Milk ejection in dairy cows with full-day cow-calf contact

Effects of milking permission interval in an automatic milking system

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Milk ejection in dairy cows with full-day cow-calf contact. Effects of milking permission interval in an automatic milking system

Mjölknedsläpp hos mjölkkor med ko-kalvkontakt. Effekter av tidsintervallet mellan tillåtna mjölkningar i ett automatiskt mjölkningssystem

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Abstract

Cow-calf contact (CCC) systems have received increased attention as alternatives to early cow-calf separation in dairy production. However, the effects of CCC on milk ejection during automatic milking are not fully understood. The aim of this study was to investigate the effects of full-day CCC and different milking permission intervals on milk ejection in dairy cows housed in automatic milking systems (AMS).

A total of 40 cows (22 Swedish Red, 18 Swedish Holstein) were enrolled individually at calving and allocated to four treatment groups in a 2 x 2 factorial design based on management system and milking permission interval: CCC with 4 h milking permission (CCC4), conventional management (CONV) with 4 h milking permission (CONV4), CCC with 10 h milking permission (CCC10), and CONV with 10 h milking permission (CONV10). Conventional cows were separated from their calves 12 h after calving, whereas CCC dams remained in full physical contact with their calves for up to 13 weeks before an 8-day fenceline period and subsequent separation. All cows were housed in the same loose-housing barn with one AMS unit with controlled cow traffic through an automatic selection gate, while CCC dams had access to a separate cow-calf area where unrestricted interaction and suckling were possible.

Milk ejection was assessed using milk fat concentration in strip milk collected immediately after machine milking. Approximately 20 ml of milk was collected by hand from each quarter into bronopol-coated tubes and analysed for milk fat concentration at quarter level. Sampling was performed on two occasions: within the first 12 days in milk and approximately 10 days before the start of fenceline weaning. During the first sampling occasion, milk samples were collected at all milkings during a 24-hour period, whereas two milkings per cow were sampled during the second occasion. Milkings were classified as normal when all analysed quarters exceeded 8.4% milk fat concentration, and as impaired when all analysed quarters were below 8.4%. Milkings with quarter-level values both above and below the threshold were classified as inconsistent.

Cows managed with CCC generally showed a numerically lower milk fat concentration throughout both sampling occasions. At the first sampling occasion, CCC4 and CCC10 dams showed a significantly lower proportion of milkings classified as normal and a higher proportion classified as inconsistent compared with CONV10 cows. In contrast, no clear differences were observed between the 4 h and 10 h milking permission intervals. At the second sampling occasion, there was a numerical increase in all treatment groups in the proportion of normal milkings, however no significant differences were detected between groups. The results suggest that full-day CCC may influence milk ejection during machine milking in dairy cows housed in an AMS, whereas milking permission interval alone appeared to have limited effect under the conditions of this study.

Keywords: cow-calf contact, milk ejection, fat concentration, strip milk, automatic milking

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Abbreviations

Abbreviation	Description
AMS	Automatic milking system
CCC	Cow-calf contact
CONV	Conventional
DIM	Days in milk
VMS	Voluntary milking system

1. Introduction

Animal welfare has become an increasingly important issue in society, as public and consumer concern about practices in dairy production has grown (EFSA 2015; Weary & von Keyserlingk 2017). In particular, cow-calf contact (CCC) systems have received growing attention from the public (Sirovica et al. 2022) and among dairy farmers (Eriksson et al. 2022) interested in alternatives to early separation practices. CCC systems are often promoted as welfare-enhancing due to their support of maternal contact and natural cow-calf behaviours (Johnsen et al. 2016).

At the same time, CCC systems present challenges related to milk production. In such systems, milk removal occurs through a combination of calf suckling and machine milking. Reduced amount of saleable milk is frequently mentioned as a concern by dairy farmers who are considering adopting CCC (Neave et al. 2022; Bertelsen & Vaarst 2023), and early separation of cow and calf is therefore often regarded as economically efficient (Flower & Weary 2003). Although being suckled by the calf most often reduces the amount of milk harvested for sale from high producing dairy cattle (Fröberg et al. 2007; Barth 2020; Churakov et al. 2023; Zipp & Knierim 2024; van Zyl et al. 2025), the milk consumed by the calf contributes to its growth and development. In the context of CCC, a reduction in the amount of harvested milk may hence be regarded as a net loss to the farmer when the reduction exceeds the amount of milk consumed by the calf.

Lower milk harvest at machine milking in CCC systems cannot always be explained by calf milk intake alone, as losses may be aggravated by incomplete milk removal during machine milking (Mendoza et al. 2010; Barth 2020; Neave et al. 2024). Efficient milk removal requires an effective milk ejection response, which is regulated by the milk ejection reflex (Bruckmaier & Blum 1998). During this reflex, oxytocin is released from the posterior pituitary gland in response to tactile stimulation of the teats, enabling milk stored in the mammary gland to become available for removal during milking (Bruckmaier & Wellnitz 2008). Milk ejection can be impaired by factors that inhibit oxytocin release, such as stress or insufficient teat stimulation (Bruckmaier & Blum 1998; Bruckmaier 2005). In addition, the presence of a calf or sensory cues from the calf during machine milking has been shown to reduce oxytocin release (Akers & Lefcourt 1984; Tancin et al. 2001). It has therefore been suggested that nursing cows may retain a proportion of milk during machine milking, potentially for their calves, resulting in increased amount of residual milk remaining in the udder (Krohn 2001).

In addition, in some CCC systems (i.e., half- and full-day contact) suckling by the calf may occur at irregular times between machine milkings which can increase the variation in udder fill at the time of milking (Barth 2020; Neave et al. 2024).

Udder fill affects the readiness of the udder for milk removal, as greater udder fill increases the intramammary pressure which in turn has been associated with a shorter time between tactile stimulation and milk ejection (Bruckmaier & Hilger 2001). In automatic milking systems (AMS), the timing of milking is controlled by system-defined minimum milking intervals that determine whether a cow is permitted to be milked, so-called milking permissions (Jacobs & Siegford 2012). Shorter milking permissions result in shorter intervals between milkings and therefore provide less time for milk accumulation in the udder before machine milking (Bruckmaier & Hilger 2001). Industry recommendations may further encourage short milking permissions; for example, DeLaval suggests a four-hour milking interval for cows between 0–100 days in milk (DIM) (DeLaval 2016). The combination with irregular calf suckling and short milking permissions may therefore further reduce udder fill at the time of machine milking - potentially exaggerating the negative effect of CCC on efficient milk ejection.

Assessing whether milk ejection during machine milking is affected by factors such as CCC and differences in minimum duration between subsequent milking requires reliable indicators of how effectively milk is removed from the udder during machine milking. Milk fat concentration increases throughout machine milking and is highest toward the end of milking, due to milk with higher fat concentration becoming available from the alveolar compartment later in the milking process (Ontsouka et al. 2003; Rico et al. 2014). Because the release of this alveolar milk depends on milk ejection, the fat concentration toward the end of milking can be used as an indicator of how effectively milk ejection occurred during machine milking. Fat concentration in strip milk collected directly following machine milking therefore provides an indicator of the degree of udder emptying, with lower fat concentrations indicating incomplete removal of alveolar milk (Jenni et al. 2024). Post-milking strip milk sampling has occasionally been used to evaluate milk ejection in CCC studies, with Rell et al. (2024) reporting lower milk fat concentrations in cows nursing their calves compared to cows that did not have contact with their calf.

Despite evidence that CCC cows may retain milk during machine milking, the effects on milk ejection of CCC depending on different milking permission settings in AMS, alone and in combination, have received limited attention. Therefore, the aim of this study was to investigate how two levels of milking permission (4 h and 10 h) affected milk ejection in CCC dams compared to conventional (CONV) cows kept in the same unit. In particular, the study focuses on how these factors influenced the fat concentration of milk collected at the end of machine milking, with sampling conducted at the quarter level.

1.1 Research questions

This study addresses the following research questions:

- Does CCC affect milk ejection in dams housed with their calves in AMS, as compared to CONV managed cows?

- Does shorter milking permission at 4 instead of 10 hours affect milk ejection in CCC and CONV cows housed in an AMS?

1.2 Hypothesis

It was hypothesized that cows managed in a CCC system would have lower milk fat concentration in strip milk collected after milking than CONV managed cows, suggesting less complete udder emptying during machine milking. It was further hypothesized that a 4-hour milking permission would be associated with lower milk fat concentration in strip milk collected after milking than a 10-hour interval.

2. Literature review

2.1 Cow-calf contact systems in dairy production

Cow-calf contact is defined as any system allowing physical interaction between a cow and a calf after the immediate postpartum period (Sirovnik et al. 2020). Calves may remain with their biological dam (dam-rearing) or be reared with at least one alien cow nursing multiple calves (foster cow system) (Johnsen et al. 2016; Eriksson et al. 2022). Additionally, CCC systems can be further classified according to other aspects of management, including the duration of daily contact between cow and calf, the timing of contact relative to machine milking, and if it is the cow or the calf that initiates contact (Sirovnik et al. 2020).

In full-day CCC systems, cows and calves are housed together for almost 24 hours per day, with temporary separation typically limited to machine milking or feeding procedures (Sirovnik et al. 2020). Since cow and calf share the same environment, continuous social interaction and nursing on demand are possible (Johnsen et al. 2016). This system has been applied in different housing systems, including loose housing with cubicles and a calf creep area, and has been combined with automatic milking systems or milking parlours (Fröberg et al. 2011; Wagner et al. 2013). Among European farms practising CCC, full-day permanent contact was the most used daily contact length (Eriksson et al. 2022), although management practices differed considerably between farms.

In contrast to full-day CCC systems, part-time contact systems limit the duration of daily interaction between cow and calf beyond separation for milking or during feeding times (Sirovnik et al. 2020). Similar management principles have previously been described as restricted suckling, in which calves are typically allowed access to the dam during short, defined periods, often organised around milking sessions (Johnsen et al. 2016). For instance, Roth et al. (2009) allowed calves to suckle for approximately 15 minutes before milking, whereas Fröberg et al. (2008) provided about 30 minutes of suckling after milking, both twice daily. After each suckling session, the calf is returned to individual or group calf housing, resulting in a more controlled pattern of milk removal by the calf as compared with full CCC systems. This management practice is common in tropical dual-purpose cattle systems, where calves are allowed to suckle for limited periods in connection with milking (Sandoval-Castro et al. 2000; Fröberg et al. 2007). In addition, part-time contact can also be organised so that contact occurs either during the night or during the day (Sirovnik et al. 2020), so called half-day contact. For instance, cows and calves may spend the daytime together while being separated during the night (Veissier et al. 2013).

Regardless of whether the system is full-day or part-time, it may allow either full or partial contact between cow and calf. Full contact permits unrestricted suckling

and interaction, whereas partial contact limits behaviours such as suckling (Sirovnik et al. 2020). In addition, Sirovnik et al. (2020) classify systems as cow-driven or calf-driven depending on which animal that primarily initiates contact. This can be achieved through calf creep areas or automated selection gates that restrict access for either the cow or the calf (Johnsen et al. 2016). In barns with AMS, cow-driven traffic can be organised in different ways based on how cows move between the calf and other resources. These include pre-milking traffic, where cows pass the robot when returning from the calf to feeding or resting areas; post-milking traffic, where cows pass the robot before accessing the calf; and free cow-calf traffic, where cows can move freely between areas (Sirovnik et al. 2020).

2.1.1 Effect of cow-calf contact on milk yield

Between different studies evaluating machine milk yield, the CCC systems used have often differed in their design (Bar-Peled et al. 1995; Fröberg et al. 2007; Mendoza et al. 2010; van Zyl et al. 2025). Similarly, milk yield outcomes have varied considerably across studies (Bar-Peled et al. 1995; Fröberg et al. 2007; Mendoza et al. 2010; Churakov et al. 2023), suggesting that the type of CCC used in the study influence the amount of saleable milk obtained at milking. For instance, restricted suckling systems have in some cases been associated with higher saleable milk yield in suckled cows compared to cows without calf contact, such as such as when low-producing Zebu-Holstein crosses were milked once daily while calves suckled twice per day (Fröberg et al. 2007). In a study evaluating milk yield in high-producing Holstein cows total milk production, calculated as machine-milked milk plus calf intake, was higher for cows that were both milked and suckled by two foster calves, as compared to cows without calf contact but milked three or six times per day (Bar-Peled et al. 1995). However, it is worth noting that the amount of milk obtained at machine milking was much less in the suckled group. This finding aligns with Mendoza et al. (2010), who reported a lower machine milk yield in cows subjected to 30 min of suckling (by their own or other calves) twice daily during the first eight weeks after calving, compared with cows whose calves were artificially reared. In contrast, Churakov et al. (2023) reported an estimated reduction in total milk production for cows kept with their calves in AMS for 16 weeks postpartum, compared to cows separated from their calf within 24 h after calving and housed without calf contact in the same AMS unit. This pattern was also observed by Zipp & Knierim (2024), who evaluated CCC in dual-purpose German Black Pied cows, comparing no contact, half-day contact, and full-day contact during nine weeks postpartum, with cows milked twice daily. During this period, machine milk yield was reduced by ~45% in half-day contact and ~62% in full-day contact compared to no contact. However, half-day cows, which were separated from their calves overnight and reunited after morning milking, showed similar machine milk yield to no contact cows at mornings milking, whereas yields were lower at afternoon milking.

After separation of the calf, machine milk yield generally increases in CCC dams, but it does not always reach the same level as for CONV cows. In the study by Zipp & Knierim (2024), cows previously kept in half-day contact reached similar

machine milk yield as no contact cows after separation, whereas cows from full-day contact remained lower for the rest of the lactations. Similarly, van Zyl et al. (2025) evaluated CCC systems that differed in both contact duration and weaning strategy compared to CONV managed cows separated within 12 h after calving. In one experiment, cows and calves had full-day contact for 8 weeks followed by daytime contact until abrupt separation at ~16 weeks postpartum. In a second experiment, cows and calves remained in full-day contact for ~16 weeks before gradual weaning was initiated using nose flaps. Calves either wore nose flaps for two weeks before separation or wore nose flaps for one week followed by one week of fenceline contact before separation. In both experiments, machine milk yield increased after separation but remained lower than in CONV reared cows for varying periods. Following abrupt separation in the first experiment, machine milk yield remained lower for approximately 3 weeks post-separation. In the second experiment, cows weaned using nose flaps alone remained lower than CONV cows for up to 11 weeks post-separation, whereas cows additionally provided fenceline contact reached comparable machine milk yield to CONV cows within one week after separation. In contrast, in Norwegian dairy herds practising both full-day CCC and early separation as part of a research study, no differences in milk yield were found after separation between cows that had prolonged contact with their calves post-calving and those separated early (Sørby et al. 2024). Additionally, nighttime contact during the nursing period has been reported to not affect milk yield across the full lactation compared to cows without calf contact (Barth 2020)

One obvious explanation for the reduced amount of machine-milked milk in CCC systems is that part of the milk produced by the cow is consumed by the calf. However, calf intake alone may not fully explain this reduction, and differences in milk harvested during machine milking between cows with and without calf contact have been reported to exceed what could feasibly be consumed by the calf (Neave 2024). Krohn (2001) suggested that nursing cows may retain more residual milk in the udder, potentially influencing milk ejection during machine milking.

2.2 Normal milk ejection

Efficient milk removal in dairy cows depends on the milk ejection reflex, which allows milk stored in the mammary gland to become available during milking (Bruckmaier & Blum 1998). In the bovine mammary gland, most of the milk is stored in the mammary alveoli, where it is retained by capillary forces and hence not immediately available for removal by machine milking (Bruckmaier & Wellnitz 2008). For milk to become available for harvest, it must first be transported from the alveoli into the major ducts and gland cisterns of the udder. This occurs when myoepithelial cells surrounding the alveoli contract, forcing milk towards the cisternal compartments of the udder (Bruckmaier & Wellnitz 2008).

The contraction is initiated by the hormone oxytocin, which is released from the posterior pituitary as part of a neuroendocrine reflex (Bruckmaier & Blum 1998). The reflex is primarily triggered by tactile stimulation of the teats during milking or suckling, which activates sensory receptors and transmits nerve signals to the hypothalamus, leading to oxytocin release in the bloodstream (Bruckmaier & Blum 1998; Bruckmaier 2005). In addition to direct teat stimulation, cows may develop conditioned responses to stimuli associated with the milking routine, such as auditory signals from milking equipment or the general milking environment. These cues can stimulate oxytocin release even before milking begins (Bruckmaier & Wellnitz 2008). However, due to its short half-life, continuous oxytocin release is required to sustain myoepithelial contraction and maintain milk flow until most of the available milk has been removed (Bruckmaier et al. 1994) by suckling or machine milking.

2.3 Impaired milk ejection

Impaired milk ejection can be defined as a situation in which the milk ejection reflex is delayed, incomplete, or temporarily absent. This can be caused by peripheral inhibition, where oxytocin is present in the bloodstream but does not act properly in the mammary gland (Bruckmaier & Wellnitz 2008). However, such peripheral inhibition has only been observed, to the author's knowledge, under experimental conditions and may be induced by the administration of α-adrenergic receptor agonists or agents that block oxytocin receptor (Bruckmaier et al. 1997; Wellnitz & Bruckmaier 2001). Impaired milk ejection can also happen due to central inhibition, where the pituitary gland releases too little or no oxytocin in response to stimulation (Bruckmaier & Wellnitz 2008). The release of oxytocin can be insufficient due to several factors, for example inadequate teat stimulation (Bruckmaier & Blum 1998; Bruckmaier 2005), leading to impaired milk ejection. In addition, the neuroendocrine reflex can be inhibited by stress-related factors that interfere with central oxytocin secretion. For example, impaired oxytocin release resulting in impaired milk ejection often occur both during disturbances in the milking environment and at the first machine milkings of primiparous cows (Bruckmaier 2005). Furthermore, the presence of a calf, or exposure to sensory cues from the calf, during machine milking has in some contexts been shown to reduce oxytocin release. For example, Akers & Lefcourt (1984) reported lower oxytocin concentrations during machine milking in cows housed together with their calves during the first postpartum week compared to cows separated shortly after calving. Likewise, Tancin et al. (2001), who compared suckling and machine milking in cows kept with their calves, found that oxytocin release was lower during machine milking in the presence of the calf than during suckling, and that complete inhibition of oxytocin release during machine milking was observed in some cows. A field study conducted on Swiss dairy farms reported impaired milk ejection primarily in cows kept with full-day CCC, whereas such conditions were not observed in conventional systems without calf contact (Rell et al. 2024).

When oxytocin release is inhibited, the milk ejection reflex does not occur properly, and more alveolar milk remains in the mammary gland. Accumulation of milk in the mammary gland in turns triggers a local negative feedback mechanism that inhibits further milk secretion (Weaver & Hernandez 2016); as such, regular milk removal is crucial to maintain high milk production (Wall & McFadden 2012). Accordingly, several studies have shown that increasing milking frequency for CONV managed cows, for example from two to three or four milkings per day, results in higher machine milk yield (Allen et al. 1986; Soberon et al. 2011; Hanling et al. 2021). Delyus et al. (2020) reported similar results but also found that the latency to milk ejection (time from teat stimulation to milk flow onset) was longer when the interval between milkings was shorter (4.5–6.0 h) compared with longer intervals (7.5–9.0). Bruckmaier & Hilger (2001) investigated milk yield while using milking intervals of 4, 8 and 12 h and demonstrated that greater udder filling due to longer milking intervals increased intramammary pressure and reduced the delay between teat stimulation and the onset of milk ejection. This relationship may be explained by the degree of udder fill, as shorter milking intervals allow less time for milk accumulation.

In addition to the duration of time between milkings, the degree of udder filling is also influenced by physiological factors, for example stage of lactation (Bruckmaier & Hilger 2001). As milk production declines after peak lactation, less milk accumulates in the udder between milkings towards the end of the lactation, and cows in late lactation may therefore be more prone to delayed milk ejection after onset of tactile stimulation if the milking intervals are kept constant (Bruckmaier & Hilger 2001; Wieland & ten Have 2025). Similarly, primiparous cows generally have a lower milk yield compared to multiparous cows, particularly during early lactation (Siewert et al. 2019), which may result in lower udder fill and thereby contribute to a delayed milk ejection response (Bruckmaier & Hilger 2001). However, the smaller udder size and cisternal capacity of primiparous cows (Pfeilsticker et al. 1996) may compensate for the lower milk volume by maintaining intramammary pressure high despite lower milk yield. At the same time, because residual milk constitutes a larger proportion of the udder contents in cows with smaller cisternal capacity, incomplete udder emptying may have a greater impact on feedback inhibition of milk secretion in primiparous cows (Albaaj et al. 2018; Deacon et al. 2023). Furthermore, in CCC systems specifically, udder fill may also be affected by calf suckling occurring between machine milkings. Depending on system, suckling can increase the variation in udder fill at the time of machine milking (Barth 2020; Neave et al. 2024), potentially contributing to increased variability in the milk ejection response.

2.4 Methods to measure milk ejection

2.4.1 Residual milking

Residual milking was introduced early in dairy physiology research as a method to assess milk ejection efficiency (Swanson & Hinton 1951; Adams & Allen 1952). In this approach, cows are machine milked and after completion of normal milking, exogenous oxytocin is administered to induce maximal milk ejection.

After a short delay, the cows are milked a second time and the obtained milk is defined as residual milk, this milk represents the fraction of alveolar milk not released during normal machine milking (Adams & Allen 1952; Bruckmaier & Blum 1998). The method has been widely used to quantify residual milk in relation to factors potentially affecting milk ejection, including milking frequency (Ferneborg et al. 2017), breed differences (Negrão & Marnet 2006), tactile stimulation and music during milking (Lechuga et al. 2023), and nursing versus non-nursing management systems (De Passillé et al. 2008).

2.4.2 Milk flow curves

Milk flow curves have long been used to describe the milking response of dairy cows and to quantify milk flow dynamics during machine milking (Beck et al. 1951). The milk flow during machine milking typically follows a characteristic pattern (Figure 1, black line), with an initial increase in flow after cluster attachment, followed by a plateau phase with stable high milk flow, and then a decline toward the end of milking (Tančin et al. 2006; Sandrucci et al. 2007). In cases of impaired milk ejection bimodal milk flow curves occur (Figure 1, double line), in which milk flow starts with the removal of cisternal milk, then temporarily declines or cease until the alveolar milk has been transferred into the cistern and flow resumes (Dodenhoff et al. 1999). Bimodal milk flow patterns are therefore commonly used to identify delayed or impaired milk ejection, which is often associated with insufficient teat stimulation prior to cluster attachment (Tancin & Bruckmaier 2001; Sandrucci et al. 2007; Moore-Foster et al. 2019). However, milk flow curves can also detect if milk ejection is completely absent, in which case only the cisternal milk fraction is removed (Bruckmaier & Blum 1998), and the milk curve will display a single peak representing the cisternal milk fraction. This makes this approach particularly useful for detecting disturbances in the timing of milk ejection.

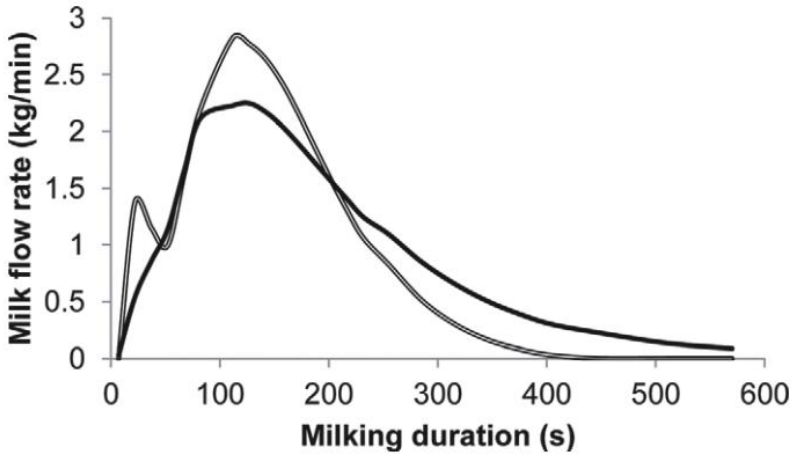


Figure 1. Examples of milk flow curves. The solid black line represents a typical normal milk flow curve, when normal milk ejection has occurred. The grey double line represents a bimodal milk flow curve, indicating delayed milk ejection. When milk ejection does not occur only the smaller first peak is observed, representing milk extracted from the major milk ducts and the cisternal compartment (Edwards et al. 2014).

2.4.3 Milk fat concentration

Another approach to evaluate milk ejection is based on the fact that milk fat concentration increases throughout machine milking, due to milk stored in the cisternal compartment generally containing a lower fat concentration than milk stored in the alveolar compartment (Johansson et al. 1952; Ontsouka et al. 2003; Rico et al. 2014). This relationship has long been recognised, and early studies showed that milk obtained by hand stripping after machine milking was particularly rich in fat (Wilson & Cannon 1934). Similar findings have been reported more recently by Ontsouka et al. (2003), who at week four of lactation reported the fat concentration to be 22.4 ± 4.1 g/L in cisternal milk, while it was 84.3 ± 4.6 g/L in the final fraction of milk obtained during normal machine milking and 130.7 ± 9.4 g/L in the residual milk.

Previous studies have reported lower fat concentration in the composite milk, representing the average fat concentration of all milk removed during normal machine milking, from CCC cows compared to non-nursing cows, which has been suggested to indicate impaired milk ejection (Zipp et al. 2018; Barth 2020). Rell et al. (2024) instead compared the fat concentration in strip milk collected after the completion of normal machine milking, representing the final milk fraction remaining in the udder rather than average fat concentration of the entire milking. The authors reported that the end of milking milk fat concentration averaged 7.1g/100 g in cows nursing their calves and 8.7 g/100 g in non-nursing cows. Fat concentration in strip milk collected after machine milking has therefore been proposed as a reliable indicator of udder emptying, where lower fat concentrations suggest incomplete removal of alveolar milk (Jenni et al. 2024).

2.4.4 Application of methods in the present study

In the present study, milk ejection was assessed using milk fat concentration in strip milk collected immediately after machine milking. This approach provides an indirect measure of udder emptying, as higher fat concentrations indicate a greater removal of alveolar milk (Ontsouka et al. 2003; Jenni et al. 2024).

It should be noted that this MSc thesis only covers part of the study period of the overall experimental study, which covered also the weaning and post-separation period. However, due to time constraints for the present work, only two of the sampling occasions for end of milking milk fat concentration were included in the analysis; one in early lactation shortly after calving and one approximately 10 days prior to the fenceline weaning period. In addition to the outcomes presented here, milk flow curve data were recorded throughout the study, and residual milking was performed on one occasion pre-weaning and two occasions post-separation. The outcomes were not included here, due to the scope and analytical limitations of the present work.

3. Method

3.1 Study site and animals

This study was conducted at the Swedish Livestock Research Centre, Uppsala, Sweden, from January to May 2026. The study was carried out in accordance with ethical approval granted by the Swedish Ethical Committee on Animal Experiments (SLU-ID: Dnr 5.8.18-1279/2023).

In total, 40 cows were included in the study and managed under commercial dairy farm conditions. The cows were divided into four subgroups depending on degree of calf contact (CCC vs. CONV) and milking permission setting (4 vs. 10 hours). To ensure animal welfare and calf safety, cows with confirmed udder infection with *Staphylococcus aureus* during previous lactations, those that were severely lame, as well as cows that were aggressive either toward their calf or toward humans in the calving box were not considered for inclusion. The cows that entered the study calved from 4 January to 28 February 2026 and were followed throughout early lactation. One CONV cow was moved to a sick pen at six DIM and excluded three days later due to *S. aureus* udder infection, this occurred before any milk sampling could be performed. To obtain 10 cows per group, an additional CONV cow calving within the described interval was enrolled as a replacement. The final study population evaluated in this work consisted of 22 Swedish Red cows (8 primiparous and 14 multiparous) and 18 Swedish Holstein cows (7 primiparous and 11 multiparous).

3.2 Experimental design

The study was designed as a 2 x 2 factorial experiment to evaluate the effects of management system (CCC vs. CONV) and milking permission interval (4 vs. 10 hours) on end of milking milk fat concentration during early lactation. Milking interval treatments were implemented by regulating the milking permission interval in the VMS. The milking permission interval defined the minimum time required between two milkings before a cow was allowed access to the milking unit. The experimental timeline of the full study period, including sampling occasion for the current work is illustrated in Figure 2.

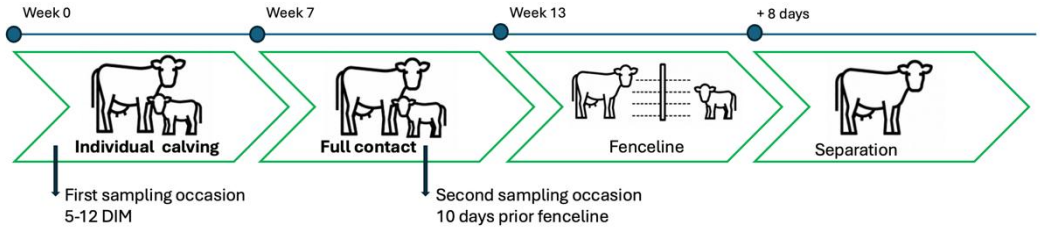


Figure 2. Experimental timeline of CCC management in the larger study. All cows entered the study individually at calving during study week 0–7 and remained in full physical contact with their calves for up to 13 weeks (the last calving CCC cow had a 6-week period of full-day contact). The contact period was followed by an 8-day fenceline period, during which suckling was prevented but visual and limited tactile contact were maintained. After the fenceline period, calves were separated from their dams. Sampling for the current work was conducted on two occasions; the first occurred between 5 and 12 days in milk (DIM) for each individual cow and the second 10 days prior to the start of the fenceline period.

A total of 40 cows were allocated to one of four treatment groups, balanced for parity, breed, calving date, calf sex, and calf type (purebred or beef cross) (Table 1). As the cows entered the study on different dates, the length of the study period for the individual cows depended on their calving date. Once all CCC dams had calved, all cows assigned to the CCC treatment had the possibility to remain in full physical contact with their calves for an additional 6 weeks. Thereafter, CCC calves were housed in the calf creep for eight days during the fenceline period, during which suckling was no longer possible, but visual and limited tactile contact across a permeable barrier were maintained. Due to the continuous enrolment of cows during the calving period, the CCC calves differed in age, ranging from 6 to 13 weeks, at the onset of the fenceline period; however, all calves were provided with milk during this period regardless of age. After the fenceline period, calves were separated from their dams and transferred to the farm's usual calf housing. According to the farm's standard routine, calves were provided with milk until at least 10 weeks of age. For calves older than 10 weeks at separation, farm staff decided whether they were abruptly weaned or continued to receive milk following the farm's usual step-down weaning procedure.

Table 1. Characteristics of the enrolled cows in each treatment group. Forty cows were allocated to four subgroups defined by management system (cow-calf contact vs. conventional) and milking permission interval (4 hour vs. 10 hour). One conventional cow was excluded prior to sampling due to Staphylococcus aures udder infection and is not included in the table.

	CCC4[1]	**CONV4[2]**	**CCC10[3]**	**CONV10[4]**
n	10	10	10	10
Primiparous, n	4	4	4	3
Parity, median (IQR)	2.0 (1–3)	2.0 (1–3)	2.0 (1–3)	2.5 (1–4)
Cow breed, n				
SR[5]	5	6	4	7
SH[6]	5	4	6	3
DIM[7] at first sampling, median (IQR)	9.0 (6–10)	8.0 (7–9)	8.5 (7–11)	9.5 (9–11)
Calv sex, n[8]				
Heifer	5	5	6	4
Bull	5	6	5	6
Calf type, n[8]				
Purebred	7	7	8	6
Beef cross	3	4	3	4

[1]*Cow calf contact with 4 hours milking permission intervals.*
[2]*Conventional cows with 4 hours milking permission intervals*
[3]*Cow calf contact with 10 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*
[5]*Swedish red.*
[6]*Swedish Holstein.*
[7]*Days in milk.*
[8]*The number of calves per group can exceed 10 due to twin births.*

3.3 Housing

After calving, all cows were housed individually in calving pens until milked six times, with milking occurring twice daily. Conventional cows were separated from their calves 12 hours postpartum, according to the farm's standard routine, whereas CCC dams remained in full physical contact with their calves.

After the calving pen period all cows were moved to and housed in the same loose-housing barn. While CCC dams could have full contact with their calves in a separate CCC area, the CONV cows were physically separated from the CCC calves throughout the study period (Figure 3). The barn was designed to allow controlled cow traffic and integration of a VMS unit (Delaval VMS™ Classic, DeLaval International AB, Tumba, Sweden). Cow movement within the barn was regulated through a one-way traffic design centred around an automatic selection gate (see description below).

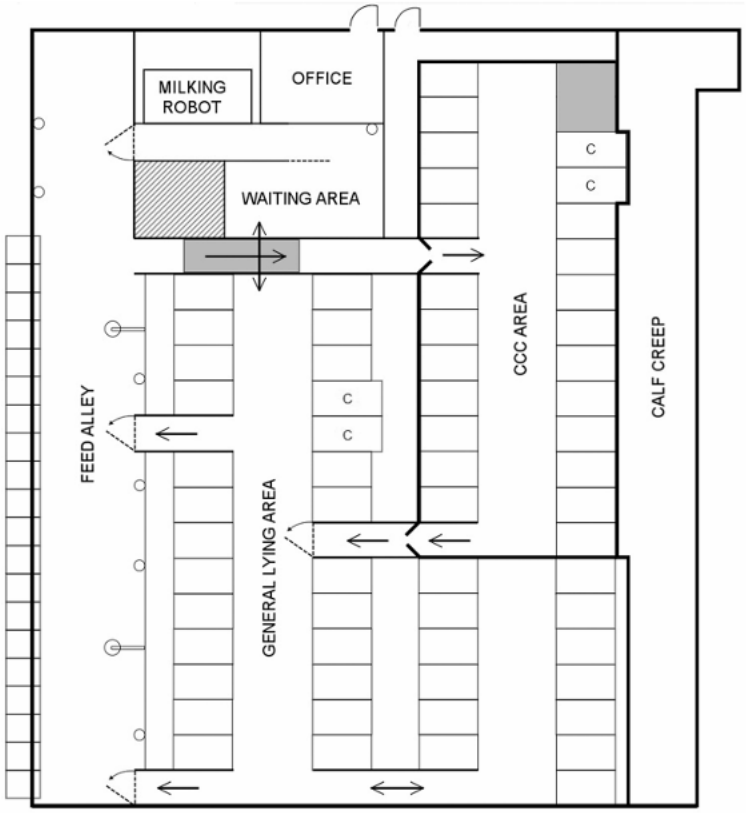


Figure 3. Overview of the barn layout and cow traffic. The barn includes a feed alley, an automatic selection gate (grey area with crossed arrows), a waiting area and a milking robot, a CCC area accessible only to CCC dams and calves, a calf creep accessible only to CCC calves and a general lying area accessible to all cows. All cows passed through the automatic selection gate when leaving the feed alley. Cows with milking permission were directed to the waiting area and milking robot, whereas cows without milking permission were directed either to the CCC area (CCC dams only) or to the general lying area (CONV cows). Dams with CCC could exit the CCC area at any time through one-way gates to the general lying area or to the feed alley. Arrows show how cows could move through the barn, with one-headed arrows indicating one-way traffic and two-way arrows free passage The letter "C" indicates concentrate feeding stations. Adapted from Wegner & Ternman (2023).

In the feed alley, all cows (both CCC and CONV) had *ad libitum* access to partial mixed ration, seven water cups and two mechanical brushes (DeLaval Swinging Cow Brush SCB, DeLaval Internation AB, Tumba, Sweden). Partial mixed ration was provided in 20 individual feeding bins (CRFI, BioControl AS; Rakkestad, Norway), with fresh feed delivered five times per day using a rail-suspended distribution wagon (DeLaval FS16000, DeLaval International AB, Tumba, Sweden).

Exit from the feed alley was possible only through an automatic selection gate (DeLaval Smart Selection Gate SSG, DeLaval International AB, Tumba, Sweden), which regulated cow movement within the barn according to treatment

assignment and milking permission status. Cows with milking permission were directed to the waiting area and milking robot if no more than three cows were already present. If more than 14 h had passed since the previous milking, cows were directed to the waiting area if no more than six cows were already present. If more than 18 h had passed since the previous milking, cows were directed to the waiting area regardless of the number of cows already present. When lacking milking permission CCC cows were instead directed to the CCC area, while CONV cows were directed to the general lying area; the same happened for cows with milking permission when the waiting area was full.

The waiting area consisted of a holding area with slatted flooring where cows could stand prior to milking, equipped with one water bowl. Cows were milked in an AMS unit (Delaval VMS^{TM} Classic, DeLaval International AB, Tumba, Sweden) where they received a small amount of concentrate feed during milking. After completed milking, cows exited the robot directly back into the feed alley.

The CCC area consisted of 22 free stalls and two concentrate feeding stations (DeLaval feed station FSC400, DeLaval International AB, Tumba, Sweden), and allowed physical interaction, including suckling, between CCC dams and calves. Contact between CCC dams and calves was cow-driven (Sirovnik et al. 2020), meaning that CCC dams were the primary initiators of contact and could voluntarily restrict contact by leaving the CCC area through a one-way gate. Calves had exclusive access to a deep-bedded calf creep, allowing them to eat solid feed and rest undisturbed by older cows. The creep was bedded with straw and provided access to roughage, two water bowls, two concentrate feeding stations (DeLaval concentrate station calves, DeLaval International AB, Tumba, Sweden), and two mechanical calf brushes (DeLaval mini swinging brush for calves MSB, DeLaval International AB, Tumba, Sweden).

The general lying area was accessible to all cows regardless of treatment and contained 37 free stalls and two concentrate feeding stations (DeLaval feed station FSC400, DeLaval International AB, Tumba, Sweden). All free stalls were bedded with chopped straw, and manure was removed manually from the stalls by farm personnel multiple times per day. Mechanical scrapers were used in the alleys to remove manure. In the CCC area, the mechanical scrapers were operated manually to avoid injuries in the calves.

3.4 Milking settings and robot management

In the AMS unit, the vacuum level was set to 46 kPa. Milking was performed using DeLaval Smart Pulsation, a system that automatically adapts pulsation settings to individual cows based on recorded milk flow data derived from both historical and real-time measurements, using predefined pulsation ratios (60:40, 65:35, 70:30, and 74:26; representing the proportion of milking phase to massage phase). Automatic cluster detachment occurred when milk flow dropped below 0.42 kg min^{-1}. The teat cups were equipped with DeLaval Clover^{TM} liner

(DeLaval International AB, Tumba, Sweden), which were changed after approximately 2 500 milkings. These settings remained constant during the entire experimental period and were used for all cows regardless of treatment group; the only AMS unit setting that differed between group was the milking permission interval. In cases where cows did not tolerate attachment of the teat cups by the robotic arm, manual assistance was provided when necessary. This could include manual teat cleaning and manual attachment of teat cups to complete the milking.

Handling of cows in relation to robot visits was standardized and did not differ between treatment groups. To facilitate robot visits, cows observed leaking milk while having milking permission could either be fetched to the feed alley or guided to the selection gate. Additionally, if cows remained in the waiting area for an extended period without voluntarily entering the AMS unit, they could be gently encouraged to enter the AMS unit. These interventions were performed while staff carried out other routine tasks in the pen and were recorded in a software application. Cows that repeatedly remained in the waiting area for extended periods without entering the robot were not allowed access to the waiting area during the night. In such cases, staff ensured that these cows were milked during the final hours before leaving the barn for the night and shortly after returning in the morning.

3.5 Milk sampling and milk fat analysis

Milk samples were collected at quarter level at two sampling occasions points per cow. The first sampling was conducted between 5 and 12 days after individual calving. The second sampling was performed approximately 10 days prior to the transition the fenceline contact phase.

The first sampling occasion spanned a 24-hour period, during which sampling occurred at all milkings. For cows that were milked only once during this period, the sampling period was prolonged until milk samples from two separate milking were obtained. Only two milkings were included during the second sampling occasion, as residual milk collection was performed after the cow's second entry into the milking robot following milk fat sampling.

Milk was collected in tubes coated with bronopol (2-bromo-2-nitropropane-1,3-diol) by hand stripping, approximately 20 ml from each quarter. Bronopol was added as a preservative to prevent microbial growth and stabilize milk composition. For each sample, cow ID, quarter, sampling date and time, and sampling occasion were recorded. After the sampling occasion milk samples were kept at 4°C until analysis, which occurred within four days.

Milk samples were analysed for milk fat concentration with Fourier Transform Infrared Spectroscopy (CombiScope^{TM}, FTIR 600 HP dairy analyser, Delta Instruments, Shelton, USA), tubes containing too little milk were not analysed leading to missing milk fat concentration values on individual quarters. All

laboratory analyses were performed at the Department of Applied Animal Science and Welfare (THV) laboratory at SLU.

3.6 Data handling

Data on milking events for each cow were obtained from the herd management software (DelPro, DeLaval International AB, Tumba, Sweden). The actual milking interval for all days during the full-day CCC period was calculated for each cow and summarized as mean and standard deviation (SD) per treatment group.

The quarter-level milk fat concentration (%) values obtained from laboratory analyses were assembled in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). For each sampling occasion, the mean value of milk fat concentration and SD of milk fat concentration were calculated per treatment group. First, mean values per cow and sampling occasion were obtained by first averaging quarter-level values per cow-milking, followed by averaging the obtained values per sampling occasion (multiple milkings per cows and sampling occasion). After this, the mean cow-level values were used to calculate the mean and SD per treatment group and sampling occasion. Additionally, minimum and maximum milk fat concentration values were determined across all milkings and across individual quarters per treatment group. In addition, mean ± SD milk fat concentration was calculated in an identical way, per treatment group, by the factors used to balance the groups (breed, parity, calf sex, and calf breed), within each treatment group.

To classify individual milkings into normal, impaired, or inconsistent milk ejection, the coefficient of variation (CV) for milk fat concentration on quarter level was calculated for each cow-milking using R (R Foundation for Statistical Computing, Vienna, Austria). The mean CV across all milkings (22.3%) was used as the cut-off to separate milkings with low and high variation, to identify cows with inconsistent values between quarters during individual milkings. Regardless of the CV value, a milking was classified as having normal milk ejection if the milk fat concentration in all analysed quarters exceeded 8.4%, while it was classified as impaired milk ejection if the fat concentration was lower than 8.4% in all analysed quarters (Ontsouka et al. 2003). Milkings with quarter-level values both above and below the 8.4% threshold were classified as mildly inconsistent when the CV remained below the 22.3% cut-off value, and as inconsistent when the CV exceeded the 22.3% cut-off value.

For the statistical analyses, milkings classified as mildly inconsistent and inconsistent were grouped together as inconsistent milkings to avoid excessively small subgroup sizes. If quarter levels were missing for more than two quarters the individual milking was excluded from classification. Proportions per cow and sampling occasion were calculated based on the remaining classified milkings. If

fewer than one classified milkings remained for a sampling occasion, the cow was excluded from statistical analyses.

3.7 Statistical analysis

Statistical analyses were performed in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). For each cow, the proportion of milkings classified as normal, inconsistent and impaired milk ejection was analysed as continuous variables separately for the first and second sampling occasion. Individual value plots were generated in Minitab (Minitab LLC, State College, PA, USA) to visualize the distribution of the proportion of milkings classified as normal, impaired, and inconsistent milk ejection within each treatment group and sampling occasion.

Differences between treatment groups (CCC4, CONV4, CCC10 and CONV10) were evaluated using the non-parametric Kruskal-Walli's test. Observations were ranked across all groups using average ranks in the case of ties. The sum of ranks for each group (Ri) was calculated, and the test statistic was obtained as:

$$H = \frac{12}{N\ (N+1)} \sum \frac{R_i^2}{n_i} - 3(N+1)$$

where N is the total number of observations, R_i is the sum of the ranks for group i, and n_i is the number of observations in group i. To account for tied ranks, a tie-correction factor (D) was calculated as:

$$D = 1 - \frac{\sum(t^3 - t)}{(n-1)n(n+1)}$$

where t represents the number of tied observations for each set of tied ranks and n is the total number of observations. The original Kruskal-Wallis test statistic was subsequently adjusted to:

$$H_{adj} = \frac{H}{D}$$

The adjusted test statistic (H_{adj}) was used to determine statistical significance. P-values were obtained from the chi-square distribution with degrees of freedom equal to the number of groups minus one.

If a significant effect was detected ($p < 0.05$), pairwise comparisons were performed using the exact Mann-Whitney U test. For each pairwise comparison, observations from the two groups were combined and ranked using average ranks

in the case of ties. The Mann-Whitney U statistics for the two groups were calculated according to:

$$U_1 = n_1 n_2 + \frac{n_1(n_1 + 1)}{2} - T_1$$

$$U_2 = n_1 n_2 + \frac{n_2(n_2 + 1)}{2} - T_2$$

where T_1 and T_2 represents the rank sums of the two groups, and $n_1$and $n_2$represent the number of observations in each group. The smaller of U_1 and U_2 was used as the Mann-Whitney U statistic. Because the number of observations was below the threshold for applying the normal approximation ($n \geq 20$), exact two-sided p-values were obtained from of the Mann-Whitney U critical value tables. A Bonferroni correction was applied to account for multiple pairwise comparisons within each analysis, resulting in a corrected significance level of $\alpha = 0.0083$. In addition, the rank-biserial correlation (*r*) was calculated to assess the strength of differences between groups according to:

$$r = 1 - \frac{2U}{n_1 n_2}$$

where correlation values were interpreted as small ($r < 0.3$), moderate ($r = 0.3$-0.5), or large ($r > 0.5$) difference between compared groups.

4. Result

The actual milking intervals during the full-day CCC period varied between treatment groups, with mean ± SD values of 9.4 ± 1.2 h for CCC4, 8.3 ± 1.3 h for CONV4, 14.9 ± 2.1 h for CCC10, and 12.4 ± 1.3 h for CONV10.

At the first sampling occasion, seven cows were not allowed into the waiting area during night-time, as they previously had been observed standing for long periods in the waiting area without entering the milking unit (CCC4: 1; CONV4: 1; CCC10: 4, CONV10: 1). These cows were instead guided to the waiting area, and if necessary also into the milking unit, during the final hours before staff left the barn in the evening, and again shortly after staff returned in the morning. During the second sampling occasion, this applied to only one cow in CONV10.

4.1 Missing data

At the first sampling occasion, 9 of 384 quarter samples were excluded for milk fat content determination due to insufficient sample volume (CCC4: 3 of 104; CONV4: 0 of 120; CCC10: 6 of 80; CONV10: 0 of 80); the missing quarters originated from 6 cow-milkings (CCC4: 2 of 26; CCC10: 4 of 20). At the second sampling occasion, 25 of 320 quarter samples were excluded for the same reason (CCC4: 5 of 78; CONV4: 0 of 80; CCC10; 13 of 80; CONV10: 5 of 80), with the missing quarters originating from 11 cow-milkings (CCC4: 3 of 20; CCC10; 5 of 20; CONV10: 3 of 20). The lower number of samples in CCC4 during the second occasion was due to one CCC4 cow being dried off in the left front quarter following mastitis due to *Escherichia coli.* Additionally, at the second sampling occasion, one CCC4 dam was excluded from statistical analysis for one milking due to insufficient milk volume for fat analysis in three out of four quarter samples, resulting in only one classified milking for that cow. Furthermore, one CCC10 dam was excluded entirely from statistical analysis at this sampling occasion because neither of the two milkings had sufficient quarter samples for fat analysis.

4.2 First sampling occasion

Milk fat concentration in strip milk at the end of milking differed numerically between treatments (Table 2). The highest group mean value was observed in CONV10 (13.1 ± 2.9%), and the lowest in CCC10 (7.6 ± 2.6%). For individual milkings, cow-level mean values ranged from 2.0% (CCC10) to 19.2% (CONV10), and for individual measured quarter-level values ranged from 1.2% (CCC10) to 21.1% (CCC4).

Milk fat concentration by factors used to balance treatment groups varied between categories (Table 3). As before, CONV10 showed the numerically highest mean values, whereas CCC10 showed the lowest. Holstein cows consistently had lower milk fat concentration in all treatment groups, while no other factor showed a

consistent pattern between degree of calf contact or milking permission interval. The association between machine milk yield and milk fat concentration for individual quarter samples at the first sampling occasion are presented in Figure 4 for each treatment group.

Table 2. Milk fat concentration (%) in strip milk at the end of milking by treatment group at the first sampling occasion, performed at 5—12 DIM. Mean ± SD values relate to the group level. Min-max values represent the range of cow-level mean values across all milkings (Milking min-max), and the range of values obtained from quarter-level analyses (Quarter min-max).

Treatment	n	Milk fat (%) mean ± SD	Milking min-max	Quarter min-max
CCC4[1]	10	8.2 ± 2.9	3.1 – 15.8	1.3 – 21.1
CONV4[2]	10	10.1 ± 1.9	6.5 – 14.7	3.1 – 19.0
CCC10[3]	10	7.6 ± 2.6	2.0 – 17.9	1.2 – 20.7
CONV10[4]	10	13.1 ± 2.9	9.5 – 19.2	6.7 – 19.6

[1]*Cow-calf contact with 4 hours milking permission intervals.*
[2]*Conventional cows with 4 hours milking permission intervals.*
[3]*Cow-calf contact with 10 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*

Table 3. Milk fat concentration (%) in strip milk at the end of machine milking during the first sampling occasion, performed at 5—12 DIM. Mean ± SD and values relate to the group level and summarized by treatment group and factors used when balancing treatment groups.

	Category	CCC4[1]	CONV4[2]	CCC10[3]	CONV10[4]
Breed	SR[5]	8.3 ± 3.8	10.6 ± 2.1	8.1 ± 3.3	13.6 ± 3.3
	SH[6]	8.1 ± 2.0	9.4 ± 1.6	7.3 ± 2.2	12.0 ± 1.1
Parity	Primiparous	6.8 ± 2.1	10.8 ± 1.7	8.1 ± 3.7	11.2 ± 0.6
	Multiparous	9.1 ± 3.1	9.7 ± 2.0	7.3 ± 2.2	14.0 ± 3.1
Calf sex	Bull	9.6 ± 3.1	10.2 ± 2.0	7.6 ± 3.0	12.8 ± 2.7
	Heifer	6.8 ± 2.0	10.0 ± 2.1	7.6 ± 2.4	13.6 ± 3.4
Calf breed	Purebred	8.2 ± 3.5	9.8 ± 2.0	7.3 ± 2.7	11.5 ± 0.9
	Beef cross	8.2 ± 0.4	10.6 ± 1.9	8.4 ± 2.6	15.4 ± 3.4

[1]*Cow calf contact with 4 hours milking permission intervals.*
[2]*Conventional cows with 4 hours milking permission intervals*
[3]*Cow calf contact with 10 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*
[5]*Swedish Red.*
[6]*Swedish Holstein.*

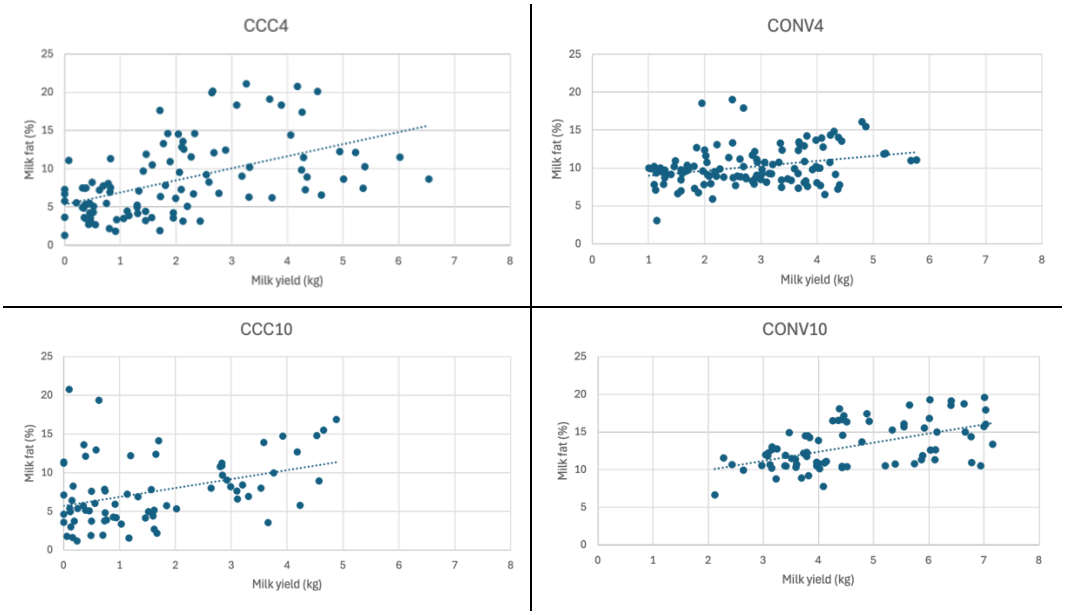


Figure 4. Relationship between quarter-level milk yield and quarter-level milk fat concentration in strip milk at the end of milking during the first sampling occasion, performed at 5—12 DIM. The relationship is shown separately for each treatment group, with abbreviations referring to cow-calf contact (CCC) and conventional management (CONV) and numbers (4 vs. 10) indicating the milking permission interval in hours. Each dot represents one quarter sample, so that each cow-milking is represented by up to four dots.

4.3 Second sampling occasion

Milk fat concentration in strip milk at the end of milking differed numerically between treatments (Table 4). Again, the highest group mean was observed in CONV10 (14.8 ± 1.8%), and the lowest in CCC10 (8.1 ± 4.0%). For individual milkings, cow-level mean values ranged from 1.2% (CCC10) to 19.7% (CONV10), and for individual measured quarter-level values ranged from 0.9% (CCC10) to 21.6% (CONV10).

Milk fat concentration by factors used to balance treatment groups varied between categories (Table 5). This time, both CONV4 and CONV10 generally showed cows consistently having higher milk fat concentration compared to CCC4 and CCC10. Primiparous cows consistently had lower milk fat concentration in all treatment groups, while no other factor showed a consistent pattern between degree of calf contact or milking permission interval. The association between machine milk yield and milk fat concentration for individual quarter samples are presented in Figure 5, split by treatment group. Milk fat concentration in relation to DIM for the second sampling occasion is presented in Figure 6.

Table 4. Milk fat concentration (%) in strip milk at the end of milking by treatment group at the second sampling occasion. Mean ± SD values relate to the group level. Min-max values represent the range of cow-level mean values across all milkings (Milking min-max), and the range of values obtained from quarter-level analyses (Quarter min-max).

Treatment	n	Milk fat (%) mean ± SD	Milking min-max	Quarter min-ma
CCC4[1]	10	9.4 ± 3.2	1.7 – 16.0	1.6 – 21.0
CCC10[2]	10	8.1 ± 4.0	1.2 – 14.1	0.9 – 18.2
CONV4[3]	10	13.2 ± 3.0	7.0 – 17.9	5.9 – 20.0
CONV10[4]	10	14.8 ± 1.8	11.4 – 19.7	3.4 – 21.6

[1]*Cow-calf contact with 4 hours milking permission intervals.*
[2]*Conventional cows with 4 hours milking permission intervals.*
[3]*Cow-calf contact with 10 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*

Table 5. Milk fat concentration (%) in strip milk at the end of machine milking during the second sampling occasion. Mean ± SD and values relate to the group level and summarized by treatment group and factors used when balancing treatment groups.

	Category	CCC4[1]	CONV4[2]	CCC10[3]	CONV0[4]
Breed	SR[5]	10.9 ± 2.3	14.1 ± 2.7	7.4 ± 3.7	14.8 ± 2.1
	SH[6]	8.0 ± 3.6	11.7 ± 3.1	8.5 ± 4.6	15.0 ± 0.8
Parity	Primiparous	8.2 ± 1.8	11.0 ± 2.7	3.4 ± 1.9	13.6 ± 1.2
	Multiparous	10.3 ± 3.8	14.6 ± 2.2	10.4 ± 2.2	15.4 ± 1.7
Calf sex	Bull	9.6 ± 3.5	14.0 ± 3.8	7.0 ± 4.1	14.6 ± 1.9
	Heifer	9.3 ± 3.3	12.4 ± 2.0	9.4 ± 4.0	15.3 ± 1.6
Calf breed	Purebred	10.1 ± 3.1	12.3 ± 2.9	7.4 ± 4.7	14.1 ± 1.2
	Beef cross	7.9 ± 3.6	14.5 ± 2.9	9.4 ± 2.2	15.9 ± 2.0

[1]*Cow calf contact with 4 hours milking permission intervals.*
[2]*Conventional cows with 4 hours milking permission intervals*
[3]*Cow calf contact with 10 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*
[5]*Swedish Red.*
[6]*Swedish Holstein*.

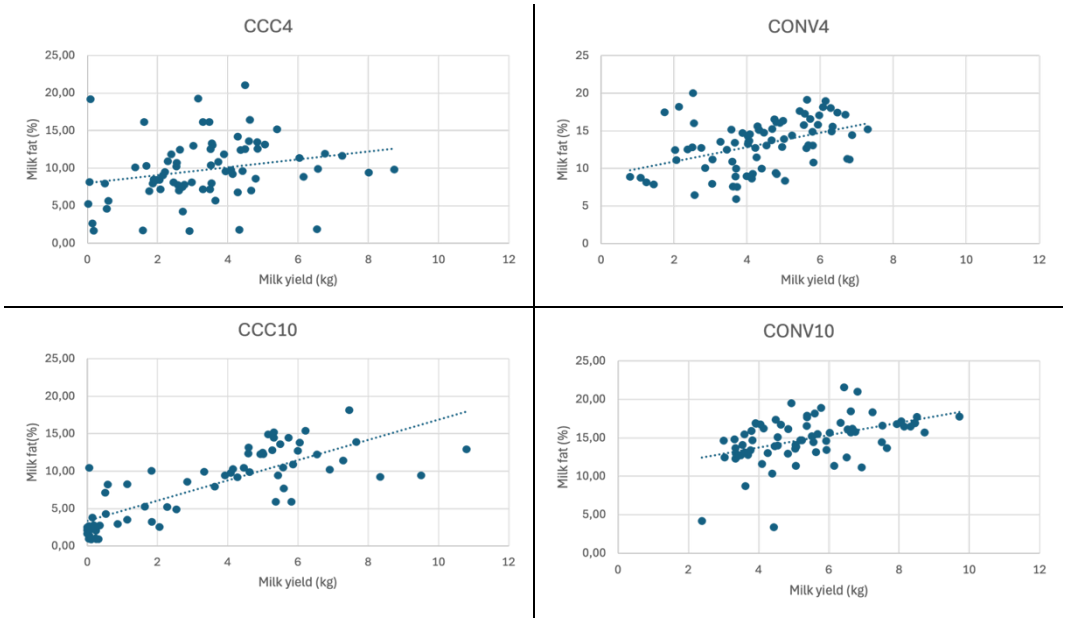


Figure 5. Relationship between quarter-level milk yield and quarter-level milk fat concentration in strip milk at the end of milking during the second sampling occasion. The relationship is shown separately for each treatment group, with abbreviations referring to cow-calf contact (CCC) and conventional management (CONV) and numbers (4 vs. 10) indicating the milking permission interval in hours. Each dot represents one quarter sample, so that each cow-milking is represented by up to four dots.

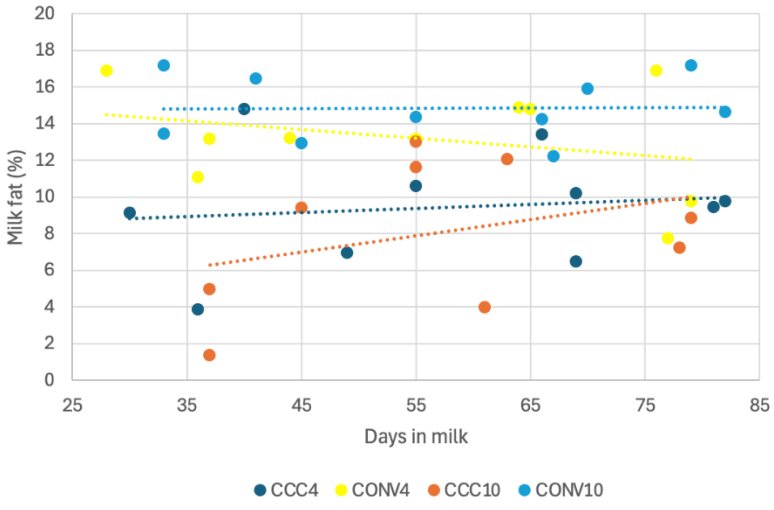


Figure 6. Relationship between days in milk and milk fat concentration in strip milk at the end of machine milking during the second sampling occasion. The relationship is shown separately for each treatment group, with abbreviations referring to cow-calf contact (CCC) and conventional management (CONV) and numbers (4 vs. 10) indicating the milking permission interval in hours. Each dot represents one cow-level mean values.

4.4 Classification of milkings

For both sampling occasions combined, in total 48 of 62 milkings with a CV value above the median (22.89) were classified as inconsistent, whereas 18 of 111 milking with a CV value below the median were classified as mildly inconsistent. At the first sampling occasion 10 milkings were classified as mildly inconsistent (CCC4: 1 of 26; CONV4: 6 of 30; CCC10: 2 of 20; CONV10: 1 of 20) and 32 milkings were classified as inconsistent (CCC4: 15 of 26; CONV4: 5 of 30; CCC10: 11 of 20; CONV10: 1 of 20). For the second sampling occasion 6 milkings were classified as mildly inconsistent (CCC4: 3 of 20; CONV4: 3 of 20) and 18 milkings were classified as inconsistent (CCC4: 8 of 19; CCC10: 7 of 18; CONV10: 3 of 20). The distribution of classifications (normal, mildly inconsistent, inconsistent and impaired) per cow within each treatment group is shown in Figure 7 (first sampling occasion) and Figure 8 (second sampling occasion).

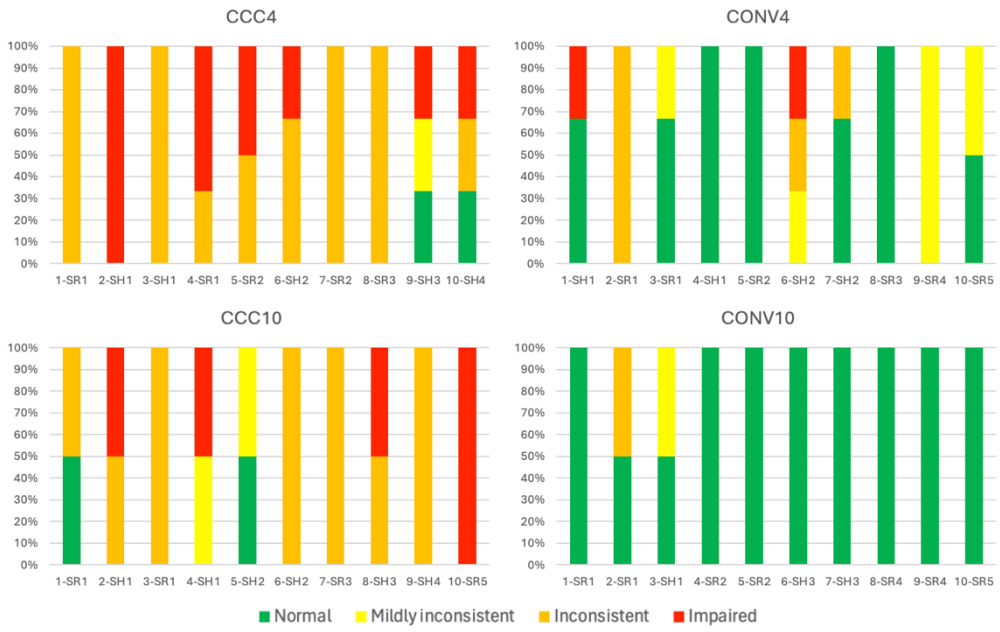


Figure 7. Distribution of classifications (normal, mildly inconsistent, inconsistent, and impaired) per cow for the first sampling occasion within each treatment group (CCC4, CONV4, CCC10, CONV10). The abbreviations refer to cow-calf contact (CCC) and conventional management (CONV), and the numbers (4 and 10) indicate milking permission intervals (hours). Each bar represents the proportion of classifications for an individual cow. Labels on the x-axis represent individual cows (1-10), where breed is indicated as Swedish Holstein (SH) or Swedish Red (SR), and numbers (1-5) indicate parity.

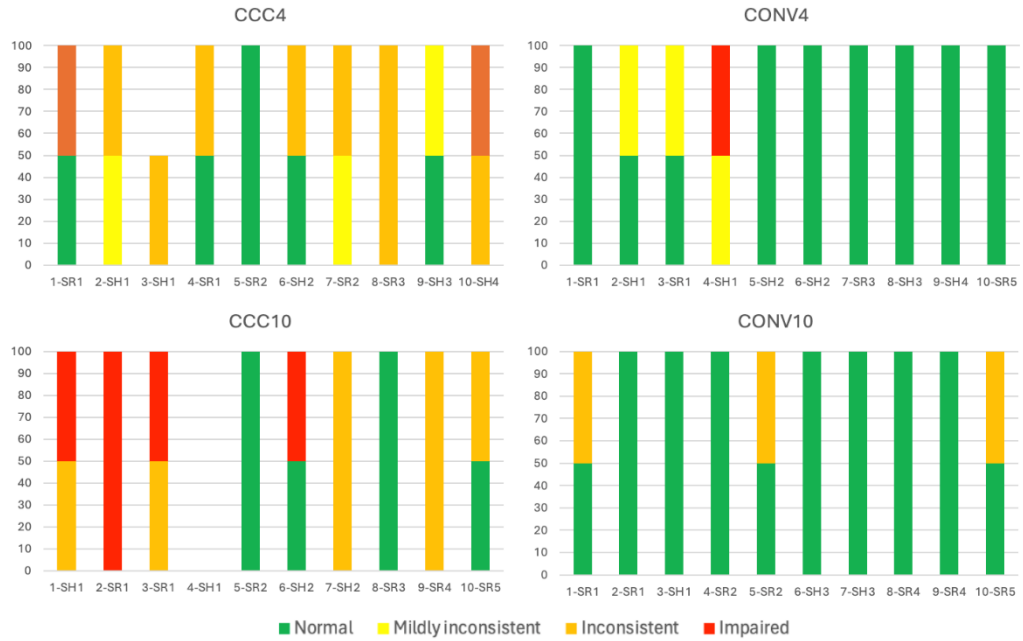


Figure 8. Distribution of classifications (normal, mildly inconsistent, inconsistent, and impaired) per cow for the second sampling occasion within each treatment group (CCC4, CONV4, CCC10, CONV10). The abbreviations refer to cow-calf contact (CCC) and conventional management (CONV), and the numbers (4 and 10) indicate milking permission intervals (hours). Each bar represents the proportion of classifications for an individual cow. Labels on the x-axis represent individual cows (1-10), where breed is indicated as Swedish Holstein (SH) or Swedish Red (SR), and numbers (1-5) indicate parity. One CCC4 dam (3-SH1) had only one of two milkings classified, as three out of four quarter milk samples from the other milking were insufficient for fat analysis. One CCC10 cow (4-SH1) had neither of two milkings classified, as quarter milk samples from both milkings were insufficient for fat analysis.

4.5 Statistical differences between treatments

For the first sampling occasion, the Kruskal-Wallis omnibus tests showed that there were significant differences between the treatment groups in the proportion of milkings per cow classified as normal ($H_{adj}(3) = 21.6$, p = <0.001), impaired ($H_{adj}(3) = 9.6$, p = 0.02), and inconsistent milk ejection (H_{adj} (3) = 12.8, p = 0.01). Results from the pairwise Mann-Whitney U-test comparisons are presented in Tables 6, 7 and 8 for normal, impaired and inconsistent milk ejection, respectively. Corresponding individual value plots are presented in Appendix 1.

Table 6. Pairwise Mann-Whitney U-test comparisons of the proportion of milkings classified as normal milk ejection during the first sampling occasion, performed at 5—12 DIM. Median values and sample sizes (n) are presented for each treatment group (CCC4, CCC10, CONV4 and CONV10). Pairwise comparisons are reported as Mann-Whitney U-values with two-tailed p-values and rank-biserial correlations (r). P-values were derived from critical Mann-Whitney U distribution tables. Statistical significance was evaluated using Bonferroni-corrected significance level of α = 0.0083 and significant p-values are shown in bold.

			U-value (P-value) pairwise comparisons		
Group	n	Median	CCC10	CONV4	CONV10
CCC4[1]	10	0.0	48 (p>0.20) r = 0.04	18 (0.10<p<0.20) r = 0.64	0 (**p<0.001**) r = 1.00
CCC10[2]	10	0.0	-	9 (0.10<p<0.20) r = 0.62	2 (**p<0.001**) r = 0.96
CONV4[3]	10	66.7	-	-	25 (0.05<p<0.10) r = 0.50
CONV10[4]	10	100.0	-	-	-

[1]*Cow-calf contact with 4 hours milking permission intervals.*
[2]*Cov-calf contact with 10 hours milking permission intervals.*
[3]*Conventional cows with 4 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*

Table 7. Pairwise Mann-Whitney U-test comparisons of the proportion of milkings classified as impaired milk ejection during the first sampling occasion, performed at 5—12 DIM. Median values and sample sizes (n) are presented for each treatment group (CCC4, CCC10, CONV4 and CONV10). Pairwise comparisons are reported as Mann-Whitney U-values with two-tailed p-values and rank-biserial correlations (r). P-values were derived from critical Mann-Whitney U distribution tables. Statistical significance was evaluated using Bonferroni-corrected significance level of α = 0.0083 and significant p-values are shown in bold.

			U-value (P-value) pairwise comparisons		
Group	n	Median	CCC10	CONV4	CONV10
CCC4[1]	10	33.3	44 (p>0.20) r = 0.12	27 (0.05<p<0.10) r = 0.46	20 (0.02<p<0.05) r = 0.60
CCC10[2]	10	0.0	-	36 (p>0.20) r = 0.28	30 (0.10<p<0.20) r = 0.40
CONV4[3]	10	0.0	-	-	40 (p>0.20) r = 0.28
CONV10[4]	10	0.0	-	-	-

[1]*Cow-calf contact with 4 hours milking permission intervals.*
[2]*Cov-calf contact with 10 hours milking permission intervals.*
[3]*Conventional cows with 4 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*

Table 8. Pairwise Mann-Whitney U-test comparisons of the proportion of milkings classified as inconsistent milk ejection during the first sampling occasion, performed at 5—12 DIM. Median values and sample sizes (n) are presented for each treatment group (CCC4, CCC10, CONV4 and CONV10). Pairwise comparisons are reported as Mann-Whitney U-values with two-tailed p-values and rank-biserial correlations (r). P-values were derived from critical Mann-Whitney U distribution tables. Statistical significance was evaluated using a Bonferroni-corrected significance level of $\alpha = 0.0083$ and significant p-values are shown in bold.

			U-value (P-value) pairwise comparisons		
Group	n	Median	CCC10	CONV4	CONV10
CCC4[1]	10	58.3	45 (p>0.20) r = 0.10	33 (p>0.20) r = 0.34	13 (**0.001<p<0.005**) r = 0.74
CCC10[2]	10	50.0	-	30 (0.10<p<0.20) r = 0.40	11 (**0.001<p<0.005**) r = 0.78
CONV4[3]	10	33.3	-	-	29 (0.10<p<0.20) r = 0.42
CONV10[4]	10	0.0	-	-	-

[1]*Cow-calf contact with 4 hours milking permission intervals.*
[2]*Cov-calf contact with 10 hours milking permission intervals.*
[3]*Conventional cows with 4 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*

For the second sampling occasion, the Kruskal-Wallis omnibus tests showed that there were significant differences between the treatments groups in proportion of milkings per cow with normal milk ejection ($H_{adj}(3) = 13.2$, p = 0.004), as well as in the proportions of milkings per cow scored as having inconsistent quarter-level milk fat concentration ($H_{adj}(3) = 7.9$, p = 0.049). However, no significant difference was found between groups regarding the proportion of milkings per cow with impaired milk ejection (H_{adj} (3) = 6,9, p = 0.074). Results from the pairwise Mann-Whtiney U-test comparisons are presented in Tables 9 and 10 for normal, and inconsistent milk ejection, respectively. Corresponding individual value plots are presented in Appendix 2.

Table 9. Pairwise Mann-Whitney U-test comparisons of the proportion of milkings classified as normal milk ejection during the second sampling occasion. Median values and sample sizes (n) are presented for each treatment group (CCC4, CCC10, CONV4 and CONV10). Pairwise comparisons are reported as Mann-Whitney U-values with two-tailed p-values and rank-biserial correlations (r). P-values were derived from critical Mann-Whitney U distribution tables. Statistical significance was evaluated using a Bonferroni-corrected significance level of α = 0.0083 and significant p-values are shown in bold.

			U-value (P-value) pairwise comparisons		
Group	n	Median	CCC10	CONV4	CONV10
CCC4[1]	9	50.0	39 (p>0.20) r = 0.00	17 (0.02<p<0.05) r = 0.63	13 (0.005<p<0.01) r = 0.72
CCC10[2]	9	0.0	-	20 (0.02<p<0.05) r = 0.57	16 (0.01<p<0.02) r = 0.64
CONV4[3]	10	100.0	-	-	49 (p>0.20) r = 0.03
CONV10[4]	10	100.0	-	-	-

[1]*Cow-calf contact with 4 hours milking permission intervals.*
[2]*Cov-calf contact with 10 hours milking permission intervals.*
[3]*Conventional cows with 4 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*

Table 10. Pairwise Mann-Whitney U-test comparisons of the proportion of milkings classified as inconsistent milk ejection during the second sampling occasion. Median values and sample sizes (n) are presented for each treatment group (CCC4, CCC10, CONV4 and CONV10). Pairwise comparisons are reported as Mann-Whitney U-values with two-tailed p-values and rank-biserial correlations (r). P-values were derived from critical Mann-Whitney U distribution tables. Statistical significance was evaluated using a Bonferroni-corrected significance level of α = 0.0083 and significant p-values are shown in bold.

			U-value (P-value) pairwise comparisons		
Group	n	Median	CCC10	CONV4	CONV10
CCC4[1]	9	50.0	31 (p>0.20) r = 0.23	19 (0.02<p<0.05) r = 0.58	19 (0.02<p<0.05) r = 0.58
CCC10[2]	9	50.0	-	31 (p>0.20) r = 0.32	31 (p>0.20) r = 0.32
CONV4[3]	10	0.0	-	-	50 (p>0.20) r = 0.0
CONV10[4]	10	0.0	-	-	-

[1]*Cow-calf contact with 4 hours milking permission intervals.*
[2]*Cov-calf contact with 10 hours milking permission intervals.*
[3]*Conventional cows with 4 hours milking permission intervals.*
[4]*Conventional cows with 10 hours milking permission intervals.*

5. Discussion

The aim of this study was to investigate how full-day cow-driven CCC vs. CONV and milking permission interval (4 h vs. 10 h) affected milk ejection in dairy cows housed in an AMS with guided cow traffic.

5.1 Effect of cow-calf contact

Cow-calf contact appeared to affect milk ejection at the first sampling occasion, when CONV10 cows showed a significant higher proportion of milkings classified as normal compared with CCC dams. In addition, the descriptive milk fat concentrations supported this interpretation, as CCC dams generally showed numerically lower values than CONV cows. These findings are consistent with previous studies reporting lower milk fat concentrations in cows housed with calves, both in composite milk samples and in strip milk collected after machine milking (Zipp et al. 2018; Barth 2020; Rell et al. 2024). Since lower milk fat concentrations have been suggested to indicate less complete removal of alveolar milk (Jenni et al. 2024), these observations support the notion of altered milk ejection in CCC dams. Comparable observations were also made in an unpublished study conducted on the same farm as the present study, where CCC dams showed lower strip milk fat concentrations after machine milking than CONV cows, associated with impaired milk ejection (Almlöf, personal communication, 2024).

One possible explanation for the lower proportion of normal milkings in CCC dams is that suckling and machine milking may not stimulate milk ejection equally in cows nursing calves. Both Tancin et al. (2001) and De Passillé et al. (2008) reported stronger oxytocin release during suckling than during machine milking in cows housed with calves, suggesting that milk ejection during AMS milking may have been less effective in some CCC dams. Because stress can inhibit oxytocin release, and cows remain highly motivated to maintain contact with and nurse their calves several weeks postpartum (Johnsen et al. 2015; Jensen et al. 2024), it is possible that separation from their calf during AMS milking may also have influenced milk ejection responses in CCC dams.

Since normal milk ejection is required for efficient milk removal (Bruckmaier & Blum 1998), a consequence of the lower proportion of milkings with normal milk ejection observed in CCC dams could be a reduced amount of milk harvested by the milking system; this finding is consistent with the lower machine milk yield reported in several CCC studies (Churakov et al. 2023; Zipp & Knierim 2024; van Zyl et al. 2025). Further, Neave (2024) suggested that the reduction in machine milk yield may be too large to be explained solely by calf milk intake. Churakov et al. (2023) also estimated a lower total milk production in CCC dams compared with CONV cows. In contrast, Bar-Peled et al. (1995) reported higher total milk production in high-producing Holstein cows nursing two foster calves than in

cows without calf contact that were milked either three or six times daily, despite lower machine milk yield. Notably, Bar-Peled et al. (1995) has so far, to the author's knowledge, been the only study that directly measuring calf milk intake in CCC systems by weighing calves before and after suckling. One possible explanation for the contrasting findings is that the restricted suckling system used by Bar-Peled et al. (1995) may have allowed the udder to refill between nursing bouts and machine milkings, while still maintaining a high demand for milk through suckling by two calves. In contrast, calves in the full-day CCC system studied by Churakov et al. (2023) could remove milk throughout the day, potentially resulting in lower udder fill at machine milking. Differences in both udder fill and the extent of milk removal may therefore have contributed to the contrasting estimates of total milk production. Further complicating the relationship between calf presence, milk ejection and machine milk yield, Fröberg et al. (2007) reported increased machine milk yield for low producing cows in a restricted suckling system where calves were present during machine milking to stimulate milk ejection. The calves were also subsequently allowed to suckle after machine milking. The increased machine milk yield compared to cows separated from their calves was suggested to be related to more frequent udder emptying and more complete removal of residual milk by the calf, thereby reducing feedback inhibition of milk secretion. As calves in the present study had continuous access to the udder between milking, opportunities for complete udder emptying were not linked to machine milking in the same was as in the system studied by Fröberg et al. (2007), which may explain why milk ejection appeared to be affected differently.

In addition to the lower proportion of normal milkings, CCC dams also showed a significant higher proportion of inconsistent milkings compared with CONV10 cows at the first sampling occasion. Inconsistent milkings may reflect uneven milk removal between udder quarters during individual milkings, suggesting variation in milk availability or milk ejection within the udder. This pattern was observed more frequently in CCC dams than in CONV cows, and, to our knowledge, quarter-level variation in post-milking milk fat concentration in CCC systems has received limited attention in previous literature, making this a potentially novel finding. One possible explanation for this pattern may be related to calf suckling behaviour prior to machine milking. In full-day and half-day CCC systems, calves may suckle at irregular intervals between milkings (Barth 2020; Neave et al. 2024), which can alter both the amount and distribution of milk within the udder. Because cisternal milk generally contains a lower fat concentration than alveolar milk (Johansson et al. 1952; Ontsouka et al. 2003; Rico et al. 2014), suckling shortly before machine milking could theoretically increase milk fat concentration in certain quarters by removing a larger proportion of low-fat cisternal milk before machine milking. Conversely, suckling shortly after a relatively successful machine milking could potentially remove the remaining fat-rich alveolar milk, resulting in temporarily lower milk fat concentrations until milk fat has been replenished. Depending on which and how many quarters were suckled, this could theoretically contribute to substantial variation in milk fat concentration between udder quarters within the same

milking. However, since neither the timing of suckling events nor which udder quarters were suckled were recorded in the present study, these proposed mechanisms remain speculative and require further investigation. Nevertheless, inconsistent milkings were also observed in CONV cows, although less frequently than in CCC dams, indicating that variation in quarter-level milk fat concentration may occur even in the absence of calf contact.

At the second sampling occasion, the proportion of milkings with normal milk ejection had increased substantially in both treatments, suggesting improved milk ejection during AMS milking over time. This may be biologically important, as regular and complete milk removal is considered essential for maintaining high milk production (Wall & McFadden 2012), whereas retained residual milk can contribute to local inhibition of milk secretion (Weaver & Hernandez 2016). Thus, even if some milkings remained incomplete, having at least some milkings with normal milk ejection each day may potentially have reduced the negative effects of residual milk accumulation. The lower proportion of normal milkings observed at the first sampling occasion may partly be due to cows not yet being fully adapted to the AMS environment, since altered or unfamiliar milking conditions can disturb oxytocin release and milk ejection responses, especially in primiparous cows (Tancin & Bruckmaier 2001; Bruckmaier 2005). In addition, cows were in a very early stage of lactation during the first sampling occasion (DIM 5—12), which may also have influence milk ejection patterns. Lower udder fill has previously been associated with delayed milk ejection in cows during lactation (Bruckmaier & Hilger 2001). However, the earliest stage evaluated in that study was weeks 3—11 of lactation, whereas cows in the present study were sampled within the first days after calving. It therefore remains unclear whether the same relationship applies during this very early lactation period.

5.2 Effect of milking permission interval

The overall effect of milking permission interval on milk ejection appeared to be limited. Although statistical analyses identified some treatment effects, no clear or consistent differences were observed between cows assigned to a 4-h and 10-h milking permission intervals within either management system. The rank-biserial correlations provided additional context to these findings. Comparing the CCC groups, effect sizes were consistently small across all milk ejection classifications and sampling occasions. When comparing the CONV groups, however, a moderate effect size was observed for the proportion of milkings classified as normal milk ejection at the first sampling occasion, whereas this pattern was no longer evident at the second sampling occasion. Accordingly, no significant differences were detected between CCC4 and CCC10 or between CONV4 and CONV10 at either sampling occasion.

Previous studies have reported delayed milk ejection at short milking intervals, likely due to reduced udder fill and lower intramammary pressure (Bruckmaier & Hilger 2001). Similarly, Delyus et al. (2020) observed longer latency to milk

ejection when cows were milked more frequently. However, direct comparisons with the present study should be made with caution. In AMS, the actual interval between milkings is influenced not only by the assigned milking permission interval, but also by factors such as robot occupancy, waiting times, cow traffic, and individual cow motivation to visit the robot. During the period of full-day CCC, realised milking intervals averaged approximately 8-9 h in the 4 h treatment groups and 12-15 h in the 10 h treatment groups, meaning that cows assigned to the 4-h milking permission treatment were still milked at intervals considerably longer than those evaluated in previous studies reporting delayed milk ejection. For example, Bruckmaier & Hilger (2001) compared milking intervals of 4, 8 and 12 h, whereas Delyus et al. (2020) reported longer milk ejection latency at intervals of 4.5-6.0 h compared with 7.5-9.0 h. Thus, although the treatment groups differed in their realised milking interval, the cows assigned to the 4 h treatment may not have been milked frequently enough for effects comparable to those reported in previous studies to occur.

In the CCC groups, calf suckling between machine milkings may also have contributed to the lack of clear differences between the 4 h and 10 h treatments. Previous authors have suggested that unrestricted suckling can increase variation in udder fill at the time of machine milking (Barth 2020; Neave et al. 2024), potentially obscuring effects of milking permission interval. Consequently, differences in realised milking interval may not have translated into consistent differences in udder fill between CCC4 and CCC10 dams. A further possible explanation is the relatively low statistical power of the present study. The limited number of cows per treatment group, combined with substantial between-cow variation and relatively simple statistical analyses compared with mixed-model approaches, may have reduced the ability to detect effects of milking permission interval.

5.3 Cow-related factors associated with milk ejection

Some additional data patterns were observed in the present study, for instance descriptive differences related to parity and breed. At the first sampling occasion, Swedish Red cows showed higher milk fat concentrations in end of milking strip milk than Swedish Holstein cows across all treatment groups. This is consistent with known breed characteristics, as Swedish Red cows generally produce milk with higher fat concentration than Swedish Holstein cows (Växa 2026). More pronounced differences were observed for parity, where multiparous cows in several treatment groups tended to show higher milk fat concentrations in strip milk compared with primiparous cows, particularly within CCC10 during the second sampling occasion (primiparous: 3.4 ± 1.9 %; multiparous: 10.4 ± 2.2 %). One reason for this may be that primiparous cows are generally more sensitive to new milking environments and routines during early lactation (Bruckmaier 2005). At the same time, primiparous cows generally produce less milk than multiparous cows during early lactation (Siewert et al. 2019), which may have resulted in lower udder fill before machine milking. Since udder fill influences the milk ejection response (Bruckmaier & Hilger 2001), this may also have contributed to

the numerically lower milk fat concentration in primiparous cows. However, breed and parity were not statistically analysed in the present study, and these numerical differences should therefore be interpreted cautiously.

5.4 Methodological considerations

A strength of the present study was the controlled comparison of CCC dams and CONV cows within the same AMS environment, minimizing variation related to housing, feeding, and general management. Furthermore, milk samples were collected after milking and therefore represented strip milk rather than composite milk, providing information about the final milk fraction remaining in the udder after machine milking.

However, several limitations should be considered. First, milk fat concentration in strip milk represents an indirect measure of milk ejection and udder emptying in comparison to a direct measurement of the residual milk volume or milk ejection evaluations based on milk flow curves. Although previous studies have suggested that strip milk fat concentration is a useful indicator of udder emptying (Jenni et al. 2024), interpretation of the results remains indirect. In addition, the interpretation of strip milk fat concentration may be more complex in CCC dams than in CONV cows. The physiological basis for using strip milk fat concentration as an indicator of udder emptying is largely derived from studies of machine milking, where milk ejection is followed immediately by milk removal (Ontsouka et al. 2003; Rico et al. 2014). However, less is known about how milk fat is distributed following calf suckling. Caja et al. (2004) showed that milk ejection without subsequent milk removal increased cisternal milk volume, but that this increase gradually declined when cows were not milked. This suggests that milk distribution within the udder continue to change following milk ejection if milk is not removed immediately. Consequently, it remains unclear whether the redistribution of milk following a milk ejection without subsequent milk removal also affects the distribution of milk fat within the udder, and therefore the milk fat concentration present at the start of a subsequent milking. As a result, in the present study a recent suckling event may have influenced quarter-level strip milk fat concentration by inducing milk ejection without removing milk equally from all udder quarters. To better understand how milk fat concentration changes throughout the milking process, and whether recent suckling influences the milk fat concentration present at the start of milking, future CCC studies could collect hand-stripped milk samples both before and after machine milking while also recording the timing of suckling events relative to milking.

In addition, the classification of milk ejection in the present study was based on a predefined threshold for quarter-level strip milk fat concentration, which, to the authors' knowledge, has not previously been validated as a measure of milk ejection. The selected threshold was based on Ontsouka et al. (2003), and alternative thresholds may have resulted in different classifications of milk ejection. Several variables relevant for interpretation of milk ejection dynamics,

including residual milk volume, milk flow curves and timing of calf suckling were recorded in the larger study but were not included in the present work due to time and analytical limitations. Including these variables may have improved interpretation of how variation in udder fill influence milk ejection during machine milking.

Previous studies have shown that machine milk yield may partly or fully recover after calf separation in CCC systems (Zipp & Knierim 2024; van Zyl et al. 2025), suggesting that future studies should investigate whether milk ejection dynamics also recover following calf separation, and whether this differs among CCC systems. Such knowledge may contribute to development of management strategies that support both animal welfare and efficient milk production in dairy systems. Another study limitation was the small number of cows included in each treatment group, particularly considering the substantial variation between individual animals, limiting what inferences that could be made from the results. For example, while the comparison between CCC4 and CONV4 for the proportion of milkings classified as normal milk ejection was not statistically significant, the calculated rank-biserial correlation showed a large effect size, potentially indicating a biologically relevant difference between the groups. However, the number of CCC dams that could be included in the study was constrained, as the contact area between cows and calves contained a limited number of cubicles. In addition, limited previous research on quarter-level strip milk sampling made it difficult to estimate the sample size required to detect treatment effects when the study was designed. The proportion-based data also contained a high number of tied values, which may have reduced the sensitivity of the Mann-Whitney U-tests to detect differences between treatments, particularly given the small sample size. Future studies using similar statistical analysis methods would likely benefit from larger treatment groups and a greater number of milkings per sampling occasion, which could reduce the influence of between-cow variation and increase the statistical power to detect treatment effects. In addition, as the Bonferroni correction method that was used in this study generally increases the risk of type II errors, alternative approaches for multiple comparison correction, such as false discovery rate control, may also be beneficial in future studies.

5.5 Practical implications

The present findings may have practical implications for implementation of CCC systems in dairy production. Cow-calf contact systems are increasingly discussed as a management strategy that may support natural behaviour and improve aspects of animal welfare (Johnsen et al. 2016; Sirovica et al. 2022). However, the results of the present study suggest that CCC may also influence milk ejection and potentially milk harvest during machine milking, which may be relevant for farmers considering implementation of full-day cow-driven CCC systems in herds milked in an AMS. In addition to CCC, the present study also evaluated whether different milking permission intervals were associated with differences in milk ejection. Current recommendation from DeLaval suggest a milking permission

interval of four hours for conventional cows in early lactation (DeLaval 2016). However, practical recommendations for CCC systems remain unclear and according to discussions with DeLaval advisors, both shorter and longer milking permission intervals have been proposed as potential management strategies in herds with CCC (Ferneborg, personal communication, 2026). The rationale for shorter intervals is to increase milking frequency and milk harvest (Allen et al. 1986; Soberon et al. 2011; Hanling et al. 2021), whereas longer intervals may allow greater udder fill before machine milking and potentially facilitate milk ejection (Bruckmaier & Hilger 2001; Delyus et al. 2020). Under the conditions of the present study, no clear differences in milk ejection success were observed between the 4 h and 10 h milking permission treatments, providing limited support for either strategy. Further research is therefore needed to determine the optimal milking permission strategy for AMS herds practicing CCC.

6. Conclusion

The results of this study showed that cows managed with full-day cow-driven CCC had a lower proportion of milkings classified as normal and a higher proportion classified as inconsistent compared with CONV10 managed cows at the first sampling occasion. In contrast, no clear differences were found between the 4 h and 10 h milking permission intervals. Overall, the results suggest that CCC may influence milk ejection during machine milking in an AMS during early lactation, whereas the milking permission interval alone appeared to have limited effect under the conditions of the present study. Further large-scale studies are needed to improve the understanding of how milk fat concentration in strip milk collected at the end of machine milking reflects milk ejection in automatic milking systems with CCC.

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Popular science summary

For many dairy calves, separation from the cow occurs within the first hours after birth. However, interest in alternative systems where cows and calves are allowed to stay together has increased in recent years. These so-called cow-calf contact systems are often considered beneficial for animal welfare because they allow natural behaviours such as suckling and social bonding. At the same time, many dairy farmers are concerned that calves nursing their dams may reduce the amount of milk that can be harvested in the milking system.

One possible explanation is that cows nursing calves may not release milk as effectively during machine milking. Milk in the udder is released through a hormonal reflex triggered by teat stimulation. If this reflex is incomplete, part of the milk may remain in the udder instead of being harvested by the milking machine. Previous studies have suggested that contact with the calf may influence this process, but little is known about how this works in modern automatic milking systems.

The study was carried out at the Swedish Livestock Research Centre at SLU in Uppsala. Forty dairy cows were included and divided into four groups. Half of the cows were separated from their calves shortly after calving according to conventional management, while the other half remained in full-day contact with their calves for several weeks after birth. In addition, cows were managed with either a short or long milking permission interval in the automatic milking system.

To evaluate how completely the udder was emptied during machine milking, milk samples were collected by hand directly after milking. The fat concentration of this remaining milk was analysed. Milk fat concentration increases during milking, and milk left in the udder after milking is therefore expected to contain a high fat concentration if milk removal has been effective.

The results showed that cows housed together with their calves generally had lower milk fat concentrations in the milk remaining after machine milking. This may indicate that these cows were not emptied as completely during milking as conventionally managed cows. In contrast, the length of the milking permission interval appeared to have limited influence on the results.

The findings suggest that cow-calf contact may influence milk removal during automatic milking. However, the results should be interpreted carefully, as several factors can affect how much milk is available in the udder at the time of milking. More research is therefore needed to better understand how cow-calf contact systems can be managed in automatic milking systems while maintaining both good animal welfare and efficient milk harvesting.

Appendix

Appendix 1

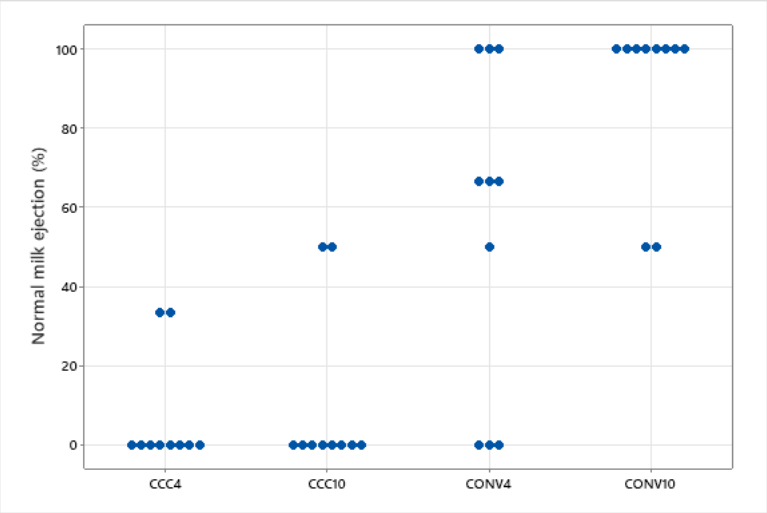


Figure 1.1 Individual value plot showing the proportion of milkings classified as normal milk ejection (%) for individual cows within each treatment group (CCC4, CCC10, CONV4, CONV10) during the first sampling occasion. The abbreviations refer to cow-calf contact (CCC) and conventional management (CONV), and the numbers (4 and 10) indicate milking permission intervals (hours). Each point represents one cow and indicates the percentage of milkings classified as normal milk ejection out of the total number of evaluated milkings during the first sampling occasion.

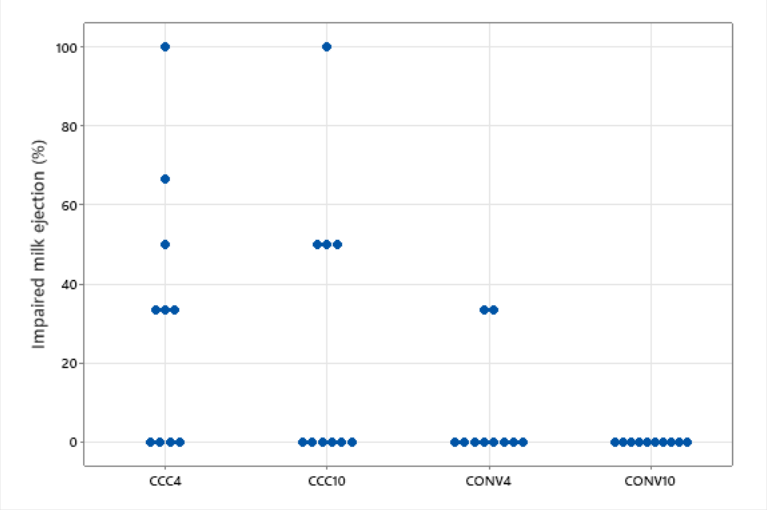


Figure 1.2 Individual value plot showing the proportion of milkings classified as impaired milk ejection (%) for individual cows within each treatment group (CCC4, CCC10, CONV4, CONV10) during the first sampling occasion. The abbreviations refer to cow-calf contact (CCC) and conventional management (CONV), and the numbers (4 and 10) indicate milking permission intervals (hours). Each point represents one cow and indicates the percentage of milkings classified as impaired milk ejection out of the total number of evaluated milkings during the first sampling occasion.

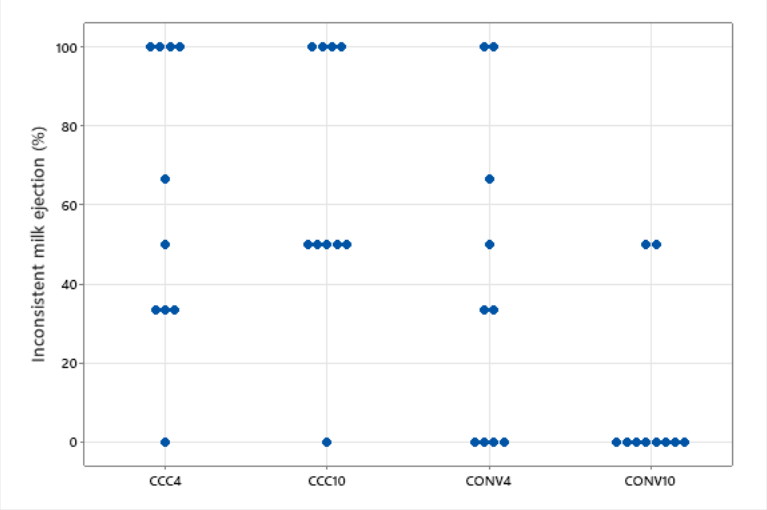


Figure 1.3 Individual value plot showing the proportion of milkings classified as inconsistent milk ejection (%) for individual cows within each treatment group (CCC4, CCC10, CONV4, CONV10) during the first sampling occasion. The abbreviations refer to cow-calf contact (CCC) and conventional management (CONV), and the numbers (4 and 10) indicate milking permission intervals (hours). Each point represents one cow and indicates the percentage of milkings classified as inconsistent milk ejection out of the total number of evaluated milkings during the first sampling occasion.

Appendix 2

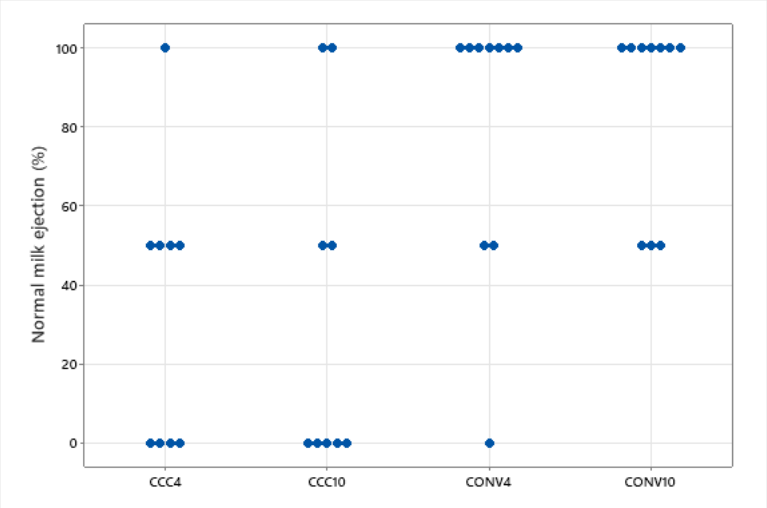


Figure 2.1 Individual value plot showing the proportion of milkings classified as normal milk ejection (%) for individual cows within each treatment group (CCC4, CCC10, CONV4, CONV10) during the second sampling occasion. The abbreviations refer to cow-calf contact (CCC) and conventional management (CONV), and the numbers (4 and 10) indicate milking permission intervals (hours). Each point represents one cow and indicates the percentage of milkings classified as normal milk ejection out of the total number of evaluated milkings during the second sampling occasion.

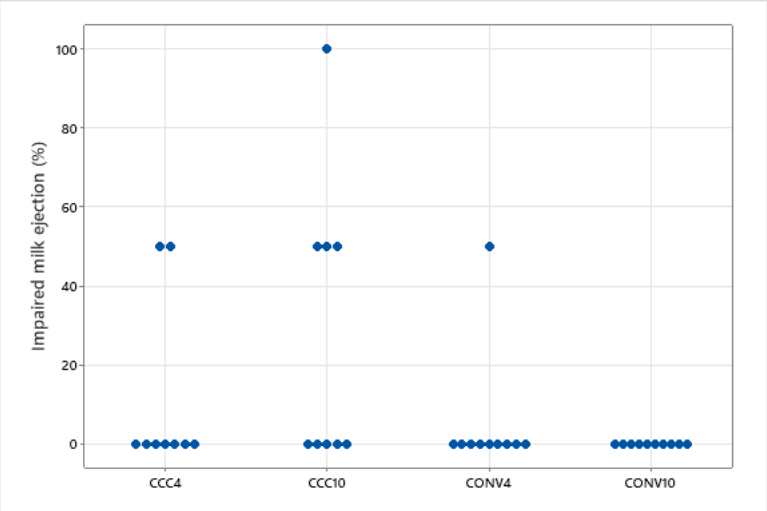


Figure 2.1 Individual value plot showing the proportion of milkings classified as impaired milk ejection (%) for individual cows within each treatment group (CCC4, CCC10, CONV4, CONV10) during the second sampling occasion. The abbreviations refer to cow-calf contact (CCC) and conventional management (CONV), and the numbers (4 and 10) indicate milking permission intervals (hours). Each point represents one cow and indicates the percentage of milkings classified as impaired milk ejection out of the total number of evaluated milkings during the second sampling occasion.

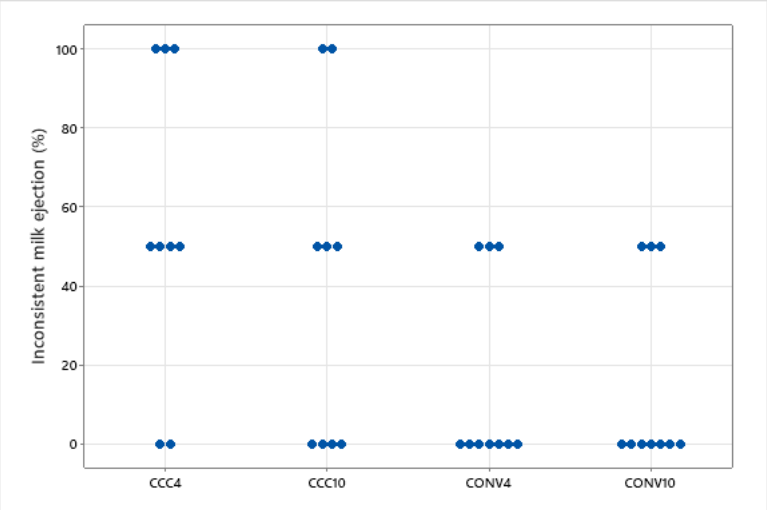


Figure 2.1 Individual value plot showing the proportion of milkings classified as inconsistent milk ejection (%) for individual cows within each treatment group (CCC4, CCC10, CONV4, CONV10) during the second sampling occasion. The abbreviations refer to cow-calf contact (CCC) and conventional management (CONV), and the numbers (4 and 10) indicate milking permission intervals (hours). Each point represents one cow and indicates the percentage of milkings classified as inconsistent milk ejection out of the total number of evaluated milkings during the second sampling occasion.

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