., 2019; Liliana et al., 2023), which together with mastitis is the major source of variation in milk protein content apart from genetics. In addition, (Gellrich et al., 2014) showed that stressful events may alter milk protein composition and Barth (2020) hypothesized that the changes in milk protein during CCC may be related to the whey protein fraction, which should be investigated further.

Milk lactose content in this study varied from 3.74% to 5.10%, with an average of 4.64 ± 0.25% (mean ± SD) with only a tendency of a treatment effect found (Table 2). Milk lactose content from Norwegian Red breed cows is on average 4.75% (TINE, 2022), meaning that the average measured in our study was similar or slightly lower. SD cows which had a longer period of 24 h/d access had a tendency of a lower overall milk lactose content, which supports other studies with lower lactose levels from nursing. Though the variation of lactose content was low, during all phases with nursing (24 h/d, 12 h/d and 6 h/d) the average lactose content was 4.61 ± 0.26% (mean ± SD), which is greater than described in literature (Zipp, 2018) but is likely the effect of breed (TINE, 2022). The variation of lactose content was lower than the variation of fat and protein content.

### Table 2. Milk composition in different access phases (24 h/d – 0 h/d) of cows in a cow-driven CCC system with long \((n = 16)\) or short \((n = 14)\) debonding. Values are presented as marginal means ± standard error

<table>
<thead>
<tr>
<th>Phase</th>
<th>Long debonding</th>
<th>Short debonding</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h/d</td>
<td>12-60h/d</td>
<td>0h/d</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>3.00 ± 0.29</td>
<td>3.07 ± 0.20</td>
<td>4.38 ± 0.34</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>3.33 ± 0.07</td>
<td>3.37 ± 0.05</td>
<td>3.61 ± 0.08</td>
</tr>
<tr>
<td>Lactose content (%)</td>
<td>4.66 ± 0.06</td>
<td>4.71 ± 0.06</td>
<td>4.70 ± 0.07</td>
</tr>
</tbody>
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content in this study, which was expected from literature (Ptak et al., 2012).

Detecting true effects in this study was challenging because of the impact of the normal lactation curve on milk production. However, using covariates for machine milk yield enabled us to detect treatment effects. Cows were allocated based on calving dates which caused unbalances in parity and calf sex among treatments. This may cause limitations to interpretations of parity and treatments effects, but was necessary to minimize calf age as well as lactation stage differences within batch. Using a control group would have illustrated the impact of CCC on milk production better, but since the primary aim of our study was to compare different strategies for a CCC system, we did not include a control group. Despite a high variation in machine milk yield and milk composition and a relatively low number of animals, we were able to demonstrate effects of the 2 debonding strategies on machine milk yield and milk composition. We were also able to detect differences within and across groups between different levels of access restrictions. Controlled cow access may be a key factor in trying to adjust to the great variation in milk yield among cows. Production data combined with smart gate data enables detection of the individual cow’s preferences and makes a potential for better profitability for the farmer while still allowing for maternal behavior in CCC systems. Future studies should aim to have a balance in parity and further investigate different debonding strategies at different calf ages.

CONCLUSIONS

This study has shown that from the 2 different initiation time and lengths of the debonding, a longer debonding initiated earlier may result in a higher machine milk yield while minor effects on milk flow and milk composition in the short term. A long debonding led to higher machine milk yield during the last 5 d of 6 h/d and 0 h/d access compared with short debonding. SCC was on a healthy level, with no difference between treatments. Milk fat content increased with reduction in access, regardless of treatment. There was a tendency of a higher milk protein content while a lower milk lactose content from cows with a shorter debonding, but with very low variation. Collectively, these results may point toward a longer debonding being more favorable for cow performance. We recommend to further explore ways of gradually reducing contact before total separation in CCC systems in order develop more knowledge on strategies that maximize performance in cow and calf, and reduces stress responses.

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