



# Effects of gradual weaning through 12-hour contact and artificial milk feeding on cow behaviour, milk yield and calf weight gain in a cow-calf contact system with automatic milking

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# Effects of gradual weaning through 12-hour contact and artificial milk feeding on cow behaviour, milk yield and calf weight gain in a cow-calf contact system with automatic milking

*Effekter av gradvis separation genom 12-h kontakt och artificiell mjölkutfodring på korsk beteende och mjölkavkastning samt viktökning hos kalvar i ett ko-kalvsystem med robotmjölkning*

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## Abstract

Cow-calf contact (CCC) systems offer an alternative to artificial calf rearing within the dairy farming practice; it improves calf growth rates and allows cow and calf to express mother-offspring behaviours. However, longer periods of CCC can cause strong behavioural responses in both cow and calf when they are inevitably later separated, and research has yet to agree on optimal management of separation and weaning practices in CCC systems. This study explored a gradual separation system spanning a total of 14 study weeks and containing three successive periods of CCC contact: 24-hour, 12-hour and 0-hour daily contact. The main study aims were to discern whether cow daily lying times were affected by transitioning from 24-hour to 12-hour contact and whether feeding calves additional milk mitigated decreases in calf weight gain often seen after physical separation from the dam. Additionally, the study recorded cow milk yields during the entire study period and investigated cows' use of the stalls adjacent to the calf confinement area in the mornings before and after initiating 12-hour CCC.

In this study, nine cow-calf pairs were allowed an initial period of 24-hour unrestricted contact lasting 3-12 weeks depending on the birth date of the calf. Study data were only collected for three weeks of the 24-hour contact period, as all calves were not in the experimental pen before this time. Then, a 6.5-week period of half-day contact followed, when calves and cows were separated during nighttime. During the last 4.5 weeks of the study, cows and calves were physically separated but still able to see, hear and smell each other. Calves were introduced to an external milk source from the start of 12-hour contact until week 13 of the study, postponing the point of weaning past the point of physical separation of calves from their dams. Cows' daily lying time was logged through scan sampling at 10-minute intervals during the week before and the week after the 12-hour contact period commenced. Cows' use of stalls close to the calf confinement area was recorded for the same period. Calves were regularly weighed to calculate average daily weight gain (ADG) for the different periods. Cow milk yield data covering the entire study period was gathered through parlour software.

Transitioning from a 24-hour to 12-hour contact period did not seem to negatively affect cow lying times – rather, the cows mostly numerically increased lying times during the first week of 12-hour CCC. Cows did not seem to anticipate calf release from confinement, as they did not congregate in the stalls closest to the calf creep before the calves were released in the mornings. The average milk yield delivered in the robot increased numerically during the 12-hour contact, and was at its highest following physical separation. The number of milkings with records of incompletely milked teats decreased numerically with decreasing amount of contact for multiparous cows. Calf weight gain was found not to differ between 24- and 12-hour contact periods, suggesting that 12-h contact in combination with artificial milk feeding was sufficient to maintain high milk intake. Contrary, ADG decreased significantly following physical separation, but the growth rate still exceeded expectations, averaging at  $0.9 \pm 0.14$  kg/day.

These results provide initial indications that gradual reduction of daily contact time may somewhat mitigate the negative impacts of abrupt separation whilst maintaining a high calf growth rate across a variety of calf ages. Temporally dividing separation and weaning and allowing calf access to milk post-separation may serve to alleviate the decrease in weight gain often seen at separation, although further research using more controlled trials is needed to solidify this theory. Cow lying time did not substantially decrease following initiation of 12-hour contact, indicating that this may be a promising strategy to decrease severe separation distress in dams. Similar studies need to be

performed on cow lying times when transitioning from 12-hour to 0-hour contact, as this study was unable to evaluate this aspect.

*Keywords:* Dam rearing, milk production, lying behaviour, ADG, half-day contact

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## Abbreviations

AMF	Automatic milk feeder
AMS	Automatic milking system
ADG	Average daily (weight) gain
CCC	Cow-calf contact
DIM	Days in milk
SLU	Swedish University of Agricultural Sciences
SH	Swedish Holstein
SR	Swedish Red



# 1. Introduction

Today, the dairy industry largely practices artificial calf rearing, where cows and calves are separated shortly after birth and calves are milk-fed and raised separately from their dams (Marcé *et al.* 2010; Kalvportalen 2019). Housing methods where cows and calves are instead kept together for varying periods of time after birth are labelled cow-calf contact (CCC, Sirovnik *et al.* 2020) systems. These CCC systems provide some benefits, such as high calf growth rates (Fröberg *et al.* 2011), but are not without disadvantages as calf growth generally decreases markedly at weaning (eg. Roth *et al.* 2009; Fröberg *et al.* 2011). Additionally, allowing cows to be suckled may result in impaired milk ejection and subsequent lower machine milk yields (de Passillé *et al.* 2008). Whilst reviewing the subject, Johnsen *et al.* (2016) acknowledged hampered calf growth post-weaning, decreased saleable milk quantities and increased post-separation stress responses as challenges associated with implementing CCC. As prolonged CCC allows for a cow-calf bond to develop, separating these established cow-calf pairs often results in pronounced behavioural responses in dams - for instance frequent vocalisation (Lidfors 1996) and reduced lying times (Krohn *et al.* 1990a). Implementing half-day instead of whole-day CCC has been suggested as a way to lesser this elevated stress response, with initial, albeit limited research results supporting this claim (Johnsen *et al.* 2016).

This study explored a CCC automatic milking system (AMS) implementing 12-hour contact as an intermediate step between unrestricted contact and physical separation of dam and calf. In addition to reduced contact, calves were manually milk fed during the full 12-h contact period and for three weeks after the calves could no longer suckle the cows. The study aimed to evaluate whether this gradual separation and weaning process influenced the dams' lying behaviour through describing daily lying times one week prior to and one week following initiation of the 12-hour contact period. Machine milk yields were assessed prior to and following provision of an additional milk source for the calves, to discern whether additional milk feeding could improve machine milk yields. Additionally, the study measured calf average daily weight gains (ADG) during the whole study period, to determine whether gradual separation in conjunction with postponed weaning off milk can mitigate the calf growth check often seen at separation in CCC systems.

## 2. Literature review

### 2.1 Defining the cow-calf bond

#### 2.1.1 The postpartum period

In broad terms, the immediate postpartum period of domestic cattle is characterised by a short period of intensely maintained cow-calf contact and the establishment of a dam-calf bond, after which dams and calves spend increasing amounts of time apart (Edwards & Broom 1982; Kiley-Worthington & De la Plain 1983).

Immediately postpartum, the cow sniffs and starts licking the newborn calf. The cow spends a large proportion of the following few hours continuing this behaviour – licking has been shown to occupy up to 50% of the first hour after birth (Edwards & Broom 1982). In addition, she vocalises through distinct, low-pitched calls (Selman *et al.* 1970; Kiley-Worthington & De la Plain 1983). These behaviours are presumed to be a crucial part of forming the cow-calf bond, as maternal recognition of the calf develops very soon after birth. In a study by Hudson & Mullord (1977), cows and calves were separated from each other for differing amounts of time following an initial postpartum contact of 5 minutes, before being reintroduced. All dams who were separated from their calves for a maximum of 5 hours were still able to distinguish their own calves from alien calves. Only one dam was separated from her calf for 12 hours, but readily accepted the calf at reintroduction. The dams in the study of Hudson & Mullord (1977) were described to exhibit signs of distress such as vocalising and restlessness during the separation period; these signs were absent in control dams who were immediately separated from their calves. The behaviours were reported to persist for up to 24 hours after separation, even though at this point in time the dam rejected their own calves when reintroduced. However, the behaviours were not quantified further in the study, so the duration of time bonded dams perform increased vocalisation after separation is not known from this study.

Bovine species are frequently described as “hider” ungulates; this indicates that the young calf mainly spends time sheltered some distance away from its dam, as

opposed to “follower” species such as sheep where the young remain in close proximity with their mothers from birth (Lent 1974). However, the classification of domestic cattle remains diffuse. Some authors have opted to label cattle as “facultative hidiers”, presumably to better reflect the species’ adaptability to differing housing conditions (Padilla de la Torre *et al.* 2016). Maremma cattle, a semi-wild type of cattle minimally affected by domestication, exhibit parts of both hiding and following behaviour (Vitale *et al.* 1986). The calf hides in tall vegetation during the first days of life, but may also on occasion be observed following the mother. Langbein & Raasch (2000) reported similar hiding behaviours in domestic suckler cattle kept at pasture. In this study, the calves showed a clear preference for lying down in a designated calf creep area supplied with tall vegetation as opposed to in the pasture with their dam. By five days of age, the percentage of time spent hiding in this vegetation tended towards a decrease, further suggesting that the “hiding” period is comparatively short-lived in cattle.

### 2.1.2 Cow-calf relations in the herd

A few days after birth, the dynamics between cow and calf shifts. Dam-initiated contact with the calf declines quite rapidly (Kiley-Worthington & De la Plain 1983; Jensen 2011), whilst calf-initiated contact with the dam seems to increase (Lidfors *et al.* 1994) or at the very least stay consistent (Kiley-Worthington & De la Plain 1983), making calves the primary initiators of CCC after the initial dam-calf bonding period. Examples of such contact behaviours are suckling, sniffing (Lidfors & Jensen 1988) and allogrooming (Kiley-Worthington & De la Plain 1983). Even though dam-initiated suckling decreases as the calf gets older, Kiley-Worthington & De la Plain (1983) reported that dams may actively seek out their calves in order to stand in its immediate proximity for periods of up to an hour.

As the first 6 months progress, calf and dam spend their time at increasing distances from one other. During the aforementioned, short post-partum hiding period, calves are mostly solitary (Langbein & Raasch 2000). Following these first days of life, calves instead gather in groups often termed “creches”, and no longer show a clear preference for sheltered areas (Sato *et al.* 1987). Calves also intermittently follow their dams when grazing. Still, cows and calves alike spend most of their time with same-aged peers and not with their dams or offspring (Kiley-Worthington & De la Plain 1983; Vitale *et al.* 1986).

### 2.1.3 Weaning and separation

Given that dairy calves are most commonly artificially milk fed and weaned at a young age, little is known about when weaning would normally occur in dairy cattle. In extensively managed Zebu (*Bos indicus*) cattle, dam-initiated weaning

occurs between 7-14 months of age (Reinhardt & Reinhardt 1981b), although weaning is not to be confused with a total discontinuation of the social bond between dam and offspring when continuously kept in the same herd. Zebu cows and their offspring have been shown to exhibit mutual preferences towards one other as licking and grazing partners for several years after weaning compared with unrelated herd members, suggesting that some aspect of a familial bond is recognised by the animals far beyond the age of weaning (Reinhardt & Reinhardt 1981a). Similar results are reported in dual-purpose Salers cattle, where preferential bonds between dams and yearling offspring have been found to persist past the subsequent calving period (Veissier *et al.* 1990).

In contrast, in countries with intensive milk production dairy calves are commonly separated from their dam shortly after calving, although age at weaning may vary between countries and farms. For instance, in Sweden, separating the calf and dam immediately postpartum is common practice (Kalvportalen 2019), provided the farm isn't certified by the Swedish organic production label KRAV (KRAV 2023) or part of the Swedish dairy company Arla's label "Ännu bättre djuromsorg" ("Even better animal care", author translation) (Arla 2020). These labels state that the calf has to be provided suckling opportunities for a minimum of either 24 (KRAV) or 12 hours (Arla) postpartum and therefore the calf stays with the dam for at least this period of time. Conventionally raised calves are generally weaned off milk at approximately eight weeks of age in Europe (Marcé *et al.* 2010), while calves under the regulation of the aforementioned labels have access to milk for at least ten (Arla) or twelve (KRAV) weeks postpartum.

## 2.2 Dam response to separation

### 2.2.1 Behaviour

As of yet, no standardised methods of assessing dam reaction to separation have been developed, although recent research may combine a variety of the behaviours to be discussed below (see eg. Neave *et al.* 2023). This section covers behavioural responses of the dam that often are evaluated in studies of dairy cow and calf separation, but does not claim to be a comprehensive review of all studied behaviours.

#### *Lying time*

Lying down is an important behaviour for dairy cows, and central to the cow welfare concept of "cow comfort" (Växa Sverige 2022). Cattle spend a considerable part of their daily time-budget lying down. For example, heifers seemingly harbour an inelastic demand of at least 12 hours daily lying time (Jensen *et al.* 2005). The

term “inelastic demand” dictates that when exposed to an experimental environment where the animals have to “pay” a certain cost to get access to a behaviour, for example through pushing through a gate or pressing a paddle, the motivation to access the behaviour for a defined amount of time does not decrease even as the cost or amount of work needed to be able to perform the behaviour increases (Kirkden & Pajor 2006).

Similar daily lying time values have been reported for lactating cows, both on Canadian AMS farms (median 11.4 h/day; Westin *et al.* 2016) and Canadian free-stall farms (mean 11 h/day; Ito *et al.* 2009). However, both studies underline a great variation in daily lying times across farms and individuals. Regardless of individual variations, lying is highly valued by the cow; when subjected to time constraints, it is prioritised over both eating and social contacts (Munksgaard *et al.* 2005).

Johnsen *et al.* (2021) piloted daily lying times for 8 cows kept in a cow-driven (see Sirovnik *et al.* 2020 for definition) CCC system on an AMS farm. Cows and calves were kept together for 31 days, after which a gradual decrease of daily contact was initiated over a period of 6 days using programmable selection gates. The authors reported mean lying times of 10.6 hours during the 24-h suckling phase and 11.3 hours during the two days following the gradual separation phase.

The separation of cow and calf after a period of CCC has been proven to affect lying times in several studies; however, most of these studies evaluate cow-calf separation at younger calf ages. Several authors have observed either less lying behaviour (Lidfors 1996; Stěhulová *et al.* 2008) or increased cow standing times (Flower & Weary 2001) immediately after cow-calf separation at timepoints varying from 4 days to 2 weeks postpartum. This occurred regardless of whether cows had visual and auditory contact with their calf post-separation - as was the case in Lidfors (1996) as well as for some cow groups in Stěhulová *et al.* (2008) - or were abruptly and completely separated from the calf (Flower & Weary 2001; Stěhulová *et al.* 2008). Weary & Chua (2000) published findings of a more unclear nature; they showed numerical lying time decreases in cows when abruptly separated at 6 h or 1 day postpartum, but an increase when separated at 4 days; the differences pre- and post-separation were not tested for significance.

Less is known about the effects of separating cows and calves at higher calf age. Krohn *et al.* (1990b) described that lying times in dams decreased significantly during the first 24 hours post-separation in a twice daily restricted suckling system where dams were kept next to their calves and then abruptly separated at 8 weeks postpartum. Similarly, Mac *et al.* (2023) evaluated separation responses in dams abruptly separated from their calves at 100 days of age and found that the dams spent significantly more time standing on the first day post-separation than during

either of the two following days. Most research into the subject describe their results as total daily standing or lying time during observation periods, but Krohn *et al.* (1990b) also reported frequencies and durations of lying bouts, reporting that the number of bouts seemed to increase whilst bout durations decreased during the first day post-separation. To summarise, decreases in lying time seem to occur post-separation across a variety of calf ages and both when abruptly separated or still allowed some degree of remote contact between the cow-calf pair, but the decreases seem short-lived. Limited evidence further points to dams lying down and standing up more often in response to calf separation, as well as lying down for shorter periods at a time, but this occurrence is not widely researched as of yet.

### *Vocalisation*

The subject of vocalisation as a behavioural response to cow-calf separation is frequently raised in CCC studies (Johnsen *et al.* 2016). Post-separation vocalisation in dairy cattle has been studied at separation timepoints ranging from 6 hours (Weary & Chua 2000) to 100 days (Mac *et al.* 2023) postpartum. When comparing abrupt separation responses at 6 hours, 1 day and 4 days postpartum, longer periods of CCC contact was found to be associated with louder and more frequent vocalisation in the dams (Weary & Chua 2000). Furthermore, increased dam vocalization following abrupt separation has been observed at least until separation at 3.5 months of calf age (Mac *et al.* 2023).

As many behavioural studies are focused on a short time period after separation (eg. 24 hours, Lidfors 1996, Weary & Chua 2000; 21 hours, Flower & Weary 2001; 51 hours, Stěhulová *et al.* 2008), little is known about how many days increased vocalisations last. In line with the findings of Weary & Chua (2000) described above, the time period of contact pre-separation may possibly affect the duration of vocalisation post-separation. Stěhulová *et al.* (2008) separated cows and calves at 4-7 days of age either abruptly or with maintained vocal, visual and olfactory contact. They found that vocalisation frequencies were most prominent during the first 9-10 hours post-separation and greatly decreased by 51 hours post-separation; vocalisation was also significantly more frequent if some level of contact between cow and calf was retained. Veissier *et al.* (2013) found that the percentage of cows vocalising after abrupt calf separation at 10 weeks of age was significantly higher than that of control cows which had been separated from their calves immediately postpartum for two whole days. Lastly, in a study by Nicolao *et al.* (2022), calves in a half-day CCC contact system were abruptly separated from dams at an approximate age of 11 weeks, although the possibility of vocal cow-calf contact was maintained. As a result, the proportion of dams frequently vocalising increased during day 1 and 2 following separation when compared to the day before separation. The proportion started to decrease by day 4 and had regressed to pre-separation values by day 7 after separation.



### *Other behaviours*

Apart from lying times and vocalisations, studies address several other behaviours in conjunction with cow-calf separation. Cows seem to generally increase their activity post-separation, including the amount they move (Hudson & Mullord 1977; Flower & Weary 2001) and putting their head outside the pen in a presumed effort to locate or reach the separated calf (Flower & Weary 2001; Stěhulová *et al.* 2008). One study reported that dams separated from calves 10 weeks postpartum showed signs of agitation such as kicking, stopping or vocalising before they entered the milking parlour and during milking for two days following separation; these behaviours were not present in control dams who had been separated from calves shortly postpartum (Veissier *et al.* 2013). Potential post-separation alteration of daily rumination time for the dams seem to vary with calf age at separation. Mac *et al.* (2023) found that cows did not significantly alter rumination times when separated from their calves at 100 DIM, while Lidfors (1996) reported a decrease in dam rumination when separated from calves 4 days postpartum compared to immediately before separation. Early on, visible eye white was suggested as a possible indicator of frustration in dairy cows, and was found to increase at separation (Sandem & Braastad 2005), but this aspect has not been investigated in further studies at the time of this review.

### 2.2.2 Milk yield

Nursing cows have most often been shown to have lower machine milk yields compared to non-nursing cows (e.g. de Passillé *et al.* 2008; Wenker *et al.* 2022; also reviewed by Johnsen *et al.* 2016). This decrease in machine milk yield spans all types of CCC systems (Johnsen *et al.* 2016) and is commonly attributed to two main factors that are addressed in detail below. There is limited evidence that higher machine milk yields can be obtained from suckled dams during certain conditions; Bar-Peled *et al.* (1998) found that Holstein cows nursing two foster calves in a restricted suckling system being milked thrice and suckled for 15 minutes thrice daily gave higher milk yields than cows milked three or six times per day. Furthermore, in dairy cows crossed with the more primordial Zebu cattle, calf presence and restricted suckling seems to improve rather than hamper the amount of milk collected for human consumption (Fröberg *et al.* 2007).

The reduction in obtained milk yields from suckled dams could be explained by the calves consuming some of the milk that would otherwise contribute to the machine milk yield (Flower & Weary 2001; de Passillé *et al.* 2008), decreasing the amount of saleable milk. The current Swedish daily milk allowance recommendation amounts to 8 L/day during the first four weeks of the calf's life (Kalvportalen 2019). This corresponds justifiably with findings of calf milk intake when offered ad libitum allowances in teat buckets, with Johnsen *et al.* (2015c) reporting an average

daily intake of 8.2 L during 0-6 weeks of age. Additionally, through weighing calves before and after isolated suckling bouts, calves in restricted suckling systems have been recorded to consume 6.5-12.5 kg of milk daily between 0-9 weeks of age (de Passillé *et al.* 2008). A study evaluating two indirect methods of estimating milk yield losses in AMS-housed CCC cows estimated that 7.3-11.3 kg of milk was lost daily on average per cow (Churakov *et al.* 2023). Theoretically, these estimates contained both the milk consumed by the calf and residual milk left in the udder in cases of incomplete milk letdown (see below).

Secondly, cows nursing their own calves show a lower increase in oxytocin levels at machine milking compared to non-nursing cows (de Passillé *et al.* 2008). Hence, they may exhibit incomplete milk ejection at milking. Oxytocin is the main regulatory hormone of the milk ejection reflex and it is generally conditioned to the site and sounds related to machine milking in dairy cows (Sjaastad *et al.* 2016), provided no CCC is allowed (Akers & Lefcourt 1984; de Passillé *et al.* 2008). Lower increase in oxytocin levels at machine milking results in incomplete milk ejection and larger volumes of residual milk remain in the udder after milking (de Passillé *et al.* 2008). Additionally, incomplete emptying doesn't only subtract the milk left in the udder from the milk yield; there is also some evidence that an increased amount of residual milk may exert an inhibitory effect on milk production (Sjaastad *et al.* 2016; Albaaj *et al.* 2018). De Passillé *et al.* (2008) suggested that the incomplete rise of oxytocin levels during machine milking is due to the presence of a cow-calf attachment, but the underlying mechanisms for this have not been scientifically studied as of yet.

After separation, the oxytocin levels at machine milking for CCC cows promptly rebound to levels comparable with non-nursing cows (Akers & Lefcourt 1984). As a result, tank milk yields increase, additionally aided by the absence of nursing by the now-separated calves. Indeed, some studies even report results suggesting that nursing cows might equal non-nursing cows in total lactation yields through an increase in milk production post-separation (Flower & Weary 2001; Johnsen *et al.* 2015a), whilst other studies report a lower milk yield persisting for some time post-separation (Metz 1987; Barth 2020). Although this effect of separation might be considered economically favourable on account of the increase in machine milk yield, the calves generally handle the transition off milk – which in many cases occurs simultaneously with separation - poorly, as will be discussed in the next section.

## 2.3 Calf response to separation

### 2.3.1 Behaviour

Calf behavioural responses to separation largely resemble those seen in dams, but seem to occur with some delay; several studies report peaks in behavioural frequencies concerning behaviours such as vocalisation and activity between 9-24 hours after abrupt separation with (Fröberg *et al.* 2011) or without (Weary & Chua 2000) simultaneous abrupt weaning. Weary & Chua (2000), who separated the calves at 6 hours, 1 day or 4 days of age, theorised that this is a result of the calves being “hidlers” – i.e. the calves are used to being left alone for some periods of time in early life, and thus do not immediately react to being separated from dams. One of the earliest papers published on the subject describes behaviours of very young calves immediately following abrupt separation as follows:

“The general pattern shown by all calves separated at 96 h [age] was sniffing at the litter, walls and steel bars for about half an hour, whereafter they laid down and fell asleep. Some calves stood up and looked around before the observation period of 2 h was ended.” (Lidfors 1996)

However, some responses have still been reported immediately following abrupt separation also in young calves; Flower & Weary (2001) found that two-week-old calves had increased standing time when observed 0, 1 and 6 hours post-separation, compared to 1 hour pre-separation. Calves in the study were also found to place their heads outside the pen and move more than calves abruptly separated at 24 hours postpartum. These findings reflect those of Weary & Chua (2000), who demonstrated higher rates of activity, increased standing time and head placement outside the pen in calves separated at 4 days of age compared to calves separated at 6 hours or 24 hours postpartum. However, as described above, young calves up to at least four days show a delay after abrupt separation before their behavioural responses peak.

Apart from general increased activity, a number of studies report increased vocalisation post-separation; but, during the first hours post-separation, calves remain comparatively quiet (Lidfors 1996; Flower & Weary 2001; Fröberg *et al.* 2011). In Fröberg *et al.* (2011), vocalisation frequencies peaked as late as 24 hours post-separation and weaning. Similarly, Flower & Weary (2001) recorded very few calf calls until 18 hours had passed since abrupt cow-calf separation. It should be noted that calf vocalisation may also in part be due to a simultaneous weaning, as Thomas *et al.* (2001) found that calves deprived of milk or receiving limited allowances vocalised more than calves with high or *ad libitum* milk access. This will be discussed further in 2.4.

### 2.3.2 Growth check

Undisputedly, CCC calf weight gain during the suckling period exceeds that of calves reared artificially on limited milk allowances, particularly when unrestricted CCC is implemented (Johnsen *et al.* 2016). Several unrestricted CCC systems report ADGs of 1.2 kg during suckling periods of 4-8 weeks (Grøndahl *et al.* 2007; Johnsen *et al.* 2021), and even instances of growth rates up to 1.4 kg/d have been documented (Mac *et al.* 2023). This improved growth is often attributed to a higher milk consumption relative to milk allowances offered in artificial rearing (Flower & Weary 2001; Roth *et al.* 2009; Meagher *et al.* 2019).

A high weight gain in the young dairy calf may be considered advantageous for numerous reasons. High milk allowances – and consequent greater ADG – have been shown to reduce age of puberty onset in heifer calves (Shamay *et al.* 2005) and thereby theoretically allows for a lower age at first calving, improving cost-effectiveness for the farmer (Kalvportalen 2019). Bar-Peled *et al.* (1997) found evidence to support this theory, reporting that heifers raised on thrice daily restricted suckling showed a markedly improved weight gain and a significant decrease in age at first calving compared to control heifers raised on milk replacer. It has also been suggested that *ad libitum* whole-milk feeding of heifer calves can improve fat-corrected milk production during their first lactation (Shamay *et al.* 2005), but research on this topic remains limited.

As stated previously, one commonly observed issue in CCC systems is a sudden reduction of ADG post-weaning (Veissier *et al.* 1990; Roth *et al.* 2009; Fröberg *et al.* 2011). This phenomenon is not exclusive to CCC calves but is more prominent in dam-reared calves than in those reared artificially, as shown by Roth *et al.* (2009). In this study, CCC calves showed a median ADG of more than 1 kg/day during all three months preceding weaning and separation. During the month following weaning and separation, median ADG decreased below 0.5 kg/day. These authors also reported a significantly lower concentrate intake in CCC calves during the milk-feeding period. Since solid feed intake is essential for development of the forestomachs in the calf (Sjaastad *et al.* 2016), the study presumed that these calves were thus less equipped to utilize solid feed at weaning and therefore showed a more pronounced decrease in weight gain post-weaning compared to the artificially reared calves. These findings are consistent with results of a similar study performed by Fröberg *et al.* (2011), who found that concentrate intake and ADG in CCC calves were affected for at least two weeks post-weaning. Before abrupt weaning and separation, CCC calves had an ADG of 1.434 kg/day – in the two weeks following weaning and separation, the ADG averaged only 0.033 kg/day. Corresponding values for calves artificially raised on high milk allowances were 0.906 and 1.120, respectively. As this growth check is undesirable, strategies

involving more of a gradual weaning and separation process have been suggested as a way to increase the intake of solid feed pre-separation.

## 2.4 Methods of decreasing stress at separation

The following section addresses methods of decreasing stress at separation and their effects on cow and calf behaviour and performance. In contrast to other parts of this review with the exception of section 1.1., this part also includes studies using beef cattle breeds, as most research in this subject has been carried out on beef rather than on dairy breeds.

The two main alternative weaning methods discussed in this section consist of introducing an intermediate step before the final separation of cow and calf. Calves are either physically separated but maintain visual and auditory contact with their dams— so-called fenceline separation (Price *et al.* 2003) – or are kept with the dams but are prohibited from suckling through the fitting of a nose flap, often referred to as two-step weaning (Haley *et al.* 2005). After varying periods of this restricted contact, cows and calves are then completely separated. Commonly, these practices have been researched in beef cattle where the calves only consume milk from their dams – thus, calves are weaned at the same time as the fenceline or nose flaps are introduced.

### 2.4.1 Fenceline separation

Fenceline-separated beef calves have been found to have a higher ADG post-separation compared to abruptly separated calves and may maintain this advantage for up to 10 weeks after separation (Price *et al.* 2003), even though there might still be a decrease in ADG following complete separation from the dam (Enríquez *et al.* 2010). When compared to abruptly separated calves, beef calves vocalise less post-separation, both when still in fenceline contact with the dam (Price *et al.* 2003) and when later separated completely from the dam (Enríquez *et al.* 2010).

In the first study on fenceline separation in dairy cattle, calves were similarly described as having a relatively low vocal response at separation (Johnsen *et al.* 2015b). More specifically, calves showed very low rates of high-pitched vocalisations compared to calves separated from their dams by a solid wall. High-frequency calls can be considered contact-seeking calls, observed for instance when cow and calf are separated and looking to reunite to nurse or suckle (Padilla de la Torre *et al.* 2015). Johnsen *et al.* (2015b) also reported on dam responses, finding that behavioural responses of dams at fenceline separation resembled those of dams separated from calves by a solid wall. Both groups displayed increases in reinstate-

ment behaviours such as high-pitched vocalisation, alertness and reaching heads outside the pen post-separation.

### 2.4.2 Two-step weaning

Two-step weaning through nose flaps is somewhat more ambiguous than fenceline separation. Beef calves weaned in two steps utilising nose flaps have been found to vocalise and walk less post-separation, but spend more time lying down and eating than control calves who have been abruptly separated and weaned (Haley *et al.* 2005). Dams have also been found to vocalise less following physical separation if their calves had been fitted with nose flaps before separation, although no record was made of their initial response to their calves wearing nose flaps (Lambertz *et al.* 2015). In contrast to fenceline weaning, nose flaps have been found to be either of no significance to (Lambertz *et al.* 2015), or detrimental to calf weight gain in beef cattle; the latter has been proven both during the nose flap wearing period (Haley *et al.* 2005; Enríquez *et al.* 2010), post-separation (Enríquez *et al.* 2010) and in dairy calves (Wenker *et al.* 2022a). Additionally, a study equipping approximately 8-month old beef calves with nose flaps found that 90% of animals had developed nasal abrasions after seven days of wearing them, and in 45% of animals the irritation persisted at least a week after removal of the flaps (Lambertz *et al.* 2015). Thus, while the use of nose flaps reduces some of the unwanted responses following separation, this practice may negatively affect the welfare of the calves.

### 2.4.3 Postponed weaning

Temporal division of separation and weaning can also be achieved by continuing to artificially feed calves milk after they no longer have contact with the dam. In recent years, a few studies on CCC systems have used automatic milk feeders (AMF) to continue providing milk post-separation (Johnsen *et al.* 2015a; b; 2018). Results indicate that CCC calves that have learnt to drink milk from an AMF may have improved weight gains (Johnsen *et al.* 2015a) and produce fewer high-pitched vocalisations post-separation (Johnsen *et al.* 2015b, 2018) than peers not using the AMF, suggesting a reduction in perceived stress at physical separation when weaning and separation do not coincide.

## 3. Materials and methods

This study was performed at the Swedish Livestock Research Centre (Lövsta) in Uppsala as part of the larger research project “Cow and calf together”, approved by a regional ethics committee (ID: 5.8.18-18138/2019).

### 3.1 Animals

Initially, the group of animals selected for the larger project included 11 dairy cow-calf pairs. One calf had to be euthanised at 5 d of age due to disease; as a result, the dam was also excluded from this study before weaning commenced. Another cow was diagnosed with *E. coli* mastitis mid-study, severely affecting milk yields and possibly also affecting calf milk intake. Milk yield and weight gain data concerning this pair was therefore excluded from data analyses. The cow was still included in analyses concerning lying times, as she was not showing signs of mastitis until two weeks had passed since the last date of lying time observations. The 9 cow-calf pairs included in milk yield and ADG measurements consisted of two breeds (Swedish Red SR, n=3 and Swedish Holstein SH, n=6); cows were of varying parities (1-4, median 2). The additional individual included in lying time measurements was a third-parity SR cow, bringing the total of cows assessed with regards to daily lying times to 10. The included calves spanned an age range of 55 days. Due to other research purposes within the project utilising the same animals (see 3.3), all calves were female.

### 3.2 Husbandry

The experimental pen consisted of a feed alley, a freestall area, a calf creep and an AMS (DeLaval VMS™ V300, DeLaval International, Tumba, Sweden) as detailed in figure 1. Cow traffic was free between all areas except for the calf creep, which was inaccessible to cows. Calves had access to all areas excluding the AMS until the implementation of restricted contact, when they were confined to the calf creep during separation periods (see 3.3 for details). Upon entering the AMS, cows were milked if at least six hours had passed since their last milking. If more than 12 hours

passed since a cow had last been milked, the milking robot alerted barn staff and the cow was manually fetched to be milked.

The freestall area consisted of a central alley lined by two rows of stalls, one facing the calf creep and one facing the feed alley. Six out of 11 calf creep-oriented stalls faced metal gates, which formed part of the calf creep wall; the remaining 5 stalls faced a solid partition between the freestall area and the calf creep. The opposite row facing the feed alley contained 12 stalls, of which two were partitioned from the feed alley by a solid barrier. Thus, the total number of stalls amounted to 23, resulting in a stocking density of <50% during time periods when the calves were kept in the calf creep. Freestalls were floored with rubber matting and a combined layer of wood shavings and peat, which was topped up 8-10 times daily by an automatic dispenser (JH miniStrø COW, MAFA i Ängelholm AB, Ängelholm, Sweden). Floors in the system were rubber. Automatic manure scrapers located in the feed and central alleys functioned continuously throughout the day.

The feed alley allowed access to a 2.5-metre water trough, 9 feed stations with feed bins (CRFI, BioControl AS, Rakkestad, Norway) and a mechanic swinging cow brush (DeLaval SCB, DeLaval International, Tumba, Sweden). The feed stations provided cows with a partial mixed ratio of grass-clover silage, concentrate (8-10 kg/cow) and straw (4% inclusion). Additional concentrate was available in the milking robot, which dispensed 0.5-1 kg of concentrate per passing up to a maximum amount of 2 kg/cow/day.

The calf creep measured approximately 48.5 m<sup>2</sup> and was bedded with a deep layer of wood shavings throughout. It also contained an approximately 4.7 m<sup>2</sup> area where calves could be temporarily contained for handling procedures. This area was also where the calves were fed and weighed. In the creep, calves had *ad libitum* access to silage, roughage and minerals. Calf feeders (DeLaval concentrate station calves, DeLaval International AB, Tumba, Sweden) provided access to controlled amounts of concentrate. Water was readily available through a push-paddle waterer or a water-filled teat bucket, as the water trough in the feed alley was deemed too high for calves to access initially. The calf creep was manually supplied with wood shavings and personnel were instructed to do this often enough to ensure a dry bed at all times.



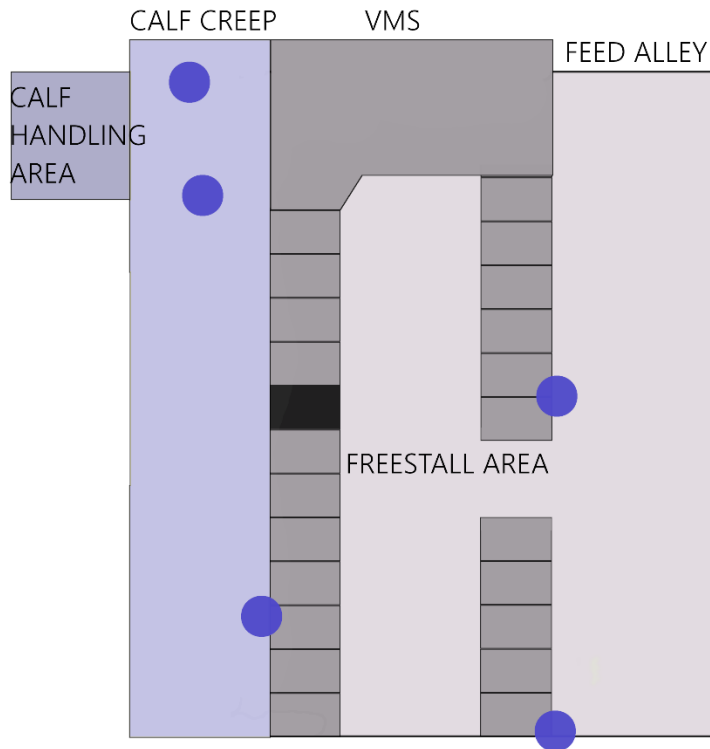


Figure 1. Schematic picture of the experimental pen. Camera locations are marked with blue dots. Picture based on original work by Claire Wegner, [Ladugården / Externwebben \(slu.se\)](https://www.ladugarden.se/externwebben)

### 3.3 Study design

#### 3.3.1 Study framework

Cows calved in individual calving pens and were moved to the AMS system 3-10 (median 4) days after calving. Cow-calf pairs were enrolled into the study during a recruitment period of 9 weeks prior to commencing the study, leading to different pairs having differing amounts (3-12 weeks) of unrestricted contact depending on when the calf was born. The start of the actual study was marked by the last cow-calf pair entering the pen and spanned a total of 14 weeks between March and July 2023. In-study, the 24-hour contact period lasted for 3 weeks. Then, the 12-hour contact period was initiated; calf age at this point averaged  $54 \pm 21$  (25-80) days. During this period, calves were restricted to the calf creep between approximately 19:00 in the evenings and 07:00 in the mornings. This period lasted for 6.5 weeks, after which a 0-hour contact period was initiated. At this point in time, calves

averaged  $100 \pm 21$  (71-126) days of age. Calves were kept in the calf creep adjacent to their dams for an additional 4.5 weeks but were unable to suckle their dams or be in physical contact with them. After this, the study was terminated and cows and calves alike were removed from the experimental pen.

### 3.3.2 Calf confinement

During the study, when allowed access to the whole pen, calves could pass between the calf creep and the cow pen through an opening in the creep measuring approximately 3 metres in width. This opening was then blocked by movable metal gates during the periods of confinement to the calf creep, thus still allowing visual, olfactory and auditory contact between cow and calf. Physical contact was limited due to the placement of the freestall neck rails. Visual and olfactory contact was unlimited regardless of whether calves were confined or not, but the neck rails did not allow cows to touch or lick the calves and vice versa if the calf creep opening was blocked. Cows were at no point able to access the calf-creep – thus, the CCC system could be described as calf-driven (as defined by Sirovnik *et al.* 2020), meaning CCC was primarily regulated by the behaviour of the calves.

### 3.3.3 Milk feeding procedures

During the 12-hour contact period, calves were confined to the aforementioned smaller area adjacent to the calf creep for milk feeding prior to release in the mornings. Calves were confined to this area in two groups of 4-5 individuals and kept in the area for approximately 10 minutes, during which they were offered *ad libitum* tempered, whole milk in teat buckets. Calves were introduced to the teat buckets during 3-4 training occasions held before initiation of 12-h contact; these consisted of filling the buckets with electrolyte water or milk and gently directing the calf to a teat whilst receiving positive attention from the handler. As the training progressed, older calves were only shown the milk in buckets and not directed to the teat. After the 12-hour contact period had commenced, the date at which each calf started to independently consume milk from a teat bucket was registered.

After initiation of 0-hour contact, calves were offered *ad libitum* milk through the same procedure, but instead twice daily at approximately 06:00-07:00 and 18:00-19:00 for a week. After this, weaning began; milk was offered once daily and the daily milk allowance was limited to six litres per calf. This lasted for four days, after which milk allowances were further reduced by two litres every four days until calves were completely weaned off milk. One calf never learned to drink from a bucket and also became agitated during confinement for group feeding during the 12-hour contact period; she was therefore no longer included in group feedings for the remainder of the study due to concerns for calf and personnel safety.

## 3.4 Data collection

### 3.4.1 Lying times

The experimental pen was supplied with a total of 6 fisheye cameras (Samsung SNF-8010VM, Samsung Techwin Co., Ltd., Seoul, South Korea) continuously recording the pen. Cameras were placed to offer a bird's-eye view perspective (see figure 1 for camera placements in the pen) of different parts of the pen. This video material was then retrospectively observed using the program BackUp Viewer (v2 1.4.6\_M190708, Hanwha Techwin Co. Ltd., Seongnam-si, South Korea). Lying time for cows was estimated through scan sampling at 10-minute intervals (Martin & Bateson 2007), in accordance with earlier validation of sampling of cattle behaviour (Mitlöhner *et al.* 2001), and ten minutes of lying time was logged for each scan on which an individual cow was found to be lying down. Observations spanned 7 days prior to and 7 days following initiation of the 12-hour contact period. Initially, the study aimed to do the same for the 7 days prior to and 7 days following 0-hour initiation, but due to equipment malfunctions, no video material from this period was available. Hence, this aim had to be abandoned and instead lying time data collection was focussed on the 24-to-12-hour transition period. Cows were identified using spray-painted symbols on the back and sides and logged as either lying down or standing up during each scan. In rare cases where the symbols had rubbed off, a collection of pictures was used to aid in identifying cows. If a cow was found to be in the process of lying down or getting up during a scan, the result of the previous scan was checked and the behaviour was logged according to the prior state of the cow, as that behaviour was presumed to have continued until the scan in question. Scans were performed by a single observer. To assess intra-observer reliability, a randomly selected 24-hour period was scored twice; once at the beginning and once the end of the data collection period. The Pearson correlation coefficient acquired for these two periods concerning the proportion of scans lying was  $r(1438) = 1, p = 0$ , indicating a high intra-observer reliability.

### 3.4.2 Calf weight gain

Calf weights were gathered on a total of 15 different occasions using a portable calf scale (PM200, Marechalle Pesage, Chauny, France). Weighing generally occurred once weekly during the 15 weeks of the study, with the exception of study week 9, when no weights were recorded, and study week 2, when weights were recorded twice on two successive days.

### 3.4.3 Milk data

Milk yield data was recorded automatically by the AMS on quarter level and logged in a spreadsheet for further processing. Data was then converted to daily total milk

yield for each individual cow. One cow was removed from the VMS system due to lameness three weeks into the 0-hour contact period. Thus, no milk data was available for this individual past the aforementioned date. Milk yield data for the remaining 8 cows spanned the entire study period of 2023-03-27 to 2023-07-03.

#### 3.4.4 Stall use

An initial prospect of the study was to investigate prevalence of so-called “anticipatory behaviour” in the dams during the 12-hour period, centred around calf release from the calf creep in the mornings. This was based on anecdotal observations made by research staff that were milk feeding the calves, of dams seemingly expecting the calf release in the mornings and actively seeking out the area close to the calf creep prior to the time of release. To further explore this and determine a suitable time period for analysis, two days were studied continuously between 00:00 and 12:00 – the last day of the 24-hour period and another day one week after initiation of the 12-hour period. The 24-hour day was used as baseline to determine if 12-hour observations differed substantially during any given time period. No difference in cow behaviour was apparent in the minutes preceding release; however, the patterns of cow use of the calf creep-adjacent stalls seemed to change somewhat in the period between the two studied days when taking the whole observation period of 12 hours into account. Therefore, the week before and the week after initiation of 12-hour contact was instead studied through scan sampling at 10-minute intervals between 00:00 and 12:00 to describe this possible variation and relabelled as stall use rather than anticipatory behaviour. A calf creep-adjacent stall was logged as in use provided at least one cow hoof was inside the stall as opposed to in the central alleyway of the system. During each scan, the proportion of stalls in use was recorded.

### 3.5 Data analysis

Data analysis was performed using either Minitab® (v 21.3.1.0., Minitab, LLC) or Microsoft Excel (v. 2310, Microsoft Corporation). Calf weight gain was the only outcome for which any statistical inference was included, as *a priori* hypotheses were absent for the other studied factors.

#### 3.5.1 Lying times

Lying time observations were summarised for each individual cow and day, assigning each cow 10 minutes of lying time for each scan on which she was observed to be lying down. To evaluate possible individual variations in daily lying patterns or responses to initiation of 12-hour contact, a weekly average of lying

time was calculated for each observational week (week 1: 24-hour contact, week 2: 12-hour contact) per dam. In order to illustrate temporal changes in lying times during the observation period, daily means for the whole cow group were likewise calculated for each of the two observational weeks and also for each observation day.

### 3.5.2 Calf weight gain

Calf ADG was calculated for the full 24-hour, 12-hour and 0-hour contact periods from available body weight data. The resulting values were assessed for normality through the Anderson-Darling test function in Minitab and found to be normally distributed. To discern whether there were differences in mean ADG between the 24- and 12-hour, the 24- and 0-hour period or the 12- and 0-hour contact periods, paired t-tests were used. The significance level was established at  $p < 0.05$  and the null hypothesis stated that there were no discernible differences in mean ADG between the groups. To correct for the number of statistical tests performed using the same data, the Bonferroni correction was used, settling the final significance level at  $p < 0.017$ .

### 3.5.3 Milk data

Milk data was developed similarly to lying time data to allow for assessment of individual variations and responses during the three contact periods. For example, mean daily milk yields for each contact period were calculated on both group and individual level. Mean number of daily milking events and mean number of daily milking events for which the robot recorded lower than expected milk yield for at least one teat (noted as incomplete milking) were acquired on individual and group level for each contact period (24-, 12- or 0-hour). Additionally, number of incomplete milkings were summarised per contact period for each individual cow and averaged per day. Finally, to visualise any possible effects on milk yields obtained in the robot depending on contact period (24-, 12 or 0-hour), milk yield curves over the full study period were assembled per dam using seven-day rolling averages of the available milk yield data. To further evaluate if additional milk feeding of the calves affected the robot milk yield, the date when calves started to consume milk from an external milk source was superimposed on the milk yield curves of their dams.

### 3.5.4 Stall use

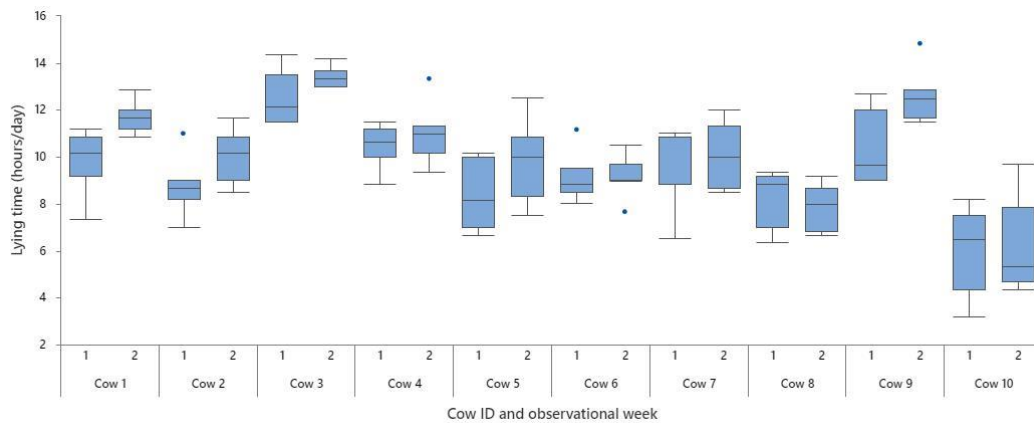
The proportion of stalls in use was summarised for each scan time point and week and averaged to achieve one mean percentage of stalls in use per time point for week 1 and week 2, respectively. These means were then used to create two temporal curves of mean percentage of stalls in use during the different time points

of the two weeks studied to assess any differences in stall use with regards to the time of day. The total average of calf creep-adjacent stalls in use during the studied periods was also acquired for each of the two weeks.

## 4. Results

### 4.1 Lying time

During week 1 of lying time observations, cows averaged  $9.2 \pm 2.03$  hours of lying time per day. Individual variations are presented in figure 2. Lying times during week 2 averaged somewhat higher at  $10.2 \pm 2.33$  hours daily lying time. The minimum reported daily lying time was 3.17 hours and occurred during week 1, whilst the maximum reported daily lying time was 14.83 hours and appeared during week 2. The highest mean lying time of any individual day – 10.58 hours - occurred during the day following initiation of 12-hour contact. All but two cows numerically increased in mean lying time during week 2.



*Figure 2. Individual lying times (hours/day) of cows during week 1 (last week of 24-hour CCC) and week 2 (first week of 12-hour CCC) of lying time observations.*

## 4.2 Calf weight gain

### 4.2.1 Descriptive results

During the period of 24-hour contact, calves averaged at an ADG of  $1.17 \pm 0.239$  kg/day, with the highest ADG measured at 1.57 kg/day, and the lowest at 0.79 kg/day.

During the 12-hour contact period, mean ADG was  $1.19 \pm 0.190$  kg/day; maximum and minimum ADG recorded was 1.48 kg/day and 0.85 kg/day, respectively. Five calves numerically increased in ADG during the 12-hour period compared to the 24-hour period. The remaining four calves decreased in ADG. As a result, the average difference in ADG between the 24-hour and the 12-hour contact periods was  $0.0 \pm 0.22$  kg/day.

After transitioning from the 12-hour to the 0-hour contact period, calves on average gained  $0.89 \pm 0.141$  kg/day. This amounted to a mean reduction of  $0.3 \pm 0.06$  kg weight gained per day. Individual calf ADG varied between 0.70-1.15 kg/day during the 0-hour period. One calf maintained the same ADG as during the 12-hour period; all other calves decreased in ADG. The greatest observed decrease amounted to 0.54 kg less weight gained per day when compared to the ADG of the 12-hour period. At no point in time did a calf decrease in weight from one weighing to the next, regardless of contact period. Individual calf ADG for each period can be found in figure 3.

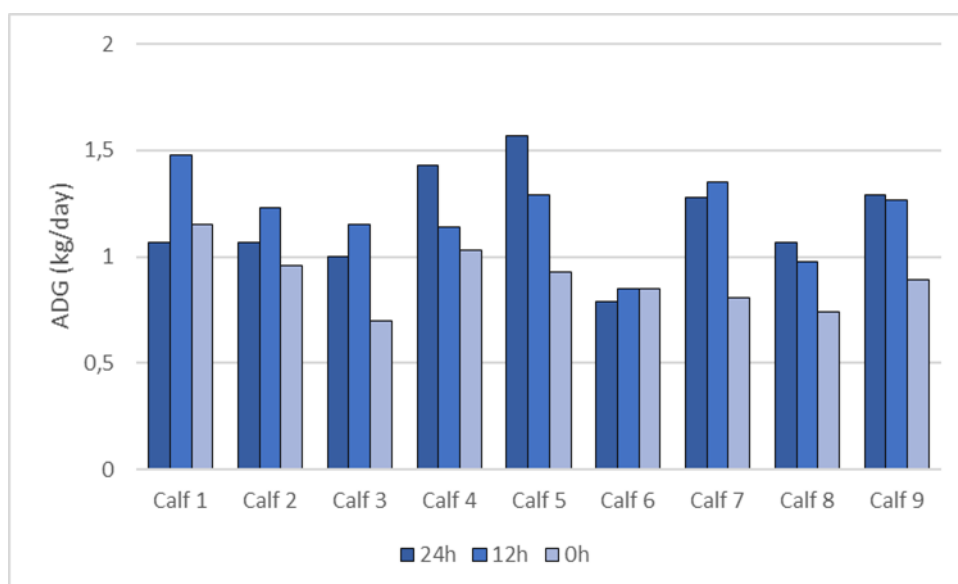


Figure 3. Individual calf ADG (kg/day) averaged across 24-, 12- and 0-hour CCC periods.



## 4.2.2 Hypothesis testing

No statistical significance was found concerning difference in calf ADG during the 24-hour contact versus the 12-hour contact period ( $t(8) = -0.26, p = 0.805$ ). There was a statistically significant difference in ADG between 12-hour and 0-hour contact periods ( $t(8) = 5.37, p = 0.001$ ), as well as between 24-hour and 0-hour contact periods ( $t(8) = 3.45, p = 0.009$ ), meaning that calves gained significantly less weight during this period than during either the 24-hour or the 12-hour period.

## 4.3 Milk data

### 4.3.1 Milk yield

On average, the whole cow group yielded  $30.0 \pm 10.87$  kg of milk/day to the robot during the entire study period. Yields were numerically highest ( $36.1 \pm 8.50$  kg/day) during the 0-hour contact period and lowest ( $24.6 \pm 9.83$  kg/day) during the 24-hour contact period. During the 12-hour contact period, the daily yield averaged  $28.7 \pm 10.88$  kg. Minimum individual daily yields for each contact period (24-h, 12-h, 0-h) were 6.16, 6.62 and 11.83 kg/day. Corresponding maximum individual daily yields amounted to 55.14, 59.53 and 57.54 kg/day. Average individual milk yields during each period are presented in figure 4 below, while figure 5 shows individual cow milk yield curves for the entire study period.

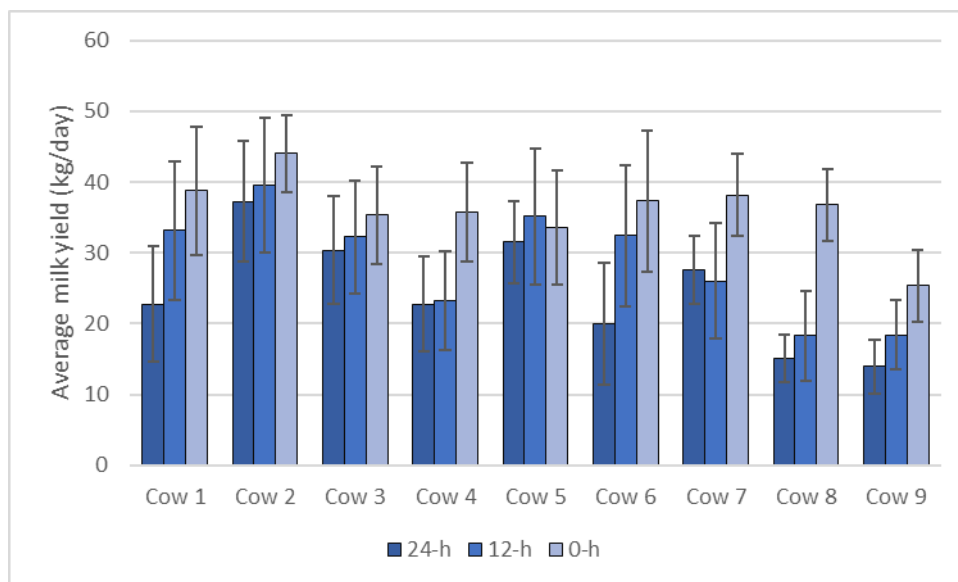


Figure 4. Individual cow milk yields (kg/day) averaged across 24-hour, 12-hour and 0-hour CCC periods. Error bars represent the standard deviation.

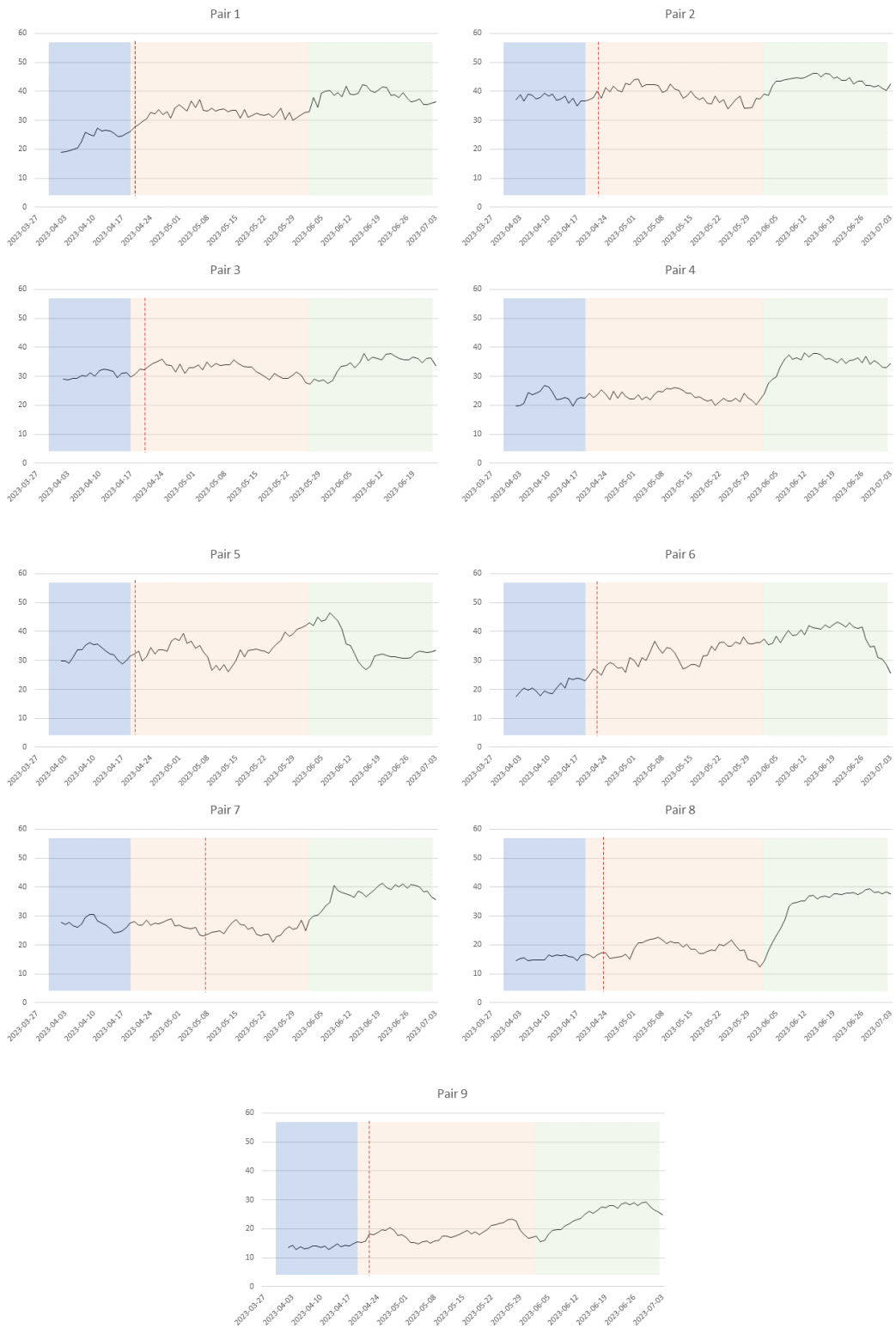


Figure 5. Individual cow daily milk yields (kg/day) throughout the study period, represented by 7-day running averages. The date on which each dam's calf was first observed drinking additional milk from a teat bucket is marked by a vertical line. Each CCC period is identified by a different colour (blue = 24-hour contact, orange = 12-hour contact, green = 0-hour contact).

### 4.3.2 Additional milk data

#### *Number of milkings*

The number of milkings for an individual cow varied between 1 and 4 milkings per day during the 24-hour and 12-hour contact periods, and between 1 and 5 during the 0-hour contact period. The mean number of milkings per day was highest during the 0-hour contact period, averaging at  $2.7 \pm 0.71$  milkings/day. The 24-hour contact period averaged  $2.5 \pm 0.77$  milkings/day, and the 12-hour contact period  $2.3 \pm 0.71$  milkings/day.

#### *Incomplete milking*

Occurrences of milking events for which at least one teat had lower than expected robot milk yield decreased numerically with each consecutive contact period for most dams. During 24-hour contact, the mean number of incomplete milkings per cow and day was  $0.7 \pm 0.75$ . During 12-hour contact, this declined to  $0.4 \pm 0.68$  incomplete milkings/cow/day and finally, during 0-hour contact, the corresponding value was  $0.1 \pm 0.47$  incomplete milkings/cow/day. Individual means for each contact period are displayed in figure 6. During the whole study period, individual cows varied between 0-3 incomplete milkings on a given day.

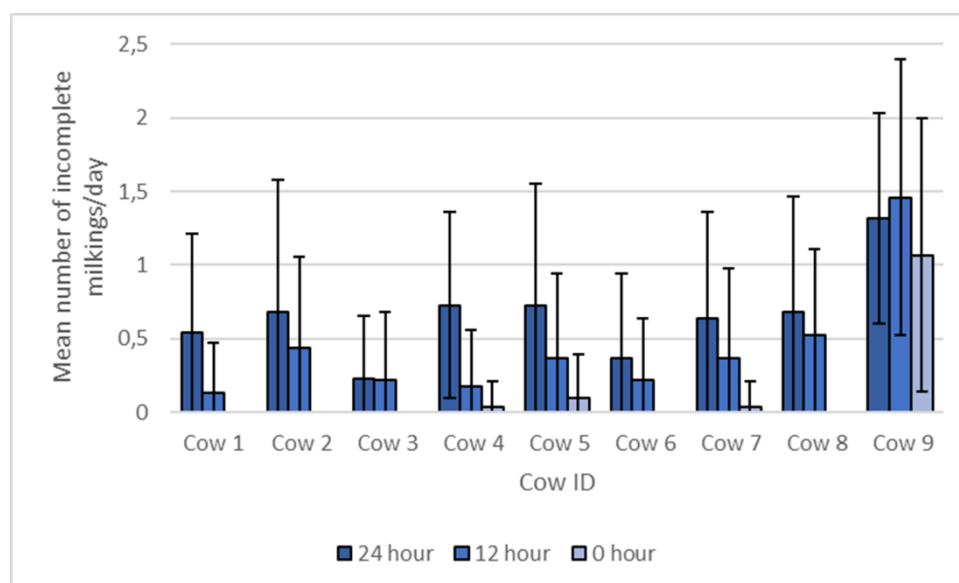


Figure 6. Individual cow occurrences of incomplete milkings averaged per day across each CCC period. Error bars represent the standard deviation.

### 4.4 Stall use

During the last week of 24-hour contact, the mean proportion of calf creep-adjacent stalls in use during any given time studied amounted to  $31.8 \pm 6.36\%$ . Correspon-

ding values during the first week of 12-hour contact were  $32.6 \pm 9.39\%$ . The mean percentage of calf creep-adjacent stalls in use during different time points during the day are presented in figure 7 below.

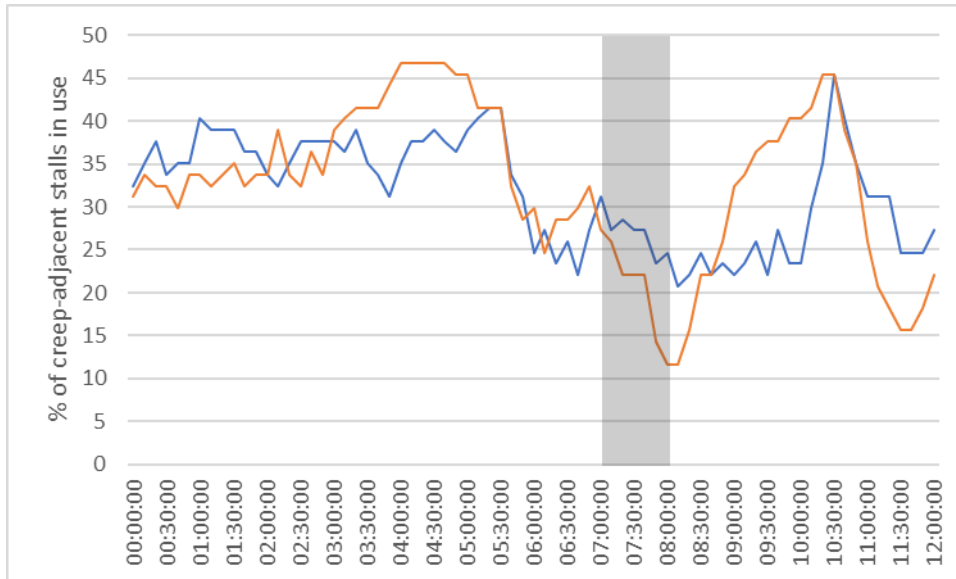


Figure 7. Average percentage of calf creep-adjacent stalls (a row of stalls facing a calf creep) in use by cows ( $n=10$ ). The blue line represents the last week of 24-hour contact; the orange line the first week of 12-hour contact, where calves were enclosed in a separate calf creep during night hours. The approximate release time of calves from the calf creep is marked by a grey bar.

## 5. Discussion

The main aims of this study were to discern whether cow daily lying times in a CCC system with gradual separation were affected by transitioning from 24-hour to 12-hour contact, and whether feeding calves additional milk mitigated decreases in calf weight gain often seen after physical separation from the dam. Additionally, the study recorded cow milk yields during the entire study period, from 24-hour to 0-hour contact. Finally, the study investigated cows' use of the stalls adjacent to the calf confinement area in the mornings before and after initiating 12-hour CCC.

The aims of this study were largely explorative, and this study should be considered a pilot, as the outcomes have rarely been evaluated in earlier comparable studies. One *a priori*-hypothesis was formed regarding calf weight gain as this has been thoroughly investigated in several previous studies. The results provide initial indications that gradual reduction of daily contact time may somewhat mitigate the negative impacts of abrupt separation whilst maintaining a high calf growth rate across a variety of calf ages. Temporally dividing separation and weaning and allowing calf access to milk post-separation may serve to alleviate the decrease in weight gain often seen at separation, although further research using more controlled trials is needed to solidify this theory. Cow lying time did not substantially decrease following initiation of 12-hour contact, indicating that this may be a promising strategy to decrease severe separation distress in dams. Similar studies need to be performed on cow lying times when transitioning from 12-hour to 0-hour contact, as this study was unable to evaluate this aspect.

### 5.1 Effects of CCC on the cow

#### 5.1.1 Lying times & stall use

Individual cow lying times showed a large individual variation, where daily measures varied between roughly 3-15 hours. Some possible explanations for this can be found in the literature. Daily lying times have been found to be positively associated with higher cow parities and number of days in milk (DIM) in Holstein cows (Vasseur *et al.* 2012; Solano *et al.* 2016), the latter association being

especially evident in first-lactation heifers (Solano *et al.* 2016). In contrast, a high milk yield may instead negatively influence daily lying times, as shown by Solano *et al.* (2016) and Fregonesi & Leaver (2002). A lower frequency of udder emptying may also contribute to decreased lying times (Österman & Redbo 2001). Cows included in this study were not balanced with regards to any of the aforementioned aspects. It is therefore natural that they should express some individual variation, seeing as they were of varying parities and expressed a total spread of 55 days in DIM. Theoretically and most likely, the cows might also have differed in total milk production, although this cannot be said for certain due to unknown amounts of milk being consumed by calves in the system. How often cows were nursed or milked likely also varied between cows and affected the frequency of udder emptying.

The initiation of 12-hour contact did not appear to decrease cow lying time. Rather, the numerically highest mean group daily lying time befell the day after 12-hour contact was initiated. With this said, log book reports from the study report a general unrest among the cows during at least the first morning of the 12-hour period. It could therefore also be argued that the high lying times observed on this day were actually compensation for an increased activity level during the preceding night and morning. Most individual cows increased their lying time during week 2; the mean group daily lying time during 12-hour contact was 1 hour greater than during 24-hour contact, a fact which counters the aforementioned argument as the cows settled into the new routine after only a day or two according to log book notes. As no statistical inference was applied to this data, its importance should not be overstated. However, Johnsen *et al.* (2021) have published findings of a somewhat similar nature, describing a numerical increase in lying time for cows when separated from their calves through gradually reducing daily contact over a period of six days. Another study that has been conducted on lying times in CCC systems reported no significant difference in lying times between cows in a 24-hour contact CCC system and control cows without calf contact (Wegner & Ternman 2023). Regardless, as the lying time was not reduced even the first day after 12-hour contact was initiated, there is little support in the current study for behavioural responses indicating strong stress in the dams at this time point. It would have been interesting to evaluate dam behavioural responses at the initiation of the 0-hour period; this should be studied further after the video data has been secured to evaluate the effects of complete physical separation in a gradual separation system.

As demonstrated by Munksgaard *et al.* (2005), lying is a highly prioritised cow behaviour and therefore may not be best suited to judge stress responses in cows as a singular variable. Nevertheless, decreased lying times as a short-lived behavioural response has been described in multiple studies on abrupt cow-calf separation (Lidfors 1996; Flower & Weary 2001; Stěhulová *et al.* 2008). Future research might

take into account further behavioural responses, such as dam vocalisation – something not possible in this study due to the absence of audio recordings - or general activity level prior to and following contact period changes to obtain multiple indicators for stress-related responses.

The percentage of stalls closest to the calf creep in use by cows visually differed somewhat between week 1 (last week of 24-hour contact) and week 2 (first week of 12-hour contact), but due to time constraints, only the first 12 hours of each day could be surveyed and included in the study. Further research is needed to disprove or confirm any changes in cow stall use behaviour when transitioning from a 24- to a 12-hour CCC system.

### 5.1.2 Milk yield

Cows in this study generally provided the highest machine milk yields during 0-hour contact. Twelve-hour CCC boasted slightly increased milk yields when compared visually to 24-hour CCC, although the difference was small between these two periods. Notably, two cows showed mean increases of up to 18.5 kg/d between 12- and 0-hour contact periods. This is well above what an average calf might be expected to consume in a day based on values from restricted suckling systems (de Passillé *et al.* 2008; Fröberg *et al.* 2008) as well as earlier estimates of milk yield losses in 24-hour CCC systems with automatic milking (Churakov *et al.* 2023). This might be explained by a couple of factors. These cows may have been more or less routinely suckled by alien calves, leading to larger amounts of milk lost in some individuals cross-suckled by several calves than other cows suckling only one calf. Increases may also have been due to a more complete milk letdown at machine milking, aided in turn by either a better degree of udder filling shortening time to milk letdown (Bruckmaier & Hilger 2001) or improved oxytocin levels enabling a more complete udder emptying (Bruckmaier *et al.* 1994). A combination of these effects may be most plausible, but as no record of either residual milk post-milking or cross-suckling was made in this study, which effects were actually present cannot be ascertained.

Another fact to take into account is that earlier research has proven that even when only allowed restricted suckling, calves are still able to consume large amounts of milk in short time spans; when allowed to suckle only twice daily, calves are still able to consume around 10 - 12.5 L of milk daily (de Passillé *et al.* 2008; Fröberg *et al.* 2008). Ergo, the 12-hour contact period in itself should theoretically not cause an increase in milk yields purely as a result of calves consuming less milk. Rather, any effect seen, however small, might be attributed to the additional milk source provided to calves at approximately the same time point as the initiation of the 12-hour contact period. A way to validate this in further research might be to employ

a group of control dams with calves which would not be provided additional milk and compare milk yield changes between the groups when transitioning from 24- to 12-hour contact.

Occurrences of incomplete milkings appeared visually quite similar between 24- and 12-hour periods for most cows, although the degree to which occurrences decreased varied to some extent between cows. In nearly all individuals, incomplete milkings dropped to near-zero levels during the 0-hour contact period, with the only exception being a primiparous heifer maintaining high levels of incomplete milking occurrences throughout the study. Impaired milk ejection in heifers due to stress-induced oxytocin inhibition is not uncommon, but usually occurs only in early lactation and then rapidly decreases as the heifer becomes accustomed to milking (Van Reenen *et al.* 2002). This was not the case for the primiparous cow in this study, as she remained consistently high in mean occurrences of incomplete milkings throughout the study period. Reasons for incomplete milking occurring may be several. Essentially, the robot logs this if one or more teats yield a lesser amount of milk than expected. This might be due to factors such as impaired milk ejection, the cow kicking off a teat cup mid-milking, or a low degree of udder quarter filling, for example due to a cow having been recently nursed by one or several calves before milking. Therefore, without investigating reasons for every occurrence logged, no conclusions can really be drawn from available data in this study, but might be of interest to explore further.

Lastly, the variation in milk yields and response to differing periods of CCC raises the question of whether there are cow-level factors such as parity, breed, or DIM which may significantly affect these outcomes. Post peak lactation, a programmed cell death starts to occur in the udder, gradually lessening milk yields (Sjaastad *et al.* 2016). Also, as mentioned previously, some evidence suggests that residual milk might hamper milk production (Sjaastad *et al.* 2016; Albaaj *et al.* 2018). As a result, cows with large amounts of residual milk in the udder may struggle with improving milk yields in CCC systems if separated after peak lactation. Identifying if some cows might be more suited for CCC systems than others would be valuable information for a farmer looking to build an economically sustainable CCC system, but to this author's knowledge, this is as of yet a largely unexplored subject. The question also remains at which age calves should be either weaned or transitioned to artificial milk feeding to allow for optimal increases in cow milk yields post-separation.



## 5.2 Effects of CCC on the calf

### 5.2.1 Average daily weight gain

During 12-hour contact, the difference in weight gain from the 24-hour CCC period was not significant. Despite providing an additional milk source and implementing a gradual separation procedure, calves decreased significantly in ADG during 0-hour contact when compared to earlier contact periods. With this said, only two calves maintained an ADG of less than 750 g/day post-separation, which is exactly the goal value set by the Swedish organisation Växa for optimising age at first calving (Växa 2022), although somewhat blunt as it averages a long and varying growth period (3-13 months). Furthermore, the mean post-separation ADG value of 0.9 kg/day in this study succeeds that of many earlier reported growth rates. Fröberg *et al.* (2011) studied calves weaned and separated simultaneously after 8 weeks unrestricted CCC, and recorded a mean ADG of only 0.033 kg/day in the two weeks following separation. Other research reports post-separation ADG values more closely corresponding to those found in this study. Veissier *et al.* (2013) reported a mean ADG of 0.8 kg/day when calves were abruptly weaned and separated at 10 weeks of age. It should be noted that calves in the current study were physically separated from dams at a later age than in the aforementioned studies, varying between 10-18 weeks of age, and weaned even later, at 13-21 weeks of age, as this may have affected the baseline growth rates of the included calves.

As briefly touched upon in the literature review, some initial research has been conducted into providing external milk sources for CCC calves post-separation. Johnsen *et al.* (2015a) studied calves in a CCC system involving an initial contact of 6 weeks, then a four-day fenceline separation period before total separation. Some calves, termed “nursing calves”, were given access to an automatic milk feeder (AMF) at total separation from the dam and never introduced to its use. Other, so-called “combined calves” had AMF access throughout the study period and also received gentle training to use the AMF similar to the method used in this study. They found that very few calves of the nursing group ever used the AMF - only three individuals - whereas nine out of the 10 combined calves did. Thus, a short training period when introducing an external milk source may improve the odds of calves in fact also using it. Another possibility is that the age or contact type at which calves are introduced to the milk source affects the likelihood that the calf utilises the milk source. Furthermore, whilst our study employed no control group and could therefore not statistically confirm that the external milk source had an effect on calf weight gain, Johnsen *et al.* (2015a) found that calves using the AMF post-separation gained more weight than calves that did not.

### 5.3 Conclusion

This study concludes that transitioning from whole-day to half-day CCC may mitigate some unfavourable effects commonly observed at abrupt separation, such as decreases in calf growth rates and short-term decreases in cow daily lying times. The study invites further research into gradual separation practices in CCC systems, as well as into introducing calves to an external milk source pre-separation to dampen the effects of later separation on calf ADG and behavioural response. The small sample size of both cows and calves urges caution when interpreting all results obtained in this study. Nonetheless, they may provide a sound foundation for further research on the subject.

Cows numerically increased lying times in response to the change in daily CCC duration. Calf growth rates were maintained during 12-hour CCC. The rates decreased after being physically separated from the dam, but were still high overall at 0.9 kg/day. Additionally, mean milk yields were lowest during 24-hour contact, intermediate during 12-hour contact and highest during 0-hour contact. Cows did not seem to anticipate calf release into the pen in the mornings following commencement of 12-hour CCC. Finally, incomplete milkings occurred in all contact periods, but to a much greater extent during 24- and 12-hour contact in all non-primiparous cows.

Our results found cows did not express decreased lying times after initiating a 12-hour CCC contact period, suggesting they did not experience strong signs of distress. Further research is needed to determine the degree to which cows in a gradual separation system react to later complete and physical separation from their calves.

## References

- Akers, R.M. & Lefcourt, A.M. (1984). Effect of presence of calf on milking-induced release of prolactin and oxytocin during early lactation of dairy cows. *Journal of Dairy Science*, 67 (1), 115–122. [https://doi.org/10.3168/jds.S0022-0302\(84\)81274-6](https://doi.org/10.3168/jds.S0022-0302(84)81274-6)
- Albaaj, A., Marnet, P.G., Hurtaud, C. & Guinard-Flament, J. (2018). Adaptation of dairy cows to increasing degrees of incomplete milk removal during a single milking interval. *Journal of Dairy Science*, 101 (9), 8492–8504. <https://doi.org/10.3168/jds.2018-14451>
- Arla (2020). *Arlabönder går före och sätter ny standard för djuromsorg*. Arla. <https://www.arla.se/om-arla/nyheter-press/2020/pressrelease/arlaboender-gaar-foere-och-saetter-ny-standard-foer-djuromsorg-3034178/> [2023-11-17]
- Bar-Peled, U., Robnson, B., Maltz, E., Tagari, H., Folman, Y., Bruckental, I., Voet, H., Gacitua, H. & Lehrer, A.R. (1997). Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. *Journal of Dairy Science*, 80 (10), 2523–2528. [https://doi.org/10.3168/jds.S0022-0302\(97\)76205-2](https://doi.org/10.3168/jds.S0022-0302(97)76205-2)
- Bar-Peled, U., Aharoni, Y., Robnson, B., Bruckental, I., Lehrer, R., Maltz, E., Knight, C., Kali, J., Folman, Y., Voet, H., Gacitua, H. & Tagari, H. (1998). The effect of enhanced milk yield of dairy cows by frequent milking or suckling on intake and digestibility of the diet. *Journal of Dairy Science*, 81 (5), 1420–1427. [https://doi.org/10.3168/jds.S0022-0302\(98\)75706-6](https://doi.org/10.3168/jds.S0022-0302(98)75706-6)
- Barth, K. (2020). Effects of suckling on milk yield and milk composition of dairy cows in cow–calf contact systems. *Journal of Dairy Research*, 87 (S1), 133–137. <https://doi.org/10.1017/S0022029920000515>
- Bruckmaier, R.M., Schams, D. & Blum, J.W. (1994). Continuously elevated concentrations of oxytocin during milking are necessary for complete milk removal in dairy cows. *Journal of Dairy Research*, 61 (3), 323–334. <https://doi.org/10.1017/S0022029900030740>
- Bruckmaier, R.M. & Hilger, M. (2001). Milk ejection in dairy cows at different degrees of udder filling. *Journal of Dairy Research*, 68 (3), 369–376. <https://doi.org/10.1017/S0022029901005015>
- Churakov, M., Eriksson, H.K., Agenäs, S. & Ferneborg, S. (2023). Proposed methods for estimating loss of saleable milk in a cow-calf contact system with automatic milking.

*Journal of Dairy Science*, 106 (12), 8835–8846. <https://doi.org/10.3168/jds.2022-23099>

- Edwards, S.A. & Broom, D.M. (1982). Behavioural interactions of dairy cows with their newborn calves and the effects of parity. *Animal Behaviour*, 30 (2), 525–535. [https://doi.org/10.1016/S0003-3472\(82\)80065-1](https://doi.org/10.1016/S0003-3472(82)80065-1)
- Enríquez, D.H., Ungerfeld, R., Quintans, G., Guidoni, A.L. & Hötzel, M.J. (2010). The effects of alternative weaning methods on behaviour in beef calves. *Livestock Science*, 128 (1), 20–27. <https://doi.org/10.1016/j.livsci.2009.10.007>
- Flower, F.C. & Weary, D.M. (2001). Effects of early separation on the dairy cow and calf: 2. Separation at 1 day and 2 weeks after birth. *Applied Animal Behaviour Science*, 70 (4), 275–284. [https://doi.org/10.1016/S0168-1591\(00\)00164-7](https://doi.org/10.1016/S0168-1591(00)00164-7)
- Fregonesi, J.A. & Leaver, J.D. (2002). Influence of space allowance and milk yield level on behaviour, performance and health of dairy cows housed in strawyard and cubicle systems. *Livestock Production Science*, 78 (3), 245–257. [https://doi.org/10.1016/S0301-6226\(02\)00097-0](https://doi.org/10.1016/S0301-6226(02)00097-0)
- Fröberg, S., Aspegren-Güldorff, A., Olsson, I., Marin, B., Berg, C., Hernández, C., Galina, C.S., Lidfors, L. & Svennersten-Sjaunja, K. (2007). Effect of restricted suckling on milk yield, milk composition and udder health in cows and behaviour and weight gain in calves, in dual-purpose cattle in the tropics. *Tropical Animal Health and Production*, 39 (1), 71–81. <https://doi.org/10.1007/s11250-006-4418-0>
- Fröberg, S., Gratte, E., Svennersten-Sjaunja, K., Olsson, I., Berg, C., Orihuela, A., Galina, C.S., García, B. & Lidfors, L. (2008). Effect of suckling ('restricted suckling') on dairy cows' udder health and milk let-down and their calves' weight gain, feed intake and behaviour. *Applied Animal Behaviour Science*, 113 (1), 1–14. <https://doi.org/10.1016/j.applanim.2007.12.001>
- Fröberg, S., Lidfors, L., Svennersten-Sjaunja, K. & Olsson, I. (2011). Performance of free suckling dairy calves in an automatic milking system and their behaviour at weaning. *Acta Agriculturae Scandinavica, Section A — Animal Science*, 61 (3), 145–156. <https://doi.org/10.1080/09064702.2011.632433>
- Grøndahl, A.M., Skancke, E.M., Mejdell, C.M. & Jansen, J.H. (2007). Growth rate, health and welfare in a dairy herd with natural suckling until 6–8 weeks of age: a case report. *Acta Veterinaria Scandinavica*, 49 (1), 16. <https://doi.org/10.1186/1751-0147-49-16>
- Haley, D.B., Bailey, D.W. & Stookey, J.M. (2005). The effects of weaning beef calves in two stages on their behavior and growth rate. *Journal of Animal Science*, 83 (9), 2205–2214. <https://doi.org/10.2527/2005.8392205x>
- Hudson, S.J. & Mullord, M.M. (1977). Investigations of maternal bonding in dairy cattle. *Applied Animal Ethology*, 3 (3), 271–276. [https://doi.org/10.1016/0304-3762\(77\)90008-6](https://doi.org/10.1016/0304-3762(77)90008-6)

- Ito, K., Weary, D.M. & von Keyserlingk, M.A.G. (2009). Lying behavior: Assessing within- and between-herd variation in free-stall-housed dairy cows. *Journal of Dairy Science*, 92 (9), 4412–4420. <https://doi.org/10.3168/jds.2009-2235>
- Jensen, M.B., Pedersen, L.J. & Munksgaard, L. (2005). The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. *Applied Animal Behaviour Science*, 90 (3), 207–217. <https://doi.org/10.1016/j.applanim.2004.08.006>
- Jensen, M.B. (2011). The early behaviour of cow and calf in an individual calving pen. *Applied Animal Behaviour Science*, 134 (3), 92–99. <https://doi.org/10.1016/j.applanim.2011.06.017>
- Johnsen, J.F., Beaver, A., Mejdell, C.M., Rushen, J., de Passillé, A.M. & Weary, D.M. (2015a). Providing supplementary milk to suckling dairy calves improves performance at separation and weaning. *Journal of Dairy Science*, 98 (7), 4800–4810. <https://doi.org/10.3168/jds.2014-9128>
- Johnsen, J.F., Ellingsen, K., Grøndahl, A.M., Bøe, K.E., Lidfors, L. & Mejdell, C.M. (2015b). The effect of physical contact between dairy cows and calves during separation on their post-separation behavioural response. *Applied Animal Behaviour Science*, 166, 11–19. <https://doi.org/10.1016/j.applanim.2015.03.002>
- Johnsen, J.F., de Passille, A.M., Mejdell, C.M., Bøe, K.E., Grøndahl, A.M., Beaver, A., Rushen, J. & Weary, D.M. (2015c). The effect of nursing on the cow–calf bond. *Applied Animal Behaviour Science*, 163, 50–57. <https://doi.org/10.1016/j.applanim.2014.12.003>
- Johnsen, J.F., Zipp, K.A., Kälber, T., Passillé, A.M. de, Knierim, U., Barth, K. & Mejdell, C.M. (2016). Is rearing calves with the dam a feasible option for dairy farms?—Current and future research. *Applied Animal Behaviour Science*, 181, 1–11. <https://doi.org/10.1016/j.applanim.2015.11.011>
- Johnsen, J.F., Mejdell, C.M., Beaver, A., de Passillé, A.M., Rushen, J. & Weary, D.M. (2018). Behavioural responses to cow-calf separation: The effect of nutritional dependence. *Applied Animal Behaviour Science*, 201, 1–6. <https://doi.org/10.1016/j.applanim.2017.12.009>
- Johnsen, J.F., Johanssen, J.R.E., Aaby, A.V., Kischel, S.G., Ruud, L.E., Soki-Makilutilla, A., Kristiansen, T.B., Wibe, A.G., Bøe, K.E. & Ferneborg, S. (2021). Investigating cow–calf contact in cow-driven systems: behaviour of the dairy cow and calf. *Journal of Dairy Research*, 88 (1), 52–55. <https://doi.org/10.1017/S0022029921000194>
- Kalvportalen (2019). *Kalvportalen - fakta och rådgivning om kalvar och kalvhälsa*. <https://www.kalvportalen.se/> [2023-09-21]
- Kiley-Worthington, M. & De la Plain, S. (1983). *The behaviour of beef suckler cattle: (Bos Taurus)*. Birkhäuser. (Tierhaltung, 14)

- Kirkden, R.D. & Pajor, E.A. (2006). Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. *Applied Animal Behaviour Science*, 100 (1), 29–47. <https://doi.org/10.1016/j.applanim.2006.04.009>
- KRAV (2023). 5.2.6 *Utfodring av kalvar*. <https://regler.krav.se/unit/krav-article/b0c21d62-235a-4754-beff-1bc0a056408a> [2023-10-11]
- Krohn, C.C., Jonassen, B. & Munksgaard, L. (1990a). Investigations on cow-calf relations. 2. Effect of no suckling or suckling for 5 days on the behaviour, milk yield and udder health of cows in different types of housing. *Beretning fra Statens Husdyrbrugsforsøg*, (No. 678). [https://dcapub.au.dk/pub/sh\\_beretning\\_678.pdf](https://dcapub.au.dk/pub/sh_beretning_678.pdf) [2023-11-20]
- Krohn, C.C., Jonassen, B. & Munksgaard, L. (1990b). Undersøgelser vedrørende ko-kalv samspil 3. Indflydelse af 6-8 ugers patteperiode på koens adfærd, mælkeydelse, yversundhed og reproduktion. *Beretning fra Statens Husdyrbrugsforsøg*, (773). [https://dcapub.au.dk/pub/sh\\_meddelelse\\_773.pdf](https://dcapub.au.dk/pub/sh_meddelelse_773.pdf)
- Lambertz, C., Bowen, P.R., Erhardt, G. & Gauly, M. (2015). Effects of weaning beef cattle in two stages or by abrupt separation on nasal abrasions, behaviour, and weight gain. *Animal Production Science*, 55 (6), 786–792. <https://doi.org/10.1071/AN14097>
- Langbein, J. & Raasch, M.-L. (2000). Investigations on the hiding behaviour of calves at pasture [Untersuchungen zum Abliegeverhalten bei Kälbern auf der Weide]. *Archiv für Tierzucht*, 43, 203–210
- Lent, P.C. (1974). Mother-Infant Relationships in Ungulates. In: *The Behavior of Ungulates and Its Relationship to Management*. IUCN Publications, Morges. 14–55.
- Lidfors, L. & Jensen, P. (1988). Behaviour of free-ranging beef cows and calves. *Applied Animal Behaviour Science*, 20 (3), 237–247. [https://doi.org/10.1016/0168-1591\(88\)90049-4](https://doi.org/10.1016/0168-1591(88)90049-4)
- Lidfors, L.M. (1996). Behavioural effects of separating the dairy calf immediately or 4 days post-partum. *Applied Animal Behaviour Science*, 49 (3), 269–283. [https://doi.org/10.1016/0168-1591\(96\)01053-2](https://doi.org/10.1016/0168-1591(96)01053-2)
- Lidfors, L.M., Jensen, P. & Algers, B. (1994). Suckling in free-ranging beef cattle — temporal patterning of suckling bouts and effects of age and sex. *Ethology*, 98 (3–4), 321–332. <https://doi.org/10.1111/j.1439-0310.1994.tb01080.x>
- Mac, S.E., Lomax, S. & Clark, C.E.F. (2023). Behavioral responses to cow and calf separation: separation at 1 and 100 days after birth. *Animal Bioscience*, 36 (5), 810–817. <https://doi.org/10.5713/ab.22.0257>
- Marcé, C., Guatteo, R., Bareille, N. & Fourichon, C. (2010). Dairy calf housing systems across Europe and risk for calf infectious diseases. *Animal*, 4 (9), 1588–1596. <https://doi.org/10.1017/S1751731110000650>
- Martin, P. & Bateson, P. (2007). *Measuring Behaviour: An Introductory Guide*. 3. ed. Cambridge: Cambridge University Press. <https://doi.org/10.1017/CBO9780511810893.001>

- Meagher, R.K., Beaver, A., Weary, D.M. & von Keyserlingk, M.A.G. (2019). Invited review: A systematic review of the effects of prolonged cow–calf contact on behavior, welfare, and productivity. *Journal of Dairy Science*, 102 (7), 5765–5783. <https://doi.org/10.3168/jds.2018-16021>
- Metz, J. (1987). Productivity aspects of keeping dairy cow and calf together in the post-partum period. *Livestock Production Science*, 16 (4), 385–394. [https://doi.org/10.1016/0301-6226\(87\)90007-8](https://doi.org/10.1016/0301-6226(87)90007-8)
- Mitlöhner, F.M., Morrow-Tesch, J.L., Wilson, S.C., Dailey, J.W. & McGlone, J.J. (2001). Behavioral sampling techniques for feedlot cattle. *Journal of Animal Science*, 79 (5), 1189–1193. <https://doi.org/10.2527/2001.7951189x>
- Munksgaard, L., Jensen, M.B., Pedersen, L.J., Hansen, S.W. & Matthews, L. (2005). Quantifying behavioural priorities—effects of time constraints on behaviour of dairy cows, *Bos taurus*. *Applied Animal Behaviour Science*, 92 (1), 3–14. <https://doi.org/10.1016/j.applanim.2004.11.005>
- Neave, H.W., Jensen, E.H., Durrenwachter, M. & Jensen, M.B. (2023). Behavioral responses of dairy cows and their calves to gradual or abrupt weaning and separation when managed in full- or part-time cow-calf contact systems. *Journal of Dairy Science*, In press. <https://doi.org/10.3168/jds.2023-24085>
- Nicolao, A., Veissier, I., Bouchon, M., Sturaro, E., Martin, B. & Pomiès, D. (2022). Animal performance and stress at weaning when dairy cows suckle their calves for short versus long daily durations. *Animal*, 16 (6), 100536. <https://doi.org/10.1016/j.animal.2022.100536>
- Österman, S. & Redbo, I. (2001). Effects of milking frequency on lying down and getting up behaviour in dairy cows. *Applied Animal Behaviour Science*, 70 (3), 167–176. [https://doi.org/10.1016/S0168-1591\(00\)00159-3](https://doi.org/10.1016/S0168-1591(00)00159-3)
- Padilla de la Torre, M., Briefer, E.F., Reader, T. & McElligott, A.G. (2015). Acoustic analysis of cattle (*Bos taurus*) mother–offspring contact calls from a source–filter theory perspective. *Applied Animal Behaviour Science*, 163, 58–68. <https://doi.org/10.1016/j.applanim.2014.11.017>
- Padilla de la Torre, M., Briefer, E.F., Ochocki, B.M., McElligott, A.G. & Reader, T. (2016). Mother–offspring recognition via contact calls in cattle, *Bos taurus*. *Animal Behaviour*, 114, 147–154. <https://doi.org/10.1016/j.anbehav.2016.02.004>
- de Passillé, A.M., Marnet, P.-G., Lapierre, H. & Rushen, J. (2008). Effects of twice-daily nursing on milk ejection and milk yield during nursing and milking in dairy cows. *Journal of Dairy Science*, 91 (4), 1416–1422. <https://doi.org/10.3168/jds.2007-0504>
- Price, E.O., Harris, J.E., Borgwardt, R.E., Sween, M.L. & Connor, J.M. (2003). Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behavior and growth rate<sup>1</sup>. *Journal of Animal Science*, 81 (1), 116–121. <https://doi.org/10.2527/2003.811116x>

- Reinhardt, V. & Reinhardt, A. (1981a). Cohesive relationships in a cattle herd (*Bos indicus*). *Behaviour*, 77 (3), 121–151.
- Reinhardt, V. & Reinhardt, A. (1981b). Natural sucking performance and age of weaning in zebu cattle (*Bos indicus*). *The Journal of Agricultural Science*, 96 (2), 309–312. <https://doi.org/10.1017/S0021859600066089>
- Roth, B.A., Barth, K., Gygax, L. & Hillmann, E. (2009). Influence of artificial vs. mother-bonded rearing on sucking behaviour, health and weight gain in calves. *Applied Animal Behaviour Science*, 119 (3), 143–150. <https://doi.org/10.1016/j.applanim.2009.03.004>
- Sandem, A.-I. & Braastad, B.O. (2005). Effects of cow–calf separation on visible eye white and behaviour in dairy cows—A brief report. *Applied Animal Behaviour Science*, 95 (3), 233–239. <https://doi.org/10.1016/j.applanim.2005.04.011>
- Sato, S., Wood-Gush, D.G.M. & Wetherill, G. (1987). Observations on creche behaviour in suckler calves. *Behavioural Processes*, 15 (2), 333–343. [https://doi.org/10.1016/0376-6357\(87\)90017-9](https://doi.org/10.1016/0376-6357(87)90017-9)
- Selman, I.E., McEwan, A.D. & Fisher, E.W. (1970). Studies on natural suckling in cattle during the first eight hours post partum I. Behavioural studies (dams). *Animal Behaviour*, 18, 276–283. [https://doi.org/10.1016/S0003-3472\(70\)80038-0](https://doi.org/10.1016/S0003-3472(70)80038-0)
- Shamay, A., Werner, D., Moallem, U., Barash, H. & Bruckental, I. (2005). Effect of nursing management and skeletal size at weaning on puberty, skeletal growth rate, and milk production during first lactation of dairy heifers. *Journal of Dairy Science*, 88 (4), 1460–1469. [https://doi.org/10.3168/jds.S0022-0302\(05\)72814-9](https://doi.org/10.3168/jds.S0022-0302(05)72814-9)
- Sirovnik, J., Barth, K., Oliveira, D. de, Ferneborg, S., Haskell, M.J., Hillmann, E., Jensen, M.B., Mejdell, C.M., Napolitano, F., Vaarst, M., Verwer, C.M., Waiblinger, S., Zipp, K.A. & Johnsen, J.F. (2020). Methodological terminology and definitions for research and discussion of cow-calf contact systems. *Journal of Dairy Research*, 87 (S1), 108–114. <https://doi.org/10.1017/S0022029920000564>
- Sjaastad, O.V., Hove, K. & Sand, O. (2016). *Physiology of Domestic Animals*. 3. ed. Oslo: Scandinavian Veterinary Press.
- Solano, L., Barkema, H.W., Pajor, E.A., Mason, S., LeBlanc, S.J., Nash, C.G.R., Haley, D.B., Pellerin, D., Rushen, J., de Passillé, A.M., Vasseur, E. & Orsel, K. (2016). Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in freestall barns. *Journal of Dairy Science*, 99 (3), 2086–2101. <https://doi.org/10.3168/jds.2015-10336>
- Stěhulová, I., Lidfors, L. & Špinková, M. (2008). Response of dairy cows and calves to early separation: Effect of calf age and visual and auditory contact after separation. *Applied Animal Behaviour Science*, 110 (1), 144–165. <https://doi.org/10.1016/j.applanim.2007.03.028>



- Thomas, T.J., Weary, D.M. & Appleby, M.C. (2001). Newborn and 5-week-old calves vocalize in response to milk deprivation. *Applied Animal Behaviour Science*, 74 (3), 165–173. [https://doi.org/10.1016/S0168-1591\(01\)00164-2](https://doi.org/10.1016/S0168-1591(01)00164-2)
- Van Reenen, C.G., Van der Werf, J.T.N., Bruckmaier, R.M., Hopster, H., Engel, B., Noordhuizen, J.P.T.M. & Blokhuis, H.J. (2002). Individual differences in behavioral and physiological responsiveness of primiparous dairy cows to machine milking. *Journal of Dairy Science*, 85 (10), 2551–2561. [https://doi.org/10.3168/jds.S0022-0302\(02\)74338-5](https://doi.org/10.3168/jds.S0022-0302(02)74338-5)
- Vasseur, E., Rushen, J., Haley, D.B. & de Passillé, A.M. (2012). Sampling cows to assess lying time for on-farm animal welfare assessment. *Journal of Dairy Science*, 95 (9), 4968–4977. <https://doi.org/10.3168/jds.2011-5176>
- Växa (2022). *Inkalvningsålder*. <https://www.vxa.se/fakta/styrning-och-rutiner/hallbara-atgarder/avel/inkalvningsalder/> [2023-11-29]
- Växa Sverige (2022). *Kokomfort*. <https://www.vxa.se/fakta/styrning-och-rutiner/hallbara-atgarder/byggnader-och-teknik/kokomfort/> [2023-10-06]
- Veissier, I., Lamy, D. & Le Neindre, P. (1990). Social behaviour in domestic beef cattle when yearling calves are left with the cows for the next calving. *Applied Animal Behaviour Science*, 27 (3), 193–200. [https://doi.org/10.1016/0168-1591\(90\)90056-J](https://doi.org/10.1016/0168-1591(90)90056-J)
- Veissier, I., Caré, S. & Pomiès, D. (2013). Suckling, weaning, and the development of oral behaviours in dairy calves. *Applied Animal Behaviour Science*, 147 (1), 11–18. <https://doi.org/10.1016/j.applanim.2013.05.002>
- Vitale, A.F., Tenucci, M., Papini, M. & Lovari, S. (1986). Social behaviour of the calves of semi-wild Maremma cattle, *Bos primigenius taurus*. *Applied Animal Behaviour Science*, 16 (3), 217–231. [https://doi.org/10.1016/0168-1591\(86\)90115-2](https://doi.org/10.1016/0168-1591(86)90115-2)
- Weary, D.M. & Chua, B. (2000). Effects of early separation on the dairy cow and calf: 1. Separation at 6 h, 1 day and 4 days after birth. *Applied Animal Behaviour Science*, 69 (3), 177–188. [https://doi.org/10.1016/S0168-1591\(00\)00128-3](https://doi.org/10.1016/S0168-1591(00)00128-3)
- Wegner, C.S. & Ternman, E. (2023). Lying behaviour of lactating dairy cows in a cow-calf contact freestall system. *Applied Animal Behaviour Science*, 259, 105851. <https://doi.org/10.1016/j.applanim.2023.105851>
- Wenker, M.L., van Reenen, C.G., Bokkers, E.A.M., McCrea, K., de Oliveira, D., Sørheim, K., Cao, Y., Bruckmaier, R.M., Gross, J.J., Gort, G. & Verwer, C.M. (2022a). Comparing gradual debonding strategies after prolonged cow-calf contact: Stress responses, performance, and health of dairy cow and calf. *Applied Animal Behaviour Science*, 253, 105694. <https://doi.org/10.1016/j.applanim.2022.105694>
- Wenker, M.L., Verwer, C.M., Bokkers, E.A.M., te Beest, D.E., Gort, G., de Oliveira, D., Koets, A., Bruckmaier, R.M., Gross, J.J. & van Reenen, C.G. (2022b). Effect of type of cow-calf contact on health, blood parameters, and performance of dairy cows and calves. *Frontiers in Veterinary Science*, 9. <https://www.frontiersin.org/articles/10.3389/fvets.2022.855086> [2023-09-28]

Westin, R., Vaughan, A., Passillé, A.M. de, DeVries, T.J., Pajor, E.A., Pellerin, D., Siegford, J.M., Vasseur, E. & Rushen, J. (2016). Lying times of lactating cows on dairy farms with automatic milking systems and the relation to lameness, leg lesions, and body condition score. *Journal of Dairy Science*, 99 (1), 551–561.  
<https://doi.org/10.3168/jds.2015-9737>

## Popular science summary

In dairy farming, cows need to continuously give birth to calves to produce milk. Commonly, calves and cows are separated soon (hours-days) after birth. Calves are then raised separately from their mothers and fed milk replacer or whole-milk provided in buckets or milk feeders with artificial teats. Alternate rearing methods where cow and calf are allowed varying degrees of contact for longer periods of time are labelled cow-calf contact (CCC) systems, and can be beneficial in several ways. Under natural conditions a calf may suckle their mother for up to 14 months of age and form social bonds with their mother persisting beyond this period. Thus, when kept together, both cow and calf are able to express important dam-calf behaviours such as suckling or grooming one another, positively affecting animal welfare. Calves also gain weight at a very high rate when allowed to suckle their dam. This is beneficial for the farmer, as quicker growth allows for female calves to reach maturity and start producing milk earlier, whilst heavier male calves are more valuable if sold for meat production purposes.

However, there are challenges to keeping cows and calves in CCC systems. Keeping cows and calves together for longer periods of time strengthens the bond between them. When they are later inevitably separated, both cows and calves express strong behavioural reactions to the separation. Both cows and calves vocalise more frequently; presumably, cows in an effort to contact the calf, and calves partially also because of hunger as they are no longer allowed to suckle. Cows may decrease the amount of time spent lying down in a day during the first days after they are separated from their calves, indicating severe distress. Research is ongoing to determine ways to minimise this response. It causes unnecessary stress for the animals as well as economical losses, as calves generally decrease dramatically in weight gain following this separation. Another issue concerns reduced amounts of milk made available from cows kept in CCC systems. The calf drinks large amounts of milk – up to 12.5 L/day – that might otherwise have been sold. Additionally, suckled cows generally have a higher risk of impaired milk let-down at machine milking, which leads to incomplete emptying of the udder and further loss of saleable milk and resulting in economic losses for the farmer.

This study was performed on a batch of 10 pairs of cows and calves and aimed to investigate whether cows in a CCC system changed their lying behaviour when

subjected to a gradual separation process. Additionally, it evaluated whether calves allowed to drink milk not only from their dams but also from teat buckets would experience less weight gain after separation from their dam, and if this might improve cow machine milk yields. Cows and calves were initially kept together and allowed unrestricted access to one another. After some time in this system, calves were confined to a part of the system called the calf creep during nighttime. The calf creep was inaccessible to cows, whilst calves could move freely between the calf creep and the rest of the system during daytime. Cows and calves were still able to see, hear and smell each other during periods of calf confinement. This so-called 12-hour contact period lasted for 6.5 weeks. The calves were trained to drink milk from artificial teat buckets before initiating this period to make them less dependent on their mothers for nutrition. Artificial milk feeding continued throughout this period and into the next, called the 0-hour contact period. During this final period the calves were confined to the calf creep during the full day and were not allowed to suckle or be in physical contact with the dams. This period lasted a total of 4 weeks, during which bucket milk feeding continued for approximately 3 weeks. After this, the study was terminated.

During the study, data was obtained concerning how much weight the calves gained each week and how much saleable milk the cows yielded. Video material of the study pen was used to assess daily cow lying times during the week before (week 1) and the week after (week 2) commencing 12-hour contact. It was also used to determine the degree to which cows spent their time in proximity to the calf creep during the first 12 hours of each day during the aforementioned weeks.

The results of the study showed that there was little difference in how much milk was available to sell between the 24- and 12-hour contact period, while a much higher amount of sellable milk was obtained during the 0-hour contact period. The amount of incomplete milkings – meaning that cows for some reason did not yield as much milk to the milking robot as expected, for example due to having been recently nursed or not fully releasing the milk in the udder to be accessed by the robot – was at its highest during the 24-hour period and lowest at the 0-hour period. Calves gained comparable amounts of weight during 24- and 12-hour periods, but decreased in weight gain after being separated and no longer allowed to suckle their dams. Despite this, the rate of weight gain was still considered quite high compared to average values. Cows did not decrease lying times when 12-hour contact was initiated; rather, they seemed to spend more time lying down. Whether cow lying behaviour was affected by transitioning from 12- to 0-hour contact could not be assessed in this study due to missing video material.

To summarise, the effects seen in this study indicate that transitioning from 24-hour to 12-hour contact in CCC systems may lessen unwanted effects often occurring at

abrupt separation, such as calf growth stalling and cows spending less time lying down. Additionally, feeding calves artificially during the gradual separation process and continuing this for some time after final separation from their dams may aid the calves in maintaining a beneficial growth rate even after separation.

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