Welfare implications of prolonged cow-calf contact in dairy farming

Margret Lisanne Wenker

Propositions

1. Direct separation of cow and calf ignores the need to express maternal behaviour.
   (this thesis)

2. Keeping calves together with their dam in conventional dairy systems does not guarantee welfare benefits.
   (this thesis)

3. To prevent a 2 degrees Celsius global temperature increase, plant-based foods need to become the default in the human diet.

4. Emotional empowerment should be prioritized over cognitive skills in children's education.

5. Success and failure are toxic illusions created by society.

6. Speciesism is just as irrational as any other discrimination.

Propositions belonging to the thesis, entitled

Welfare implications of prolonged cow-calf contact in dairy farming

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Margret Lisanne Wenker

Thesis committee

Promotor

Dr E.A.M. Bokkers
Associate Professor, Animal Production Systems Group
Wageningen University & Research

Co-promotors

Dr C.G. van Reenen
Senior researcher, Animal Health & Welfare Group
Wageningen Livestock Research

Dr C.M. Verwer
Researcher
Louis Bolk Institute, Bunnik

Other members

Prof. Dr T.B. Rodenburg, Wageningen University & Research
Dr M. Vaarst, Aarhus University, Tjele
Dr K. Barth, Johann Heinrich von Thünen Institute, Westerau
Dr R. Jorritsma, Utrecht University

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Margret Lisanne Wenker
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“It’s not about humanizing them.
It’s about recognizing them.”

\- Carol Shilor -

Contents

Chapter 1

General Introduction

Public acceptability of management practices, as well as good health and welfare of calves and cows in dairy production systems, are essential for safeguarding the sustainability of the dairy industry, now and in the future. To secure consumer trust and political support, practices need to reflect public values and the animals' needs (von Keyserlingk et al., 2013). In this thesis I present results of a comparative study on different calf rearing systems that either do or do not allow dairy cow and calf to be together for a prolonged period after birth. Alternative calf rearing systems with cow-calf contact are a response to public concerns with regard to standard calf rearing practices that involve early cow-calf separation (Textbox 1) (Brombin et al., 2019). This study assessed the implications of different types of cow-calf contact for dairy cow and calf welfare in comparison to a conventional rearing system without cow-calf contact. Here, good animal welfare is defined as functioning well (i.e. good health), feeling well (i.e. positive mental state), and being able to express natural behaviour (Duncan, 2005; Fraser et al., 1997; Hemsworth et al., 2015; Mellor, 2012).

Textbox 1. Standard calf rearing management practices on commercial dairy farms

Dairy cattle are one of the domestic animal species that is withheld from maternal care by separating the cow and the new-born calf shortly after birth. After removing the calf from the dam, the dairy calf is generally housed in a single pen (indoor or outdoor) for some days up to several weeks before it is moved to a group pen with other calves. The calves are fed either whole milk or milk replacer (generally about 4 to 8 L/calf/day) from automated milk feeders, open buckets, teat buckets or milk bars with multiple teats, and are weaned usually between 6 to 12 weeks of age. About 35 to 50% of all calves stay on the dairy farm and is raised as future dairy cow, whereas bull calves and surplus female calves leave the farm after two weeks to be fattened at a veal farm. The dam usually returns to the lactating cow herd after a short recovery period of some hours in the maternity pen and is being milked two or three times a day for dairy production. Generally, the yearly cycle of giving birth, removing the calf, and milking the cow until her next calf is born repeats itself two to three times on average. Thereafter, the dam is sent for slaughter and is replaced by one of her heifer calves in the future dairy herd.

1. Social concerns regarding dairy calf rearing

So far, the dairy industry has enjoyed a positive public image (European Commission, 2005; Placzek et al., 2020; Ventura et al., 2013). However, the lay public is becoming increasingly aware of how their food is produced (Cembalo et al., 2016), and are faced with sometimes disturbing videos documenting contentious practices that may affect how the livestock sector is perceived in general (Weary and von Keyserlingk, 2017). The traditional practice of quickly removing the new-born calf from the dam appears to conflict with public values and this is one of the reasons[1] that the dairy industry is criticized at times (Placzek et al., 2020; Weary and von Keyserlingk, 2017). Recent surveys performed in several countries around the world seem to suggest that lay citizens may be concerned about separating the calf from the cow from an animal

[1] Other reasons for public criticism involve for instance the intensification of dairy production that resulted in disruptive effects on the environment, rural populations, biodiversity, and animal welfare (von Keyserlingk et al., 2013)

welfare point of view (see review by Placzek et al., 2020). Relevant studies in this respect were executed in, for example, the Netherlands (Boogaard et al., 2011), Germany (Busch et al., 2017), the United States of America (Ventura et al., 2013), Canada (Ventura et al., 2016) and Brazil (Cardoso et al., 2017; Hötzel et al., 2017). At least among the respondents that were consulted in these surveys (from several hundred to more than a thousand), a substantial part expressed concerns and objection to the absence of cow-calf contact in conventional calf rearing practices. Nonetheless, it is important to distinguish between the consumer and the citizen. Generally, when individuals participate in attitude surveys they play the role of citizen making socially responsible decisions. When buying food, however, those same individuals become consumers choosing with their wallet where product prices become their highest priority, which can result in purchases that are not always in line with their personal values (von Keyserlingk et al., 2013). Nevertheless, the current and future sustainability of the dairy industry could be affected by a gap between societal expectations and actual farm practices (Broom, 2021, 2010). Therefore, the dairy industry is increasingly responding to public concerns in order to maintain and preserve their license to produce, which also extends to methods and aspects of calf rearing.

2. Early cow-calf separation as standard practice

Early cow-calf separation is generally considered economically beneficial, important for safeguarding the health of cow and calf, and ethically preferable (Beaver et al., 2019a; Flower and Weary, 2003; Neave et al., 2021). I will briefly explain the rationale behind the conventional practice of removal of the calf from the dam shortly after birth, followed by an evaluation of current calf rearing practices from an animal welfare point of view.

2.1 Reasoning behind early cow-calf separation

On economic grounds, preventing the calf from suckling may result in more saleable milk for the dairy farmer (Flower and Weary, 2003). Calves can suckle up to 15 L/d (Roth et al., 2009), hence decreased volumes of harvested milk are regularly reported during the suckling period (Meagher et al., 2019). Milking efficiency is another argument used in favor of early cow-calf separation, as efficient milking requires that cows let down their milk soon after the milking equipment is attached (Flower and Weary, 2003). Although Fröberg et al. (2008) reported that the latency to milk let-down was similar between suckled and non-suckled cows, milk ejection problems in the milking parlor can occur when cows are additionally suckled (Johnsen et al., 2016; Zipp et al., 2018) and are undesirable from both a worker's and animal's perspective (Beggs et al., 2018; Flower and Weary, 2003). Moreover, removal of new-born calves to prevent suckling is thought to facilitate cow's return to estrus and thus enhance the likelihood of a short calving interval (Carruthers and Hafs, 1980; Flower and Weary, 2003).

Furthermore, manual feeding of calves may enhance the control and monitoring of feed intake. Dairy farmers maintain that individual feeding of calves ensures adequate colostrum intake that is essential for the passive transfer of immunity, and facilitates the detection of health problems (Flower and Weary, 2003). Besides, there is a potential for an increased risk of disease transmission between cow and calf when the young calf is kept together with older animals in areas designed for cows (Johnsen et al., 2016; Sumner and von Keyserlingk, 2018; Ventura et al., 2013) . Especially, for paratuberculosis the most susceptible animals in a herd are the newborn calves and young stock that can get infected via the fecal–oral transmission route (i.e. contact with adult cow manure) (Sweeney et al., 2012).

In addition, dairy farmers maintain practical reasons (e.g. current barn design) that relate to economic and health arguments in favor of early separation. For instance, some consider the slatted floors with manure scrapers in cow barns (which may be slippery and have too wide openings between slats) as calf-unfriendly due to the increased risk of accidents (Vaarst et al., 2020). Some reconstructions in existing cow barns might be necessary to safely house calves together with the dam, and those adjustments may be costly (Knierim et al., 2020). Besides, in pasture-based systems lack of proper shelter and fencing for young calves is a frequently mentioned concern (Neave et al., 2021).

Finally, dairy farmers argue on compassionate grounds that early separation minimizes the distress response in both the cow and calf by preventing the formation of a maternal bond (Flower and Weary, 2003). Correspondingly, several studies showed that prolonging contact between cow and calf resulted in an increased behavioural response by the cow and her calf when abrupt separation eventually occurred (Flower and Weary, 2001; Hudson and Mullord, 1977; Johnsen et al., 2018; Stěhulová et al., 2008; Weary and Chua, 2000). Moreover, dairy farmers worry that prolonging cow-calf contact will result in difficulties with untamed calves, which can negatively affect the human–animal relationship and therefore cause stress during handling later in life (Neave et al., 2021; Vaarst et al., 2020).

2.2 Current calf rearing practices and animal welfare

In the last half century, dairy farms moved away from more naturalistic environments and diets to more specialized management practices that provide control for farm personnel, such as restricted milk feeding and individual housing of calves in early life (Beaver et al., 2019b). Yet, there is growing evidence suggesting that this early life environment of conventionally reared dairy calves may limit their physical, behavioral, and cognitive development (see review by Costa et al., 2019a).

First of all, calves in conventional dairy systems are fed two to three times per day restricted amounts of milk (approximately 10 % of their body weight) with the majority of dairy farmers around the world feeding 6 L or less in two meals per day (e.g. Murray et al., 2016; Staněk et al., 2014; Vasseur et al., 2010), which is in contrast to calves' natural milk intake that is estimated at a milk consumption of about 20 % of their body weight (Khan et al., 2011). Consequently, several studies reported that conventionally fed calves show impaired growth rates and behaviour indicating feelings of hunger due to restrictive milk allowances (de Paula Vieira et al., 2008; Khan et al., 2011; Rosenberger et al., 2017). Moreover, conventionally fed calves can at times show abnormal oral behaviour directed toward fixtures in the pen or other calves due to limited sucking possibilities in current feeding practices (Latham and Mason, 2008; Margerison et al., 2003), as most of the commonly used (teat)buckets leave the calves little time to satisfy their motivation to suck due to the high drinking speed with no or minimal suckling possibilities (de Passillé, 2001). Moreover, open bucket feeding results in an unnatural drinking position of the calf's head which, when accompanied by suboptimal milk temperatures (as milk cools off in the bucket rather quickly), may compromise the esophageal reflex to close of the esophageal groove (Sjaastad et al., 2010). Consequently, there might be an increased risk of milk entering the underdeveloped rumen rather than the abomasum, which may cause indigestion, diarrhea, ruminal bloat, and reduced growth (Burgstaller et al., 2017; Sjaastad et al., 2010).

Another key calf welfare issue is the individual housing of young calves in first weeks of life, which prevents much of calves' social behaviour. Dairy calves are highly motivated to have social contact in early life (Chua et al., 2002; Ede et al., 2021; Holm et al., 2002), which indicates that contact with conspecifics is a priority for calves' welfare. Moreover, social play behaviors are suggested to promote positive affective states (Ahloy-Dallaire et al., 2018; Boissy et al., 2007), but cannot be performed by individually housed calves. In addition, individual housing in early life is known to impair calves' behavioural development and cognitive ability including learning deficits (Duve and Jensen, 2012; Gaillard et al., 2014; Jensen and Larsen, 2014; Meagher et al., 2015).

Young calves are highly susceptible to disease due to a naïve immune system, hence dairy farmers sometimes face high risks of morbidity and mortality in young calves (e.g. Santman-Berends et al., 2021, 2019; Windeyer et al., 2014). This not only has significant economic consequences for farmers (Mohd Nor et al., 2012), but also raises concern regarding animal welfare (Ortiz-Pelaez et al., 2008). Dutch dairy farmers indicated that the evolution of the Dutch dairy industry towards a more intensive farming system was the most important reason for increased mortality rates, as increasing herd sizes has led to less time and priority for the calves (Santman-Berends et al., 2014). One crucial aspect in calf management is timely provision of adequate amounts of high quality colostrum to provide the young calf with immunologic protection in the first weeks of life (Barrington et al., 2002), which can be at risk when care of new-born calves is not prioritized. On most dairy farms calves are fed colostrum by bottle to guarantee sufficient colostrum intake, although a recent survey among Dutch dairy farmers revealed that 50 % of calves born during nighttime risk delayed colostrum administration (Robbers et al., 2021).

Early cow-calf separation itself prevents much of the cow's natural maternal behaviour. Maternal behaviour of cows can be expressed through licking, nursing, attentiveness and proximity towards the young, and protecting it from potential threats (Grandinson, 2005; von Keyserlingk and Weary, 2007). Despite the belief that dairy cows have been selected against some aspects of maternal behaviour, such as docility to humans when the calf is removed and milk let-down in absence of their calf (Edwards and Broom, 1982), cows still perform maternal behaviour when given the opportunity (Illmann and Špinka, 1993; Jensen, 2011; von Keyserlingk and Weary, 2007). The opportunity to express natural behaviour is one of the key points in the concept of animal welfare (Fraser et al., 1997; Špinka, 2006; von Keyserlingk et al., 2009). Natural behaviour generally involves species-specific patterns that are associated with positive affective states, as those behavioural patterns help the animal to satisfy needs, provide them with emotionally positive experiences, and can bring long-term benefits (e.g. social skills) (Špinka, 2006). Consequently, preventing expressions of such behavioural patterns may cause distress (Boissy et al., 2007; Špinka, 2006).

Taken together, there is a need for improved calf rearing systems and perhaps rearing calves in contact with the dam may be relevant in this respect. However, it is currently unknown if, and to what extent, the above-mentioned calf welfare issues are associated with maternal deprivation of calves, and thus could be solved by providing cow-calf contact. For instance, leaving calves with the dam to suckle colostrum on their own can also lead to failure of passive transfer considering that not all calves stand up and find the udder in the first hours after birth (Besser et al., 1991; Ventorp and Michanek, 1992). At the same time, suckling colostrum from the dam has been found to increase the amount of immunoglobulins absorbed by the calves, suggesting that suckling in itself may promote passive transfer (Quigley et al., 1995; Stott et al., 1979). Besides, rearing calves together with their dams may address some welfare concerns, as it would allow for early socialization of calves, freely suckling of milk, and maternal-filial interactions to occur (Gygax and Hillmann, 2018; Whalin et al., 2021). Interestingly, the European Food and Safety Authority states that the needs of young calves are most effectively met by the presence and actions of their mothers, and lack of maternal care is regarded as risk factor for poor animal welfare (EFSA, 2006). Yet, reintroducing maternal care in current dairy farming appears to conflict with the industry's perceived best practices regarding maternal, social, physical, and nutritional restrictions (Beaver et al., 2019b). Therefore, there is a need for objective and comparative research into the pros and cons of conventional practices and alternative calf rearing systems that allow for contact between the calf and the dam, from an animal health and welfare perspective.

3. Prolonged cow-calf contact as alternative rearing system

Alternative rearing systems that allow calves to be raised by their dam are receiving increasing interest from the public, dairy farmers, and researchers (Johnsen et al., 2016; Kälber and Barth, 2014; de Oliveira et al., 2020). Some dairy farmers have developed rearing systems where the calf typically stays together with the dam in the dairy herd (either on pasture or inside the barn) for several days up to mostly numerous weeks whilst the calf suckles milk (freely) from the dam and the dam is additionally milked twice daily. Due to the large variation in how this prolonged cow-calf contact can be established, I will briefly clarify a common standard set of terminologies before illustrating the potential welfare benefits of such systems.

3.1 Definitions and terminology for prolonged cow-calf contact

Alternative calf rearing systems in which cows and calves stay in contact for an prolonged period of time, so called cow-calf contact (CCC) systems, vary considerably in terms of the type and duration of physical contact allowed between dams and calves (Johnsen et al., 2016). As suggested by Sirovnik et al. (2020), cow-calf contact can be defined as any housing or management system that allows for physical contact between a dam and her own calf (i.e. dam-calf rearing) or between a foster cow and her foster calf (i.e. foster cow rearing). The focus of this thesis is on dam-calf rearing.

In terms of physical contact, this can be either full contact or partial contact. Full CCC allows unrestricted physical contact between the cow and her calf, which includes the expression of natural behaviour such as suckling, licking, and resting in contact. On the contrary, a partial CCC system allows only for limited physical contact and suckling is prevented (e.g. by housing the calf behind a fence-line or using udder nets to cover the cow's udder) (Sirovnik et al., 2020).

The duration of daily contact in CCC systems can be defined as either whole day or part-time contact. Whole day contact implies that the cow and the calf have the possibility to have physical contact for almost 24 h daily, except during milking hours. In contrast, part-time contact suggests CCC for only half a day (either during the day or the night, usually in between the two milkings) or during two (or more) short periods daily (usually around milking time (e.g. 2 x 30 min), while the rest of the day the cow and calf are separated) (Sirovnik et al., 2020).

Generally, CCC is allowed throughout the milk feeding period after which debonding (i.e. weaning and separation) occurs (Sirovnik et al., 2020). Weaning entails the either abrupt or gradual process of permanent deprivation off milk (Mills and Marchant-Forde, 2010), which under natural conditions occurs gradually at eight to ten months of age (Reinhardt and Reinhardt, 1981a). Separation describes the prevention of physical contact between the cow and her calf, which can occur either abruptly (i.e.

all physical contact is suddenly prevented, which is still the most commonly used in dairy production) or stepwise (i.e. first a reduction of the amount of daily contact between cows and calves prior to permanent separation) (Sirovnik et al., 2020). Examples of stepwise separation methods applied in CCC practices are placing the calf behind a fence-line to reduce the amount of contact (Johnsen et al., 2015a) or fitting calves with nose-flaps to prevent suckling prior to permanent separation (Loberg et al., 2008). Despite weaning at a relatively young age which is known to still induce stress in both cow and calf (Lambertz et al., 2015; Stěhulová et al., 2017), those methods are thought to mimic a key feature of the natural weaning process, namely the phase when the calf can no longer suckle milk although other forms of physical contact still occur (Špinka, 2006).

3.2 Potential benefits of maternal contact for animal welfare

Rearing calves in contact with cows seems a promising development to improve calf rearing management. So far, most research focused on rearing systems that allow full CCC for several weeks (see review by Meagher et al., 2019). As full CCC systems allow calves to drink according to their natural needs, one frequently reported effect of full CCC is increased calf growth reflected by growth rates of, for example, 1.20 kg/d (Grøndahl et al., 2007) and 1.41 kg/d (Ivemeyer et al., 2016) compared to the aimed growth rate of 0.85 kg/d in conventional rearing systems (Lely, 2016). Interestingly, during the first four days post-partum just the physical presence of the mother induced a similar effect, as bucket fed calves kept with their dam who wore an udder net (i.e. partial contact as suckling was prevented) had greater growth rates compared to early-separated calves fed similar amounts of milk. In fact, their growth rate in the first four days was similar to that of freely suckling calves (Krohn et al., 1999).

Prolonged CCC not only affects calves' physical development, but may also influence their behavioural development. Full CCC was found to minimize the frequency of abnormal behaviour in calves: besides a reduction in tongue rolling (Fröberg and Lidfors, 2009), several studies reported rare incidences of cross-sucking other calves and nonnutritive sucking of objects within the pen in full CCC systems (Roth et al., 2009; Veissier et al., 2013). Most likely, because suckling the dam results in larger volumes of milk intake (estimated at 9 L/calf/day during 9 weeks of part-time contact (de Passillé et al., 2008) and 15 L/calf/day during 13 weeks of full contact (Roth et al., 2009)), fulfillment of the sucking reflex, and an increased frequency of milk meals (Beaver et al., 2019b). Besides, calves reared with their dam showed more resting and rumination behaviour in the first eight weeks of life than group-reared calves without CCC (Fröberg and Lidfors, 2009). In social tests, the range of calves' social behaviour appears to be more diverse and active when meeting another conspecific (e.g. sniffing, head butting, rubbing, tail wagging, and other social play) compared to calves reared without CCC (Buchli et al., 2017; Flower and Weary, 2001; Jensen et al., 1999; Wagner et al., 2013). When integrating heifers into the dairy herd, those reared in full CCC tented to be more submissive than heifers reared without CCC

(Wagner et al., 2012). Possibly, this strategy reduced the amount of received aggression and gave heifers better access to resources, as reflected by trends of shorter latencies to lie down and longer duration of feeding (Wagner et al., 2012). In addition, longer and more frequent lying bouts in heifers reared with CCC on the second day after integration were interpreted as a faster adjustment to the cow barn, as calves reared with full CCC were familiar with the housing conditions contrary to conventionally reared calves (Zipp and Knierim, 2015). Allowing for prolonged CCC does not only promote the expression of natural behaviour in calves but also in their dams, as those alternative rearing systems allow cows to perform maternal behaviour (Jensen, 2011; Johnsen et al., 2021).

In recent surveys, health benefits of full CCC for both cow and calf were explicitly mentioned by farmers who adopted CCC on their farms (Neave et al., 2021; Vaarst et al., 2020). Moreover, a recent review showed that, with respect to cow health, the majority of studies indicated that suckling is protective against mastitis (Beaver et al., 2019a). Half the articles addressing neonatal diarrhea reported no differences between calves in full CCC compared to conventionally reared calves, although 6 out of 16 studies demonstrated beneficial effects of suckling (Beaver et al., 2019a).

4. Challenges and knowledge gaps for cow-calf contact

A recent systematic review by Beaver and colleagues (2019a) documented contradictory evidence about the animal health implications of rearing calves with or without early separation from the dam, including mixed results on calf morbidity and mortality. Thus, there is a clear need to further examine the health of cows and calves in CCC systems in comparison with rearing systems without CCC.

One major welfare challenge in CCC systems is the moment and way of debonding (i.e. weaning and separation) that is generally accompanied by strong signs of distress in both cow and calf, as reflected by increased activity and high-pitch vocalizations (Flower and Weary, 2001; Johnsen et al., 2015b; Veissier et al., 2013). These strong distress responses are attributable to the rather abrupt methods at a relatively young age (i.e. 8-12 weeks) compared to the natural weaning process that gradually occurs up to 8-10 months of age (Sirovnik et al., 2020). Now that CCC systems are increasingly implemented on European farms (e.g. in Denmark, the Netherlands, Germany, Norway, and France[2]), efforts to improve the conditions for cow-calf pairs throughout the debonding phase are needed to avoid poor welfare and increase the social acceptance of those systems (Jensen, 2017).

Another major challenge in CCC systems is the practical feasibility on current dairy farms. Existing barns are designed for adult cattle in terms of barn climate,

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[2] See the outcomes of European research projects like GrazyDaiSy, ProYoungStock, and Cow'n'Calf that identified and assessed innovative CCC rearing systems implemented by dairy farmers across Europe

feeding/water places, flooring, lying areas, animal traffic, and fencing. Therefore, keeping young calves within the dairy herd (when allowing full CCC) is considered to be challenging and requires (sometimes costly) adaptations inside the barn (Knierim et al., 2020; Vaarst et al., 2020). Moreover, ad libitum suckling by calves throughout the milk feeding period reduces machine-harvested milk yield, so the loss in farm income may be substantial (Johnsen et al., 2016; Knierim et al., 2020).

Potentially, a partial CCC system that prevents suckling could solve both bottlenecks. Firstly, in a partial CCC system calves will be fed by the animal caretaker, which results in the calf being nutritionally independent from the dam. Nutritional independence can reduce stress at debonding (Johnsen et al., 2018) and allows for a gradual weaning schedule (Khan et al., 2011). Secondly, it could be a potential compromise to meet farmers' concerns and increase the social acceptance of the dairy sector by permitting social interactions among the cow and her calf whilst keeping the non-suckling calf nearby and not within the dairy herd. Previous research reported that even in the absence of suckling, cow-calf pairs express behaviours suggestive of a mother-young bond (Johnsen et al., 2015c).

The need for broader and more systematic multidisciplinary investigations into pros and cons of CCC systems has been widely recommended (Beaver et al., 2019a; Johnsen et al., 2016; Krohn, 2001; Meagher et al., 2019). For instance, if we wish to enhance the "naturalness" of existing dairy systems through CCC, understanding preferences and motivations can inform us as to what behaviour is important to the animal, and what modifications facilitate those behavioural expressions (Beaver et al., 2019b). To date, no work has assessed to what extent dairy cows that were separated from their calf are still motivated to reunite with their young and to what extent the motivation of cows is affected by suckling. Besides behavioural consequences, there is limited comparative research available that evaluates the effect of partial CCC on biological functioning (e.g. animal health and performance) even though this is another key element of animal welfare. Last but not least, more knowledge on the efficacy of stepwise debonding methods to gradually reduce CCC and wean calves following different types of prolonged CCC is needed to minimize stress responses when debonding occurs.

5. Aim and outline of this thesis

The aim of this thesis is to assess how type of cow-calf contact (CCC) in calf rearing systems affects dairy cow and calf welfare in comparison to a rearing system without CCC. To this end, the objectives are to:

- assess cows' motivation to reunite with their calf
- examine the calf-directed affiliative behaviour of cows
- evaluate the health and performance of cow and calf
- compare gradual weaning and separation strategies to reduce stress during debonding

To study these objectives, two experiments were conducted (Figure 1). **Chapter 2** describes the experiment that was conducted at the Dairy Research and Education Centre of the University of British Colombia in Agassiz (Canada). In this experiment, I assessed the effects of early separation and suckling on the motivation of dairy cows to reunite with their calf. **Chapter 3, 4,** and **5** describe results of the experiment that

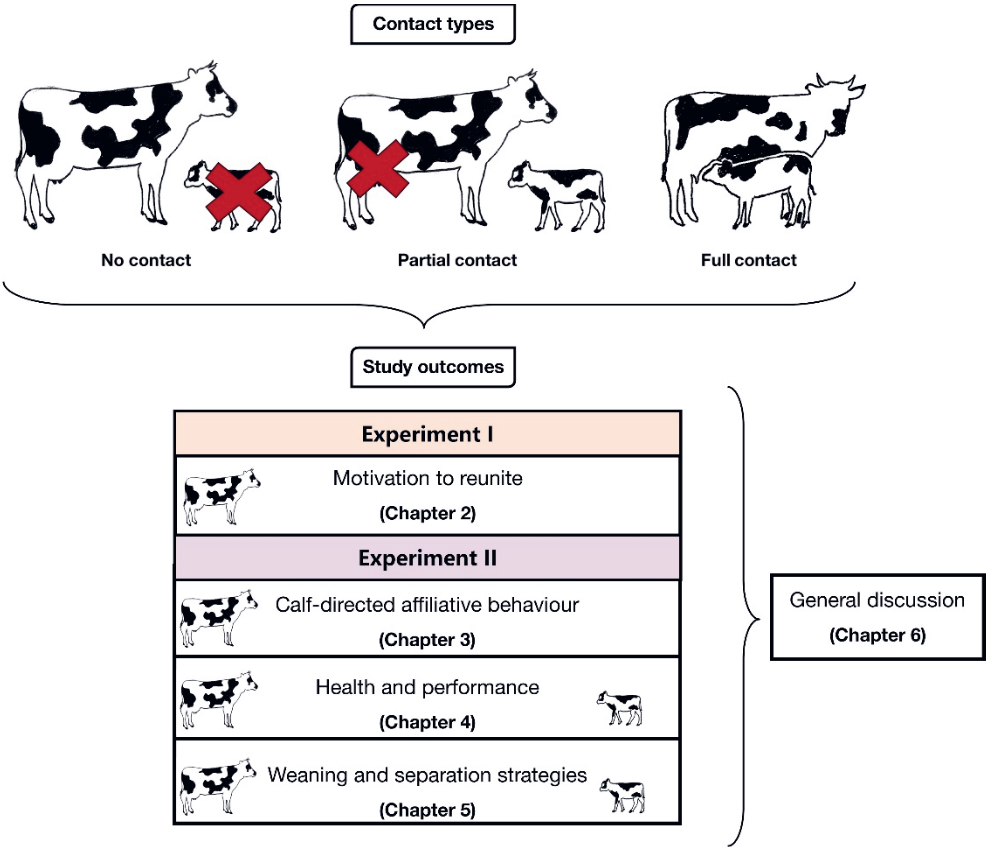

Figure 1. Overview of the thesis structure.

was conducted at the Knowledge Transfer Centre in Zegveld (the Netherlands). **Chapter 3** describes the calf-directed affiliative behaviour of cows allowed to have either partial or full contact with their calf. In **Chapter 4** the effect of type of CCC on the health and performance of cow-calf pairs is evaluated. For calves reared with either no CCC, partial CCC, or full CCC, I assessed the clinical health, physiological and immunological blood parameters, structural growth, and fecal microbiota composition. In addition, I compared the milk production and composition, disease incidence, first insemination moment, and the pre- and postpartum metabolic status of the dams. **Chapter 5** focusses on strategies to reduce stress responses in cow-calf pairs during debonding after prolonged CCC. Cow-calf pairs with partial and full contact were either subjected to gradual debonding by reducing physical contact before calves were fully weaned or by reducing physical contact after calves were fully weaned. The animals' performance, stress responses, and health status were compared to cow-calf pairs without CCC. Finally, all results are brought together and discussed in **Chapter 6**. Furthermore, recommendations are provided that could improve CCC rearing practices and may provide an enabling environment for the transition to CCC systems in dairy farming.

Chapter 2

Effect of cow-calf contact on cow motivation to reunite with their calf

Margret L. Wenker[1,2,3], Eddie A.M. Bokkers[2], Benjamin Lecorps[1], Marina A.G. von Keyserlingk[1], Cornelis G. van Reenen[2,3], Cynthia M. Verwer[4], Daniel M. Weary[1]

[1] Animal Welfare Program, University of British Columbia, Vancouver, Canada
[2] Animal Production Systems group, Wageningen University & Research, Wageningen, the Netherlands
[3] Livestock Research, Wageningen University & Research, Wageningen, the Netherlands
[4] Louis Bolk Institute, Bunnik, the Netherlands

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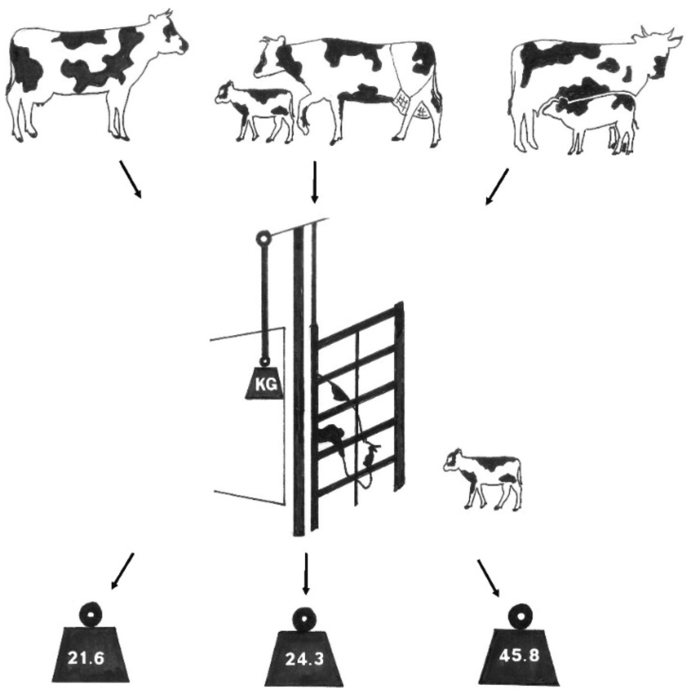
Abstract

Early cow-calf separation prevents much of cows' natural maternal behaviour. Early separation is thought to prevent the development of a cow-calf bond. To assess this bond, we measured motivation of dairy cows to reunite with their calf. To vary the degree of bonding, some cows were allowed continued contact with their calf and others were separated from their calf soon after birth, following standard practice on most farms. Among cows allowed continued contact, some were able to suckle their calf and others were prevented from suckling (by covering the cow's udder with an udder net). Cows were habituated to the weighted-gate apparatus before calving by daily training with the (un-weighted) gate. After calving, cow willingness to use the gate was assessed by determining if she would push open the gate to access to her own calf. Testing occurred once daily, with weight on the gate gradually increased. After passing through the gate, the dam's calf-directed behaviour was recorded. Suckled cows pushed a greater maximum weight (45.8 ± 7.8 kg) than separated cows (21.6 ± 6.7 kg) and non-suckled cows (24.3 ± 4.5 kg), with no differences between separated and non-suckled cows. Once reunited, latency to make nose contact and duration of licking did not differ between treatments. We conclude that motivation for calf contact is greater for cows that are suckled.

Graphical abstract



1. Introduction

Maternal behaviour of cows can be expressed through licking, nursing, attentiveness and proximity towards the young, and protecting it from potential threats (Grandinson, 2005; von Keyserlingk and Weary, 2007). On most dairy farms the cow's ability to express maternal behaviour is limited, as standard practice is to remove the calf within a few hours after birth (Stěhulová et al., 2008), but cows still perform maternal behaviour when given the opportunity (Illmann and Špinka, 1993; Jensen, 2011; von Keyserlingk and Weary, 2007). To date no work has assessed to what extent dairy cows are still motivated to reunite and interact with their young. An essential component of maternal behaviour is nursing the calf (Grandinson, 2005), but there is some evidence that the mother-young bond may establish in the absence of suckling: cow-calf pairs spent similar time in proximity regardless of the cows' ability to suckle her calf (Johnsen et al., 2015c). It is unknown to what extent the maternal motivation to reunite with the calf is affected by suckling.

Motivation tests can be used to evaluate the value animals attribute to an experience or resource (Kirkden and Pajor, 2006). Once an animal has learned to perform a task, the effort (price) for each access can be increased (Jensen and Pedersen, 2008; Webb et al., 2014). Pushing a weighted door to gain access to a reward is one method to assess motivation (Petherick and Rutter, 1990; Tucker et al., 2018). This technique has been used to assess the importance of a nest box in chickens (Kruschwitz et al., 2008), a water bath in farmed mink (Mason et al., 2001), pasture access (von Keyserlingk et al., 2017) and access to an automated brush (McConnachie et al., 2018) in dairy cattle. The more weight an animal pushes, the stronger the motivation to access that particular resource (Kirkden and Pajor, 2006).

The aim of this study was to assess the motivation of dairy cows with different levels of cow-calf contact to reunite with their calf. We hypothesized that cows routinely kept with their calf would be more motivated than control cows that has been separated from their calf soon after birth; previous work has shown that early separation greatly diminishes the bond between cow and calf (Flower and Weary, 2001). Following Johnsen et al. (2015c) who found no effect of suckling on the cow-calf bond, we hypothesized that among cows kept with their calf, the ability to nurse would not affect cow motivation.

2. Materials and methods

2.1 Animals and treatments

Holstein cows that recently gave birth (n = 34; mean parity 3 ± 0.3 lactations), were assigned randomly without replacement to one of the three treatments within each block of three successive calvings. The treatments were: i) separated from their calf within 2 h after birth and allowed no contact outside of testing sessions (n = 11, separated), ii) allowed to spend nights with their calf but fitted with an udder net to prevent suckling (n = 11, non-suckled), or iii) allowed to spend nights with their calf and to be suckled (n = 12, suckled). All cows were milked twice a day. Nightly cow-calf contact was allowed from approximately 18:30 h until 06:30 h. See Appendix 1 for more details. All methods were carried out in accordance with relevant guidelines and regulations.

2.2 Motivation test

A one-way push gate (adapted from von Keyserlingk et al., 2017) in the test pen allowed access to the calf. The push gate could be opened by physically pushing against a plate attached to the gate. See Appendix 2 for an illustration of the push gate.

2.2.1 Training

The training period was divided into two phases. In the first phase, 3 weeks before expected calving date, cows were trained to open the push gate to access fresh feed (6 repetitions per day). The second training phase started 2 to 4 d after calving; cows were trained to reunite with their calf via the push gate (1 repetition per day). During this stage, fresh feed was freely available inside the test pen before accessing the push gate; thus cows learned that opening the gate would provide access to the calf only and not to fresh feed. To be included in the experiment, cows had to push open the gate successfully in both phases. See Appendix 1 for more details.

2.2.2 Testing

The bond between cow and calf develops rapidly after birth (Flower and Weary, 2001), as such that responses to separation are much stronger after just 4 d of continued contact (relative to separation in the hours after birth; Weary and Chua, 2000). For this reason, we started testing 5 to 8 d after calving. Cows were tested individually, after the afternoon milking (i.e.17:00 - 18:30 h); the order in which cows were tested was randomized.

The test started with the gate closed but with no weight attached to the pulley system. Weight was then increased every day upon success: initially to 2.3 kg, then to 9 kg followed by 9 kg increments each day. If a cow was unsuccessful in pushing the gate open, she was retested at that same weight for the following 2 d. Testing ended when a cow failed to open the gate for three consecutive days or if she pushed the maximum weight of 90 kg. After each test session, cow and calf were returned to their corresponding pen depending upon treatment. Maximum weight pushed for each cow was recorded, and a digital camera was used to record calf-directed behaviour after reunion (i.e. latency to make nose contact, duration of licking).

2.3 Statistical analysis

Three cows were excluded from the analyses: one cow from the separated group (she did not meet the learning criterion in the second training phase; all other cows met this criterion), one cow in the non-suckled group (her calf learned to evade the udder net and started suckling half way the experiment), and one cow in the suckled group (her calf was born premature and never learned to suckle), resulting in a final sample of 31 cows (10 in both the separated and non-suckled groups, and 11 in the suckled group). All statistical analyses were performed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC), treating cow as the experimental unit. Residuals were visually assessed for normality. Significance was declared at $P < 0.05$.

2.3.1 Weight pushed

To test the effect of treatment on the maximum amount of weight pushed, the least significant difference mean comparison test in PROC GLM procedure was used. Residuals were inspected for normality and outliers.

2.3.2 Calf-directed behaviour at reunion

Behavioural observations of 7 separated cows, 10 non-suckled cows, and 11 suckled cows were included; 3 separated cows did not open the gate to reunite with their calf during the test. As cows that pushed the gate successfully had more test sessions than the unsuccessful ones, latency to make nose contact and duration of licking were averaged for each cow. None of the recorded behaviours were normally distributed, so treatment differences were analyzed using a Wilcoxon rank-sum test.

3. Results

3.1 Weight pushed

The mean maximum weight pushed ± SEM (in kg) did not differ between separated cows (21.6 ± 6.7) and non-suckled cows (24.3 ± 4.5; $p = 0.78$); whereas, suckled cows pushed a greater maximum weight (45.8 ± 7.8) than separated cows ($p = 0.03$) and non-suckled cows ($p = 0.01$) (Figure 1).

3.2 Calf-directed behaviour at reunion

The latency for cows to make nose contact (median [95% CI] in s) did not differ between separated cows (10.5 [5.0-120.0]), non-suckled cows (10.3 [3.7-78.0]), or suckled cows (22.4 [2.7-64.6]; $p = 0.97$). In addition, no difference was found in the duration of licking (median [95% CI] in s) between separated cows (43.4 [0.0-70.5]), non-suckled cows (18.1 [0.0-85.5]) or suckled cows (12.8 [1.2-27.3]; $p = 0.30$).

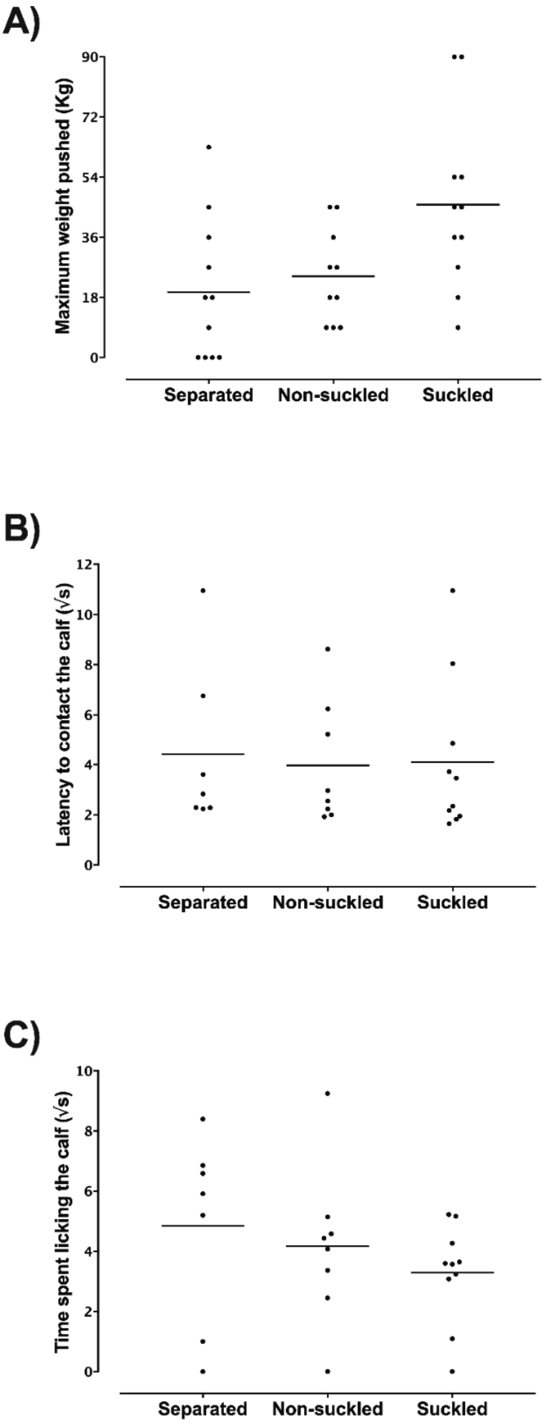


Figure 1. Cow motivation to reunite with their calf, and behaviours expressed upon reuniting, in relation to treatment; nightly cow-calf contact was allowed for non-suckled and suckled cows, but separated cows only spent time with their calf during the test. Results are shown separately for maximum weight (kg) pushed by dairy cows to reunite with their own calf **(A)**, and for those cases in which the cow successfully opened the gate, latency (sqrt(s)) to contact the calf **(B)** and time (sqrt(s)) spent licking the calf **(C)**. Values for each calf are shown separately (as black circles), with median values shown as a solid line.

N.B. Panel A shows 11 cows for separated treatment, versus 10 cows in the non-suckled and 11 in the suckled treatment. One of the four cows in the separated treatment that failed to open the weighted gate in test phase (i.e. appearing as 0 kg pushed in the plot), also failed to open the gate during the second training phase and on this basis was excluded from our statistical analysis. Measures of how cows interacted with their calves during the test session (i.e. latency to approach the calf (panel B) and time spent licking the calf (panel C)) are only available for the cows that actually opened the weighted gate during the test session, resulting in a sample size of 7 for the separated treatment, versus 10 cows in the non-suckled and 11 in the suckled treatment.

4. Discussion and conclusion

Our results showed that suckled cows were more motivated to reunite with their calf than were separated cows and cows that were not separated but also not suckled. Previous work suggested that a strong cow-calf bond can be established even in the absence of suckling (Johnsen et al., 2015c), but the results of the present study indicate that suckling increases the cow's motivation to reunite with her calf. This increased motivation may be due to a stronger bond between the cow and calf. One of the hormones involved in mother-young bonding is oxytocin (Uvnäs-Moberg, 1998), and this is known to be increased in suckled cows compared to non-suckled cows (Lupoli et al., 2001). Oxytocin and the endogenous opioids released during suckling have a rewarding effect (Nelson and Panksepp, 1998), and suckling has been considered as one of the most hedonic maternal activities (Olazábal et al., 2013).

In the present study, motivation to reunite with the calf did not differ between separated and non-suckled cows. Close contact with the calf in the first few hours after birth is considered essential to establish a maternal bond, and provides the dam with olfactory and gustatory input to recognize on her offspring (Edwards and Broom, 1982). It has been suggested that as little as 5 min of contact directly after birth is enough to establish a mother-young bond that could withstand 12 h of separation (Hudson and Mullord, 1977), although interactions with the young are considered important to maintain high maternal motivation (Poindron, 2005).

Cows in all three treatments expressed similar amounts of calf-directed behaviour once reunited, preventing any meaningful conclusions based upon these measures.

There are a number of limitations to the current study. Previous studies using a similar push gate showed that dairy cows are highly motivated to access pasture (von Keyserlingk et al., 2017) and an automated brush (McConnachie et al., 2018), but differences in the way these gates were installed prevents meaningful comparison in the weights pushed across studies. We suggest future studies directly monitor the force applied to the gate, rather than the weights lifted as reported here, as the force applied to the gate will be more easily compared across studies. The current study used a between-subject design, such that each cow was allocated to a single treatment. Cows varied considerably in their responses, both in the weight pushed and the behaviours shown when reunited with their calves, and this individual variation potentially obscured treatment differences with our design. In addition, we had expected that the separation treatment would act as a type of null control, with cows showing little or no motivation to access their calf. Our finding that some of the separated cows worked to access their calf, and engaged in a considerable degree of calf directed behaviour upon being reunited, was an unexpected and important finding, but also diminishes our ability to use this treatment as a type of null control. Future work should consider using larger samples, or within-subject designs comparing each cow's motivation to access the calf versus other valuable resources (such as pasture or food (von Keyserlingk et al., 2017)).

Acknowledgements

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Appendix 1. Detailed materials and methods

Animals and experimental setup

This research was conducted at the UBC Dairy Research and Education Centre in Agassiz (Canada) from June 2018 to November 2018. The experimental cows had an average body weight of 676 ± 14 kg. The treatments only differed in the level of cow-calf contact that was allowed during the trial. Non-suckled dams were fitted an udder net (model Nr 88439503, DeLaval, Tumba, Sweden) to cover the udder directly following parturition. During the day (between 06:30 h and 17:00 h), cows were moved to a separate pen without visual contact with the calves (see section Housing). All cows were milked twice a day at approximately 07:00 h and 17:00 h in a double 12 stall parallel milking parlour. Both non-suckled and suckled cow-calf pairs were reunited every day after afternoon milking. Cows were fed a total mixed ration (TMR; shown as percent of dry matter, consisting of 90.9% alfalfa hay, 80.8% grass, 29.8% corn silage, and 89.2% concentrates) twice a day at approximately 08:30 h and 16:00 h. All calves were provided ad libitum fresh milk twice a day at approximately 07:00 h and 16:00 h using a portable milk bar (Milk Bar 10 calf feeder, Coburn, USA). All calves were trained to use the milk bar by guiding them to the nipple in the first week of life. In the calf creep area (see section Housing) ad libitum water, hay, and TMR was provided.

Housing

Two identical free stall pens were used to house the cows containing each 12 lying stalls deep bedded with sand (115 cm wide x 205 cm long), 6 electronic feeding bins, and 1 electronic water bin (Insentec, Marknesse, the Netherlands) (Figure 1A). During the day (between 06:30 h and 17:00 h) all experimental cows were housed in the same pen (i.e. ‘home pen’), the group size never exceeded 12 animals. All experimental calves were kept in a sawdust-bedded creep area (8.5 m long x 3.0 m wide) (Figure 1A). At night (between 18:30 h and 06:30 h) separated cows stayed in the home pen and their calves stayed in the calf creep area. Non-suckled and suckled cow-calf pairs were moved to an adjacent pen (i.e. ‘contact pen’). Plywood plates (160 cm high) were placed between the two free stall pens to prevent visual contact.

Calving management

Calving took place in individual indoor maternity pens bedded with sawdust. Separated calves were removed from the dam and placed in the calf creep area within 2 h after birth (median [minimum, maximum] in min: 15 [5, 110]). After parturition separated cows spent on average 15.8 h (± 0.6) in the maternity pen before they returned to the home pen. Non-suckled and suckled calves stayed with the cow in the maternity pen for on average 32.0 h (± 0.6) and were moved to the calf creep in the morning. All fresh cows were moved to the home pen after morning milking. All calves were bottle fed 4 L of colostrum within 6 h after birth. In the maternity pen non-suckled and suckled calves were also bottle fed 4 L fresh milk twice a day until they moved to the calf creep area.

Push gate apparatus

In the test pen (Figure 1B) a one-way push gate (adapted from von Keyserlingk et al., 2017) was installed and a pulley was used to load the push gate with weights (see electronic supplementary material for a video). A plywood plate (0.6 x 0.9 m) was attached to the gate for cows to place their head against when pushing the gate. The sides of the entrance to the gate were both covered with plywood boards (1.2 m x 2.4 m) to prevent cows from being visually distracted once approaching the gate.

Training

In the first training phase dry cows were trained on a daily basis to go through the push gate (on average 6 repetitions) to access a bucket with fresh TMR (for 30 s; Figure 1B). Training started with the gate completely open (i.e. gate angle 45°) for at least 2 repetitions to get familiar with the task, then the gate was closed progressively by 15° until it was fully closed with 2.3 kg attached (Table 1). A cow had to complete each step before she was allowed to progress to the next one. If a cow failed, the gate was returned to its previous position to repeat this learning step again. Training was considered successful when a cow opened the fully closed gate with 2.3 kg for 3 consecutive repetitions. Each daily training session lasted approximately 15 min per cow. All cows passed this training phase within 5 d.

The second training phase after calving lasted 3-5 d; cows were trained to reunite with their calf via the push gate. After afternoon milking, all experimental cows were brought to a waiting pen adjacent to the test pen (Figure 1C). From there, cows were individually brought to the test pen. The calf was fitted a rope halter inside the calf creep area and was brought into the alley behind the push gate. Here, it was restrained (rope of 1 m) at approximately 2 m from the push gate. In this training phase cows had only one repetition each day for each of the 3 learning steps (Table 1). For the last learning step TMR was put inside the test pen on the opposite side of the push gate (Figure C); thus, cows could choose to go to the left to the red bucket with fresh TMR or to push open the gate and thus access her calf. Once they opened the push gate, cows were able to spend 2 min with their calf after which they were returned to either the home pen or contact pen depending on the treatment. For non-suckled and suckled cows the three training days were also used to get them familiar with the routine of having cow-calf contact overnight and being separated during the day. To be included in the study, cows had to pass the push gate voluntarily in learning step III (Table 1) within the maximum set time of 5 min after entering the test pen.

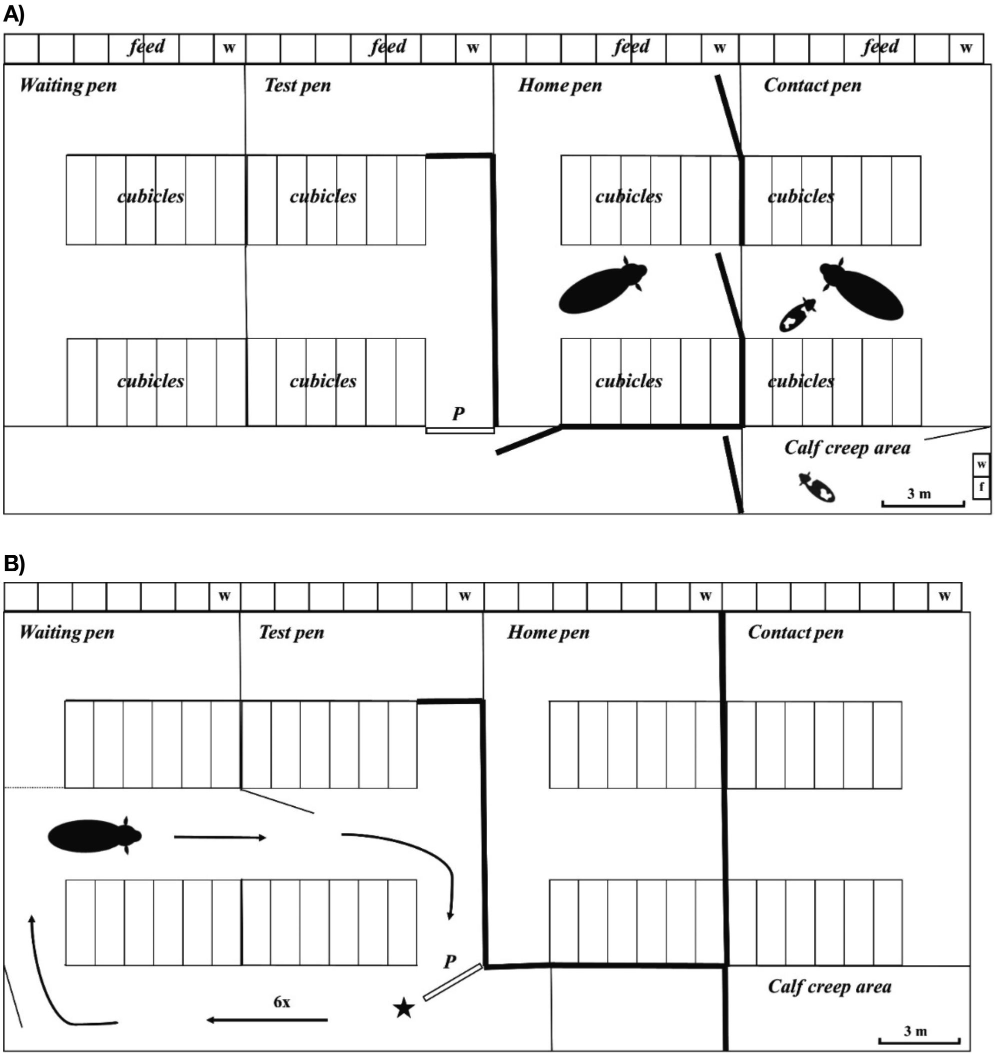

Figure 1. Experimental set-up. **A)** During the day (between 06:30 h and 17:00 h) cows were kept as one dynamic group in the 'home pen' (9.5 m x 11.5 m), all calves were housed in the sawdust-bedded calf creep area (9.5 m x 3 m). At night (between 18:30 h and 06:30 h), non-suckled and suckled cow-calf pairs were moved to the 'contact pen'. Separated animals stayed in the home pen. **B)** In the first training phase cows were trained to open the push gate apparatus. A red bucket with fresh TMR was used as reward, and was placed in the alley behind the push gate. ★ = TMR, P = push gate, w = water, f = feed. ▬▬ = plywood, ····· = route separated cow-calf pairs to their pens, - - - - = route non-suckled and suckled cow-calf pairs to contact pen.

C)

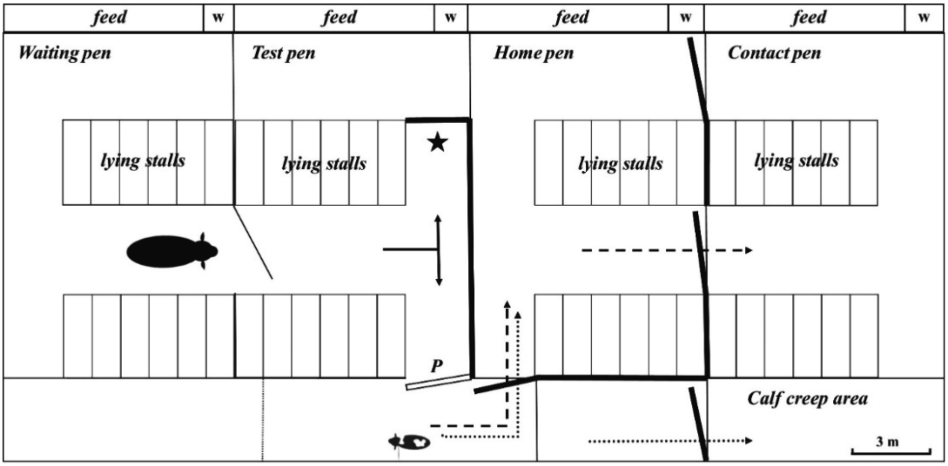

Figure 1 continued. Experimental set-up, **C)** In the second training phase cows were trained to reunite with their calf. The calf was tethered in the alley behind the push gate and TMR was present in the test pen (learning step III). After passing the second training phase, the test started the following day. After training and testing separated cows were returned to the home pen and their calves to the calf creep area; non-suckled and suckled cow-calf pairs would be brought to the contact pen. ★ = TMR, P = push gate, w = water, f = feed. ▬▬ = plywood, ····· = route separated cow-calf pairs to their pens, - - - - = route non-suckled and suckled cow-calf pairs to contact pen.

Table 1. Details on the training to operate the push gate.

Training phase	Learning step	Gate angle	Reward
First training phase	Step 1[a]	45°	30 s to eat TMR
	Step 2	30°	30 s to eat TMR
	Step 3	15°	30 s to eat TMR
	Step 4	0°	30 s to eat TMR
	Step 5	0° + 2.3 kg	30 s to eat TMR
Second training phase	Step I	30°	2 min with calf
	Step II	15°	2 min with calf
	Step III[b]	15°	2 min with calf

[a] All cows made at least 2 repetitions in this step before we proceeded to the next step.

[b] In this training step TMR was present in the test pen.

Behavioural observations

During the test a digital camera (Sony Handycam HDR-CX560) fixed on a tripod was placed in the alley at 7 m away from the calf to record latency to make nose contact and duration of licking. Latency to make nose contact with the calf was defined as time (in s) from the cow moving her shoulder through the gate until her nose contacted any part of the calf's body. Duration of licking the calf was defined as time (in s) that the cow licked any part of the calf's body.

Appendix 2. Push gate illustration

Figure 1. Illustration of the push gate apparatus.

Chapter 3

Calf-directed affiliative behaviour of dairy cows in two types of cow-calf contact systems

Margret L. Wenker[1,2], Cornelis G. van Reenen[1,2], Daiana de Oliveira[3], Kirste McCrea[3], Cynthia M. Verwer[4], Eddie A.M. Bokkers[1]

[1] Animal Production Systems group, Wageningen University & Research, Wageningen, the Netherlands
[2] Livestock Research, Wageningen University & Research, Wageningen, the Netherlands
[3] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Uppsala, Sweden
[4] Louis Bolk Institute, Bunnik, the Netherlands

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Abstract

There is an interest in alternative rearing systems that allow for prolonged cow-calf contact (CCC). Yet, a better understanding of cows' affiliative behaviour in those systems is needed. We evaluated the effect of type of CCC on calf-directed affiliative behaviour in dairy cows. Cows were permitted to have either: i) partial contact (PC) with their calf; calves were housed in a pen adjacent to the cow area allowing limited physical contact on initiative of the dam but no suckling (n = 18), or ii) full contact (FC) with their calf including suckling; calves were housed together with the dams in a free stall barn (n = 20). Proximity and physical contact between the cow and her own calf were recorded between 0-48 h postpartum in an individual maternity pen, and from 1-5 weeks postpartum in a free stall barn. Data were analyzed with generalized linear models, except for behaviour with excess of zero-valued data where a Kruskal Wallis test was used. Principal component analysis (PCA) was carried out to identify consistency of behaviour in the maternity pen and free stall barn. After parturition, latency to onset of allogrooming did not differ among treatments (mean ± SE, 8 ± 3 min, P = 0.39). Throughout the first 48 hours postpartum, no treatment differences were found in percentage of observed time spent allogrooming the calf (PC: 7.7 ± 1.3%, FC: 9.5 ± 1.5%), standing in proximity (≤1 m radius) (PC: 22.9 ± 2.1%, FC: 21.2 ± 2.1%), or lying in proximity (PC: 30.5 ± 4.3%, FC: 32.5 ± 3.2%) (P > 0.10). However, in the following 5 weeks, relative to PC cows, FC cows spent more time on average in close proximity to their calf (10.9 ± 0.1% versus 3.1 ± 0.4%, P < 0.001), and on allogrooming (2.1 ± 0.2% versus 0.5 ± 0.1%, P < 0.001). PCA revealed four components (explaining 76% of the variance). Lying in close and standing in far proximity in the maternity pen loaded (positive, negative, respectively) onto component 1, whereas physical contact and standing in close proximity in the free stall barn loaded negatively onto component 2. Standing in close proximity in the maternity pen loaded onto component 3, and standing 1-2 m near the calf in the free stall barn loaded onto component 4. Our results indicate that, in comparison with FC, PC decreases the expression of calf-directed affiliative behaviours in dairy cows, except in the 48 hours following parturition. The partial CCC set-up limited the calf's accessibility, whereas calves in full CCC could initiate contact as well. Nonetheless, large inter-individual differences in calf-directed affiliative behaviour were found that lacked consistency.

1. Introduction

Cattle are known to form long-lasting social bonds in small stable groups (Bouissou et al., 2001; Reinhardt and Reinhardt, 1981b). A social bond can be defined as a preferential mutual, affectionate relationship characterized by, among others, spatial proximity, synchronized behaviour, and allogrooming (Bouissou et al., 2001; Newberry and Swanson, 2008). These affiliative behaviours are primarily of a positive nature, provide opportunities for social support in challenging situations, and are accompanied by specific calming and rewarding physiological reactions (Newberry and Swanson, 2008; Rault, 2012). Hence, allowing for the formation and maintenance of social bonds between cattle, also under commercial conditions, is considered important in ensuring their welfare (EFSA, 2009).

Generally, the first established social bond in life is the bond between mother and young, which is essential for survival of a new-born calf under natural conditions (Newberry and Swanson, 2008). The mother's affiliative behaviour is essential for bonding, which is driven by complex internal processes (e.g. hormones around parturition) and external factors (e.g. presence of a new-born), and is known to be affected by maternal experience (Olazábal et al., 2013; von Keyserlingk and Weary, 2007). However, on most commercial dairy farms, calves are separated from the dam within 24 h postpartum; a practice which raises public concern (Busch et al., 2017; Hötzel et al., 2017; Ventura et al., 2013).

Alternative systems, where dairy cows and their calves can bond by staying in contact for a prolonged period of time, are receiving interest from various stakeholders due to the opportunities it may provide for increasing the social acceptance of the dairy sector, for example by allowing the expression of natural behaviours, and better responding to consumer demands (Beaver et al., 2019a; Brombin et al., 2019). These so-called cow-calf contact (CCC) systems can differ in the type of physical contact allowed between the dam and her calf, and are generally described as full or partial CCC systems (Sirovnik et al., 2020). Full CCC (i.e. unrestricted physical contact including suckling) typically involves keeping calves together within the herd, which allows cow-calf pairs to express affiliative behaviours, such as licking each other, suckling, and resting in contact (Johnsen et al., 2016; Wagenaar and Langhout, 2007). Yet, dairy producers that allow full CCC may face several challenges, such as loss of saleable milk, stress at separation, milk ejection disturbances, hygiene issues, and a poor human-calf relationship (Johnsen et al., 2016). One way to overcome those challenges is to reduce the duration of daily contact via part-time CCC systems, in which cow and calf have contact only during specific moments of the day (e.g. half-day or only briefly after milking) (Sirovnik et al., 2020). However, those systems are considered as labor intensive (Johnsen et al., 2016). Another option would be to allow partial CCC, where contact can be restricted by preventing suckling and limiting the physical contact (e.g. housing the calf behind a fence adjacent to the dams or using an udder net for the dam) (Sirovnik et al., 2020). Calves' nutritional independence from the dam seems to reduce stress at separation (Johnsen et al., 2018) and minimize loss of saleable milk due to suckling (de Passillé et al., 2008), whereas limiting physical contact could benefit practicability of CCC while still allowing for social interaction among cow and calf. Nevertheless, suckling is considered to be the most important and common care-giving behaviour in cattle (Lévy, 2016; von Keyserlingk and Weary, 2007). Recent work has shown that the social bond seems to grow stronger when suckling is allowed, as suckled dams showed an increased motivation to reunite with their own calf compared to non-suckled dams (Wenker et al., 2020). However, a better understanding of the social interactions in various CCC systems is needed before recommendations for specific systems can be made (Meagher et al., 2019).

Therefore, the objective of the present study was to evaluate the extent to which the type of CCC alters calf-directed affiliative behaviour of dairy cows over time. It was hypothesized that full CCC cows would show more affiliative behaviours towards their calf than partial contact cows in the weeks following calving, and that this effect would be less profound in the hours succeeding parturition.

2. Materials and methods

This study was conducted at the Knowledge Transfer Centre in Zegveld (the Netherlands) from February 2019 to July 2020. All applicable international, national, and/or institutional guidelines for the care and ethical use of animals were followed. The experimental design was approved by the Central Committee on Animal Experiments (The Hague, the Netherlands; approval number 2017.D-0083).

2.1 Animals and treatments

Thirty-eight Holstein Friesian cows were included in this study with a parallel group design. Cows were included at calving if they gave birth to a single heifer calf without substantial calving difficulties or health problems. In order for calves to have an similar aged peer, every two cows that calved successively were assigned to the same treatment, and to have either: i) partial contact (PC) with their calf; calves were housed in a pen adjacent to the cow area allowing physical contact on initiative of the dam but no suckling (n = 18), or ii) full contact (FC) with their calf including suckling; calves were housed together with the dams in a free stall barn (n = 20). The mean parity of PC cows was 3.5 ranging from 1 to 6 versus a mean parity of 2.5 for FC cows that ranged from 1 to 7. Treatment order for every set of two cows was randomized. CCC was allowed for 10 weeks. In the present study, we report data from cow-calf pairs during the first 5 weeks postpartum. However, this study was part of a large longitudinal experiment that followed the animals for longer: cows were studied until 12 weeks postpartum and calves were studied up to 6 months of age.

2.2 Calving management

Based on signs of imminent calving, cows were moved into an individual indoor straw-bedded maternity pen (3.0 m wide × 5.1 m long). Cows that were about to calve were video-monitored and the calving was assisted if necessary. Despite farm staff's regular checks of calving signs, seven cows (two in the PC group and five in the FC) calved in the dry-cow pen resulting in the calf being born on the slatted floor. Those cow-calf pairs were still included in the trial, but behaviours were only scored once a cow-calf pair was present in the maternity pen. Immediately after birth, navels were dipped with 2% iodine. Calves were briefly separated from the dam in order to measure the birth weight on a full-body calf scale (Type 8700, Welvaarts, the Netherlands). PC calves were placed in a cuddle-box (consisting of four plywood plates of 1.2 m wide × 0.8 m high) inside the maternity pen. The cuddle-box (see Appendix 1 for an illustration) prevented suckling, while still allowing tactile, visual, audible, and olfactory contact, and was placed in one of the corners across from the feeding rack. The cow could lick and sniff her calf when the calf was standing or lying, by moving her head into the box to reach the calf. When the barn temperature was below 10 °C, calves were provided with a heating lamp.

Cows in the maternity pen were milked twice daily with a mobile milking machine (Mini-milker, Kurtsan, Turkey). All calves were bottle fed with on average 3 L of colostrum from their own mother within 2 h after birth. Calves in the PC treatment group received an additional 2 L colostrum by bottle at 8–12 h, as well as at 20–24 h after birth. After the first colostrum meal by bottle (to standardize first colostrum consumption), FC calves were allowed to suckle the remaining colostrum directly from the dam's udder. Camera footage confirmed farm staff's suspicion that seven FC calves (but none of the PC calves) suckled their first colostrum before farm staff could bottle-feed them, however 4 of them did still receive some colostrum by bottle. Both PC and FC calves stayed with their dam in the maternity pen for about 72 h, after which they moved to designated group pens in the free stall barn.

2.3 Housing and feeding

The PC and FC cow-calf groups were housed inside a free stall barn in two dynamic group pens, one for each treatment (Figure 1). All experimental cows were milked twice a day at approximately 08:00 h and 18:00 h in the milking parlor with a five-point open tandem side and 11 side-by-side places. Cows were fed grass silage (early spring cuttings) once a day at approximately 09:30 h. Feed was pushed automatically (MoovPro, JOZ, the Netherlands) to the feeding rack 8 times a day. Additionally, cows could eat up to 10 kg of concentrates per day that were provided partly in the milking parlor and by an individual concentrate feeder. In both group pens cows had access to an automated brush (swinging cow brush, DeLaval, the Netherlands). When the barn temperature was below 10 °C, all young calves were fitted with a calf jacket for the first three weeks of life.

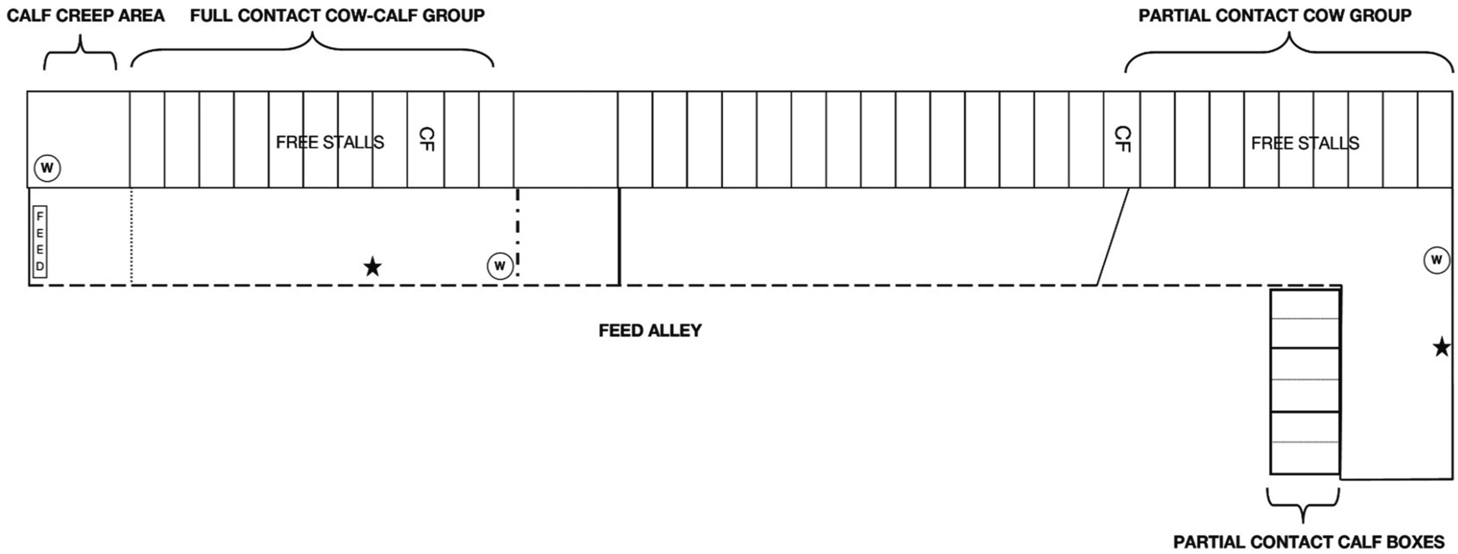

Figure 1. The full contact (FC) and partial contact (FC) cow-calf groups were housed in two dynamic group pens inside a free stall barn. The free stall barn was naturally ventilated with open sidewalls and had perlite-bedded lying stalls (1.1 m × 3.0 m). The alley was covered with rubber flooring and cleaned 8 times a day by an automated scraper. Within the FC group pen, FC calves had access to a calf creep area (3.3 m × 4.8 m) with a straw-bedded lying area (3.3 m × 1.9 m), water bucket, hay and concentrates. A metal bar hindered cows to access this area. PC calves were housed individually (1.0 m × 1.6 m) in a straw-bedded calf box for the first two weeks, after which they were pair housed in the same box (2.0 m × 1.6 m) with their similar-aged peer. The calf boxes were placed behind a wall (1.2 m high) to limit physical contact and prevent suckling. The area behind the wall where cows could stand to interact with their calf was 2.5 m × 6.0 m. W = water, CF = individual concentrate feeder, ★ = automated brush

Figure 2. Partial cow-calf contact set-up. Calves were housed in a calf box (individually for the first two weeks and pair-housed afterwards) behind a 1.2 m high wall, cows could move their heads over the wall to lick and sniff the calf.

PC calves were kept in a straw-bedded calf box (Topcalf Duo-Flex, Schrijver, the Netherlands) behind a wall (1.2 m high) adjacent to the PC cow group pen. This set-up allowed for visual, auditory, olfactory, and limited tactile contact between cow-calf pairs (Figure 2). Cows could move their head over the wall and when the calf was standing on the other side, cow-calf pairs could sniff and lick each other. One calf box could house two calves individually (1.0 m × 1.6 m), but also offered the opportunity to pair house them (2.0 m × 1.6 m) by removing the partition wall in the middle of the box. PC calves were housed individually for the first two weeks, after which they were pair housed with their similar-aged peer in the same box. In each calf box ad lib water, hay and concentrates (Topfok Kalf, de Samenwerking, the Netherlands) were provided as soon as the calf moved into the free stall barn (at 3 days of age). The PC group never exceeded more than six cow-calf pairs. Calves in the PC group were provided bulk tank milk in individual teat buckets following a fixed feeding schedule (see Appendix 2) after all three colostrum meals were consumed. Milk was provided around 08:00 h, 13:00 h, and 18:00 h. Bulk tank milk was heated up to 41 °C using a milk taxi (Milchtaxi 2.0, Holm & Laue, Germany) before being fed to the calves.

FC calves were housed together with the dams in the FC group pen, but had access to a calf creep area (inaccessible for the dams). The calf creep area provided them with a straw-bedded lying area and ad lib water, hay and concentrates from the day the new-born calves moved into the free stall barn. The FC group never exceeded more than eight cow-calf pairs. Except when cows were milked, FC calves could suckle their dams and, if allowed, other dams.

2.4 Behavioural observations

Four neighbouring maternity pens were equipped with two cameras each (Hikvision; Model DS-2CE16H5T-ITE). Cameras were installed in bird's eye view across from each other above the pens. The behaviour of PC and FC cow-calf pairs was video recorded during the first 48 hours after parturition. From these recordings (in the maternity pen) the behaviours described in Table 1 were continuously monitored at 3-min intervals during five observation periods (i.e. 0–4 h, 12–14 h, 22–24 h, 36–38 h, 46–48 h postpartum) by one of the two observers using The Observer (XT 14) software (Noldus Information Technology, the Netherlands). By observing 3 min and skipping the subsequent 3 min, 50% of each observation period was watched. Due to technical problems, no recordings were made of eight cows (three from the PC group and five cows from the FC group) in the maternity pen and were therefore excluded from this dataset.

Two similar cameras were used for both the PC and FC group pen inside the free stall barn to record cow behaviour. For the PC group pen, the cameras were placed in bird's eye view across from each other to have a front and back view of the calf-boxes. In the FC group pen, two cameras were installed onto the barn ceiling, in bird's eye view, and adjacent to each other to visualize the complete pen. In the free stall barn, the behaviours described in Table 2 were monitored by two observers when calves were approximately 7 days (week 1), 21 days (week 3), and 35 days (week 5) of age using The Observer software. There was one observation day for each week, and this occurred on Sunday in order to reduce behavioural disturbances due to activities of farm staff as much as possible. For each observation day in the free stall barn, behavioural recordings between 04:00–22:00 h were analyzed by one of the two observers using instantaneous sampling with a 3-min sampling interval. As mentioned earlier, in each treatment group two new-born calves that were close in age were paired as similar aged peers. Focal sampling was applied to each of the two calves (and their dams) on observation days based on the birth date of the first calf. For all video observations, both the intra-observer agreement and inter-observer agreement were calculated (kappa coefficient > 0.90 and > 0.85, respectively).

2.5 Data handling and statistical analyses

All statistical analyses were performed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC), treating the cow-calf pair as the experimental unit. Residuals of all outcome variables (i.e. in the maternity pen: latency to onset of allogrooming and proportion of observed time spent on allogrooming, lying in close proximity, standing

Table 1. Description of recorded behaviours during the first 48 hours in the maternity pen for both FC (full contact) and PC (partial contact) cow-calf pairs (adapted from Jensen (2011) and Johnsen et al. (2015c)). Measurement unit: seconds.

Behaviour	Definition	Modifier
Lying	Dam is lying on her sternum or side, head may be rested or raised	Distance between any part of the dam's body (except her tail) and her calf: ≤ 1 m, > 1 m
Standing	Dam's body supported by four legs, standing upright	Distance between any part of the dam's body (except her tail) and her calf: ≤ 1 m, > 1 m
Feeding	Dam's head through feeding rack, above the feed or taking grass silage into mouth	Distance between any part of the dam's body (except her tail) and her calf: ≤ 1 m, > 1 m (for FC)
Allogrooming	Dam's muzzle in close proximity (< 5 cm) of calf, or in contact with calf's body to lick, sniff or rub any body part; could be scored simultaneously with nursing	
Nursing	Calf's muzzle is under the dam's belly in the udder area (for FC)	
Other activities	Dam is engaged in any other behaviour not listed above (e.g. drinking, social interaction other cows)	

Table 2. Description of recorded behaviours during the five weeks postpartum in the free stall barn for FC (full contact) and PC (partial contact) cow-calf pairs. Measurement unit: number of scans.

Behaviour	Definition	Modifier
Proximity [1]		
0–1 m	Distance between any part of the dam's head and her calf < 1 m	Cow posture: lying/standing
1–2 m	Distance between any part of the dam's head and her calf 1–2 m	Cow posture: lying/standing
> 2 m	Distance between any part of the dam's head and her calf > 2 m	Cow posture: lying/standing
Contact		
No contact	No physical contact between the dam and her calf	
Physical contact [2]	Dam's muzzle in close proximity (< 5 cm) of calf's body or in contact with the body	Calf: own/alien calf/both
Udder contact	Dam stands while the calf's muzzle is under the dam's belly in the udder area (for FC)	Calf: own/alien calf/both

[1] For PC cow-calf pairs proximity was estimated from the wall (at the calf's pen) to the dam's head. When a FC calf was lying the calf creep area, proximity was estimated from the metal bar to the FC dam's head.
[2] For FC cows physical and udder contact could occur simultaneously, but if there was udder contact and physical contact at the same time, this was scored as udder contact.

in close proximity; in the free stall barn: proportion of observed time spent on allogrooming, standing in 1-2 m proximity, standing in < 1 m proximity) were visually assessed for normality.

2.5.1 Data handling for maternity pen observations
In total 13 PC and 10 FC cow-calf pairs were included for the analysis of the latency to onset of allogrooming in the maternity pen. Latencies were missing for 15 cow-calf pairs, as 2 PC and 5 FC calves were born in the dry-cow pen, and video footage of the first 4 hours for 3 PC and 5 FC calves was missing due to technical problems (see Appendix 3). Latencies were calculated as the time from birth to the onset of the behaviour within the first 4 hours postpartum and were log transformed to normalize the data.

Descriptive analyses of the proportion of observed time spent on affiliative behaviours in the first 48 hours were derived by summarizing the durations that a behaviour was registered for all observed time periods divided by the total observed time that an animal was visible. These behaviours were thus expressed as proportions of observed time. This method allowed us to also include the calves born in the dry-cow pen that were observed once they were moved to the maternity pen and therefore had a shorter observation period (Barrier et al., 2012), resulting in data of 16 PC and 15 FC cow-calf pairs being analyzed. Due to the technical problems, there was no video material available for 2 PC and 5 FC calves (see Appendix 3).

2.5.2 Statistical analysis for maternity pen observations
Generalized linear model analyses were performed to analyze the observed behaviours in the maternity pen (i.e. latency to onset of allogrooming, proportion of observed time spent allogrooming, lying in close proximity, and standing in close proximity) using the PROC GLM procedure. The systematic part of the model (referred to as model 1) consisted of the following fixed effects:

$\mu + Treatment_i + Batch_j + Parity_k + (Treatment_i \times Parity_k)$ [1]

Here, μ was a base level and $Treatment_i$ = type of CCC (i = partial contact, full contact), $Batch_j$ = 16-week time period in which a calf was born (j = 1, 2, 3, 4), and $Parity_k$ = parity of the dam (k = primiparous or multiparous) were main effects. Batches were defined retrospectively to control for seasonal differences and varying group sizes in the two pens over time. Hence, the duration of the experiment was split up into batches of 16 weeks based on calving dates, so that every treatment was represented in a batch and batches represented the various seasons. Since parity is known to have an effect on cow's affiliative behaviour (von Keyserlingk and Weary, 2007), parity and the two-way interaction between parity and treatment were included in the statistical model. For the analysis of behavioural data expressed as proportions of time, the (logistic regression) model comprised a multiplicative dispersion factor with respect to the binomial variance function. Analyses of logistic models were based on maximum quasi likelihood with overdispersion parameters estimated from Pearson's generalized chi-square statistic (McCullagh and Nelder, 1989). An interaction was considered not significant when $P \geq 0.05$. Interactions that were not significant were excluded from the analysis. For all fixed effects either F-tests in analysis of variance or quasi likelihood ratio tests in logistic models were used, and significance was declared at $P < 0.05$.

2.5.3 Data handling for free stall barn observations

Due to technical problems with the video recordings in the free stall barn, 4 PC and 8 FC cow-calf pairs had one or more observation days missing (see Appendix 3). Hence, 14 PC and 12 FC cow-calf pairs with a complete series of data for all weeks were included. Descriptive analyses of the observed time spent on affiliative behaviours in the free stall barn were derived by summarizing the total amount of scans that a behaviour was scored divided by the total number of scans. The total number of scans was corrected for the scans that cows were being milked. For FC cows, the proportion of time spent standing near their calf (≤ 1 m) was corrected for the proximity due to nursing by subtracting for each week the number of scans scored as udder contact from the total number of scans scored as standing in close proximity.

2.5.4 Statistical analysis for free stall barn observations

Since the proportion of time spent in contact with any calf in the free stall barn contained an excess of zero-valued data, treatment differences were analyzed using a non-parametric Kruskal-Wallis test.

The proportion of time spent in proximity to the own calf was analyzed with a generalized linear mixed model for repeated measures, using the PROC GLIMMIX procedure. The systematic part of the model comprised the following fixed effects:

$\mu + \text{Treatment}_i + \text{Batch}_j + \text{Parity}_k + \text{Week}_l + (\text{Treatment}_i \times \text{Parity}_k) + (\text{Treatment}_i \times \text{Week}_l)$ [2]

in the same notation as before in model 1, and additionally Week_l = age of calf expressed in weeks (l = 1, 3, 5) as main effect. The model also included two-way interactions between treatment and parity, and between treatment and week. The random part of the model contained random animal effects. For the animal effects, a first-order autoregressive model (based on the actual distance between time points) was adopted to introduce correlation in the model between repeated measurements on the same animal. Similar to the analysis of behavioural proportions obtained in the maternity pen, this model comprised a multiplicative dispersion factor with respect to the binomial variance function. Again, an interaction was considered not significant when $P \geq 0.05$. Interactions that were not significant were excluded from the analysis. For all fixed effects either F-tests in analysis of variance or quasi likelihood ratio tests in logistic models were used, and significance was declared at $P < 0.05$.

2.5.5 Principal component analysis of cow behaviour

To examine patterns of intercorrelations between calf-directed affiliative behaviours, and to assess whether cows were consistent in their behaviour across the two contexts (i.e. after parturition in the maternity pen versus the following weeks in the free stall barn), a principal component analysis (PCA; Joliffe, 2002) was carried out. Eight behavioural parameters (expressed as proportions of time) were included in a PCA, five parameters that were recorded in the maternity pen, and three parameters that were recorded in the free stall barn. In the maternity pen: lying ≤ 1 m distance from the calf, lying > 1 m distance from the calf, standing ≤ 1 m distance from the calf, standing > 1 m distance from the calf pen, and allogrooming in the maternity pen. In the free stall barn: standing within 1 m from the calf, standing between 1 and 2 m from the calf, and physical contact with the calf. Calf-directed affiliative behaviours of the free stall barn were averaged for the three observation days. Those eight behaviours were selected as most relevant, because all cows could express these behavioural responses regardless the treatment group. In total 31 cows (i.e. 16 PC and 15 FC cows; see Appendix 3) were included in the PCA, as for those animals' data was available in both the maternity pen and free stall barn. PCA was performed on residuals of an analysis of variance model with treatment and parity as fixed effects. This allowed us to examine the correlation structure adjusted for treatment and parity, thereby focusing on covariation of behaviours within treatment and within parity, i.e. due to individual differences. Residuals of proportions were obtained using a logistic regression model comprising a multiplicative overdispersion factor with respect to the binomial variance function. After extraction, principal components were scaled by their standard deviations (square roots of associated eigenvalues) and subjected to varimax rotation. According to the Kaiser criterion, factors with eigenvalues larger than 1 were retained for further consideration. Loadings higher than (+/-) 0.50 were considered for interpretation.

3

3. Results

3.1. Affiliative behaviours in the maternity pen

After parturition, the latency to start allogrooming did not differ between PC and FC cows (mean ± SE in min, 7.5 ± 2.8 min, $P = 0.39$) (Table 3). Throughout the first 48 hours postpartum, time spent allogrooming the calf averaged approximately 8.6 ± 1.0% and did not differ among PC and FC cows ($P = 0.17$). Similarly, no significant differences were found between PC and FC cows for the time spent in close proximity to their calf, as all cows spent on average 22.1 ± 2.7% of the time standing and 31.5 ± 1.0% of the time lying within 1 m radius to their calf ($P = 0.10$, $P = 0.67$, respectively) (Table 3). Detailed results regarding the effects of batch and parity on affiliative behaviour in the maternity pen are summarized in Appendix 4.

Table 3. Behavioural observations in the first 48 hours postpartum in the individual maternity pen. Response parameters are shown separately for the two treatments (partial cow-calf contact, full cow-calf contact). Latencies are shown as minutes (mean ± SE) after birth, and the times that cows spent in proximity (≤ 1 m radius) to the calf and allogrooming the calf are shown as proportion of time (mean ± SE in%) of total time visible. For the number of included observations for each behaviour see Appendix 3.

Behaviour	Treatment			
	Partial contact	Full contact	F-value	P-value
Latency				
Allogrooming	10.2 ± 4.6	4.1 ± 2.3	0.78	0.39
Proportion of time				
Standing in close proximity	22.9 ± 2.1	21.2 ± 2.1	2.83	0.10
Lying in close proximity	30.5 ± 4.3	32.5 ± 3.2	0.18	0.67
Allogrooming	7.7 ± 1.3	9.5 ± 1.5	2.01	0.17

3.2. Affiliative behaviours in the free stall barn

In the free stall barn, FC cows spent on average 2.8 ± 0.4% of the time (mean proportion ± SE) standing in 1 to 2 m proximity to their calf versus 3.1 ± 0.5% for PC cows. Only in the first week treatment differences were found ($P = 0.045$). Overall, individual levels of this behaviour ranged from 0 to 17.0% for PC cows and from 0 to 11.7% for FC cows. PC cows spent more time standing in 1 to 2 m proximity to their calf in the first week postpartum (5.5 ± 1.3%) compared to the third (2.7 ± 0.9%; $P = 0.02$) and fifth week postpartum (1.3 ± 0.4%; $P = 0.001$), whereas FC cows showed a more steady pattern over time (week 1: 2.4 ± 0.5%, week 3: 3.4 ± 1.1%, week 5: 2.3 ± 0.6%; $P > 0.31$) (Figure 3A).

Moreover, FC cows spent on average 10.9 ± 0.1% of the time standing within 1 m proximity to their calf compared to 3.1 ± 0.4% for PC cows. Treatment differences were found in week 3 and 5 ($P < 0.001$). Inter-individual variation for the time spent in close proximity ranged from 0 to 17.8% for PC cows and 2.2 to 22.6% for FC cows. As shown in Figure 3B, PC cows spent more time standing in close proximity to their calf in the first week postpartum (6.3 ± 1.2%) compared to the third (2.2 ± 0.6%; $P < 0.001$) and fifth week postpartum (1.3 ± 0.4%; $P < 0.001$). However, no difference between week 3 and week 5 was found ($P = 0.18$). In contrast, FC cows showed an increase in standing close to the calf in the third week (12.8 ± 1.7%, $P = 0.01$) compared to the first (8.5 ± 1.2%) and fifth week (8.5 ± 1.6%) (Figure 3B). Detailed results regarding the effect of batch and parity on the observed time spent in proximity are summarized in Appendix 4.

In addition, FC cows spent on average more time in physical contact with their calf (2.1 ± 0.2%) compared to PC cows (0.5 ± 0.1%; $P < 0.001$). Among individuals, time

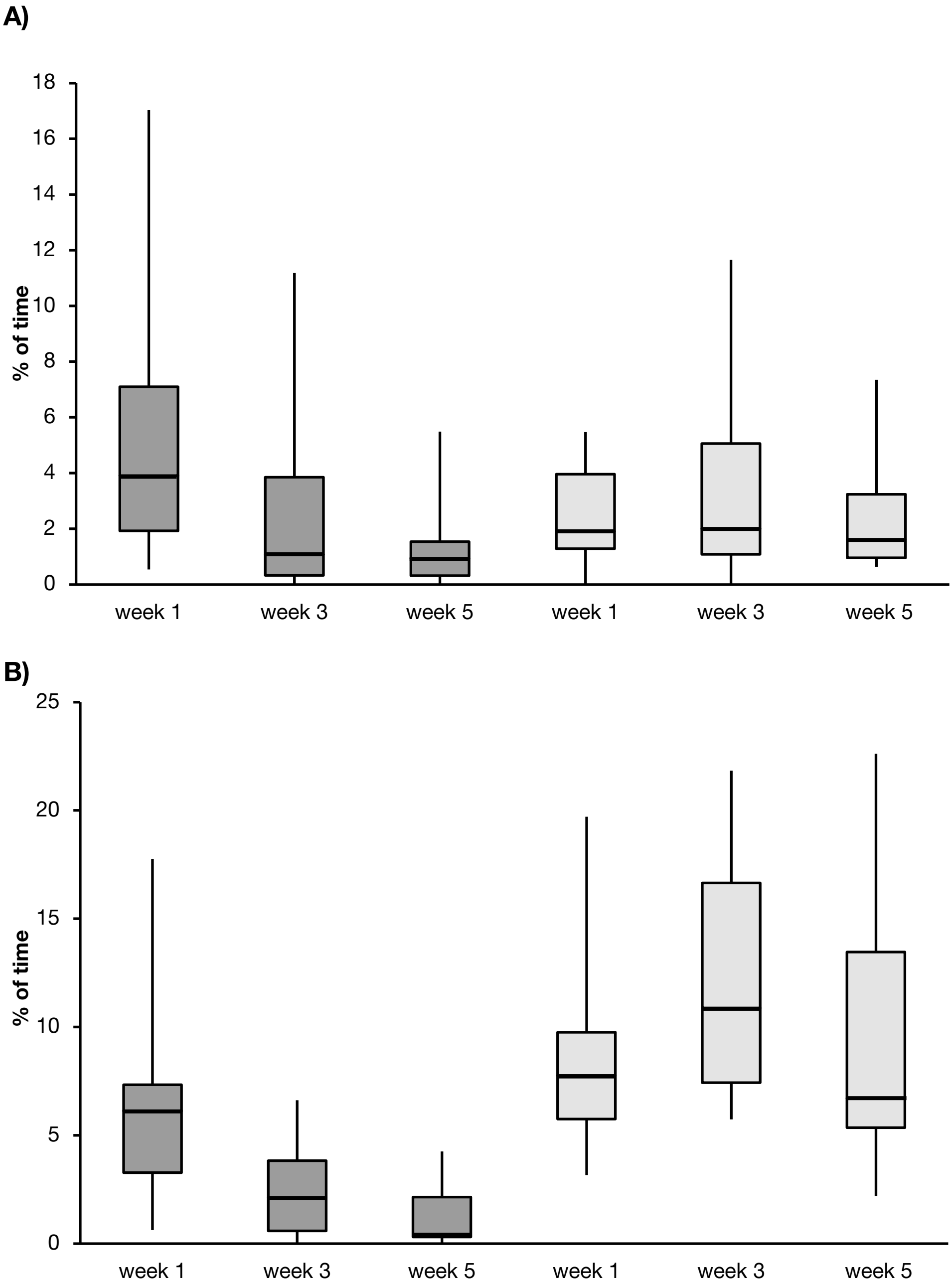

Figure 3. Calf-directed affiliative behaviour at three different calf ages expressed by dairy cows that were allowed to have either partial contact (dark grey) (n = 14) or full contact (light grey) (n = 12) with their calf in the free stall barn. Results are shown separately for **A)** time spent standing within 1 to 2 m proximity to the own calf, for **B)** time spent standing in close proximity (< 1 m radius) to the own calf.

C)

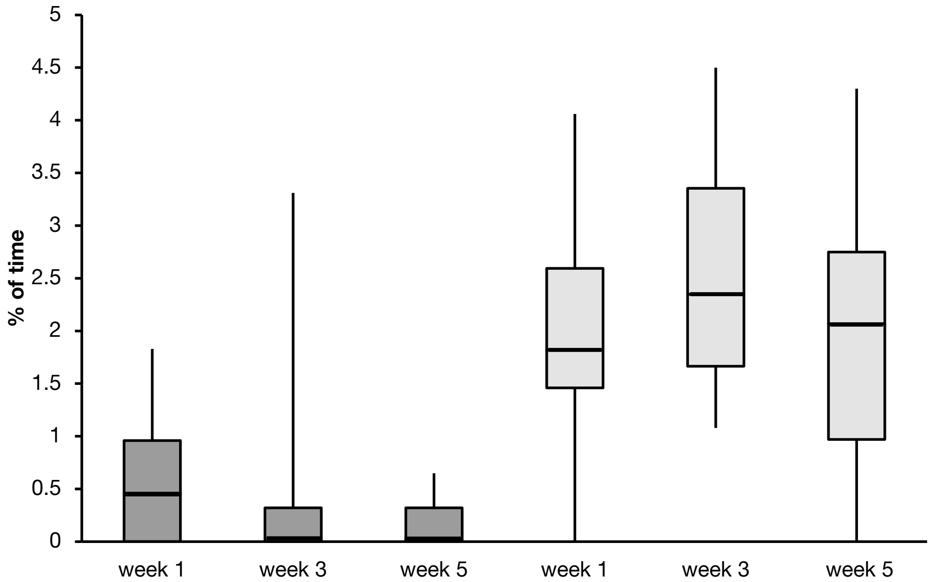

Figure 3 continued. Calf-directed affiliative behaviour at three different calf ages expressed by dairy cows that were allowed to have either partial contact (dark grey) (n = 14) or full contact (light grey) (n = 12) with their calf in the free stall barn. **C)** time spent in physical contact with the own calf. The boxplots show median (bold horizontal line within the box), 25th and 75th percentile (top and bottom of box), and range (tips of vertical whiskers).

spent in physical contact with the own calf ranged from 0 to 3.3% for PC cows and 0 to 4.5% for FC cows. Throughout time, both PC and FC cows showed a rather stable pattern in their average time spent in physical contact with their own calf (P = 0.16, P = 0.50, respectively) (Figure 3C). No treatment differences were found for the average time spent in physical contact with an alien calf (0.1 ± 0.0%, P = 0.63).

3.3. Cross-situational consistency of affiliative behaviours

The PCA of the residuals of calf-directed affiliative behaviours expressed in the maternity pen and the free stall barn revealed four principal components with eigenvalues > 1 which accounted for 76% of the variance of the data (Table 4). Notably, behaviours recorded in the maternity pen consistently loaded on different factors than behaviours recorded in the free stall barn (Table 4). Factors 1 and 3 were determined by affiliative behaviours expressed in the maternity pen, whereas factors 2 and 4 were dominated by behaviours exhibited in the free stall barn. Factors 1 and 3 summarized different types of proximity of the cow to the calf in the maternity pen, related to lying and standing close to the calf, respectively. Measures of proximity in the free stall barn exclusively loaded on factor 2 (Table 4).

Table 4. Loadings a on the first four components extracted by principal component analysis (PCA), after varimax rotation, of residuals b of cow's affiliative behaviours towards their own calf (n = 31), and the eigenvalues and proportions of total variation explained by each component.

Variable	Location	PC1	PC2	PC3	PC4
Lying in ≤ 1 m from calf	Maternity pen	**0.94**	0.02	0.08	0.13
Lying in > 1 m from calf	Maternity pen	**-0.55**	-0.20	**-0.66**	-0.28
Standing in ≤ 1 m from calf	Maternity pen	-0.16	0.03	**0.92**	-0.01
Standing > 1 m from calf	Maternity pen	**-0.82**	0.06	0.12	0.06
Allogrooming	Maternity pen	0.04	0.10	0.10	0.13
Standing in 0-1 m proximity	Free stall barn	-0.10	**-0.86**	-0.22	0.16
Standing in 1-2 m proximity	Free stall barn	0.08	0.06	0.04	**0.94**
Physical contact	Free stall barn	0.27	**-0.64**	0.49	-0.27
Eigen values		2.36	1.47	1.23	1.04
Variance explained (%)		29.52	18.41	15.37	13.02

[a] Loadings greater than 0.50 are indicated in bold.

[b] Residuals from an analysis of variance model with treatment and parity as fixed effects.

4. Discussion

The objective of this study was to evaluate the effect of different types of CCC on calf-directed affiliative behaviour of dairy cows. We focused on the dam's affiliative behaviour towards her calf, as partial CCC was created by preventing the calf to roam freely among the cows, which resulted in a cow-driven CCC system. Our results showed that the type of CCC did not affect cows' behaviour in the hours succeeding parturition. However, in the following weeks FC cows spent a higher proportion of time performing affiliative behaviours towards their calf than PC cows.

At parturition the expression of affiliative maternal behaviour is controlled by endocrine mechanisms (Lévy, 2016). Licking of the new-born calf is considered essential in establishing a mother-young bond (von Keyserlingk and Weary, 2007). After birth, all experimental cows accepted and interacted with their calf. The cuddle box in the maternity pen did not seem to hinder PC cows to interact with their calf, as both CCC groups showed similar calf-directed affiliative behaviour in the first 48 hours postpartum. In contrast, Green et al. (2020) recently showed that postpartum fence-line separation from the calf elevated stress behaviour in the dams reflected by increased alertness to the calf and high-frequency calls. In our study the average time spent in close proximity or allogrooming did not differ among the groups, so there was no indication of increased alertness towards the calf for the PC cows. Although we did not document vocalizations, the elevated stress levels found by Green et al.

could be the result of the design of the fence-line. The fence-line only allowed occasional physical contact between the dam and her calf, whereas in our cuddle-box the calf was easily accessible.

In the weeks following parturition, the preference for the related calf plus the times spent in close proximity to and in contact with the own calf suggest that a bond was formed between the cow-calf pairs regardless of the type of contact (Bouissou et al., 2001; Gubernick, 1981). In the first week, PC cows spent a larger proportion of the observed time in 1-2 m proximity to their calf than FC cows. This may have been the result of the automated brush that was positioned 2.5 m across the PC calf boxes. Fresh cows are known to use the brush frequently in the first week postpartum and this usage decreases over time (Mandel and Nicol, 2017). Nonetheless, for PC dams the proportion of observed time spent in less than 1 m proximity to and in physical contact with their calf was lower and gradually decreased over time, in contrast to FC dams that showed rather steady patterns throughout time. Dairy cattle have been classified as a 'hider species' where the mothers actively seek contact throughout the first days of life while the new-born hides itself (Lent, 1974). In the succeeding weeks the initiative to make contact shifts towards the calf (Jensen, 2011; Tucker, 2009). The higher levels of calf-directed behaviour in FC cow-calf pairs in the free stall could be the result of contact initiated by the FC calves that were roaming free in contrast to PC calves that were restrained in their calf pen and could not actively seek out the dam. Since FC cow-calf pairs could also spend time lying together in close proximity (besides the reported time spent standing in close proximity), their actual total time spent in close proximity may have been even higher than described in this study. Previous work found that affiliative behaviours did not differ among suckled and non-suckled cow-calf pairs (Johnsen et al., 2015c). Those pairs had half-day contact and were observed only in the two hours following reunion. Contrary to the current study, Johnsen et al. (2015c) housed both treatments in the same group pen in which calves roamed freely, allowing all calves to be the initiator of contact as well. We suggest for future studies to identify the contact-initiator in CCC systems and include calves' affiliative behaviour as well. Our partial CCC set-up was designed to meet some major concerns of dairy producers. By housing PC calves aside the cow herd, suckling and direct contact with manure of adult cows was prevented, while it allowed for individual feeding of calves and certain cow-calf interactions. However, in cases where the PC calf was lying in the back of the calf pen, this pen limited PC cows to interact with their calf as they could not reach the calf. Therefore, PC cows may have received less reinforcement to socially interact compared to FC cows that could more easily make contact with their calf (Meagher et al., 2019).

Possibly, the combination of those factors affected the cow-calf bond and reduced the PC dams' affinity with her calf. Nevertheless, recent descriptive work of Johnsen et al. (2021) showed that limiting physical contact in a cow-driven full CCC system did also affect the cow's affiliative behaviour. In that particular study the cows had to pass selection gates to access a meeting area for social interactions (including suckling) with their calf, and were allowed to have either free access or limited access depending on a successful milking in the automatic milking system. Numerically the limited group showed less successful visits and a lower duration of allogrooming their calf compared to the unlimited group, although they did not differ in suckling duration (Johnsen et al., 2021). This indicates that limiting physical contact in cow-driven CCC systems affects the cow's affiliative behaviour in various ways. On the other hand, oxytocin is known to be an important hormone involved in social bonding (Carter et al., 1992; Kendrick, 2000) and suckled dams have been found to have higher oxytocin levels in response to suckling/milking than non-suckled dams (Lupoli et al., 2001). In addition, suckled dams showed an increased motivation to reunite with their own calf compared to non-suckled dams, which indicates that the social bond seems to grow stronger when suckling is allowed (Wenker et al., 2020). Therefore, the greater expression of calf-directed affiliative behaviours in FC dams may also have been the result of the suckling opportunity that strengthened the mother-young bond.

Nevertheless, the current results show large variations in calf-directed affiliative behaviour between individual cows regardless of the CCC treatment, which implies that certain cows express a greater interest in being near or interacting with their calf than others. Since animal welfare relates to the quality of life as experienced by the individual animal (Winckler, 2019), individual responses should not be overlooked when investigating pure group mean responses (Richter and Hintze, 2019), especially since interactions with offspring are suggested to have a positive hedonic value for mammalian mothers (Olazábal et al., 2013). Individual differences in maternal care among cattle have been previously described and are known to be affected for example by breed (Le Neindre, 1989; Le Neindre and Sourd, 1984), cow's body condition and calf characteristics (i.e. sex, birth weight) (Stěhulová et al., 2013), parity (Edwards and Broom, 1982; Vandenheede et al., 2001), and received maternal care as calf (Le Neindre, 1989). Furthermore, individual maternal differences also seem consistent throughout lactation (Dwyer, 2008; Stěhulová et al., 2013). Interestingly, the present study showed that cows were not consistent in their affiliative behaviours (in terms of proximity to their calf) across context, i.e. in the maternity pen and the free stall barn. PCA clearly showed that affiliative behaviours expressed in the maternity pen loaded on different factors than affiliative behaviours expressed in the free stall barn. This means, for example, that cows spending a relatively long time in close proximity to their calf in the maternity pen did not necessarily show the same behavioural pattern in the free stall barn. In addition, the loading pattern (i.e. the extent to which behavioural variables correlated with a component) obtained after PCA seemed to suggest that different types of proximity may exist, for example, proximity determined either by standing or lying close (within 1 m) to the calf in the maternity pen (see Table 4, PC1 and PC3), or proximity defined in terms of standing close (within 1 m) or less close (between 1 and 2 m) from the calf in the free stall barn (see Table 4, PC2 and PC4). Collectively, these findings could imply that different affiliative behaviours (exhibited in different contexts) are driven by different motivational states. Behaviour is conceptually organized in so-called motivational systems that are each activated by specific motivational states with different underlying neurobiological systems (Koolhaas et al., 1997). Generally, animals adapt their behaviour to satisfy different motivations and to perform optimally in a given situation/environment, so maternal animals do not necessarily act according to fixed patterns but make decisions based on contextual information, emotional and internal states (including multiple motivations) (Olazábal et al., 2013). Possibly, the motivation to stand between 1-2 m from the calf in the free stall barn may have been controlled by a different motivational state than standing within 1 m from the calf; perhaps the former involves the motivation to be close to another resource in the barn (e.g. automated brush or drinker) at the same time, whereas the latter involves predominantly maternal traits. Similarly, being in close proximity in the maternity pen may be driven by a different neurochemical brain state compared to standing in close proximity in the free stall barn (Koolhaas et al., 1997). Overall, individual differences and independent dimensions underlying those differences give insight into the complexity and variety of the animals' behaviour.

A limitation of the current study is that technical problems with the cameras and digital video recorder resulted in missing data for several cow-calf pairs. However, those problems occurred randomly, so missing observations arose by chance which still allowed for an unbiased comparison between the treatment groups. More research is needed to identify other factors or traits underlying cows' variation in calf-directed affiliative behaviour. Moreover, further assessment of the effect of full and partial CCC systems on stress responses at unanswered contact attempts or when debonding (i.e. weaning and separation phase) is recommended.

5. Conclusion

This study shows that, except for the hours succeeding parturition, type of cow-calf contact (CCC) affects the expression of calf-directed affiliative behaviour in dairy cows. Partial CCC resulted in less calf-directed affiliative behaviours compared to full CCC, except in the 48 hours following parturition. This may be due to the fact that the partial CCC set-up limited the accessibility of the calf or because in the full CCC set-up calves could also initiate contact. Moreover, large inter-individual differences were found and the expression of calf-directed affiliative behaviour in the free stall barn could not be predicted based on the behavioural responses expressed in the maternity pen.

Acknowledgements

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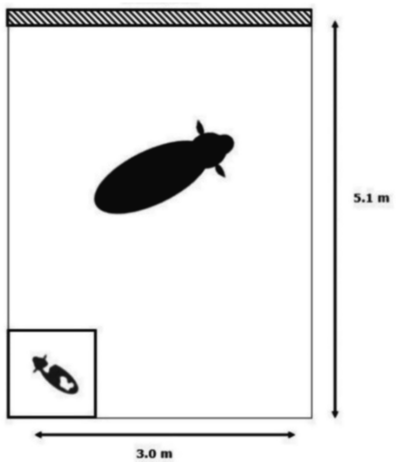
Appendix 1. Cuddle box illustration

A)



B)

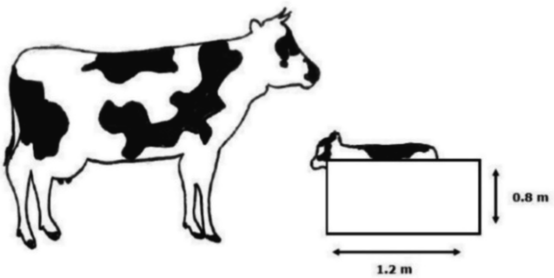


Figure 1. Illustration of the cuddle box used in the maternity pen for the partial cow-calf contact treatment, **A)** view from above, the cuddle box was placed in one of the corners across from the feeding rack, and **B)** side view of a calf inside the cuddle box.

Appendix 2. Milk feeding schedule

Table 1. Fixed feeding schedule for each individual partial contact calf fed bulk tank milk.

Week of age	Number of meals per day	Amount of milk per meal (L)	Total amount of offered milk per day (L)
1	3	2.5	7.5
2	3	3	9
3	3	3.5	10.5
4	3	3.5	10.5
5	2	3.5	7
6	2	3	6
7	2	2	4
8	1	1	1

Appendix 3. Missing observations

Table 1. Number of observed cow-calf pairs per treatment that were included in the statistical analysis. The partial contact treatment included 18 cow-calf pairs and the full contact treatment consisted of 20 cow-calf pairs in total.

Statistical analysis	Partial contact	Full contact	Reason
Maternity pen			
Latency allogrooming	n = 13	n = 10	No video footage in first 4 hours postpartum of 3 PC and 5 FC calves due to technical problems, and 2 PC and 5 FC that were born on slatted floor were excluded
Proportion of time allogrooming	n = 16	n = 15	No video footage of 2 PC and 5 FC calves throughout the first 48 h postpartum
Proportion of time in proximity	n = 16	n = 15	No video footage of 2 PC and 5 FC calves throughout the first 48 h postpartum
Free stall barn			
Proportion of time in physical contact	n = 14	n = 12	No complete series of observations days for 4 PC and 8 FC cow-calf pairs due to video footage that was missing on one or more days because of technical problems
Proportion of time in proximity	n = 14	n = 12	No complete series of observations days for 4 PC and 8 FC cow-calf pairs due to video footage that was missing on one or more days because of technical problems
Principal component analysis	n = 16	n = 15	For 2 PC and 5 FC calves behavioural observations in the maternity pen were missing due to technical problems, there was data available for all cows regarding the free stall barn observations as the data was averaged for the different observation days

Appendix 4. Results of fixed effects for batch and parity

Table 1. Results (mean ± SE) for fixed effect batch in the GLM(M) analyses.

Outcome variable	Batch					
	1	2	3	4	F-value	P-value
Maternity pen						
Latency allogrooming (min)	11.27 ± 5.32	7.74 ± 5.97	2.58 ± 1.23	4.70 ± 1.74	0.26	0.85
Proportion of time allogrooming	0.09 ± 0.02	0.09 ± 0.01	0.07 ± 0.03	0.09 ± 0.04	0.37	0.78
Proportion of time standing in close proximity	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.03	0.34 ± 0.07	1.19	0.33
Proportion of time lying in close proximity	0.30 ± 0.07	0.34 ± 0.04	0.27 ± 0.04	0.36 ± 0.05	0.38	0.77
Free stall barn						
Proportion of time in 0-1 m proximity	0.06 ± 0.01	0.09 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.23	0.87
Proportion of time in 1-2 m proximity	0.02 ± 0.00	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.80	0.51

Table 2. Results (mean ± SE) for fixed effect parity in the GLM(M) analyses.

Outcome variable	Parity			
	Primiparous	Multiparous	F-value	P-value
Maternity pen				
Latency allogrooming (min)	1.21 ± 0.57	8.85 ± 3.34	0.09	0.77
Proportion of time allogrooming	0.08 ± 0.02	0.09 ± 0.01	1.43	0.24
Proportion of time standing in close proximity	0.27 ± 0.02	0.21 ± 0.02	8.99	0.01
Proportion of time lying in close proximity	0.32 ± 0.05	0.31 ± 0.03	0.09	0.77
Free stall barn				
Proportion of time in 0-1 m proximity	0.09 ± 0.02	0.06 ± 0.01	0.16	0.69
Proportion of time in 1-2 m proximity	0.03 ± 0.01	0.03 ± 0.00	0.03	0.88

Chapter 4

Effect of type of cow-calf contact on health, blood parameters, fecal microbiota, and performance of dairy cows and calves

Margret L. Wenker[1,2], Cynthia M. Verwer[3], Eddie A.M. Bokkers*[1], Dennis E. te Beest[4], Gerrit Gort[4], Daiana de Oliveira[5], Ad Koets[6], Rupert M. Bruckmaier[7], Josef J. Gross[7], Cornelis G. van Reenen[1,2]

[1] Animal Production Systems group, Wageningen University & Research, Wageningen, the Netherlands
[2] Livestock Research, Wageningen University & Research, Wageningen, the Netherlands
[3] Louis Bolk Institute, Bunnik, the Netherlands
[4] Biometris, Wageningen Plant Research, Wageningen University & Research, Wageningen, the Netherlands
[5] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Uppsala, Sweden
[6] Bacteriology, Host Pathogen Interaction & Diagnostics, Wageningen Bioveterinary Research, Lelystad, the Netherlands
[7] Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

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Abstract

Prolonged cow-calf contact (CCC) may have the potential to improve dairy calf welfare. However, it is currently unknown how different types of CCC affect the animals' biological functions. We evaluated health and performance parameters of dairy calves and their dams reared in three different systems, where cows: i) had no contact with their calf (NC); the calf was removed from the dam directly after birth (n = 10), ii) were allowed to have partial contact (PC) with their calf; the calf was housed in a calf pen adjacent to the cow area allowing physical contact on initiative of the dam but no suckling (n = 18), or iii) were allowed to have full contact (FC) with their calf, including suckling; calves were housed together with their dams in a free stall barn (n = 20). Throughout the first 7 weeks postpartum, data were collected on the health status, fecal microbiota, hematological profile, immune and hormonal parameters, and growth rates of calves, and on the health status, metabolic responses, and the performance of the dams. Overall, FC calves had more health issues ($P = 0.02$) and a tendency for higher antibiotic usage ($P = 0.07$) than NC calves. Additionally, FC calves showed elevated levels of erythrocytes, hematocrit, hemoglobin, and leukocytes on day 49 compared to NC calves ($P < 0.001$). Calf fecal microbiota changed over time and we found preliminary evidence that fecal microbiota are affected by type of CCC, as reflected by differences in relative abundances of taxa including *Lactobacillus* in FC calves compared to NC and PC calves. Moreover, FC calves had a greater average daily gain in body weight than NC and PC calves ($P = 0.002$). Cow health was not affected by type of CCC, although in the first 7 weeks of lactation FC cows had a lower machine-gained milk yield accompanied by a lower fat percentage than NC and PC cows ($P < 0.001$). These results indicate that full contact posed a challenge for calf health, presumably because the housing conditions of FC calves in this experimental context were suboptimal. Secondly, ad libitum suckling led to higher weight gains and negatively affected milk fat content besides machine-gained milk yields. Overall, partial contact may be a feasible compromise to optimize calf rearing in terms of responding to public concerns by allowing limited CCC without impairing calf health and cow production. More research into strategies to improve cow-calf housing and management in full CCC systems is warranted.

1. Introduction

Under natural conditions maternal care is essential for the fitness and survival of cattle offspring (Stěhulová et al., 2013). However, on most commercial dairy farms it is standard practice to remove newborn calves from the dam within 24 hours postpartum. Then, farmers must care for the calves themselves, hence calf health and survival are chiefly reliant on the quality and quantity of animal care provided by people (Johnson et al., 2011; Marcé et al., 2010; Santman-Berends et al., 2014). Calf rearing is an important aspect of dairy herd management given that heifer calves are the future replacements for the milking cows. Young stock management practices that enhance calf health and performance can improve the future dairy herd's productivity and longevity (Buczinski et al., 2021; Hultgren and Svensson, 2009; Soberon et al., 2012). Nevertheless, dairy farms are sometimes faced with high rates of morbidity and mortality in young calves (Santman-Berends et al., 2021, 2019; Windeyer et al., 2014). This not only has significant economic consequences for farmers (Mohd Nor et al., 2012), but it also raises concern regarding animal welfare (Ortiz-Pelaez et al., 2008). Moreover, there is increasing public concern regarding the deprivation of maternal care in young dairy calves, which could pose a threat to the dairy industry's social acceptability (Hötzel et al., 2017; Ventura et al., 2013). Re-introducing prolonged maternal care into the current dairy production system as an alternative rearing practice is receiving increasing interest from various stakeholders (Johnsen et al., 2016; Sirovnik et al., 2020), and has been proposed to be beneficial for animal health and welfare (Flower and Weary, 2003; Johnsen et al., 2016).

Those alternative calf rearing systems that allow dairy cows and their calves to stay in contact for a prolonged period of time, so-called cow-calf contact (CCC) systems, can differ in the type of physical contact between dam and calf and are generally described as full or partial CCC systems (Sirovnik et al., 2020). Full CCC (i.e. unrestricted physical contact including suckling) typically involves keeping calves together within the herd, which allows cow-calf pairs to express natural behaviours, like suckling and resting in contact. Previous work found that full contact improves calf growth rates (Johnsen et al., 2016), positively affects udder health (Krohn, 2001), and promotes the expression of natural behaviour (Meagher et al., 2019). However, a recent review showed inconsistent and contradictory results for the effect of suckling on calf health, for example with regard to cryptosporidiosis, pneumonia, and mortality. The studies addressing calf diarrhea pointed to beneficial or no effects of suckling (Beaver et al., 2019a). From the dairy producers perspective, there are some major concerns regarding full CCC, such as loss of saleable milk, milk ejection disturbances, and difficulties with calf monitoring (Flower and Weary, 2003). Those concerns may be overcome by allowing partial CCC, where contact is restricted by limiting the physical contact and preventing suckling (e.g. housing the calf adjacent to the dams' pen rather than inside the pen) (Sirovnik et al., 2020). However, to date no work investigated the consequences of prolonged partial CCC for animal health and performance (i.e. biological functioning). Characterizing important biological systems, such as the gut microbiota (Arrieta et al., 2014), and biomarkers, such as cortisol, IGF-1, cholesterol, and immunoglobulins (Marcato et al., 2018) can provide a deeper insight in the animal's development and disease resistance (Pletcher and Pignone, 2011). Overall, there is a need for broader and more systematic investigations before specific recommendations for CCC systems can be made (Meagher et al., 2019).

Given the contradictory literature and existing knowledge gaps regarding the effect of maternal contact on the animals' biological functioning, the objective of this study was to evaluate the effect of different types of CCC on clinical health, blood parameters (i.e. immunological, hormonal, metabolic, hematological profiles), fecal microbiota, and performance of dairy cow and calf.

2. Materials and methods

This study was conducted at the Knowledge Transfer Centre in Zegveld (the Netherlands), a dairy research farm, from February 2019 to July 2020. All applicable international, national, and institutional guidelines for the care and ethical use of animals were followed. The experimental design was approved by the Central Committee on Animal Experiments (The Hague, the Netherlands; approval number AVD4010020174307).

2.1 Animals and experimental design

Forty-eight Holstein Friesian cows were included in this study with a parallel group design. Cows were included at calving when they gave birth to a single heifer calf without substantial calving difficulties or health problems. The mean parity was 2.9 and ranged from 1 to 7. In order for calves to have a similar aged peer, every two cows that calved successively were assigned to the same treatment to have either: i) no contact (NC) with their calf; calves were removed directly after birth and the calf was housed in a calf barn (n = 10), ii) partial contact (PC) with their calf; calves were housed in a pen adjacent to the cow area allowing physical contact on initiative of the dam but no suckling (n = 18), or iii) full contact (FC) with their calf including suckling; calves were housed together with the dams in a free stall barn (n = 20). Treatment order for every set of two cows was randomized in each block of six successive calvings. CCC was allowed for 10 weeks, although from 7 weeks onwards gradual weaning and separation strategies were applied. Cows were followed until 12 weeks postpartum and calves were studied up to 6 months of age.

2.2 Calving management

Based on signs of imminent calving, cows were moved into an individual indoor straw-bedded maternity pen (3.0 m wide × 5.1 m long) situated inside the free stall barn. Cows that were about to calve were continuously video-monitored and the calving was assisted if necessary. Despite regular checks of calving signs by farm staff, eight cows (one NC, two PC, and five FC cows) calved in the dry cow pen but were moved into an individual maternity pen and still included in the trial. Immediately after birth, navels were dipped with 2% iodine and the birth weight of the newborn calf was measured on a full-body calf scale (Type 8700, Welvaarts, the Netherlands). All NC calves were removed from the dam within 1.5 h after birth (median: 6 min, range: 1 to 63 min) and placed in an individual straw-bedded calf box (Topcalf Duo-Flex, Schrijver, the Netherlands; see section 2.3 for details) in an indoor calf barn. PC calves were placed in a cuddle-box (consisting of four plywood plates of 1.2 m wide × 0.8 m high; see Wenker et al. (2021) for details) inside the maternity pen. The cuddle-box prevented suckling, while still allowing tactile, visual, audible, and olfactory contact and was placed in one of the corners across the feeding rack. The cow could lick and sniff her calf when the calf would be standing or lying by moving her head into the box to reach the calf. FC calves stayed in full contact with their dam inside the maternity pen. When the barn temperature was below 10 °C, calves were provided with a heating lamp.

All cows were milked with a mobile milking machine (Mini-milker, Kurtsan, Turkey) twice daily in their pen. To standardize the first colostrum intake, all calves were bottle fed with (mean ± SE) 2.8 ± 0.1 L of colostrum from their own mother within 2 h (± 17 min) after birth. Calves in the NC and PC treatment group received an additional 2 L colostrum by bottle at 8 to 12 h as well as at 20 to 24 h after birth. After the first colostrum meal by bottle, FC calves could suckle the remaining colostrum directly from the dam's udder. To check colostrum quality, the brix value of the first colostrum meal was measured using an optical refractometer (0-32%, Bio Enterprise B.V., the Netherlands). Seven FC calves (but none of the NC or PC calves) started to suckle their first colostrum before farm staff could bottle-feed them. However, four of them did still drink colostrum from the offered bottle as well. All NC cows returned to the designated group pen in the free stall barn after the second postpartum milking. Both PC and FC calves stayed with their dam in the maternity pen for about 72 h, after which they moved to their designated group pens in the free stall barn.

2.3 Housing and feeding

The experimental cows were kept in dynamic groups separate from the rest of the farm herd in an indoor free stall barn in three different group pens (i.e. NC, PC, FC group) (Figure 1). All experimental cows were milked twice a day at approximately 08:00 h and 18:00 h in the milking parlor with a five-point open tandem side and 11 side-by-side places. Cows were fed grass silage (early spring cuttings) once a day at approximately 09:30 h. Feed was pushed automatically (MoovPro, JOZ, the Netherlands) to the feeding rack 8 times a day. Additionally, cows could eat up to 10 kg of concentrates per day that were provided partly in the milking parlor and by an individual concentrate feeder. When the barn temperature was below 10 °C, all young calves were fitted with a calf jacket for the first three weeks of life.

NC calves were kept in a straw-bedded calf box (Topcalf Duo-Flex, Schrijver, the Netherlands) in an indoor naturally ventilated calf barn separate from the free stall barn. One calf box could house two calves individually (1.0 m × 1.6 m), but also offered the opportunity to pair house them (2.0 m × 1.6 m) by removing the partition wall in the middle of the box. NC calves were housed individually for the first two weeks, after which they were pair housed with their similar-aged peer. Each calf was provided with ad lib water, hay and concentrates (Topfok Kalf, de Samenwerking, the Netherlands) from 3 days of age onwards.

PC calves were kept in similar calf boxes as NC calves, but inside the free stall barn behind a wall (1.2 m high) adjacent to the PC cow group pen. This set-up prevented suckling, direct contact with manure of adult cows, and housing calves within the cow herd, while it allowed for individual feeding of calves, as well as visual, auditory, olfactory, and limited tactile contact between cow-calf pairs (see Wenker et al. (2021) for an illustration). Cows could move their head over the wall and when the calf was standing, cow-calf pairs could sniff and lick each other. PC calves were also housed individually for the first two weeks, after which they were pair housed in the same box with their similar-aged peer. Ad lib water, hay and concentrates were provided from 3 days of age onwards to each individual calf. The PC group never exceeded more than six cow-calf pairs.

FC calves were housed together with the dams in the FC group pen in the free stall barn but had access to a calf creep area (inaccessible for the dams). The calf creep area provided them with a straw-bedded lying area and ad lib water, hay and concentrates from the day the newborn calves moved into the free stall barn. The FC group never exceeded more than eight cow-calf pairs.

For NC and PC calves, bulk tank milk was provided in individual teat buckets following a fixed feeding schedule (Table 1), after all three colostrum meals were consumed. Milk was provided around 08:00 h, 13:00 h, and 18:00 h. Bulk tank milk was heated up to 41°C using a milk taxi (Milchtaxi 2.0, Holm & Laue, Germany) before being fed to the calves. The amount of daily milk intake of NC and PC calves was recorded after every fed meal. FC calves could suckle their dams and, if allowed, other dams, throughout the whole day excluding milking hours.

2.4 Data collection

2.4.1 Clinical health assessment

Once a week all calves between the age of 4 to 49 days were clinically assessed using a standardized health scoring system (Table 2). The health scoring system was adapted from recent work on clinical health indicators for calves (Renaud et al., 2017a) to evaluate the respiratory system (nasal discharge, ocular discharge, cough), fecal consistency, navel inflammation, and rectal temperature on a 4-point scale. Use of antibiotics and other medicine plus any observed health problems were recorded by the farm staff in a daily logbook for both cows and calves during the entire experiment.

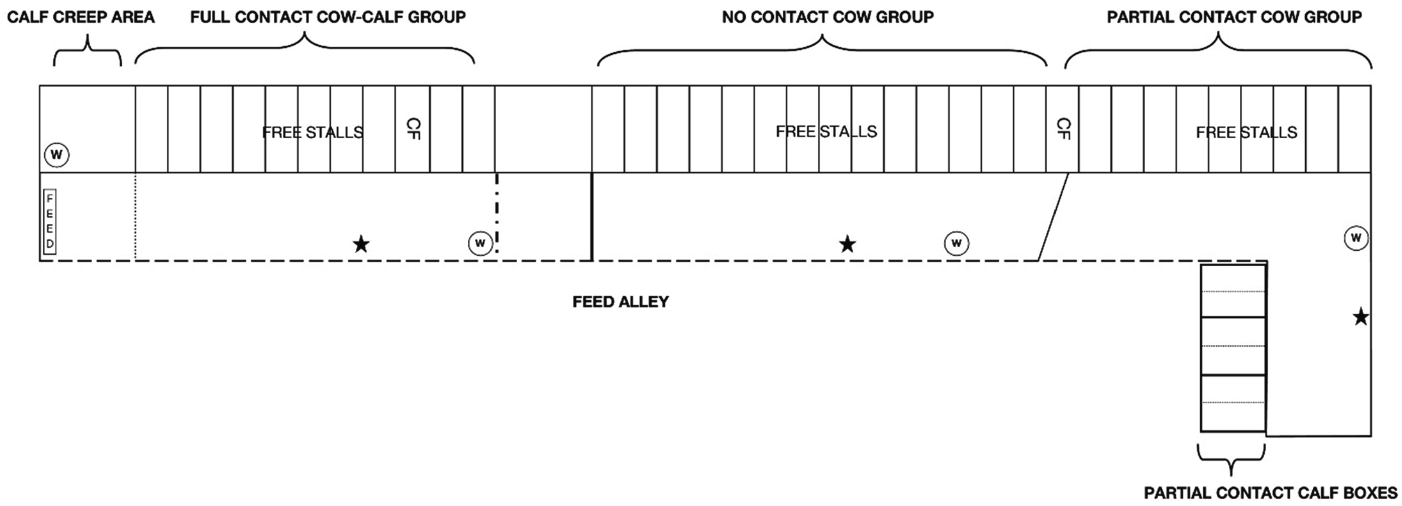

Figure 1. Experimental cows were housed in three dynamic group pens inside a free stall barn (i.e. FC, NC, PC group pen). The free stall barn was naturally ventilated with open sidewalls and had perlite-bedded lying stalls (1.1 m x 3.0 m). The alley was covered with rubber flooring and cleaned eight times a day by an automated scraper. Within the FC group pen, FC calves were kept with the dams in the group pen but had access to a calf creep area (3.3 m x 4.8 m) with a straw-bedded lying area (3.3 m x 1.9 m), water bucket, hay and concentrates. A metal bar hindered cows to access this area. PC calves were housed individually (1.0 m x 1.6 m) in a straw-bedded calf box for the first two weeks, after which they were pair housed in the same box (2.0 m x 1.6 m) with their similar-aged peer. In each individual calf box ad lib water, hay, and concentrates were provided. The calf boxes were placed behind a wall (1.2 m high) to limit physical contact and prevent suckling. NC calves were housed in an indoor naturally ventilated calf barn in similar pens as PC calves and were also pair housed at two weeks of age. W = water, CF = individual concentrate feeder, ★ = automated cow brush

4

Table 1. Fixed feeding schedule for each individual calf with no contact or partial contact to their dam fed bulk tank milk.

Week of age	Number of meals per day	Amount of milk per meal (L)
1	3	2.5
2	3	3.0
3	3	3.5
4	3	3.5
5	2	3.5
6	2	3.0
7	2	2.0
8	1	1.0

2.4.2 Performance measures

During the weekly health assessment, calf body weight was measured using a full-body calf weighing scale (Type W8700, Welvaarts, the Netherlands). Additionally, heart girth and back length were measured with a tapeline and hip height was measured using a rod (Kerbl, Germany). At 6 months of age, body weight of all calves was recorded once more using a full-body cow scale (Type 8700, Welvaarts, the Netherlands).

For the assessment of cow performance, data on machine-harvested milk yield and moment of first insemination of experimental cows were automatically collected using AgroVision dairy farm management software (AgroVision B.V., the Netherlands). Moreover, milk composition was evaluated based on milk samples collected every 3 weeks year-round. In addition to the percentage of milk fat, protein, and lactose, the somatic cell count (SCC) was analyzed (ISO 9622 and ISO 13366-2, Qlip, the Netherlands)

2.4.3 Blood sample collection and analysis

Passive transfer of immunity

Blood samples (9 mL) from calves were taken via jugular venipuncture at 24 to 48 h of age into citrate vacutainer tubes (Vacuette, Greiner BioOne, Austria). Samples were

Table 2. Description of health parameters scored on a weekly basis during the first 7 weeks of life (adapted from Renaud et al. (2017a)).

	Score			
Variable	0	1	2	3
Nasal discharge	Normal serous discharge	Small amount of unilateral discharge	Moderate amount of bilateral discharge	Copious, bilateral mucopurulent discharge
Ocular discharge	Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
Cough	No cough	Induced single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous cough
Fecal consistency	Normal consistence	Pasty, semi-formed	Pasty with large amounts of water, content adhered in the perineum and tail	Liquid with fecal content adhered in the perineum and tail
Navel inflammation	Normal	Slightly enlarged, not warm or painful	Slightly enlarged with slight pain or moisture	Enlarged with heat, pain or malodorous discharge
Rectal temperature (°C)	37.8 - 38.2	38.3 - 38.8	38.9 - 39.4	> 39.4

centrifuged for 20 min at 2000 rpm and 4 °C right after collection, and plasma was stored at −20 °C until further processing. Immunoglobulin (Ig)G concentrations were measured in plasma samples with an indirect bovine IgG specific enzyme-linked immunosorbent assay (ELISA). Wells were coated for 1 h with affinity-purified sheep anti-bovine IgG-heavy chain (Cat. No. A10-118A-13, Bethyl Laboratories, United States of America) diluted 1:100 in coating buffer (0.05 M carbonate-bicarbonate, pH 9.6, Merck KGaA, Germany). Plates were washed 6 times with 50 mM TRIS 0.14 M NaCl (Merck KGaA, Germany), incubated for 1 h in the same buffer (blocking), and then washed 6 more times. After the 6^{th} wash, 24 mg/mL of bovine reference serum (Cat. No. RS10-103-5, Bethyl Laboratories, United States of America) or diluted calf sera were added to each well, and the plates were incubated for 1 h. Wells were then washed 6 times, 100 mL of sheep anti bovine IgG-heavy chain (1:120,000) conjugated to horse-radish peroxidase (HRP) (Cat. No. A10-188P-30, Bethyl Laboratories, United States of America) were added, and plates were incubated for 1 h. After incubation, plates were washed 6 times and tetra methyl benzine (TMB) (SanBio B.V., the Netherlands) was added. Reactions were stopped after 15 min with 0.2 M H2SO4 (Merck KGaA, Germany) and the optical density at 450 nm was determined with an automated plate reader. The standard curve was generated by means of a 4-parameter curve fit and the IgG concentrations in the test samples were quantified by interpolating their absorbance from the standard curve generated in parallel with the samples.

Hematology

For the assessment of calves' hematological profile, blood samples (9 mL) were taken via jugular venipuncture 24 to 48 hours, 14 days, and 49 days of age into EDTA vacutainer tubes (Vacuette, Greiner BioOne, Austria). Calf age at the actual sample moment could deviate from the intended 14 and 49 days of age (ranging from -6 to +6 days for both time points), as the majority of calves was sampled during the weekly health and growth assessments. We followed this approach to reduce the handling of calves, as the animals' response to humans was also studied in another part of this experiment. Samples were stored and transported at 4°C prior to the analyses. Fluorescence flow cytometry (European Veterinary Laboratory, the Netherlands) was used to determine absolute numbers of different cell types in full blood, including cell count for leukocytes (WBC), granulocytes (GRA), lymphocytes (LYM), less frequently occurring and rare white blood cells (MID), erythrocytes (RBC), platelets (PLT), procalcitonin (PCT), percentage of basophils, neutrophils, eosinophils, lymphocytes, monocytes, and erythrocyte indices like hematocrit (HCT), mean corpuscular volume (MCV), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), and red cell distribution width (RDW).

Immunoglobulins and hormones

To assess calves' natural autoantibodies (N-IgA, N-IgG, N-IgM titers), and concentrations of cortisol, insulin-like growth factor 1 (IGF-1), cholesterol, and insulin at 14 and 49 days of age, blood was collected in different vacutainer tubes. EDTA samples were stored at 4 °C for a maximum of 2 h, whereas serum samples were stored at room temperature for 1 h prior to processing. All samples were centrifuged for 15 min at 3000 rpm and 4 °C and were stored at –20 °C until analysis.

Titers of N-IgG, N-IgM and N-IgA were measured in serum samples with indirect enzyme-linked immunosorbent assay (ELISA) against phosphorylcholine conjugated to bovine serum albumin (PC-BSA) according to previously published methods (Mayasari et al., 2016)(Marcato et al., 2021). Pre-diluted samples (1:10) in PBS mix (PBS + 1% horse serum (HS) + 0.05% tween) were coated with different amount of PC-BSA (PC-1011-10, Bioresearch Technologies, Canada): 1 µg/mL for N-IgG and 0.25 µg/ml for N-IgM and N-IgA. N-IgG and N-IgM were detected using 1: 20000 diluted sheep polyclonal anti-bovine IgG-heavy chain conjugated to horseradish PO (Cat. No. A10-100P, Bethyl Laboratories, United States of America), and 1: 20000 diluted rabbit polyclonal anti-bovine IgM conjugated to horseradish PO (Cat. No. A10-100P, Bethyl Laboratories, United States of America). N-IgA was detected using 1: 10000 diluted sheep polyclonal anti-bovine IgA conjugated to horseradish PO (Cat No. A10-131P, Bethyl Laboratories, United States of America). Starting dilution of standards was 1: 160 for N-IgG, 1:80 for N-IgM, and 1: 20 for N-IgA. Serial dilutions for N-IgG, N-IgM and N-IgA in serum samples started at 1: 40 (4 steps). After the last 1.5 h incubation at room temperature with the conjugates, plates were washed with demi-water. Each well of the plate was filled with 100 µL of substrate tetra methyl benzine (TMB) (Sigma Aldrich Chemie, Germany), which contained milliQ water, 1% TMB, and 10% TMB buffer. Plates were then incubated for 30 min at room temperature. After the incubation, the reaction was stopped by adding 50 µL of 2.5 N H2SO4 solution in each well. Extinctions were measured with a Multiskan reader (Lab Systems, Finland) with a wavelength of 450 nm. Titers were calculated based on log2 values of the dilution that gave extinction closest to 50% of Emax (Ploegaert et al., 2007), where Emax represents the highest mean extinction of standard positive serum present on each plate.

Hormones in blood plasma were measured by a radioimmunoassay (RIA) adapted from (Schwinn et al., 2016; Vicari et al., 2008). Plasma insulin concentrations were measured with a homologous double-antibody system using 25.7 IU/mg bovine insulin (Sigma, United States of America) for standards and for iodination and guinea-pig anti-bovine insulin (#5506, lot GP23; Bioyeda, Weizmann Institute, Israel). Precipitating anti-guinea-pig γ-globulin (Calbiochem, United States of America) was used as second antibody to separate antibody-bound from free hormone. Plasma samples were diluted (1:10) with assay buffer and ovalbumin (35 mg/mL) and paralleled with the standard curve. The intra-assay CV was 9.4% and the inter-assay CV was 5.3%.

Plasma IGF-1 concentrations were measured by extracting 50 µL plasma with 250 µL absolute ethanol and 12.5 µL of 2.4 M formic acid. Recombinant bovine IGF-1 was used as standard and for iodination. A monoclonal antibody against human IGF-1 raised in mice hybridoma cells was used as first antibody together with normal mouse serum. Sheep-anti-mouse serum (100 μL) was used together with 1000 μL 6% polyethyleneglycol to separate antibody-bound and free hormone. Plasma from a calf was diluted, so IGF-1 concentrations paralleled the standard curve. The intra-assay CV was 6.4% and the inter-assay CV was 4.3%.

Plasma cortisol concentrations were analyzed by extracting 0.1 mL plasma with 1 mL absolute ethanol. After mixing the tubes on a vortex mixer, the protein precipitate was sedimented by centrifugation at 1,500 × g for 20 min at 4 °C. Supernatants were decanted into fresh tubes, evaporated to dryness, and reconstituted in 0.5 mL PBS (0.14 M sodium chloride and 0.01 M sodium phosphate, pH 7.0) containing 0.1% gelatin. A standard curve was run in duplicate by adding cortisol at concentrations between 0.25 to 100 ng/mL. Upon addition of 0.1 mL diluted antiserum and 0.1 mL [1,2-3H] cortisol (78 Ci/mmol), each tube was mixed and incubated at 4°C for 15 h. Separation of the free hormone from the bound hormone was achieved by adding 0.4 mL of a 0.75% dextran-coated charcoal suspension. After 4 min, tubes were centrifuged (2,800 × g, 15 min, 4 °C) and 0.7 mL were pipetted from the supernatant and mixed with scintillation fluid for radioactivity counting. The intra-assay CV was 9.7% and the inter-assay CV was 6.3%.

Total cholesterol concentrations in blood serum were measured with a commercially available enzymatic kit (Cat. No. Cholesterol FS 1.1350 99 10 021; DiaSys Diagnostic Systems GmbH, Germany) with an autoanalyzer (Cobas Mira, Switzerland).

Metabolic status

Blood samples of cows were taken from the coccygeal vein by a veterinarian at 2 to 21 days before the expected calving date and at 30 to 50 days after calving. Sera were tested by the GD Animal Health Service (Royal GD, the Netherlands) for haptoglobin, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), magnesium, and urea levels to assess the metabolic status of cows in the prepartum stage (Test package for dry period, number: 11682) and for calcium, BHBA, and urea levels in the postpartum stage (Test package for fresh period, number: 11508).

2.4.4 Microbiota sampling and analysis

Rectal feces samples of calves were collected at 7 days, 28 days, 49 days, and 66 days of age. As mentioned earlier, calf age at the actual sample moment could deviate from the intended age (ranging from -4 to +3 days for the first three sample moment and from -9 to +9 days for the last sample moment), as calves were sampled during the weekly health and growth assessments. Calves were rectally finger-stimulated with sterile-gloved hand to facilitate the collection of at least 5 g feces into a 50 mL polypropylene conical bottom test tube (Cellstar, Greiner BioOne, Austria). Samples were stored at -20 °C until analysis.

Total DNA was extracted from 0.2 g fecal samples using QIAamp Fast DNA Stool Mini Kit (QIAGEN, Art.No. 51604) according the manufacturer's instructions. In addition, after resuspending the samples in InhibitEx, buffer samples were subjected to repetitive bead-beating (3 times for 30 s with 5 s cooldown in between) using Lysing Matrix B tubes (MP Biomedicals, Art.No. 116911050-CF) and the FastPrep-24 instrument (MP Biomedicals). Microbial DNA extracts were checked on a 2200 Tapestation (Agilent Technologies, United States of America).

Bacterial community composition was assessed by sequencing the combined V3–V4 hypervariable region of the 16S rRNA gene as previously published (Jurburg et al., 2019). Briefly, this region was first amplified by 25 cycles of PCR using the primers CVI_V3-forw CTACGGGAGGCAGCAG and CVI_V4-rev GGACTACHVGGGTWTCT. PCR products were checked on a 2200 Tapestation, and sequencing was performed using a V3 paired-end 300 bp sequencing on a MiSeq sequencer (Illumina Inc., United States of America). Negative controls were used in each round of amplification to confirm the sterility of reagents, and a mock community bacterial community was included in the sequencing run as a control. More details on sequence processing and bioinformatic analysis can be found in Appendix 1.

2.4.5 Hair samples and analysis

Hair samples of calves were collected on the day of birth, day 21, and day 56 of age. Samples on day 21 and day 56 reflect the level of hair cortisol metabolites between birth until day 14 and day 21 until day 49, respectively (González-de-la-Vara et al., 2011). All samples were collected from the tip of the tail by carefully clipping 2 to 3 cm of the tail hair with surgical scissors as close to the skin as possible (Burnett et al., 2015). The hair samples were stored in individually identified zip-lock plastic bags, which were kept at -20 degrees until further processing. Samples were mechanically cleaned and defatted with 5 mL n-hexane/isopropanol. Samples were dried overnight at room temperature. The dried samples were cut into small fragments approximately 1 to 2 mm with scissors. Individual 100 mg aliquots from each of the samples were milled at 30 Hz with 3 mm beads for 5 min using a TissueLyserII (Qiagen, Germany). The milled hair samples were placed in a glass test tube along with 5 mL of methanol, and the tubes were incubated at 50 °C for 18 h. After centrifuging, the liquid in the tubes was transferred to another glass vial and evaporated to dryness in a stream of nitrogen. The remaining residue was dissolved in 200 μL of Neogen extraction buffer. Extraction of all hair samples (0.5 g each) was performed with 100% methanol, after which hair cortisol metabolites were determined using a Neogen cortisol kit (Product nr. 402710, Neogen, United States of America).

2.5 Data handling

2.5.1 Total health score

A total health score (THS) was calculated based on the clinical health assessment (calf age 1 to 7 weeks), summarizing disease length and intensity for each calf (adapted from van Dixhoorn et al. (2018)). On a weekly basis, calves were classified for having clinical symptoms of common calf diseases, i.e. respiratory issues ('yes' when they had a composite respiratory score ≥ 4 (based on the sum of ocular discharge, nasal discharge, cough score)), neonatal diarrhea ('yes' when fecal score ≥ 2 (this category was included for the first 4 weeks of life as indication of neonatal diarrhea, and comprised either infectious diarrhea or feeding-related loose/liquid manure)), navel inflammation ('yes' when navel score ≥ 2), and fever ('yes' when they had a rectal temperature score of 3). Subsequently, all clinically detected health problem scores between week 1 to 7 were added to one total score (i.e. THS, dimensionless) per calf. Calves with a low THS (with few clinical symptoms lasting only a short period of time) were in good health, whereas calves with a high THS suffered from more health problems or had a slower recovery throughout the first 7 weeks of life.

2.5.2 Growth rates

Average daily gain (ADG) of calves was calculated for the body weight, hip height, back length, and heart girth between the age of 1 to 7 weeks.

2.5.3 Milk production

Since milk samples were collected triweekly and cows left the experiment at 12 weeks postpartum, milk yield and composition plus SCC data were averaged per cow during the first 7 weeks of lactation.

2.6 Statistical analysis

All statistical analyses were performed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC), except for the microbiota analysis that was performed using R (version 4.05 (R Core Team, 2021)). The animal was treated as the experimental unit. All variables and model residuals were visually checked for normality and homogeneity of variance, and response variables were log-transformed when needed.

2.6.1 Growth rates

ADG for body weight, hip height, back length, and heart girth were analyzed with a linear mixed model (using SAS procedure PROC GLIMMIX) for continuous data. The systematic part of the model (referred to as model 1) consisted of the following fixed effects:

$$\mu + \text{Treatment}_i + \text{Batch}_j + \text{Parity}_k + (\text{Treatment}_i \times \text{Parity}_k) \quad [1]$$

Here, μ was a base level and Treatment_i = type of CCC (i = no contact, partial contact, full contact), Batch_j = 16-week time period in which a calf was born (j = 1, 2, 3, 4), and Parity_k = parity of the dam (k = primiparous or multiparous) were main effects. Batches were defined retrospectively to control for seasonal differences and varying group sizes in the treatment groups over time. Hence, the duration of the experiment was split up into batches of 16 weeks based on calving dates, so that every treatment was represented in a batch and batches represented the various seasons. Given that parity is known to affect calf's growth, health, and colostrum characteristics (Gulliksen et al., 2008; Perez et al., 1990; Svensson and Liberg, 2006), parity and the two-way interaction between parity and treatment were included in the statistical model. Interactions that were not significant ($P \geq 0.05$) were excluded from the model. In addition, the model comprised a random effect for the interaction between treatment and batch. For all fixed effects, approximate F-tests were used (Kenward and Roger, 1997) and significance was declared at $P < 0.05$. Subsequent pairwise comparisons were made according to the Tukey method.

2.6.2 Total health score and antibiotic use

Data on the THS of calves were analyzed with the same linear mixed model as for growth rates (see model 1). Due to low prevalences in some treatment groups for the percentage of calves classified with clinical symptoms of specific health variables and the percentage of calves treated with antibiotics, those parameters were analyzed using a Fisher's exact method for pairwise comparisons.

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2.6.3 Immunoglobulins

To assess passive transfer of immunoglobulins after colostrum feeding, serum bovine IgG concentrations and colostral brix scores were analyzed with a linear mixed model identical to model 1. For one FC calf there was a missing IgG value, as the calf was not sampled 24-48 h after birth. Failure of passive transfer was analyzed using a Fisher's exact method for pairwise comparisons considering the few incidences.

For continuous data on natural autoantibodies (i.e. N-IgM, N-IgA, N-IgG) in serum samples of calves at 14 and 49 days of age, a total of 8 out of 96 samples were missing. Those serum samples could not be collected due to issues at blood withdrawal. Consequently, serum of in total 8 NC, 18 PC, and 15 FC calves on day 14; and 9 NC, 17 PC, and 20 FC calves on day 49 was analyzed. Here, a linear mixed model for repeated measures was performed (PROC GLIMMIX). The systematic part of the model (referred to as model 2) consisted of the following fixed effects:

$$\mu + \text{Treatment}_i + \text{Batch}_j + \text{Parity}_k + \text{Sample moment}_l + (\text{Treatment}_i \times \text{Parity}_k) + (\text{Treatment}_i \times \text{Sample moment}_l) \quad [2]$$

in the same notation as before (see model 1), and additionally Sample moment$_l$ = intended calf age at the sample moment (l = 14 or 49 days) as main effect and a two-way interaction between treatment and sample moment. Furthermore, age difference in days between the calf's age at the intended sample moment and the actual sample moment was added as co-variate among the fixed effects. Random calf effects were introduced to handle repeated measurement. Further procedures were similar to model 1, so interactions that were not significant ($P \geq 0.05$) were excluded from the model, the model comprised a random effect for the interaction between treatment and batch, approximate F-tests were used (Kenward and Roger, 1997) and significance was declared at $P < 0.05$ for all fixed effects, and subsequent pairwise comparisons were made according to the Tukey method.

2.6.4 Hematology

In total 16 out of 144 hematology profiles at either day 1, 14, or 49 were missing, as those plasma samples could not be collected due to issues at blood withdrawal. Consequently, hematology profiles of in total 9 NC, 17 PC, and 17 FC calves on day 1; 7 NC, 18 PC, and 15 FC calves on day 14; and a total of 10 NC, 16 PC, and 19 FC calves on day 49 were analyzed. A generalized linear mixed model was used for the analysis of data expressed as continuous proportions (e.g. hematocrit) using a beta distribution with logit link function, whereas other quantitative data were analyzed with an ordinary linear mixed model similar to model 2. All further procedures were identical to model 2, except that now sample moment consisted of three levels (i.e. 1, 14, 49 days); thus, a first-order autoregressive model (based on the actual distance between time points) was adopted to introduce correlation in the model between repeated measurements on the same animal.

2.6.5 Metabolite and hormone concentrations

In total 12 out of 96 blood samples of calves at 14 and 49 days of age were missing, as those samples could not be collected due to issues at blood withdrawal. Consequently, hormone concentrations of 7 NC, 18 PC, and 15 FC calves on day 14, and 10 NC, 14 PC, and 20 FC calves on day 49 were included in the analysis. In total 43 out of 144 hair samples were too dirty or too few material for cortisol extraction. This resulted in 10 NC, 14 PC, and 18 FC samples on day 0, 5 NC, 11 PC, 11 FC samples on day 21, and 10 NC, 8 PC, and 14 FC samples on day 56. Continuous data on plasma cortisol, plasma IGF-1, plasma insulin, and serum cholesterol concentrations were analyzed with a linear mixed model identical to model 2 and its corresponding procedures. The statistical model for hair cortisol concentrations was also identical to model 2, except that here sample moment consisted of three levels (i.e. 0, 21, 56 days) thus a first-order autoregressive model (based on the actual distance between time points) was adopted to introduce correlation in the model between repeated measurements on the same animal.

2.6.6 Cow health and performance

Due to low prevalences of cows with high SCC and antibiotic treatments for mastitis or endometritis, these parameters were analyzed using a Fisher's exact method for pairwise comparisons. Data on the metabolic status of cows were analyzed with a linear mixed model similar to model 1, although now the moment of sampling (i.e. number of days prepartum or postpartum) was added as co-variate among the fixed effects. Due to hemolysis incidences, two cows (i.e. one PC, one FC) were excluded from the prepartum data set and one FC cow was excluded from the postpartum data set.

Milk yield, milk composition, and number of days until first insemination were analyzed with a linear mixed model identical to model 1 and its corresponding procedures.

2.6.7 Microbiota

In total 5 NC, 12 PC, and 13 FC calves on day 7; 4 NC, 10 PC, and 10 FC calves on day 28; 6 NC, 13 PC, and 16 FC calves on day 49; and 2 NC, 9 PC, and 12 FC calves on day 66 were analyzed. The data were analyzed using unconstrained and constrained ordination analysis (PCA/RDA) of Hellinger transformed microbiota compositions. With the RDA we evaluated statistical significance of factors (i.e. treatment, parity, batch, sample moment) affecting microbiota composition. The statistical significance of separate taxa, with respect to treatment and health factors, were then evaluated using beta-binomial regressions. In this analysis the false discovery rate and the corresponding adjusted p-values were calculated using the Benjamini-Hochberg procedure.

3. Results

3.1 Growth rates of calves

FC calves had a greater ADG in body weight compared to PC and NC calves during the first 7 weeks of life ($P < 0.001$). In terms of skeletal growth rates, FC calves had a greater ADG in back length compared to PC calves ($P = 0.01$), but not compared to NC calves ($P = 0.79$). FC calves also tended to have greater ADG in heart girth compared to NC calves ($P = 0.08$), but not compared to PC calves ($P = 0.17$). No significant treatment differences were found for ADG in hip height (Table 3). At 6 months of age, no treatment differences in mean absolute body weight (± SE) were present, as FC calves weighted on average 198.6 ± 8.7 kg, PC calves weighted 192.6 ± 7.7 kg, and NC calves weighed 211.4 ± 6.9 kg ($P = 0.33$).

3.2 Health of calves and antibiotic use

Clinical observations were summarized into a THS per calf for the first 7 weeks of life, where a smaller THS reflects less health issues or a fast recovery of health issues. Prevalence of calves classified with clinical symptoms for each health variable included in the THS can be found in Table 4. FC calves had an increased mean THS (± SE) of 4.3 (± 0.6) compared to NC calves (2.1 ± 0.4) ($P = 0.02$) but did not differ in mean THS from PC calves (3.2 ± 0.4) ($P = 0.43$). No differences were found between NC and PC calves ($P = 0.18$). The THS values varied from 0 to 11 for FC calves, 0 to 8 for PC calves, and 0 to 4 for NC calves.

In the first 7 weeks of life, 21% of all calves were treated with antibiotics. The prevalence of calves treated with antibiotics tended to be higher in FC calves compared to NC calves (6 out of 20 calves versus 0 out of 10 calves, $P = 0.07$). No

Table 3. Growth performance of three groups of calves with different types of cow-calf contact (calf age 1 to 7 weeks). Different subscripts within a row indicate a significant difference ($P < 0.05$) between treatments.

	No contact			Partial contact			Full contact				
	Mean	SE	95% CI	Mean	SE	95% CI	Mean	SE	95% CI	F-value	P-value
ADG in body weight	0.72^{a}	0.05	0.34, 0.87	0.75^{a}	0.03	0.47, 1.02	1.03^{b}	0.05	0.63, 1.35	20.02	< 0.001
ADG in heart girth	0.35	0.02	0.26, 0.41	0.38	0.05	0.26, 0.57	0.43	0.02	0.29, 0.62	3.14	0.06
ADG in back length	0.25^{ab}	0.04	0.10, 0.45	0.20^{a}	0.03	0.05, 0.43	0.29^{b}	0.02	0.14, 0.45	4.87	0.01
ADG in hip height	0.25	0.02	0.19, 0.36	0.24	0.01	0.17, 0.36	0.27	0.01	0.14, 0.40	1.08	0.40

ADG: Average daily gain in kg/day for body weight and cm/day for skeletal parameters

Table 4. Prevalence of calves (%) in three treatment groups with different types of cow-calf contact that were classified at least once with clinical symptoms for various health variables. For each variable, values represent the percentage of calves within a treatment group scored at least once with a score ≥ 2 for ocular discharge, nasal discharge, cough, or navel inflammation, score > 2 for fever (calf age 1 to 7 weeks) and score ≥ 2 neonatal diarrhea (calf age 1 to 4 weeks) on a 0-3 point scale (adapted from Renaud et al. (2017a)). Different subscripts within a row indicate a significant difference ($P < 0.05$) between treatments.

	No contact	Partial contact	Full contact
Ocular discharge	0.0^{a}	16.7^{a}	70.0^{b}
Nasal discharge	50.0	55.6	85.0
Cough	40.0	44.4	40.0
Navel inflammation	10.0	27.8	30.0
Neonatal diarrhea	80.0	83.3	80.0
Fever	20.0	5.6	20.0

differences in the prevalence of calves treated with antibiotics were found between FC and PC calves (4 out of 18 calves in the latter group, P = 0.72), or between NC and PC calves (P = 0.27). No neonatal or postnatal mortality occurred among the experimental calves in the first 7 weeks of life.

3.3 Immunoglobulin concentrations in calves

Colostrum quality, as reflected by an overall mean colostral brix score (± SE) of 25.81 ± 0.74%, did not differ among treatments (P = 0.81). Similarly, mean (± SE) bovine IgG concentration in plasma after colostrum intake did not differ among treatments (FC: 24.59 ± 3.13 mg/mL; PC: 22.22 ± 2.32 mg/mL; NC: 24.63 ± 2.53 mg/mL) (P = 0.97). However, the seven FC calves that suckled their first colostrum before they were bottle-fed had greater mean bovine IgG levels (32.61 ± 6.20 mg/mL) than FC calves that received their first colostrum by bottle (18.38 ± 2.99 mg/mL) (P = 0.03). Prevalence of failure of passive transfer (defined as IgG < 10 mg/mL (Godden, 2008)) was 12.5% and occurred in 2 PC and 4 FC calves (of which 1 suckled the first colostrum meal), no treatment effect was found (P > 0.28). With respect to the THS, those six calves were not identified as outliers.

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Furthermore, mean N-IgA, N-IgG, and N-IgM did not significantly differ among treatments, although mean N-IgG declined (P < 0.001) and N-IgM increased (P = 0.002) from day 14 to day 49 for all treatments (Table 5).

3.4 Hematology of calves

An interaction between treatment and sample moment was found for RBC, HCT, MCV, HGB, WBC, GRA values (Figure 2). On day 49, RBC, HCT, and HGB values were higher in FC calves than in NC calves (P ≤ 0.001). Only for NC and PC calves, MCV values significantly decreased from day 1 to day 49 (P < 0.001). WBC values were higher on day 49 than day 1 for FC calves, and higher on day 14 compared to day 1 for PC calves (P = 0.03). For FC calves, GRA values were higher on day 49 than day 1 (P = 0.01) (Figure 2).

No treatment effect was found for MCH, MCHC, PCT, LYM, MID, and PLT values (Table 6). For all treatments MCH and MCHC were higher on day 49 than on day 1 and day 14 (P < 0.001), whereas PCT, LYM, MID, and PLT values were higher on day 14 and day 49 compared to day 1 (P ≤ 0.001). Similarly, no treatment effect was found for RDW and MPV values, but across treatments those values were lower on day 14 and day 49 compared to day 1 (P < 0.001) (Table 6). The mean percentage of monocytes was higher in NC calves compared to PC and FC calves (P = 0.01) and was higher on day 49 compared to day 1 for all treatments (P = 0.01) (Table 6). Mean percentages of lymphocytes and neutrophils did not differ among treatments, although an effect of sample moment was found (P < 0.001). For all treatments, the percentage of lymphocytes was higher on day 14 and day 49 compared to day 1, and the percentage of neutrophils was lower on day 14 and day 49 compared to day 1 (Table 6).

Table 5. Effect of type of cow-calf contact on natural autoantibodies (N-IgA, N-IgG, N-IgM titers) titers, and metabolite and hormone concentrations measured in blood of calves at two different ages.

	No contact				**Partial contact**				**Full contact**					
	day 14		day 49		day 14		day 49		day 14		day 49			
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	F-value[1]	P-value[1]
N-IgG, titer	8.23	0.49	6.90	0.28	7.97	0.22	6.77	0.28	8.23	0.36	6.28	0.30	0.76	0.48
N-IgA, titer	1.51	0.31	2.29	0.60	1.53	0.21	2.05	0.36	2.07	0.38	2.02	0.22	1.83	0.17
N-IgM, titer	2.53	0.38	2.88	0.45	2.92	0.30	3.91	0.47	2.66	0.34	4.31	0.27	2.76	0.10
Cholesterol, mmol/L	1.70	0.22	2.65	0.20	1.75	0.15	2.89	0.18	1.54	0.19	2.77	0.14	0.06	0.94
Cortisol, ng/mL	1.58	0.21	2.39	0.41	2.64	0.36	1.28	0.27	3.32	0.51	3.28	0.56	4.27	0.06
Insulin, µU/mL	23.20	10.09	22.81	9.29	28.56	4.61	24.06	6.25	19.77	3.48	30.63	5.91	0.71	0.53

Ig(G, A, M): Immunoglobulin

[1] F-value and P-value for treatment effect

3.5 Metabolite and hormone concentrations of calves

A significant interaction between treatment and sample moment was found for plasma IGF-1 concentrations, as FC calves had a higher mean IGF-1 concentration on day 49 compared to PC and NC calves ($P < 0.001$) (Figure 2). Furthermore, FC calves tended to have a higher mean plasma cortisol concentration (3.30 ± 0.38 ng/mL) compared to PC calves (2.05 ± 0.26 ng/mL) ($P = 0.05$), but did not differ from NC calves (2.06 ± 0.27 ng/mL) ($P = 0.25$). Mean serum cholesterol concentrations did not differ among treatments ($P = 0.94$), although it significantly increased from day 14 to day 49 for all treatments ($P < 0.001$). Mean plasma insulin concentrations did not differ among treatments ($P = 0.53$) or sample moment ($P = 0.85$) (Table 5). In addition, the mean hair cortisol concentration (± SE) was 7.67 ± 0.73 ng/g and did not differ between treatments ($P = 0.29$) or sample moments ($P = 0.18$) (Table 7).

3.6 Cow health and performance

In the first 7 weeks of lactation 2 out of 10 NC cows, 5 out of 18 PC cows, and 6 out of 20 FC cows had at least once a high SCC (mean SCC > 200.000 cells/ml (Oikonomou et al., 2014)) ($P > 0.68$). In total three FC cows were treated for mastitis with antibiotic injectors ($P > 0.23$). Furthermore, one NC and one FC cow were treated with non-steroidal anti-inflammatory drugs for endometritis ($P > 0.36$).

The metabolic status of the experimental cows during the dry period did not differ significantly among treatments, as reflected by an overall mean NEFA (± SE) (0.25 ± 0.03 mmol/L), BHBA (0.48 ± 0.02 mmol/L), urea (4.90 ± 0.27 mmol/L), magnesium (0.83 ± 0.02 mmol/L) and haptoglobin (0.17 ± 0.06 g/L) concentration (Table 8). In the postpartum period, mean BHBA (0.53 ± 0.03 mmol/L), urea (3.95 ± 0.26 mmol/L), and calcium (2.31 ± 0.02 mmol/L) did also not differ significantly among treatments (Table 8).

FC cows produced less milk in the milking parlor (mean daily yield ± SE: 17.01 ± 1.97 kg/d) throughout the first 7 weeks postpartum compared to PC (28.94 ± 1.10 kg/d) and NC cows (29.25 ± 2.25 kg/d) ($P < 0.001$). Moreover, milk of FC cows had a lower mean fat content (3.51 ± 0.13%) in contrast to PC (4.29 ± 0.14%) and NC cows (4.34 ± 0.15%) ($P < 0.001$). Similarly, FC cows tended to have a lower mean lactose content (4.26 ± 0.08%) compared to PC (4.50 ± 0.03%) and NC cows (4.55 ± 0.05%) ($P = 0.07$), although mean protein content did not differ among treatments (FC: 3.52 ± 0.09%, PC: 3.38 ± 0.05%, NC: 3.42 ± 0.06%; $P = 0.32$). Last, the mean of days open until first insemination (± SE) did not differ among treatments (FC: 74 ± 5, PC: 74 ± 3, NC: 70 ± 5; $P = 0.47$).

Table 6. Effect of type of cow-calf contact on hematological parameters measured in plasma of calves at three different ages.

	No contact						Partial contact					
	day 1		day 14		day 49		day 1		day 14		day 49	
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
MCH, 10^{-12}/L	0.78	0.21	0.60	0.04	1.13	0.35	0.63	0.01	0.62	0.02	0.74	0.03
MCHC, g/dl	39.13	10.16	32.35	2.44	68.85	22.43	30.43	0.59	32.69	1.04	41.84	1.87
PLT, 10^9/L	464.20	78.68	603.44	79.91	479.38	39.12	361.00	24.46	650.17	69.41	683.40	151.36
MPV, 10^{-15}/L	5.99	0.60	4.97	0.15	4.95	0.42	6.35	0.25	5.62	0.35	5.31	0.48
PCT, %	0.20	0.03	0.27	0.04	0.16	0.01	0.16	0.01	0.24	0.03	0.22	0.03
LYM, 10^9/L	2.98	0.64	3.81	0.94	3.29	0.99	3.85	0.55	5.57	0.67	4.67	0.74
MID, 10^9/L	4.79	1.19	6.92	2.22	5.40	1.92	6.56	1.60	10.05	1.41	10.58	2.38
BASO*, %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EO*, %	0.67	0.37	1.00	1.00	0.50	0.34	0.29	0.17	0.17	0.12	1.25	0.51
LYM, %	42.78	6.53	51.25	7.25	51.60	5.04	43.41	3.76	58.89	3.61	57.25	2.77
MONO, %	3.78	1.02	7.00	1.20	7.80	1.44	3.94	0.75	5.06	0.57	5.88	0.59
NEUT, %	50.56	6.70	40.88	7.87	40.10	4.83	52.35	5.18	35.89	3.59	38.38	3.17
RDW	5.77	0.63	3.97	0.30	3.65	0.60	6.19	0.27	4.93	0.39	4.09	0.48

MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet count; MPV: mean platelet volume; PCT: procalcitonin; LYM: lymphocytes, MID: less frequently occurring and rare white blood cells; BASO: basophils; EO: eosinophils; MONO: monocytes; NEUT: neutrophils; RDW: red cell width distribution

[1] P-value for treatment effect

* The statistical model for this variable did not converge

Table 6 continued. Effect of type of cow-calf contact on hematological parameters measured in plasma of calves at three different ages.

	Full contact						
	day 1		day 14		day 49		
Variable	Mean	SE	Mean	SE	Mean	SE	P-value[1]
MCH, 10^{-12}/L	0.60	0.01	0.64	0.02	0.99	0.19	0.85
MCHC, g/dl	30.41	0.58	33.76	1.61	53.15	10.83	0.63
PLT, 10^9/L	330.91	32.83	662.46	65.89	546.87	43.00	0.34
MPV, 10^{-15}/L	6.25	0.39	5.22	0.12	5.42	0.10	0.77
PCT, %	0.14	0.01	0.30	0.05	0.21	0.02	0.60
LYM, 10^9/L	2.60	0.49	3.84	0.70	4.77	0.59	0.51
MID, 10^9/L	3.40	0.65	8.30	1.97	8.66	1.70	0.54
BASO*, %	0.00	0.00	0.00	0.00	0.00	0.00	
EO*, %	0.56	0.29	0.13	0.13	0.84	0.32	
LYM, %	32.38	4.69	47.87	3.84	54.53	2.31	0.49
MONO, %	4.19	0.69	4.60	0.71	6.16	0.46	0.01
NEUT, %	62.88	4.68	47.40	3.83	38.47	2.43	0.42
RDW	5.82	0.45	4.27	0.17	4.35	0.18	0.52

MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet count; MPV: mean platelet volume; PCT: procalcitonin; LYM: lymphocytes, MID: less frequently occurring and rare white blood cells; BASO: basophils; EO: eosinophils; MONO: monocytes; NEUT: neutrophils; RDW: red cell width distribution

[1] P-value for treatment effect

* The statistical model for this variable did not converge

4

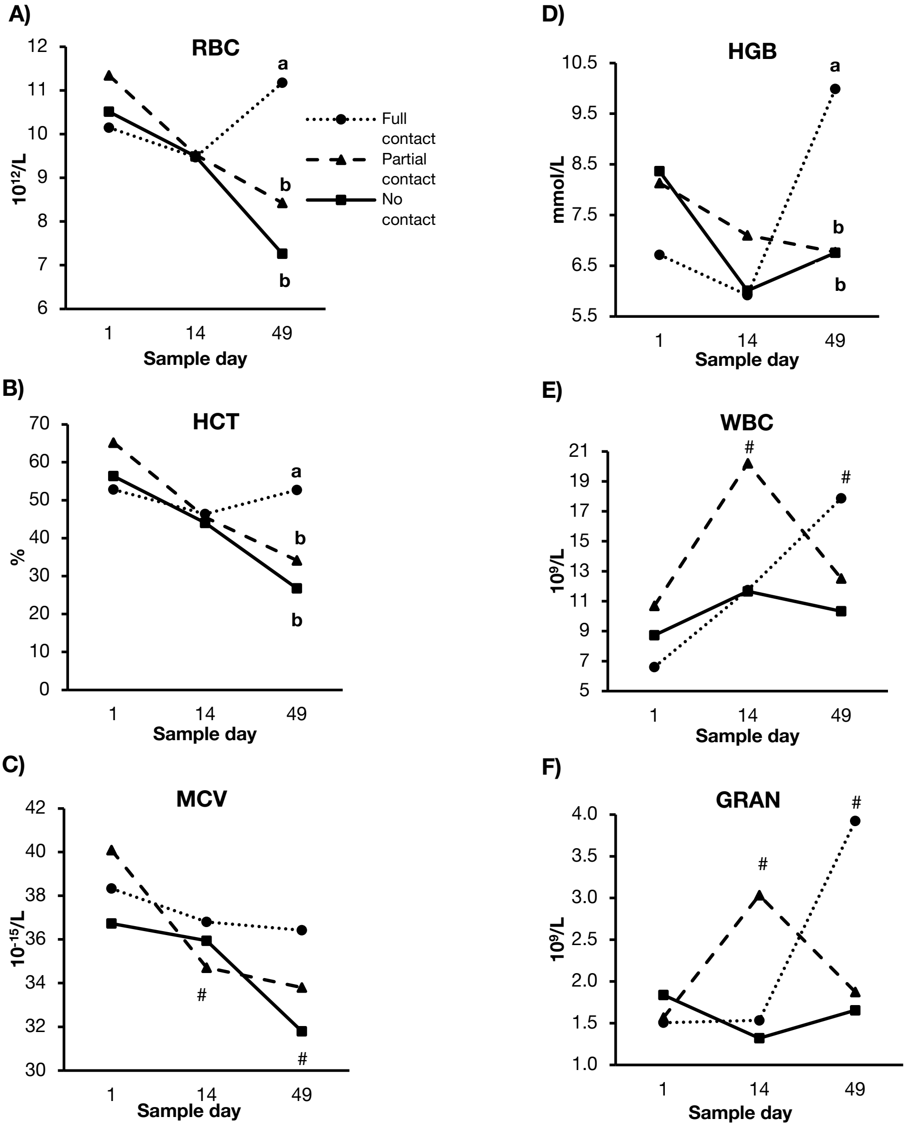


Figure 2. Interaction between treatment (no contact vs. partial contact vs. full contact) and sample moment (day 1, 14, 49) for hematological parameters (LSmeans) measured in plasma of dairy calves: **(A)** erythrocytes (RBC), **(B)** hematocrit (HCT), **(C)** mean corpuscular volume (MCV), **(D)** hemoglobin (HGB) , **(E)** leukocytes (WBC), **(F)** granulocytes (GRA). Different letters indicate significant differences ($P < 0.05$) between treatments within a sampling moment, # represent significant differences between sample days within a treatment group.

G)

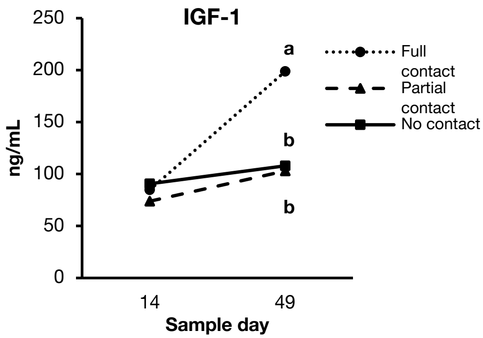


Figure 2 continued. Interaction between treatment (no contact vs. partial contact vs. full contact) and sample moment (day 14, 49) for **(G)** insulin-like growth factor 1 (IGF-1). Different letters indicate significant differences ($P < 0.05$) between treatments within a sampling moment, # represent significant differences between sample days within a treatment group.

4

Table 7. Effect of type of cow-calf contact on hair cortisol concentrations in dairy calves at three different ages.

	No contact						Partial contact						Full contact						
	Day 0		Day 21		Day 56		Day 0		Day 21		Day 56		Day 0		Day 21		Day 56		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P-value[1]
Cortisol, ng/g	7.93	1.74	3.41	0.73	3.20	0.55	9.72	2.07	8.42	1.42	8.15	3.79	9.38	2.15	10.55	3.22	4.80	0.44	0.29

[1] P-value for treatment effect

Table 8. Metabolic status of dairy cows during the dry cow period (2 to 21 days prepartum) and the fresh cow period (30 to 50 days postpartum) in three different cow-calf contact groups.

	No contact		Partial contact		Full contact			
	Mean	SE	Mean	SE	Mean	SE	F-value	P-value
Dry cow period								
NEFA, mmol/L	0.26	0.06	0.22	0.05	0.27	0.06	0.04	0.96
BHBA, mmol/L	0.50	0.04	0.46	0.05	0.47	0.04	0.25	0.79
Urea, mmol/L	4.69	0.39	5.23	0.53	4.70	0.40	0.56	0.58
Magnesium, mmol/L	0.83	0.03	0.84	0.04	0.82	0.04	0.57	0.58
Haptoglobin, g/L	0.10	0.02	0.21	0.12	0.17	0.09	0.07	0.93
Fresh cow period								
BHBA, mmol/L	0.54	0.04	0.54	0.07	0.53	0.03	0.73	0.49
Urea, mmol/L	3.36	0.61	4.79	0.52	3.65	0.31	3.29	0.05
Calcium, mmol/L	2.31	0.05	2.34	0.04	2.30	0.02	0.64	0.53

NEFA: Non-esterified fatty acids; BHBA: Beta-hydroxybutyrate

3.7 Microbiota composition

As calves aged, fecal microbiota alpha-diversity increased (see Appendix 2A) and the microbial community composition changed (see Appendix 2B). In total 35% of the variance was explained by calf age and 28% by the individual calf. Fecal microbiota RDA analysis showed that calves reared with different types of CCC were distinctly grouped at 28 (P = 0.002) and 49 days of age (P = 0.01), but not at 7 (P = 0.45) or 66 (P = 0.18) days of age (Figure 3). On day 28, the fecal microbiota composition was different in FC calves compared to NC (P = 0.02) and PC calves (P = 0.001), although NC and PC calves did not differ (P = 0.19). Likewise, the fecal microbiota composition in FC calves differed from NC (P = 0.01) and PC calves (P = 0.02) on day 49, while NC and PC calves did not differ (P = 0.82).

Univariate analysis identified differences in relative abundances between the three CCC groups on the different sample moments based on false discovery rate-corrected P-values. On day 7, NC calves had a higher abundance of *Anaerotignum* and *CAG-81* compared to PC (adjusted-P = 0.09) and FC calves (adjusted-P = 0.02). Besides, FC calves had a greater abundance of *Lactobacillus B* compared to NC and PC calves (adjusted-P = 0.06) (see Appendix 3). On day 28, FC calves had a higher abundance of *Lactobacillus B* compared to NC (adjusted-P = 0.09) and PC calves (adjusted-P = 0.04), but had a reduced abundance of *Ruthenibacterium, Alistipes A, Barnesiella, Marseille-P3106, Parabacteroides, Odoribacter, Pseudoflavonifractor,* and *Clostridium-P* compared to NC (adjusted-P < 0.10) and PC calves (adjusted-P < 0.03) (see Appendix 3). On day 49, FC calves had a higher abundance of *Butyricimonas* than NC (adjusted-P = 0.05) and PC calves (adjusted-P = 0.03), but had a reduced abundance of *Anaerotruncus, Ruminiclostridium C, Muribaculum, Ruminococcus E, RC9, Sphaerochaeta,* and *S5-A14-a* compared to NC (adjusted-P < 0.05) and PC calves (adjusted-P < 0.07) (see Appendix 3). Given that on day 66 only 2 NC calf samples were included in the analysis, differences in relative abundances were identified between PC and FC calves only. FC calves had a higher relative abundance of *Romboutsia, Turicibacter, Acholeplasma C, Acetivibrio,* and *Akkermansia* (adjusted-P < 0.09), and a reduced relative abundance of *CAG-873, Eubacterium F, Fournierella, Coprococcus B, Sphaerochaeta,* and *Barnesiella* compared to PC calves (adjusted-P < 0.09) (see Appendix 3).

After correcting the microbiota data for treatment and sample moment, the effects of health and growth parameters on taxa were assessed. Calves that suffered from respiratory issues had a higher abundance of *Olsenella* and *Slackia* compared to calves without respiratory issues (adjusted-P = 0.10). No differences in relative abundances were found for calves suffering from neonatal diarrhea (adjusted-P > 0.50) or navel inflammation (adjusted-P > 0.20) compared to calves without clinical symptoms for those diseases. In addition, no effect of weight gains (adjusted-P > 0.17) or absolute body weight (adjusted-P > 0.79) on relative abundances was found.

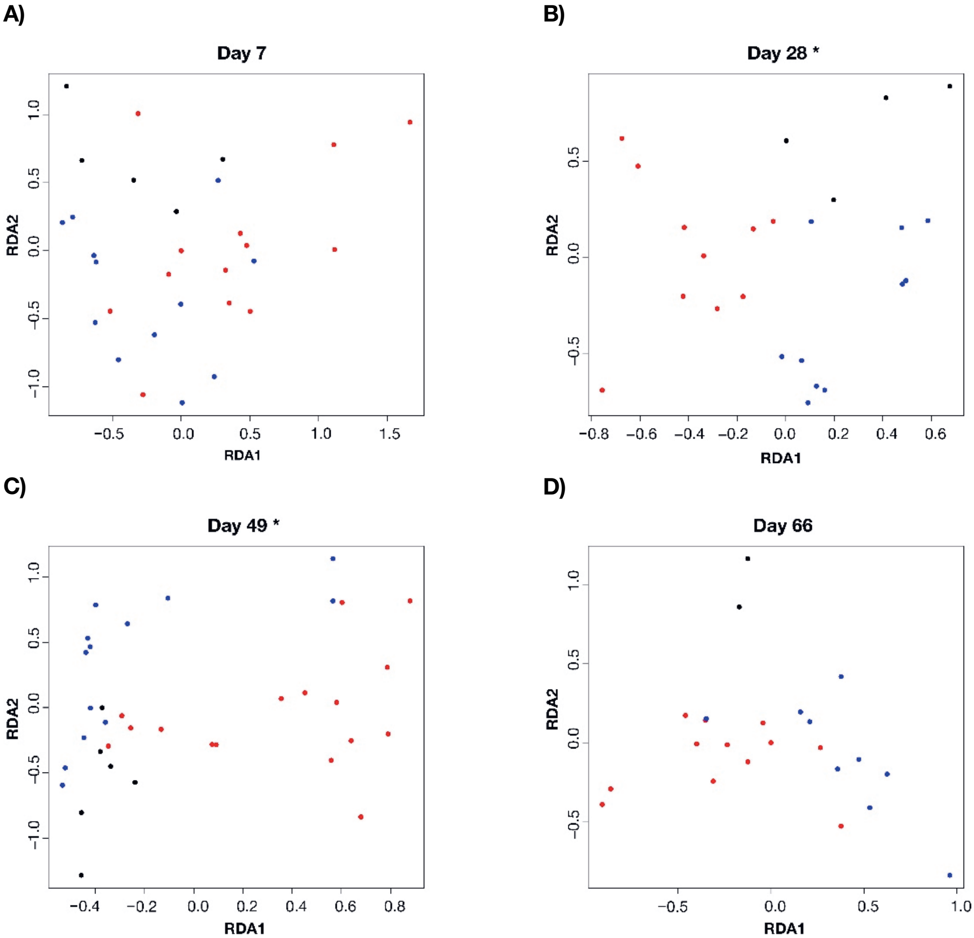


Figure 3. Redundancy analysis (RDA) of fecal microbiota in dairy calves reared with different types of cow-calf contact at **(A)** day 7, **(B)** day 28, **(C)** day 49, **(D)** day 66. The RDA is fitted conditioned on the batch effect. Individual calves with no contact are represented by black dots, partial contact by blue dots, and full contact by red dots. Asterisks indicate significant differences ($P < 0.05$) between treatment groups within a sample moment as evaluated with a permutation test.

4. Discussion

The objective of this study was to evaluate the effect of different types of CCC on the health status, blood parameters, fecal microbiota and performance of dairy calves and cows. Our results showed that FC calves appeared to have more health issues, as reflected by an increased THS and a tendency for higher antibiotic usage in the first 7 weeks of life compared to NC calves. This was supported by elevated levels of RBC, HCT, and HGB on day 49 in FC calves compared to NC calves, and elevated WBC and GRA levels in FC calves on day 49 compared to day 14. Fecal microbiota composition changed as calves aged, and differences in relative abundances of various genera were found in FC calves compared to NC and PC calves. Furthermore, FC calves had a greater body weight gain than NC and PC calves in the first 7 weeks of life, which was accompanied by higher IGF-1 concentrations on day 49 in FC calves. In this study cow health was not affected by type of CCC, although FC cows had, as expected, a lower daily machine-milked milk yield accompanied by a lower fat percentage in the first 7 weeks of lactation compared to NC and PC cows.

Ensuring adequate transfer of immunoglobulins is crucial to get off to a good start for the newborn calf (Sweeney et al., 2009). In our study quick administration of the first colostrum meal by bottle assured comparable serum IgG concentrations for all calves. Interestingly, compared to bottle feeding, suckling colostrum from the dam has been found to increase the amount of IgG absorbed by the calves, suggesting that suckling in itself may promote passive transfer (Quigley et al., 1995; Stott et al., 1979). FC calves that accidently suckled their first colostrum may have benefited from this effect (in addition to having been able to ingest a higher amount of colostrum). Furthermore, bacterial contamination of colostrum during harvesting and feeding can interfere with immunoglobulin absorption, as bacteria originating from harvesting or storing colostrum may bind free immunoglobulins in the gut lumen or directly block uptake and transport of immunoglobulin molecules across intestinal epithelial cells (Godden, 2008). Research showed that harvesting colostrum into a bucket resulted in substantial higher bacterial counts than in directly stripped colostrum (which a suckling calf would be expected to obtain) (Stewart et al., 2005). However, calves left to suckle their dams often fail to ingest adequate volumes of colostrum in time, which increases the risk for failure of passive transfer (Besser et al., 1991; Franklin et al., 2003). Therefore, close monitoring of colostrum intake in all systems including full CCC systems is highly recommended. As calves aged, immunoglobulin titers did not differ among treatment groups, although we found an increase of N-IgM titers and decline of IgG titers from day 14 to day 49. Similar patterns were found in previous research (Rajala and Castrén, 1995). From 42 days of age onwards calves are expected to have developed their own adaptive immunity (Hassig et al., 2007). Our findings, therefore, suggest that all calves were able to exhibit sufficient endogenous production of immunoglobulins over time, regardless of CCC treatment. Hematological parameters are also known to change as calves age (Mohri et al., 2007). Hence, the decrease in MCV and neutrophils accompanied by an increase in

MCH, MCHC, PLT, lymphocytes, and monocytes over time imply a normal physiological development (Mohri et al., 2007; Roland et al., 2014). Correspondingly, the overall increase in cholesterol concentrations from day 14 to day 49 reflect maturation of the gastrointestinal tract (Piccione et al., 2010).

Besides the risk for failure of passive transfer, housing conditions are a major hazard for the health of the newborn calf (Gulliksen et al., 2009). In the present study, three common calf disorders, namely umbilical cord infections, diarrhea, and respiratory issues (Mee, 2008), explain the increased THS in FC calves. Maternity pens are the first place where calves can be infected with pathogens (Maunsell and Donovan, 2008). A recent review reported inconsistencies among studies that compared no contact versus full contact with the dam in the first few days postpartum, because either beneficial, detrimental, or no effects of full contact on calf health were found (Beaver et al., 2019a). In our study the relatively high prevalence of umbilical cord infection in PC and FC calves may have been the result of the postnatal housing conditions, as those cow-calf pairs remained in the maternity pen for the first three days postpartum to strengthen the cow-calf bond, whereas NC calves were moved to an individual calf box away from the dam directly after birth. Possibly, the prolonged residence time in the maternity pen posed a challenge on the management in terms of pen hygiene, which in itself is known to increase the risk for umbilical cord infection (Mee, 2008). Those infections are harmful to the general condition and health of the calf, as bacteria can migrate to joints, lungs, and other organs, and therefore pose a risk for enteric and pulmonary infections later in life (Wieland et al., 2017). Thus, adequate maternity pen management and overall cleanliness of the calving area are of critical importance for CCC systems (Beaver et al., 2019a).

Diarrhea is mainly caused by inadequate management related to hygiene, housing, and feeding (Klein-Jöbstl et al., 2014; Vasseur et al., 2010). Previous work on prolonged CCC with suckling pointed to beneficial or no effects on calf diarrhea (Beaver et al., 2019a), although one study also found more health problems in FC calves mainly resulting from higher diarrhea incidences (Roth et al., 2009). Our study showed a high prevalence of neonatal diarrhea in all treatment groups, however we could not differentiate between infectious diarrhea or nutritional diarrhea. Perhaps, NC and PC calves were exposed to pathogens arising from milk feeding management (Klein-Jöbstl et al., 2014), whereas diarrhea incidences in the FC calves may have been caused by the large amounts of milk that they consumed (Roth et al., 2009). Nevertheless, FC calves might also have been exposed to enteric pathogens due to the group housing and contact with floors that were contaminated with adult cow manure in the FC pen (Roland et al., 2016).

The risk for respiratory disorders in young calves increases when exposed to inadequate barn climate in terms of, for example, temperature, humidity, wind speed (draft) and air quality, and inappropriate (in particular wet) bedding (Curtis et al., 2016). The high prevalence of ocular and nasal discharge accompanied by the tendency for an increased use of antibiotics in FC calves compared to NC calves in the first 7 weeks of life likely reflected an increased incidence of respiratory disorders. This corresponds with the increased RBC, HCT, HBG, WBC, GRA values found in FC calves on day 49, an age when respiratory disorders in calves are common (Svensson and Liberg, 2006). Respiratory disorders can trigger an increase in erythropoietin production, which increases the amount of erythrocytes and results in elevated RBC, HCT, HBG values (Roland et al., 2014). Moreover, leukocytes play an essential role in immune defense, and increasing levels of WBC and GRA can be indicative of an inflammation (Roland et al., 2014). FC calves were housed together with the cows and with calves of different ages, in groups of varying sizes, and in a pen that had open sidewalls. In contrast, NC and PC calves were pair housed in their own calf box that reduced contacts with other animals, and that may have protected them from unfavorable climatic conditions, like draft (van der Fels-Klerx et al., 2000). Given that existing cow pens are originally designed for adult animals rather than young calves, the potentially nonoptimal climate in those pens can pose a challenge for calf health (Johnsen et al., 2016). Although, other studies on prolonged full CCC showed beneficial or no negative effects on calf health (Grøndahl et al., 2007; Johnsen et al., 2015b; Roth et al., 2009; Wagenaar and Langhout, 2007). These inconsistencies might be due to the variability in study type and methodology, as the majority of those studies did not investigate calf health as primary outcome measure, had different barn designs, and sometimes small sample sizes. Moreover, the present experiment was conducted on a dairy farm with no previous experience with prolonged CCC. Successfully adopting new farm management systems depends, among others, on inner motivation, former experience with change, and the period of time over which new practices are implemented (Hansen and Jervell, 2015). Farmers that transformed their calf rearing system from a conventional to a full CCC system reported calf health benefits (Neave et al., 2021; Vaarst et al., 2020; Wagenaar and Langhout, 2007), but also acknowledged that it required additional infrastructure (Neave et al., 2021) and that it took time to reach the necessary change of perception on calf monitoring (Vaarst et al., 2020). We highly recommend future research to focus on identifying and optimizing suitable cow-calf housing systems and managerial changes to ensure optimal calf health in CCC systems both during and after the transition period.

Despite the higher disease incidences in FC calves, we found a greater ADG in FC calves compared to NC and PC calves throughout the milk feeding period. However, FC calves no longer differed in absolute body weight from PC and NC calves at 6 months of age. This is in contrast with other studies in which growth benefits during the suckling period were maintained for months after separation compared with separated calves (Meagher et al., 2019). Possibly, FC calves experienced a growth dip after weaning and separation (Wenker et al., *in preparation*), as weaning calves at a relatively young age from high volumes of milk while being not yet fully adapted to the solid feed is a well-known challenge in full CCC systems (Enríquez et al., 2011; Johnsen et al., 2015b). High growth rates during the milk feeding period are commonly reported in calves that suckle their dam freely for a prolonged period of time (Fröberg et al., 2011; Roth et al., 2009; Wagenaar and Langhout, 2007). We aimed to feed NC and PC calves at a rather high feeding schedule, while still following the Dutch standard practice by applying gradual weaning up to 8 weeks of age. Nevertheless, free suckling of the dam provided calves the opportunity to meet their natural requirement that is estimated at a milk consumption at about 20% of their body weight (Conneely et al., 2014; Jasper and Weary, 2002). This could explain the relatively high ADG despite the impaired health status. Besides, the higher plasma IGF-1 concentrations in FC calves, compared to PC and NC calves on day 49, reflect a greater body condition related to the higher energy intake and thus ADG (Schäff et al., 2016; Shen et al., 2004). Plasma IGF-1 concentrations are considered important for the development of the gastrointestinal tract (GIT), as in young goats increased IGF-1 concentrations due to high intake of protein and energy were accompanied by an increased rumen papillae size (Shen et al., 2004). Possibly, compared to calves that were fed with limited amounts of milk, calves reared in full CCC have a differently developed GIT attributable to ad libitum milk intake. Correspondingly, we found preliminary evidence that the succession of microorganisms colonizing the GIT was affected by type of CCC, as FC calves had different relative abundances of fecal microbiota compared to NC and PC calves. Similar results were found in 4-week old dairy calves reared with maternal contact compared to conventionally reared calves (Beaver et al., 2021). That study reported higher relative abundances of *Lactobacillus* at day 28 in calves reared with maternal contact, which is in line with our findings in FC calves that had a higher relative abundance of *Lactobacillus* on both day 7 and day 28 compared to NC and PC calves. Early colonization of *Lactobacillus* spp. can provide probiotic effects for calves and offer protection against neonatal diarrhea (Fernández et al., 2018). However, given that conventionally reared calves in (Beaver et al., 2021) were fed with waste-milk that contained residuals of antimicrobials, the changes in microbiota composition could not be attributed solely to maternal contact in that study. Nonetheless, our exploratory results imply that microbial communities developed distinctively between FC and PC calves at 28 and 49 days of age, perhaps because full CCC allowed for more vertical transmission of microbes via the dam (i.e. unimpeded reciprocal licking, contact to adult feces, and exposure to microbes on the teat skin and in maternal milk) (Oikonomou et al., 2020; Sarkar et al., 2020). Changes in microbial communities can also be affected by age, diet, antibiotics, and environmental factors (Arrieta et al., 2014; Malmuthuge and Guan, 2017). Arguably, FC calves were suckling ad libitum milk from their dams and were housed inside the cow pen, whereas NC calves were housed in a separate calf barn, PC calves were housed adjacent to the cow pen, and those two groups were fed tank milk via teat buckets. Yet, we found that alpha-diversity increased in calf fecal microbiota as animals aged, but no treatment effect on fecal microbiota composition was found in the first week (i.e. day 7). This corresponds to previous work that reported increasing diversity in GIT microbial communities as pre-weaned calves aged, except on day 7 indicated by similar microbiota in various GIT regions (Dias et al., 2018). Given that we found no treatment effect after weaning (i.e. day 66) as well, implies that pre-weaning diet was an important factor explaining the differences in colonization (Meale et al., 2016; Wiley et al., 2017). Future studies are needed to enhance our understanding of maternal factors that may affect dairy calf microbiota, and of the clinical and biological relevance of these effects.

In line with the high body weight gains of FC calves, FC cows had a lower machine-gained milk yield compared to NC and PC cows. Ad libitum milk consumption by calves is known to reduce machine-gained milk yield (Barth, 2020; de Passillé et al., 2008; Johnsen et al., 2015c). Besides, we found a reduced milk fat content in harvested milk of FC cows, which is likely to be caused by impaired alveolar milk ejection during the milking process due to suckling (Barth, 2020; Johnsen et al., 2016; Zipp et al., 2018). Reduced amounts of saleable milk with decreased fat content during the suckling period could negatively impact farm income in full CCC systems (Johnsen et al., 2016; Knierim et al., 2020). However, any reduction in saleable milk income can only truly be considered a loss if the milk intake suckled by calves exceeds the costs of what they would have been fed through other methods (e.g. bucket feeding with milk replacer, bulk tank milk, or waste milk) (Meagher et al., 2019). In addition, FC cows tended to have a decreased lactose content compared to NC and PC cows, which may be explained by the few cases of high SCC incidences. Lactose content tends to decrease when SCC increases due to clinical or subclinical udder inflammation (Costa et al., 2019b). Yet, type of CCC did not negatively affect cow health in the present study, which is in agreement with previous work that assessed udder health in suckled and non-suckled dams (Beaver et al., 2019a; Johnsen et al., 2016; Wagenaar et al., 2011; Wagenaar and Langhout, 2007). Interestingly, our study showed that the metabolic status of the cows was not affected by suckling, even though twice-daily milking in addition to ad libitum suckling was expected to increase the metabolic stress in early-lactating cows (McNamara et al., 2008). Previous work suggested that three times a day machine-milking of suckled cows resulted in a severe negative energy balance, as expressed by a heavy weight loss, elevated NEFA concentrations, and decreased glucose concentrations in their blood compared to non-suckled cows that were milked either three or six times a day (Bar-Peled et al., 1998). Blood metabolites in our study were similar to those in a recent study on cow serum metabolites (Hussein et al., 2020), although our relatively low urea concentrations in early lactation may be a result of low protein content in the feed among other management factors (Baker et al., 1995).

Given that the standard practice of early cow-calf separation is perceived as contentious by part of the public for ethical reasons (Hötzel et al., 2017; Ventura et al., 2013) and that rearing calves in full CCC with their dam does not necessarily guarantee an adequate calf health status, partial CCC might be considered as a feasible compromise for rearing dairy calves. Such a rearing system has the potential to increase the social acceptance of the dairy sector by allowing limited cow-calf interactions (von Keyserlingk et al., 2013), while at the same time meeting producers' concerns regarding calf health and the amount of harvested milk (Flower and Weary, 2003). Because stockmanship and housing conditions are crucially related to calf health (Marcé et al., 2010; Palczynski et al., 2021), we strongly recommend efforts that identify the key features of best-practices in full CCC systems to safe-guard calf health and enable a successful transition for farmers' interested in this calf rearing system. Moreover, since full CCC may provide longer-term benefits for calves' behavioral development (Meagher et al., 2019), we believe that longitudinal studies into the effects of CCC beyond the time-frame of the current experiment are also warranted.

5. Conclusion

This study shows that rearing calves in full contact with their dam compromised calf health in the first seven weeks of life, as reflected by more health issues, elevated hematological parameters, and a tendency for higher antibiotic usage compared to calves reared without contact with the dam. In comparison with partial or no contact, full contact resulted in a greater average daily body weight gain and in a different calf fecal microbiota composition with, so far, unknown biological implications. Cow health was not affected by the type of cow-calf contact. Cows that were suckled by their calves had a lower machine-gained milk yield and a lower milk fat content compared to cows in a partial or no contact system.

Acknowledgements

This study was financed by DairyNL (ZuivelNL; organization of the Dutch dairy supply chain, The Hague, the Netherlands) and the Dutch Ministry of Agriculture, Nature and Food Quality (LNV, The Hague, the Netherlands) as part of the research program 'One Health for Food' (1H4F, The Hague, the Netherlands). We thank the staff of the Knowledge Transfer Centre (Zegveld, the Netherlands) for their assistance during the experiment. Thanks to Deborah Bomfim, Bianca Costa, Anne Hoogstra, Claudia Meeusen, Fokje Steenstra, and Ilona Wardenaar for their valuable help with the experiment and data acquisition. We are grateful to Francesca Marcato from Wageningen University & Research, Frank Harders and Lars Ravesloot from Wageningen Bioveterinary Research for laboratory analyses. We thank Sabine Schnabel from Biometris for her assistance with the statistical analyses. Thanks to Kristin Sørheim from Norwegian Centre for Organic Agriculture and Yanran Cao from Norwegian University of Science and Technology for the hair cortisol analysis. We are grateful to Alex Bossers from Wageningen Bioveterinary Research for his support with the microbiota analysis.

Appendix 1. Details bioinformatic analysis microbiota

1. Library construction and sequencing

Sequence processing and preparational statistical analyses were performed in R 4.0.2 (R Core Team, 2014). The amplicon sequences were demultiplexed and subsequently filtered, trimmed, error-corrected, dereplicated, chimera-checked, and merged using the dada2 package (v.1.16.0, (Callahan et al., 2016)). By using the standard parameters except for TruncLength=(265, 235), trimLeft=(35,35), maxEE=2, and minOverlap=10. Reads were classified using the naïve Bayesian classifier and the Genome Taxonomy Data Base (GTDB v86; (Parks et al., 2021)). Using R package phyloseq (McMurdie and Holmes, 2013), the ASVs were aggregated to the Genus level. The data were filtered for low abundant taxa separately per day. Taxa that were present in at least half of the calves of a particular day were selected for that day. After filtering, the library size ranged from 43204-173453, 35888-148311, 4535-30133, and 35611-124957 for, respectively, day 7, 28, 49, and 66 (see Appendix 2A). For these days we selected 34, 81, 72, and 111 taxa containing 25%, 22%, 27%, and 21% zeroes, respectively.

1.2 Statistical analysis

The data were explored using unconstrained and constrained ordination analysis (PCA/RDA) of Hellinger transformed microbiota compositions using R package vegan (Oksanen et al., 2007). Significance was assessed using the permutation test for the RDA.

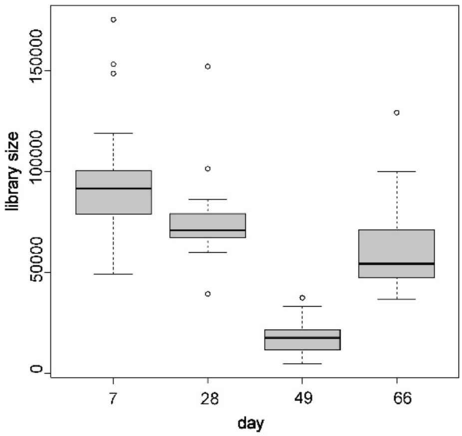
We first fitted a PCA across all days (using a set of 86 taxa that are selected for at least two days), to conclude that the day effect is the primary source of variation. Further assessment of the effect of parity, batch and treatment was done separately per day, using only the taxa selected for that day. Parity had little effect (P-values > 0.05 for all days) and could be ignored. Batch also has little effect (P-values > 0.05 for all days), but for interpretational purpose was included in evaluation of the treatment effect and health variables.

The effect of treatment on the separate taxa was evaluated using beta-binomial regression. In the beta-binomial regression one model is fitted per taxon using treatment as explanatory variable; the batch effect was also included in the model. These models were fitted per day using R package corncob (Martin et al., 2020). To account for the small sample size (and the resulting possible type I error inflation), the parametric bootstrapped was used (1e4 bootstrap samples) to estimate the P-value. Using these P-values, the false discovery rates (FDR) were calculated using the Benjamini-Hochberg procedure.

The health variables were also evaluated using beta-binomial regression. Based on the weekly health assessments, calves diagnosed with clinical symptoms for navel inflammation (i.e. navel score ≥ 2), neonatal diarrhea (i.e. feces score ≥ 2), and respiratory issues (i.e. a composite respiratory score ≥ 4 (based on the sum of ocular discharge, nasal discharge, cough score)) (Table 2). The health parameters were evaluated across days; analysis per day was not possible due to the low occurrence of some health variables. In this analysis we used a more restrictive set of taxa, only including those that were selected in at least three days (48 taxa). The health variable were included as a covariate in a model that also included calf and batch as (nested) random effects, and treatment and sample day as fixed effect. These models were fitted using R package glmmTMB (Brooks et al., 2017), which allows including random effects in the beta-binomial regression.

Appendix 2A. Supplementary figures fecal microbiota

A)



B)

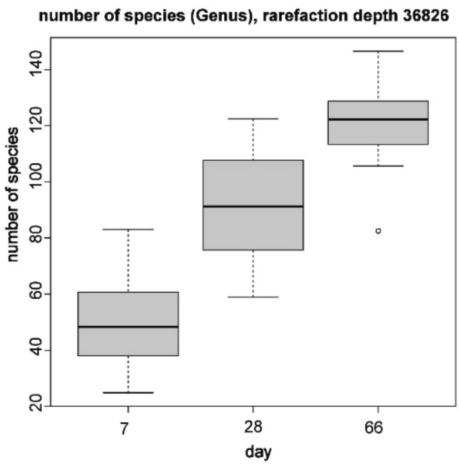


Figure 1. Boxplot diagrams for **A)** library sizes and **B)** microbiota alpha-diversity per sample moment.

Appendix 2B. PCA for all fecal microbiota samples

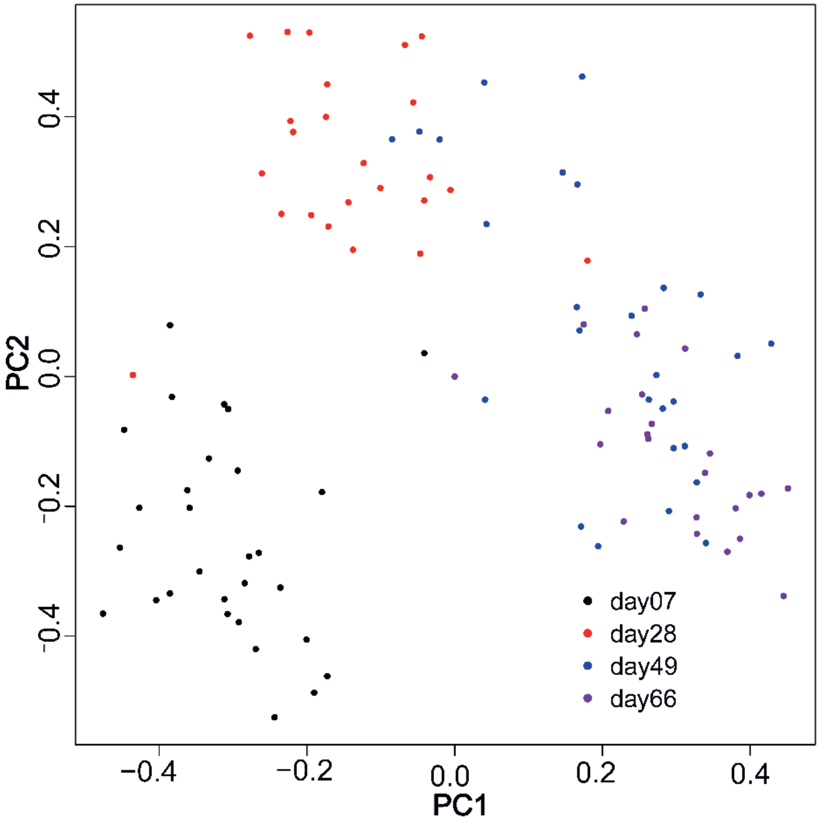


Figure 2. PCA for all fecal samples (includes taxa selected on ≥ two days, Hellinger transformed, the PCA is conditioned on calf). Per day, each dot represents an individual calf. In total 35% of the variance is explained by day and 28% by the individual animal.

Appendix 3. Differential abundances fecal microbiota

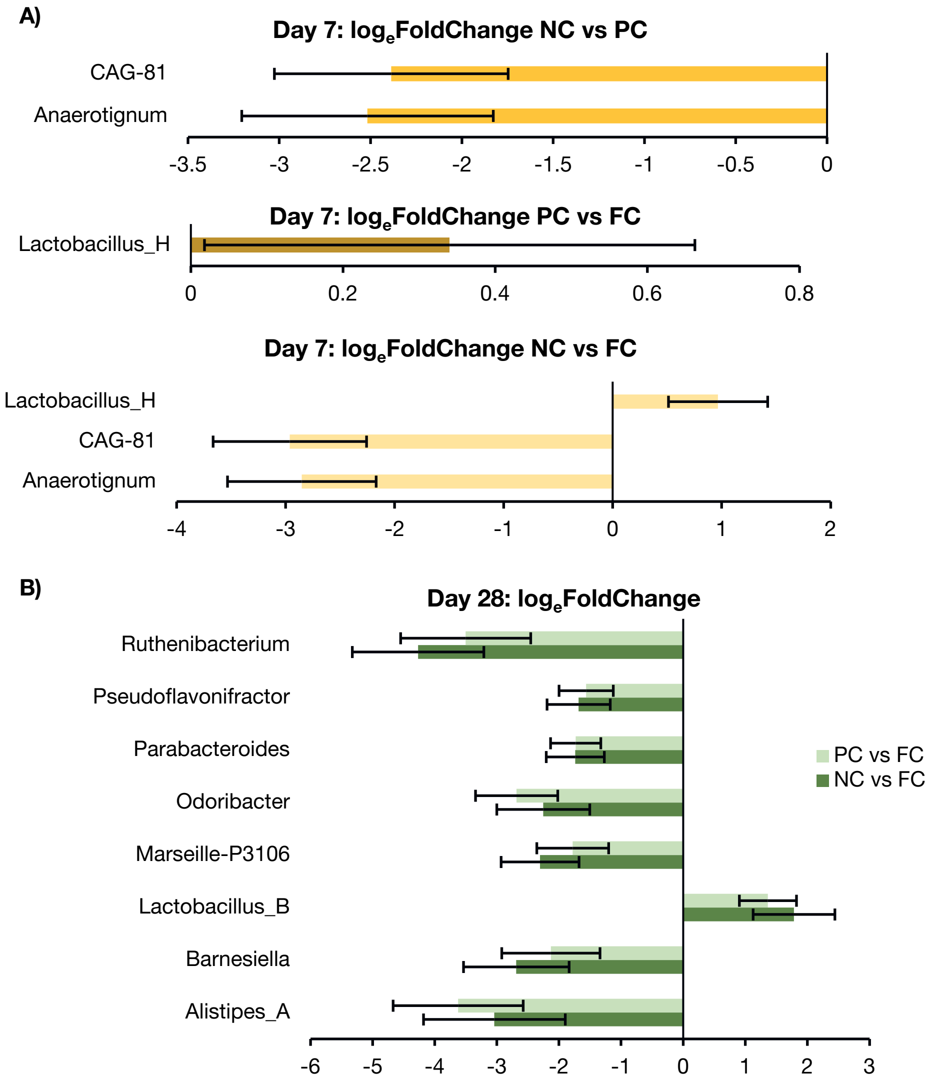
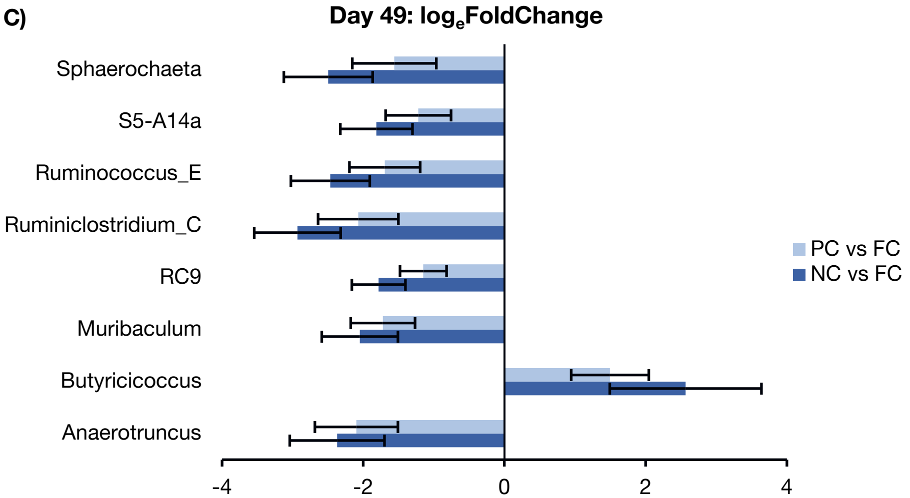


Figure 3. Differential abundances of ASVs between the different cow-calf contact groups (NC = no contact, PC = partial contact, FC= full contact) at different sample moments: **A)** day 7, **B)** day 28. A negative value of natural log fold change (± SE) meant significantly more abundance in the control group (i.e. first mentioned group) for taxon. All taxa with a FDR < 0.10 are displayed.

C)



D)

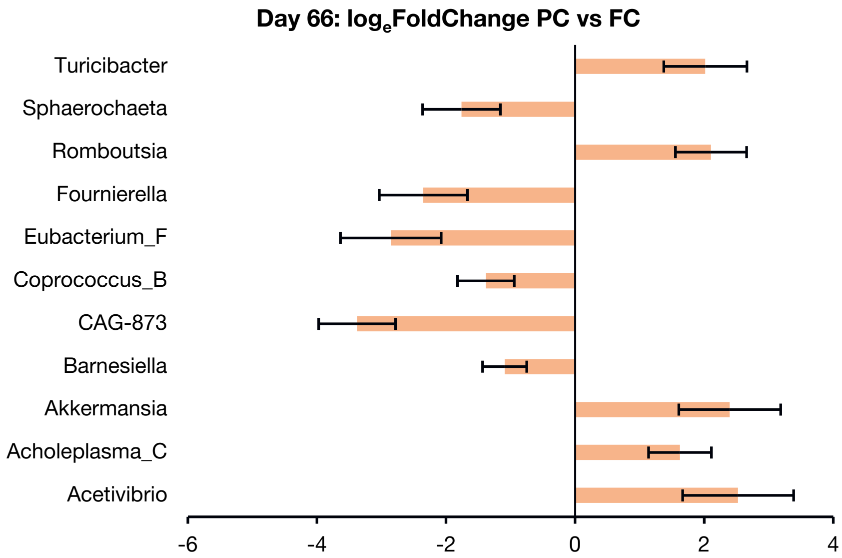


Figure 3 continued. Differential abundances of ASVs between the different cow-calf contact groups (NC = no contact, PC = partial contact, FC= full contact) at different sample moments: **C)** day 49, and **D)** day 66. A negative value of natural log fold change (± SE) meant significantly more abundance in the control group (i.e. first mentioned group) for taxon. All taxa with a FDR < 0.10 are displayed.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Chapter 5

Comparing gradual debonding strategies after prolonged cow-calf contact: Stress responses, performance, and health of dairy cow and calf

Margret L. Wenker[1,2], Cornelis G. van Reenen[1,2], Eddie A.M. Bokkers[1], Kirste McCrea[3], Daiana de Oliveira[3], Kristin Sørheim[4], Yanran Cao[5], Rupert M. Bruckmaier[6], Josef J. Gross[6], Gerrit Gort[7], Cynthia M. Verwer[8]

[1] Animal Production Systems group, Wageningen University & Research, Wageningen, the Netherlands
[2] Livestock Research, Wageningen University & Research, Wageningen, the Netherlands
[3] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Uppsala, Sweden
[4] Norwegian Centre for Organic Agriculture (NORSØK), , Tingvoll, Norway
[5] Norwegian University of Science and Technology, Department of Biological Sciences, Ålesund, Norway
[6] Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[7] Biometris, Wageningen University & Research, Wageningen, the Netherlands
[8] Louis Bolk Institute, Bunnik, the Netherlands

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Abstract

We assessed effects of two-step debonding strategies in calf rearing systems that allowed for different types of prolonged cow-calf contact (CCC) on stress responses, health and performance of dairy cows and calves. Forty-eight Holstein Friesian cow-calf pairs had either: 1) full contact including suckling, where contact was reduced before weaning via fence-line separation at day 49 (FC-FS) (n = 10); 2) full contact, where contact was reduced at day 56 by fitting calves with a nose-flap (FC-NF) (n = 10); 3) partial contact (calves were housed in a pen adjacent to the cow area allowing physical contact on initiative of the dam but no suckling), where contact was reduced before weaning by moving the calf box from the wall to hamper physical contact at day 49 (PC-BW) (n = 12); 4) partial contact, where contact was reduced the week after weaning by moving the calf box away from the wall at day 63 (PC-AW) (n = 6); 5) no contact (calves were removed from dam directly after birth and housed in a calf barn), calves were standardly weaned at day 56 (NC-S) (n = 10). Between weeks 7-10, we assessed physiological stress parameters, weight gain, and the health status of calves, plus general activity patterns based on accelerometer sensor data of cow-calf pairs before, during and after the debonding interventions. Additionally, calves were subjected to four consecutive behavioural tests (i.e. open field, novel object, human approach, handling test) prior to permanent separation at day 70 and their behavioural responses were assessed via video recordings. Machine-harvested milk yields of cows were evaluated during weeks 6-12. Data were analyzed with (generalized) linear mixed models. After reducing contact and weaning, both FC-FS and FC-NF calves spent a larger proportion of time in high activity than PC-BW, PC-AW, and NC-S calves ($P \leq 0.002$). Moreover, FC-NF calves had an impaired growth rate ($P = 0.02$). In weeks 6-9, FC-FS and FC-NF cows had lower machine-harvested milk yields than PC-BW, PC-AW, and NC-S cows ($P \leq 0.01$). We found no differences in responsiveness of calves to behavioural tests, except that NC-S calves exhibited more solitary play events compared to PC and FC calves in the novel object test ($P = 0.001$). Overall, our results imply that calves with partial CCC showed low stress responses to debonding, whereas abrupt weaning with a nose-flap during full contact seemed most stressful. Machine-harvested milk yield of FC cows seemed to recover once calves were weaned. More research into strategies to improve the process of debonding is warranted.

1. Introduction

Separating the calf from the dam shortly after birth is a routine practice on commercial dairy farms that differs from natural settings where the calf is raised by the dam (Whalin et al., 2021) and is perceived as contentious by the public (Busch et al., 2017; Ventura et al., 2013). Hence, some farmers have developed rearing systems that allow for prolonged cow-calf contact (CCC) including suckling (Johnsen et al., 2016; Vaarst et al., 2020). One major welfare challenge in those so-called full CCC systems is the moment of debonding that is generally accompanied by strong signs of distress in both cow and calf (Flower and Weary, 2001; Veissier et al., 2013).

In commercial CCC systems calves are generally weaned and separated from the dam at 8 to 12 weeks of age (Sirovnik et al., 2020), which is known to be more stressful for both the dam and the calf compared to weaning at the natural weaning age of about 8 months (de Souza Teixeira et al., 2021; Lambertz et al., 2015; Stěhulová et al., 2017). This relatively early weaning gives calves a shorter time period to learn to live independently from the mother and on a diet of solid feed only (Enríquez et al., 2011), even though this weaning age is comparable to standard rearing systems (Marcé et al., 2010). Moreover, when calves are abruptly separated from the dam after prolonged CCC, two stressful events occur at the same time: calves are weaned off of milk (i.e. need to become nutritionally independent) and lose contact with the dam (i.e. need to become socially independent) (Newberry and Swanson, 2008).

Abrupt separation after prolonged contact is thus highly stressful and should be avoided at all times, which has led to the development of gradual two-step debonding strategies (see review by Enríquez et al., 2011). Two-step debonding methods encourage calves to become nutritionally independent before separation and can, for instance, be implemented by using anti-suck devices (i.e. nose-flap that abruptly prevents suckling but allows all other forms of social interaction (Loberg et al., 2008)) or fence-lines (i.e. reduces physical contact and suckling across a fence and is generally applied for a certain period prior to complete separation (Johnsen et al., 2015a). These two-step debonding methods seem to reduce stress-responses around weaning in beef cattle at 6 to 7 months of age (Haley et al., 2005; Price et al., 2014) and in dairy cattle at 8 to 10 weeks of age (e.g. fence-line separation in Johnsen et al., 2015a; nose-flap in a foster cow system in Loberg et al., 2007). Yet, to our best knowledge no work has attempted to compare these two-step debonding methods in dam-reared calves with full CCC to identify which strategy can best minimize the adverse effects of breaking the bond after prolonged contact (Jensen, 2017).

Since calves' nutritional independence from the dam appears to reduce stress at separation (Johnsen et al., 2018), another interesting management strategy to explore is the allowance of partial CCC. A partial CCC system prevents suckling by permitting limited physical contact (Sirovnik et al., 2020). Moreover, it allows for a gradual weaning schedule, as calves can be fed manually by the farmer or via an automatic milk feeder. Recently, we showed that cows in a partial CCC system still show calf-directed affiliative behaviour but to a lesser extent than cows in a full CCC system (Wenker et al., 2021). In addition, we found an increased motivational strength of cows to reunite with their calf when full CCC was allowed compared to partial CCC, which implies that the mother-offspring bond might be less comprehensive in dairy cows that are not suckled (Wenker et al., 2020). The next step is to examine the animals' stress responses to weaning and separation when partial CCC is allowed.

Therefore, the objective of this study was to assess the effect of two-step debonding strategies in two types of cow-calf contact systems on stress parameters, health, and performance of dairy cow and calf in comparison to standard practice (i.e. early separation after birth and gradual weaning schedule). To this end, we examined responses of cow-calf pairs with either partial or full CCC subjected to two different debonding strategies by either limiting the amount of contact before the calf was fully weaned (i.e. hampering physical contact for partial CCC one week before weaning or implementing fence-line contact for full CCC the week before weaning) or after calf was fully weaned (hampering physical contact for partial CCC the week after weaning/fitting calves a nose-flap at weaning for full CCC). We hypothesized that cow-calf pairs with partial contact would show a lower stress response to the two-stage debonding strategies, as their responses were expected to be more similar to cows and calves that were managed according to the standard practice after early separation than full CCC. In contrast, cow-calf pairs with full contact were hypothesized to respond stronger to debonding than the other groups, especially the nose-flap strategy calves compared to the fence-line strategy.

2. Materials and methods

The experimental design was approved by the Central Committee on Animal Experiments (The Hague, the Netherlands; approval number AVD4010020174307). The study was conducted at the Knowledge Transfer Centre in Zegveld (the Netherlands) from February 2019 to July 2020. All applicable international, national, and institutional guidelines for the care and ethical use of animals were followed.

2.1 Animals and treatments

Forty-eight Holstein Friesian cows (mean parity of 3) were included in the experiment with a parallel group design. In order to have a similar aged peer for calves throughout the experiment, every two cows that calved successively were assigned to the same treatment. Treatment order for every set of two cows was randomized in each block of six successive calvings. Cows were allowed to have either: 1) full contact with their calf including suckling (calves were housed together with the dams in a free stall barn) and contact was reduced before weaning via fence-line separation from day 49 onwards (FC-FS) (n = 10), 2) full contact with their calf including suckling and to wean the calves suckling was prevented by fitting calves with a nose-flap (FC-NF) from day 56 onwards (n = 10), 3) partial contact with their calf (calves were housed in a calf box adjacent to the cow area allowing physical contact on initiative of the dam but no suckling) and contact was reduced before the calf was fully weaned by moving the calf box 0.5 m from the wall to prevent physical contact from day 49 onwards (PC-BW) (n = 12), 4) partial contact with their calves and contact was reduced the week after the calf was fully weaned by moving the calf box away from the wall at day 63 (PC-AW) (n = 6), or 5) no contact with their calf (calves were removed directly after birth and the calf was housed in a separated calf barn) and calves were gradually weaned via a feeding schedule and no longer received milk from day 56 onwards as reference group (NC-S) (n = 10). At 70 days of age, cow-calf pairs with partial and full CCC were permanently separated. Only female calves were included for this experiment, therefore cows were inseminated with sexed semen. A visual overview of all treatments and related events over time can be found in Appendix 1.

2.2 Housing and feeding

Based on signs of imminent calving, cows were moved into an individual indoor straw-bedded maternity pen (3.0 m wide × 5.1 m long). After birth, all full contact (FC) calves had access to their dam and could move freely inside the maternity pen, whereas all partial contact (PC) calves were placed in a cuddle-box (consisting of four plywood plates of 1.2 m wide × 0.8 m high) inside the maternity pen that prevented suckling, while still allowing tactile, visual, audible, and olfactory contact (see Wenker et al. (2021) for an illustration). This meant that all PC cows could lick and sniff their calf when the calf was standing or lying, by moving their head into the box to reach the calf. NC-S calves were removed from the dam within 1.5 h after birth (median: 6 min, range: 1 to 63 min) and placed in an individual straw-bedded calf box (1.0 m × 1.6 m; Topcalf Duo-Flex, Schrijver, the Netherlands) in an indoor calf barn. All calves were bottle fed with on average 3 L of colostrum from their own mother within 2 h after birth. After the first colostrum meal by bottle, all FC calves were allowed to suckle the remaining colostrum directly from the dam's udder. Calves in the NC-S and both PC treatment groups received an additional 2 L colostrum by bottle feeding at 8 to 12 h, as well as at 20 to 24 h after birth. More details on calving management can be found in Wenker et al. (2021). All NC-S cows moved to the designated group pen in the free stall barn after the second postpartum milking. All FC and PC cow-calf pairs stayed in the maternity pen for about 72 h, after which they were moved to designated group pens in the free stall barn.

Inside the free stall barn, experimental cows were housed in dynamic group pens (i.e. FC-FS and FC-NF in one group pen, PC-BW and PC-AW in one group pen, and NC-S in one group pen) (Figure 1). All experimental cows were milked twice a day at approximately 08:00 h and 18:00 h in the milking parlor with a five-point open tandem side and 11 side-by-side places. Cows were fed with grass silage (early spring cuttings) once a day at approximately 09:30 h. Feed was pushed automatically

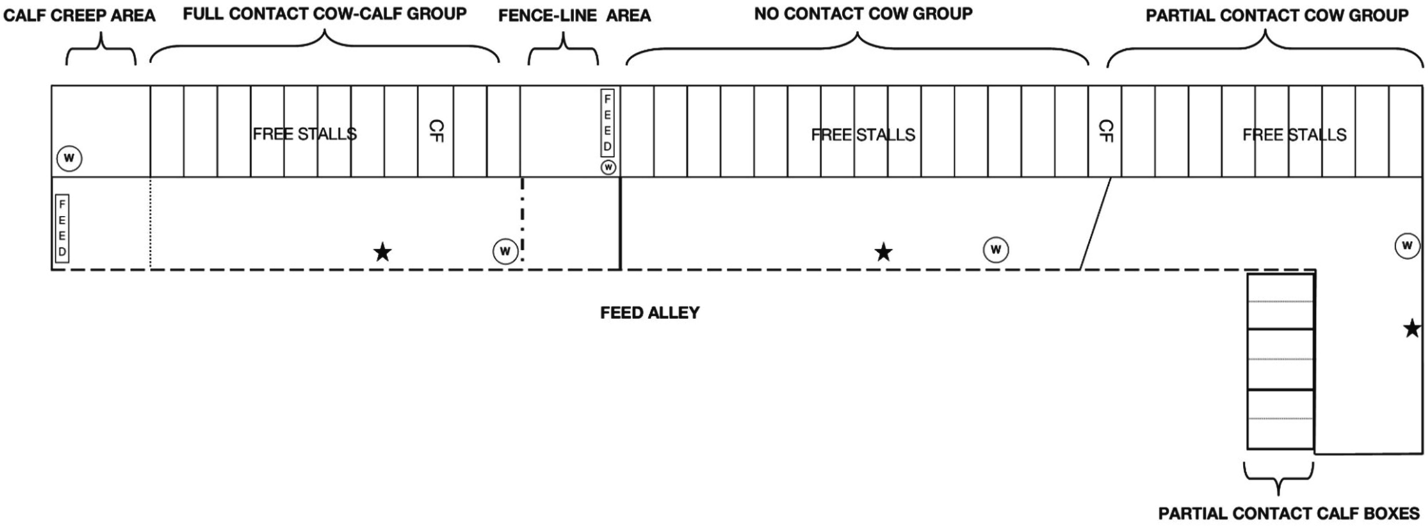

Figure 1. The full contact and partial contact cow-calf groups were housed in two separate dynamic group pens inside a free stall barn, and cows with no contact to their calves were housed in a group pen similar to those of the cow-calf contact groups. The free stall barn was naturally ventilated with open sidewalls and had perlite-bedded lying stalls (1.1 m × 3.0 m). The closed floor was covered with rubber and cleaned 8 times a day by an automated scraper. Within the full contact group pen, calves had access to a calf creep area (3.3 m × 4.8 m) with a straw-bedded lying area (3.3 m × 1.9 m), water bucket, hay and concentrates. A metal bar hindered cows to access this area. The fence-line area was used to house calves in the debonding strategy where contact was reduced before the moment of weaning via a fence (· – · – · –) (i.e. FC-FS). Here, FC-FS calves were provided with a straw-bedded lying area (3.0 m × 2.0 m), water, and solid feed. Calves with partial contact were housed individually (1.0 m × 1.6 m) in a straw-bedded calf box for the first two weeks, after which they were pair housed in the same box (2.0 m × 1.6 m) with their similar-aged peer. The calf boxes were placed behind a wall (1.2 m high) to limit physical contact and prevent suckling, while still allowing some cow-calf contact. Calf boxes were moved 0.5 m away from the wall to facilitate debonding after week 7 or 9. Calves with no contact to their dam were housed in identical calf boxes to partial contact calves in a separate naturally ventilated calf barn. W = water, CF = individual concentrate feeder, ★ = automated cow brush

(MoovPro, JOZ, the Netherlands) to the feeding rack 8 times a day. Additionally, cows could eat up to 10 kg of concentrates per day that was provided partly in the milking parlor and partly by an individual concentrate feeder. When the barn temperature was below 10 °C, all young calves were fitted with a calf jacket for the first three weeks of life.

All FC calves were housed together with the dams in the FC group pen and had access to a calf creep area (inaccessible for the dams due to a vertical metal pipe). The calf creep area provided them with a straw-bedded lying area and free access to water plus solid feed (i.e. hay and concentrates) from the day the new-born calves moved into the free stall barn. Throughout the suckling period, FC calves could freely suckle their dams throughout the day (except when cows were milked) and, if allowed, other dams as well. The FC group never exceeded more than eight cow-calf pairs.

All PC calves were kept in a straw-bedded calf box on-wheels (Topcalf Duo-Flex, Schrijver, the Netherlands) behind a wall (1.2 m high) adjacent to the PC cow group pen (see Wenker et al. (2021) for an illustration). This set-up prevented suckling, direct contact with manure of adult cows, and housing of calves within the cow herd, while it allowed for individual feeding of calves, as well as visual, auditory, olfactory, and limited tactile contact between cow-calf pairs. Cows could move their head over the wall and, when the calf was standing on the other side, cow-calf pairs could sniff and lick each other. One calf box could house two calves individually (1.0 m × 1.6 m), but also offered the opportunity to pair house them (2.0 m × 1.6 m) by removing the partition wall in the middle of the box. All PC calves were housed individually for the first two weeks, after which they were pair housed with their similar-aged peer in the same box. In each calf box ad lib water, hay, and concentrates (Topfok Kalf, de Samenwerking, the Netherlands) were provided as soon as the calf moved into the free stall barn (at 3 days of age). Calves in the PC group were provided bulk tank milk in individual teat buckets following a fixed feeding schedule (Table 1) after all three colostrum meals were consumed. Milk was provided around 08:00 h, 13:00 h, and 18:00 h. Bulk tank milk was heated up to 41 °C using a milk taxi (Milchtaxi 2.0, Holm & Laue, Germany) before being fed to the calves. The PC group never exceeded more than six cow-calf pairs.

NC-S calves were individually housed for the first two weeks in a straw-bedded calf box (identical to those of the PC calves) in an indoor naturally ventilated calf barn, after which the partition was removed and they were pair housed in the calf box with their similar-aged peer. Each calf was provided with ad lib water, hay and concentrates (Topfok Kalf, de Samenwerking, the Netherlands) from 3 days of age onwards, and was fed bulk tank milk according to the same feeding schedule as PC calves (Table 1).

Table 1. Fixed feeding schedule that allowed for gradual weaning for each individual calf fed bulk tank milk and having either no contact and partial contact with their dam.

Week of age	Number of meals per day	Amount of milk per meal (L)
1	3	2.5
2	3	3.0
3	3	3.5
4	3	3.5
5	2	3.5
6	2	3.0
7	2	2.0
8	1	1.0

2.3 Weaning and regrouping

All experimental calves were fully weaned at 56 days of age. The fixed feeding schedule (Table 1) for NC-S and all PC calves allowed for gradual weaning and they received no more milk after day 56.

To initiate gradual weaning for FC-FS calves, we used a fence-line that consisted of three metal bars that allowed the calf to stick its head (see Appendix 2 for an illustration) and suckle on initiative of the dam (i.e. the cow had to position herself next to the fence in order for the calf to have udder access) once it was placed behind the fence-line at day 49. At 56 days of age, the fence was closed off to prevent suckling by adding an extra metal bar in between the two lowest bars of the fence, which prevented the calf from sticking its head through. Behind the fence-line, calves had access to a straw-bedded lying area (3 m × 2 m) and ad libitum water, hay, and concentrates (Figure 1).

In the FC-NF treatment group, calves were fitted with a nose-flap (Quiet Wean, JDA Livestock Innovations, Canada) to prevent udder access (i.e. abrupt weaning off of milk) at 56 days of age (n = 10) (see Appendix 2 for an illustration). Those calves stayed in the cow pen until permanent separation at 70 days of age took place. The nose-flap still allowed calves to drink water and eat solid feed in the calf creep area (Figure 1) and was removed when calves moved to the young stock barn.

All calves were regrouped at 70 days of age in a young stock barn. Each pair of calves was introduced in groups of maximum six calves in straw-bedded group pens (3.0 m × 4.0 m). At 4 months of age, calves moved to a larger group pen (3.0 × 10.0 m) and were followed up to 6 months of age. All dams remained in the initial group pen until lactation week 12, after which they left the experiment and moved back into the commercial herd.

2.4 Data collection

2.4.1 Performance

Between the age of 49 to 70 days, the body weight of calves was measured once a week using a full-body calf weighing scale (Welvaarts, the Netherlands).

Machine-harvested milk yield of experimental cows was automatically collected using AgroVision dairy farm management software (AgroVision B.V., the Netherlands) between weeks 6 to 12.

2.4.2 General activity

Ear-tag accelerometer sensors (CowManager sensor, Agis Automatisering B.V., the Netherlands) were used in both cows and calves to track their general activity patterns. Sensors were attached to a radio-frequency identification ear-tag. Calves received their accelerometer sensor in the left ear at 3 to 4 weeks of age, when they were sedated for dehorning procedures. Cows were already equipped with an accelerometer sensor in their right ear for farm management purposes. The ear-tag based motion sensors contain a 3-dimensional accelerometer with proprietary software algorithms. They provided hourly measurements recorded in minutes for time spent eating, ruminating, highly active, active, and inactive, mutually exclusive times. Sensors were previously validated for both adult dairy cows (Bikker et al., 2014) and 6-week-old calves (Hill et al., 2017).

2.4.3 Clinical health check

During the weekly weighing moments, calves were clinically examined using a standardized health scoring system (see Appendix 3). The health scoring system was adapted from Renaud et al. (2017a) to evaluate the respiratory system (nasal discharge, ocular discharge, coughing), fecal consistency, navel inflammation, and rectal temperature on a 4-point scale.

2.4.4 Plasma cortisol and serum IgG concentrations

Blood samples (9 mL) of calves were taken via jugular venipuncture at 49 and 70 days of age. Calf age at the actual sample moment could deviate from the intended 49 and 70 days of age (range: 43 to 55 days of age at the first time point and 64 to 77 days of age at the second time point), as the majority of calves was sampled during the weekly health and growth assessments. We followed this approach to reduce the handling of calves, as the animals' response to humans was also studied in this experiment. Blood was collected into different vacutainer tubes (Vacuette, Greiner BioOne, Austria). EDTA-plasma samples for cortisol analyses were stored at 4 °C for a maximum of 2 h, whereas serum samples were stored at room temperature for 1 h prior to processing. All samples were centrifuged for 15 min at 3000 rpm and 4 °C, and were stored at -20 °C until further processing.

Plasma cortisol concentrations were measured in 91 out of 96 samples using a radioimmunoassay (RIA) (Schwinn et al., 2016). Plasma cortisol was analyzed by extracting 0.1 mL plasma with 1 mL absolute ethanol. After mixing the tubes on a vortex mixer, the protein precipitate was sedimented by centrifugation at 1,500 × g for 20 min at 4 °C. Supernatants were decanted into fresh tubes, evaporated to dryness, and reconstituted in 0.5 mL PBS (0.14 M sodium chloride and 0.01 M sodium phosphate, pH 7.0) containing 0.1% gelatin. A standard curve was run in duplicate by adding cortisol at concentrations between 0.25 to 100 ng/mL. Upon addition of 0.1 mL diluted antiserum and 0.1 mL [1,2-3H] cortisol (78 Ci/mmol), each tube was mixed and incubated at 4°C for 15 h. Separation of the free hormone from the bound hormone was achieved by adding 0.4 mL of a 0.75% dextran-coated charcoal suspension. After 4 min, tubes were centrifuged (2,800 × g, 15 min, 4 °C) and 0.7 mL was pipetted from the supernatant and mixed with scintillation fluid for radioactivity counting. The intra-assay CV was 9.7% and the inter-assay CV was 6.3%.

IgG concentrations were measured in 87 out of 96 serum samples with indirect enzyme-linked immunosorbent assay (ELISA) against bovine IgG. Wells were coated for 1 h with affinity-purified sheep anti bovine IgG-heavy chain (Cat. No. A10-118A-13, Bethyl Laboratories, United States of America) diluted 1:100 in coating buffer (0.05 M carbonate-bicarbonate, pH 9.6, Merck KGaA, Germany). Plates were washed 6 times with 50 mM TRIS 0.14 M NaCl (Merck KGaA, Germany), incubated for 1 h in the same buffer (blocking), and then washed 6 more times. After the 6th wash, 24 mg/mL of bovine reference serum (Cat. No. RS10-103-5, Bethyl Laboratories, United States of America) or diluted calf sera were added to each well, and the plates were incubated for 1 h. Wells were then washed 6 times, 100 mL of sheep anti bovine IgG-heavy chain (1:120,000) conjugated to horse-radish peroxidase (HRP) (Cat. No. A10-188P-30, Bethyl Laboratories, United States of America) was added, and plates were incubated for 1 h. After incubation, plates were washed 6 times and tetra methyl benzine (TMB) (SanBio B.V., the Netherlands) was added. Reactions were stopped after 15 min with 0.2 M H2SO4 (Merck KGaA, Germany) and the optical density at 450 nm was determined with an automated plate reader. The standard curve was generated by means of a 4-parameter curve fit and the IgG concentrations in the test samples were quantified by interpolating their absorbance from the standard curve generated in parallel with the samples.

2.4.5 Hair cortisol

In addition to plasma indicators of stress (Sheriff et al., 2011), we measured hair cortisol in calves which is assumed to be able to reveal more long-term stress (Burnett et al., 2015; Heimbürge et al., 2019). Hair samples of calves were collected on days 56 and 84, as those time points reflect the level of hair cortisol metabolites between day 49 and 77 (González-de-la-Vara et al., 2011). Again, calf age at the actual sample moment could deviate from the intended age (range: from 50 to 66 days of age at the first time point and 78 to 93 days of age at the second time point). Samples were collected from the tip of the tail by carefully clipping 2 to 3 cm of the tail hair with surgical scissors as close to the skin as possible (Burnett et al., 2015). The hair samples were stored in individually identified zip-lock plastic bags, which were kept at -20 °C until further processing. In total 64 out of 96 samples were mechanically cleaned and defatted with 5 mL n-hexane/isopropanol. Samples were dried overnight at room temperature. The dried samples were cut into small fragments approximately 1 to 2 mm long with scissors. Individual 100 mg aliquots from each of the samples were milled at 30 Hz with 3 mm beads for 5 min using a TissueLyserII (Qiagen, Germany). The milled hair samples were placed in a glass test tube along with 5 mL of methanol, and the tubes were incubated at 50 °C for 18 h. After centrifuging, the liquid in the tubes was transferred to another glass vial and evaporated to dryness in a stream of nitrogen. The remaining residue was dissolved in 200 µL of Neogen extraction buffer. Extraction of all hair samples (0.5 g each) was performed with 100% methanol, after which hair cortisol metabolites were determined using a Neogen cortisol kit (Product no. 402710, Neogen, United States of America).

2.4.6 Vaccination challenge

All calves were vaccinated with an inactivated Nobivac Rabies vaccine (1 mL intramuscular; WBVR, the Netherlands) at 49 days of age. Blood was collected via jugular venipuncture at 70 days of age. After incubation at room temperature for 1 h, blood samples were centrifuged for 15 min at 3000 rpm and 4 °C. Sera were stored at -20 °C until analysis. Serological responses of 43 out of 48 calves to the Rabies vaccine were analyzed by Wageningen Bioveterinary Research using the fluorescent antibody virus neutralization test (Cliquet et al., 1998) (Reference number 00-14-0871, Wageningen Bioveterinary Research, the Netherlands). Serological titers were converted to international units (IU)/mL.

2.4.7 Behavioural tests

Prior to moving the experimental calves to the young stock barn at 70 days of age (range: 64 to 79 days of age), calves were subjected to four behavioural tests applied in consecutive order (adapted from Duve et al., 2012 and Lecorps et al., 2018). The order of the tests was: Open Field Test (OFT, 4 min), Novel Object Test (NOT, 4 min), Human Approach Test (HAT) consisting of an inactive (2 min) and active approach phase (2 min), and a Handling Test (HT, 2 min). All tests took place in the same straw-bedded experimental area (7 m long, 3 m wide test pen) between 09:30 h and 12:30 h, and animals were tested individually in a predetermined random test order. The focal animal was taken from its home pen to the test pen in a calf transporter by two experimenters. The 15-min test began once the calf entered the test pen and the door was closed. For the OFT calves entered the test pen that was empty and unfamiliar. After 4 min OFT, the NOT started by dropping a novel object (black-white umbrella) over one of the fences enclosing the test arena (see Appendix 4). After 4 min NOT, the umbrella was removed by pulling it with the attached wire over the fence out of the arena. Subsequently, the calf was given 1 min to habituate to the removal of the novel object, where after the HAT started. One of the two (familiar) experimenters entered the test pen and stood immobile in the middle of the side-fence enclosing the test area (see Appendix 4) for 2 min, while avoiding eye contact with the calf. Next, the experimenter started to move and actively approach the calf with one arm stretched out attempting to stroke the calf for 2 min. After the HAT, the 2 min HT started by an animal caretaker entering the test pen who (along with the experimenter) attempted to place a halter on the calf and hold its head up. After the HT, the behavioural tests were finished and the calf was loaded on the trailer for transportation to the young stock barn and the next test animal was brought up to the test pen. Behaviour during the tests was recorded using a camera (Hikvision, model DS-2CD2145FWD-IS combined with the Hikvision DS-2FP2020 microphone) positioned 3 m above the pen.

For the OFT, time spent in locomotion, time spent in contact with the wall and floor, and frequency of escape attempts were recorded. For the NOT and HAT, both latency to first contact with the object (NOT)/human (HAT) and time spent in contact with the object (NOT)/human (HAT) were recorded. For the HT, the number of attempts to fit the halter and the latency to accept the halter were recorded. Details with respect to the ethogram can be found in Appendix 5. Due to technical problems video footage was available for 39 out of 48 calves (see Table 5). All videos were continuously observed by one trained observer who was blind to treatments, using the software Mangold Interact® (Program Version 18.1.4.4).

2.5 Data handling and statistical analyses

All statistical analyses were performed using SAS (version 9.4, SAS Institute, Institute Inc., Cary, NC), treating the animal as the experimental unit. Residuals of all variables were visually checked for normality and homogeneity of variance, and response variables were log-transformed when needed.

2.5.1 Calf performance

Average daily gain (ADG) in calf body weight was calculated over the 3-week period between weeks 7 to 10. A linear mixed model analysis was performed using the PROC GLIMMIX procedure. The systematic part of the model (referred to as model 1) consisted of the following fixed effects:

$$\mu + Treatment_i + Batch_j + Parity_k + (Treatment_i \times Parity_k) \quad [1]$$

Here, μ was a base level and $Treatment_i$ = type of CCC and corresponding debonding strategy (i = FC-FS, FC-NF, PC-BW, PC-AW, NC-S), $Batch_j$ = 16-week time period in which a calf was born (j = 1, 2, 3, 4), $Parity_k$ = parity of the dam (k = primiparous or multiparous), and a two-way interaction between treatment and parity. Batches were defined retrospectively to control for seasonal differences and varying group sizes in the treatment groups over time. Hence, the duration of the experiment was split up into batches of 16 weeks based on calving dates and they represented the various seasons. Interactions that were not significant ($P \geq 0.05$) were excluded from the model. In addition, the model comprised a random effect for the interaction between treatment and batch. For all fixed effects, approximate F-tests were used (Kenward and Roger, 1997) and significance was declared at $P < 0.05$. Subsequent pairwise comparisons were made according to the Tukey method.

Calves' absolute body weight between weeks 7 and 10 were analyzed with a linear mixed model for repeated measures (PROC GLIMMIX). The systematic part of the model (referred to as model 2) consisted of the following fixed effects:

$\mu + Treatment_i + Batch_j + Parity_k + Week_l + (Treatment_i \times Parity_k) + (Treatment_i \times Week_l)$ [2]

in the same notation as before (model 1), and additionally $Week_l$ = calf age in weeks ($l = 7, 8, 9, 10$) as main effect and a two-way interaction between treatment and week. Random calf effects were introduced to handle repeated measurements for calves. Further procedures were identical to model 1, so interactions that were not significant ($P \geq 0.05$) were excluded from the model, the model comprised a random effect for the interaction between treatment and batch, approximate F-tests were used for all fixed effects, followed by pairwise comparisons according to the Tukey method.

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2.5.2 Cow performance

Continuous data for cows' machine-harvested milk yield were analyzed with a linear mixed model identical to model 2 and its corresponding procedures, except that fixed effect $Week_l$ now included lactation weeks ($l = 6, 7, 8, 9, 10, 11, 12$).

2.5.3 General activity

For both cows' and calves' behavioural sensor data, the hourly output data from the CowManager sensor system were summarized into daily measures of general activity. Only data with 18 or more hourly recordings within 24 h were included in the analysis. The proportions of time spent inactive, active, highly active, eating and ruminating were calculated by dividing the total number of minutes of recorded behaviour by the total number of minutes recorded per day. Since a few sensors malfunctioned during the experiment, data was available for 39 out of 48 cows and 38 out of 48 calves (see Table 2 and 3). A baseline before any debonding interventions took place was calculated based on the average proportion of time spent inactive, active, highly active, eating or ruminating, between days 39 to 44 (i.e. age for calves, days in milk for cows). The behavioural responses after debonding interventions took place were based on the average proportion of time spent on all behaviours between days 0 to 4 after reducing contact, weaning, and regrouping (Johnsen et al., 2015a). Additionally, the average proportion of time spent on all behaviours after all interventions took place (i.e. between days 7 to 11 after regrouping) was calculated.

A generalized linear mixed model (fitted with PROC GLIMMIX) with an overdispersed binomial distribution and logit link function with multiplicative dispersion factor was used to analyze behavioural sensor data (i.e. proportion of time spent inactive, active, highly active, eating and ruminating). The systematic part of the model comprised the following fixed effects:

μ + Treatment$_i$ + Batch$_j$ + Parity$_k$ + Time Period$_m$ + (Treatment$_i$ $\times$ Parity$_k$) + (Treatment$_i$ $\times$ Time Period$_m$) [3]

in the same notation as in model 2 by replacing Week$_l$ for Time Period$_m$, at which Time Period$_m$ = 4-day period after a debonding intervention (m = baseline before interventions, reduced contact, weaning, regrouping, after debonding interventions). The model also included two-way interactions between treatment and parity, and between treatment and time period. As random effect the model included the interaction between treatment and batch. For the animal effects, a spatial power covariance structure (based on unequally spaced longitudinal measurements) was adopted to introduce correlation in the model between repeated measurements on the same animal. All further procedures were identical to model 2.

2.5.4 Health scores

Prior to statistical analyses, calves were classified for having clinical symptoms of respiratory issues (i.e. 'yes' when they had a composite respiratory score ≥ 4 based on the sum of ocular discharge, nasal discharge, cough score) and diarrhea (i.e. 'yes' when fecal score ≥ 2) for each week (adapted from McGuirk (2008)). Subsequently, the number of weeks classified as having clinical symptoms for each health deficit was summed per calf. Therefore, a calf that was observed 2 out of 4 weeks with clinical respiratory symptoms and 1 out of 4 weeks with clinical symptoms for diarrhea, would get an outcome of 2 for respiratory problems and 1 for diarrhea.

The prevalence of calves classified at least once with clinical symptoms for diarrhea or respiratory issues were analyzed using a Fisher's exact method for pairwise comparisons. A generalized linear mixed model with an overdispersed binomial distribution and logit link function was fitted to analyze the number of weeks that calves classified with clinical symptoms for respiratory issues and diarrhea. As random effect the model included the interaction between treatment and batch. The systematic part was identical to model 1 and its corresponding procedures.

2.5.5 Physiological stress responses

Differences between week 10 (end of debonding strategies) and week 7 (start of debonding strategy) (delta, Δ = day 70 – day 49) were calculated for serum IgG and plasma cortisol concentrations. Additionally, differences in hair cortisol values were calculated between week 12 and week 8 (delta, Δ = day 84 – day 56).

Linear mixed model analyses identical to model 1 and its corresponding procedure were performed for Δ serum IgG, Δ plasma cortisol, Δ hair cortisol, and Rabies IgG responses. Additionally, the difference in days between the calf's age at the two sample moments was added as co-variate among the fixed effects.

2.5.6 Behavioural tests

Latencies were analyzed with a linear mixed model identical to model 1, and additionally calf age as co-variate among the fixed effects. The exact calf age during the tests could deviate from the intended age of 70 days, as calves were regrouped together with their similar aged peer directly after the behavioural tests.

Generalized linear mixed model analyses with a Poisson distribution and log link function with multiplicative dispersion factor were used for behaviours expressed as frequency (e.g. vocalizations), whereas an overdispersed binomial distribution with logit link function was used for behaviours expressed as proportion of time. The systematic part of the model consisted of the same fixed and random effects as model 1, and additionally calf age as co-variate. Again, all further procedures were identical to model 1.

3. Results

3.1 Performance of cow-calf pairs

For calves' absolute body weight, an interaction was found between treatment and week ($P = 0.001$). Overall, calves' absolute body weight increased over time, except for FC-NF calves between week 8 and 9 and PC-BW calves between week 9 and 10 (Figure 2A). In terms of ADG from weeks 7 to 10, FC-NF had a lower mean ADG (± SE) (0.64 ± 0.08 kg/d) compared to FC-FS calves (0.79 ± 0.05 kg/d), PC-BW calves (0.81 ± 0.08 kg/d), PC-AW calves (0.91 ± 0.06 kg/d), and NC-S calves (0.88 ± 0.07 kg/d) ($P = 0.02$). At 6 months of age, no significant difference in absolute body weight was found among treatment groups (FC-FS calves: 183.6 ± 13.3 kg, FC-NF calves: 213.5 ± 9.5 kg, PC-BW calves: 194.0 ± 7.1 kg, PC-AW calves: 191.8 ± 11.3 kg, NC-S calves: 211.4 ± 6.9) ($P = 0.42$).

For cows' daily milk yield, an interaction was found between treatment and week ($P < 0.001$), as FC-FS and FC-NF cows had a lower machine-harvested milk yield in week 6 until 9 compared to PC-BW, PC-AW, and NC-S cows. From week 10 onwards machine-harvested milk yield no longer significantly differed between any of the treatment groups (Figure 2B).

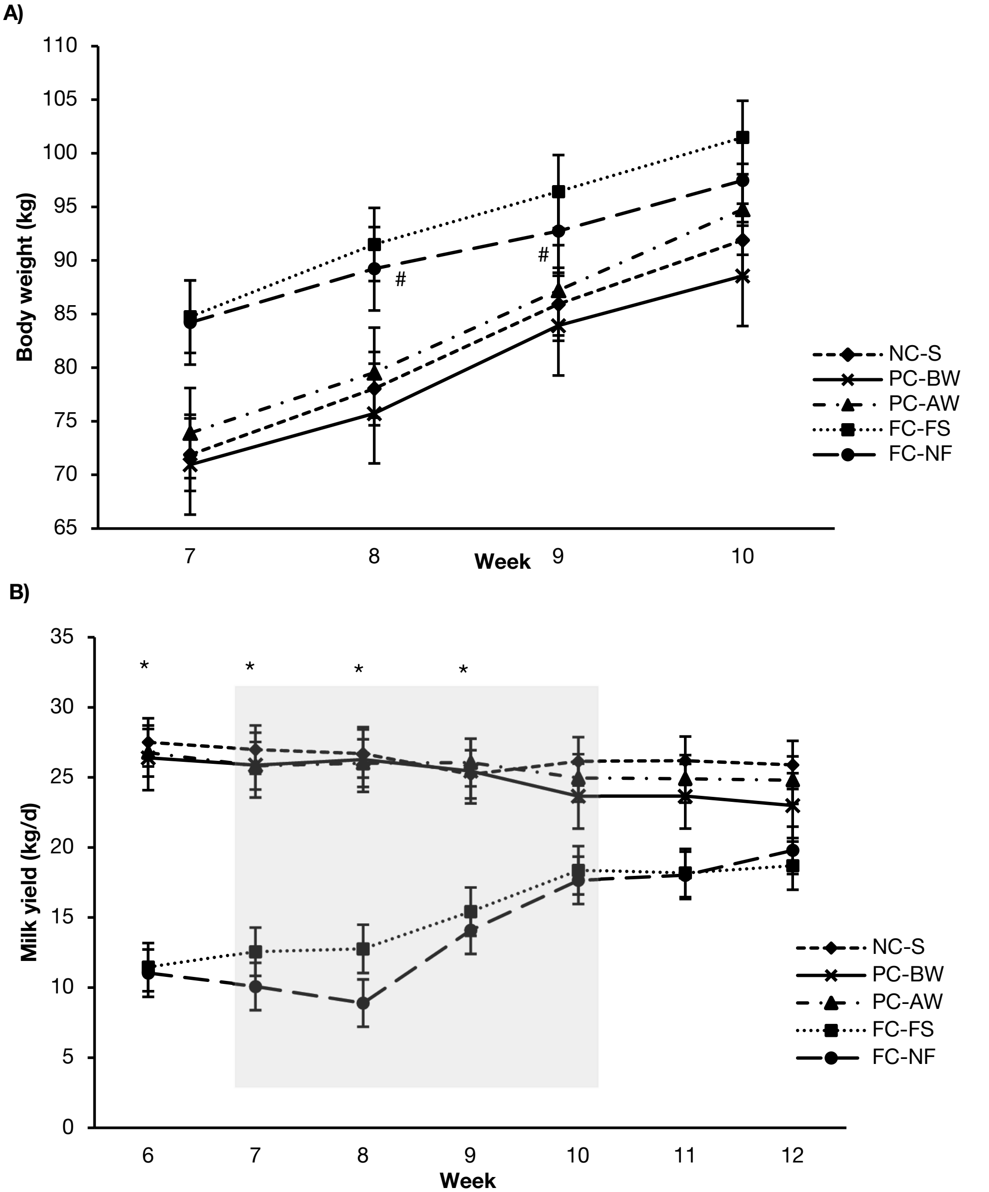

Figure 2. Interactions between treatment and week for animal performance in three types of cow-calf contact with different debonding strategies systems for **A)** growth of calves represented by absolute body weight (kg) from week 7 to 10 (LS means ± SEM), and **B)** milk production (kg/d) of cows between week 6 to 12 (LS means ± SEM). The light grey box indicates the debonding period between week 7 to 10. NC-S: no contact, standard weaning; PC-BW: partial contact, reducing contact before weaning; PC-AW: partial contact, reducing contact after weaning; FC-FS: full contact, reducing contact before weaning via fence-line separation; FC-NF: full contact, reducing contact at weaning via nose-flap insertion. Asterisks at specific time points indicate significant treatment differences ($P < 0.05$), whereas the # represents a significant effect of time within the FC-NF treatment.

3.2 General activity patterns of cow-calf pairs

For calves' general activity pattern, an interaction between treatment and time period was found for the proportion of time spent highly active, active, and ruminating ($P \leq 0.002$) (Figure 3). In the 4-day period after reducing contact, FC-FS calves spent a larger proportion of time in high activity than PC-BW and PC-AW calves. In the 4-day period after weaning, both FC-FS and FC-NF calves spent a larger proportion of time in high activity compared to PC-BW, PC-AW, and NC-S calves (Figure 3A). The proportion of time spent active was only higher in FC-FS and FC-NF calves compared to PC-BW, PC-AW, and NC-S calves in the 4-day period before interventions took place (Figure 3B). The proportion of time spent ruminating was lower in FC-FS and FC-NF calves in the 4-day period before interventions took place, after reducing contact, and after weaning compared to PC-BW, PC-AW, and NC-S calves (Figure 3C). Furthermore, the overall proportion of time spent inactive decreased over time for all calves ($P < 0.001$), and tended to be higher for FC-NF calves compared to FC-FS, PC-BW, and PC-AW calves ($P = 0.07$) (Table 2). The average proportion of time spent eating increased from the moment of weaning until the week after regrouping ($P < 0.001$). Besides, the overall proportion of time spent eating differed between treatment groups ($P = 0.03$) (Table 2).

The effect of treatment on general activity patterns of cows depended on parity regardless of the time period, as reflected by a significant interaction term treatment by parity for the proportion of time spent highly active, active, eating, and ruminating (Table 3). Overall, primiparous FC-NF and PC-AW cows spent a larger proportion of their time highly active and eating, and a smaller proportion of time ruminating compared to the other dams. Additionally, only primiparous FC-FS and FC-NF cows spent a smaller proportion of time active compared to the other dams. The proportion of time spent inactive was higher in multiparous cows compared to primiparous cows irrespective of treatment and time period ($P = 0.03$) (Table 3).

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3.3 Calves' health status and physiological stress-responses

The number of weeks that calves were classified with clinical symptoms for respiratory symptoms between weeks 7 to 10 did not differ significantly among treatment groups (mean weeks ± SE, FC-FS: 1.1 ± 0.5; FC-NF 0.9 ± 0.4; PC-BW: 0.7 ± 0.3; PC-AW: 0.7 ± 0.2; NC-S: 0.1 ± 0.1) ($P = 0.12$). Similarly, number of weeks that calves were scored with clinical symptoms for diarrhea did not differ among treatment groups (FC-FS: 1.4 ± 0.3; FC-NF: 1.6 ± 0.4; PC-BW: 0.5 ± 0.3; PC-AW: 1.0 ± 0.30; NC-S: 0.9 ± 0.4) ($P = 0.37$). Prevalence of calves classified with clinical symptoms for diarrhea and respiratory issues can be found in Table 4.

No significant treatment differences were found for delta plasma cortisol levels, delta serum IgG concentrations (both Δ = day 70 – day 49), and Rabies IgG response to vaccination challenge (Table 4). Additionally, no significant treatment differences were found for delta hair cortisol concentrations (Δ = day 84 – day 56) (Table 4).

Table 2. Behaviour of calves expressed as proportion of time (mean ± SE [95% CI]) in three types of cow-calf contact with different debonding strategies based on ear-tag accelerometer sensors. For each behaviour, bold numbers represent the overall mean ± SE [95% CI] per treatment group.

	No contact	Partial contact	
	S[1] (n = 8)	BW[1] (n = 6)	AW[1] (n = 12)
Eating	**0.17 ± 0.01 [0.07-0.31]**[bc]	**0.10 ± 0.01 [0.04-0.19]**[a]	**0.12 ± 0.00 [0.03-0.23]**[ab]
Before interventions	0.12 ± 0.01 [0.03-0.23]	0.10 ± 0.01 [0.07-0.15]	0.12 ± 0.01 [0.05-0.19]
Reduced contact	N.A.[2]	0.08 ± 0.01 [0.04-0.13]	0.10 ± 0.01 [0.02-0.20]
Weaning	0.15 ± 0.01 [0.11-0.20]	0.08 ± 0.01 [0.04-0.12]	0.11 ± 0.01 [0.01-0.19]
Regrouping	0.18 ± 0.01 [0.07-0.28]	0.13 ± 0.01 [0.04-0.22]	0.15 ± 0.01 [0.06-0.26]
After interventions	0.22 ± 0.01 [0.09-0.35]	0.14 ± 0.01 [0.05-0.22]	0.15 ± 0.01 [0.07-0.24]
Inactive	**0.33 ± 0.01 [0.23-0.44]**	**0.32 ± 0.01 [0.19-0.40]**	**0.32 ± 0.01 [0.17-0.45]**
Before interventions	0.39 ± 0.03 [0.26-0.63]	0.37 ± 0.01 [0.30-0.40]	0.32 ± 0.01 [0.21-0.44]
Reduced contact	N.A.	0.35 ± 0.01 [0.30-0.39]	0.34 ± 0.01 [0.17-0.49]
Weaning	0.35 ± 0.01 [0.26-0.43]	0.34 ± 0.01 [0.28-0.40]	0.33 ± 0.01 [0.14-0.46]
Regrouping	0.30 ± 0.01 [0.22-0.37]	0.27 ± 0.02 [0.19-0.44]	0.28 ± 0.01 [0.15-0.48]
After interventions	0.30 ± 0.01 [0.21-0.42]	0.31 ± 0.01 [0.23-0.42]	0.30 ± 0.01 [0.22-0.41]

Different subscript letters indicate significant differences between treatment groups ($P < 0.05$).

[1] S: standard procedure, early separation after birth and weaning at d56; BW: reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall; AW: reducing contact after weaning by moving partial contact calves from the wall at day 63; FS: reducing contact (at day 49) by placing full contact calves behind a fence-line; NF: reducing contact at weaning by fitting full contact calves a nose-flap at day 56.

[2] No contact calves were only subjected to weaning and regrouping; There was no reduced contact phase for FC-NF calves, as contact was only reduced at the moment the nose-flap was inserted to wean the calf.

Table 2 continued. Behaviour of calves expressed as proportion of time (mean ± SE [95% CI]) in three types of cow-calf contact with different debonding strategies based on ear-tag accelerometer sensors. For each behaviour, bold numbers represent the overall mean ± SE [95% CI] per treatment group.

	Full contact		P-value	P-value
	FS[1] (n = 5)	NF[1] (n = 7)	Treatment	Time period
Eating	**0.17 ± 0.01 [0.07-0.30]**^{c}	**0.12 ± 0.01 [0.05-0.23]**^{a}	0.03	< 0.001
Before interventions	0.10 ± 0.01 [0.05-0.15]	0.09 ± 0.00 [0.05-0.12]		
Reduced contact	0.16 ± 0.01 [0.12-0.21]	N.A.		
Weaning	0.18 ± 0.02 [0.10-0.31]	0.08 ± 0.01 [0.03-0.13]		
Regrouping	0.20 ± 0.02 [0.11-0.33]	0.15 ± 0.01 [0.07-0.25]		
After interventions	0.18 ± 0.01 [0.11-0.25]	0.18 ± 0.01 [0.08-0.25]		
Inactive	**0.30 ± 0.01 [0.14-0.52]**	**0.37 ± 0.01 [0.22-0.51]**	0.07	< 0.001
Before interventions	0.45 ± 0.02 [0.28-0.56]	0.42 ± 0.01 [0.30-0.53]		
Reduced contact	0.32 ± 0.01 [0.25-0.36]	N.A.		
Weaning	0.29 ± 0.03 [0.21-0.49]	0.38 ± 0.01 [0.27-0.56]		
Regrouping	0.22 ± 0.03 [0.10-0.33]	0.34 ± 0.02 [0.20-0.47]		
After interventions	0.27 ± 0.01 [0.22-0.34]	0.32 ± 0.01 [0.22-0.46]		

Different subscript letters indicate significant differences between treatment groups (P < 0.05).

[1] S: standard procedure, early separation after birth and weaning at d56; BW: reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall; AW: reducing contact after weaning by moving partial contact calves from the wall at day 63; FS: reducing contact (at day 49) by placing full contact calves behind a fence-line; NF: reducing contact at weaning by fitting full contact calves a nose-flap at day 56.

[2] No contact calves were only subjected to weaning and regrouping; There was no reduced contact phase for FC-NF calves, as contact was only reduced at the moment the nose-flap was inserted to wean the calf.

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Table 3. Behaviour of cows expressed as proportion of time (LS means ± SEM [95% CI]) in three types of cow-calf contact with different debonding strategies based on ear-tag accelerometer sensors.

	No contact	Partial contact	
	S[1] (n = 8)	BW[1] (n = 3)	AW[1] (n = 11)
Highly active			
Primiparous	0.10 ± 0.02 [0.07-0.17]	N.A.[2]	0.18 ± 0.04 [0.10-0.29]*
Multiparous	0.11 ± 0.02 [0.06-0.15]	0.08 ± 0.03 [0.05-0.16]	0.09 ± 0.02 [0.05-0.15]
Active			
Primiparous	0.06 ± 0.01 [0.05-0.08]	N.A.	0.06 ± 0.01 [0.04-0.08]
Multiparous	0.06 ± 0.01 [0.04-0.07]	0.09 ± 0.01 [0.06-0.13]	0.06 ± 0.01 [0.05-0.09]
Inactive			
Primiparous	0.20 ± 0.03 [0.17-0.28]	N.A.	0.22 ± 0.03 [0.16-0.29]
Multiparous	0.22 ± 0.02 [0.18-0.28]	0.27 ± 0.04 [0.21-0.38]	0.27 ± 0.03 [0.21-0.34]
Eating			
Primiparous	0.28 ± 0.07 [0.15-0.45]	N.A.	0.38 ± 0.10 [0.20-0.60]*
Multiparous	0.32 ± 0.07 [0.19-0.50]	0.14 ± 0.06 [0.05-0.32]	0.24 ± 0.07 [0.11-0.43]
Ruminating			
Primiparous	0.32 ± 0.07 [0.18-0.50]	N.A.	0.18 ± 0.06 [0.08-0.36]*
Multiparous	0.28 ± 0.07 [0.15-0.45]	0.34 ± 0.11 [0.14-0.62]	0.31 ± 0.08 [0.15-0.54]

Asterisks indicate significant treatment differences within parity ($P < 0.05$)

[1] S: standard procedure, early separation after birth and weaning at d56; BW: reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall; AW: reducing contact after weaning by moving partial contact calves from the wall at day 63; FS: reducing contact (at day 49) by placing full contact calves behind the fence-line; NF: reducing contact at weaning by fitting full contact calves a nose-flap at day 56.

[2] Due to malfunctioning of sensors primiparous cows were missing in this treatment group, so their general activity patters could not be estimated.

Table 3 continued. Behaviour of cows expressed as proportion of time (LS means ± SEM [95% CI]) in three types of cow-calf contact with different debonding strategies based on ear-tag accelerometer sensors.

	Full contact		P-value	P-value
	FS[1] (n = 8)	NF[1] (n = 9)	Treatment × Parity	Parity
Highly active			< 0.001	
Primiparous	0.11 ± 0.03 [0.06-0.18]	0.19 ± 0.04 [0.12-0.29]*		
Multiparous	0.10 ± 0.03 [0.05-0.17]	0.10 ± 0.02 [0.05-0.14]		
Active			0.01	
Primiparous	0.04 ± 0.01 [0.03-0.06]*	0.04 ± 0.01 [0.03-0.06]*		
Multiparous	0.07 ± 0.01 [0.05-0.09]	0.07 ± 0.01 [0.05-0.08]		
Inactive			0.33	0.02
Primiparous	0.22 ± 0.04 [0.16-0.30]	0.20 ± 0.03 [0.15-0.27]		
Multiparous	0.23 ± 0.03 [0.17-0.29]	0.26 ± 0.02 [0.21-0.32]		
Eating			< 0.001	
Primiparous	0.36 ± 0.09 [0.18-0.58]	0.48 ± 0.09 [0.30-0.67]*		
Multiparous	0.27 ± 0.08 [0.13-0.48]	0.27 ± 0.06 [0.15-0.43]		
Ruminating			< 0.001	
Primiparous	0.28 ± 0.08 [0.13-0.50]	0.16 ± 0.05 [0.08-0.29]*		
Multiparous	0.30 ± 0.08 [0.14-0.52]	0.25 ± 0.06 [0.13-0.41]		

Asterisks indicate significant treatment differences within parity (P < 0.05)

[1] S: standard procedure, early separation after birth and weaning at d56; BW: reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall; AW: reducing contact after weaning by moving partial contact calves from the wall at day 63; FS: reducing contact (at day 49) by placing full contact calves behind the fence-line; NF: reducing contact at weaning by fitting full contact calves a nose-flap at day 56.

[2] Due to malfunctioning of sensors primiparous cows were missing in this treatment group, so their general activity patters could not be estimated.

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Table 4. Results of debonding strategies in three types of cow-calf contact systems on **A)** physiological stress responses of calves (mean ± SE) (Δ = difference between the start- and end of interventions for serum IgG and plasma cortisol concentrations in blood (i.e. Δ = day 70 – day 49) and hair cortisol concentrations (i.e. Δ = day 84 – day 56)) and **B)** prevalences of calves classified at least once with clinical symptoms of diarrhea and respiratory issues between day 49 to day 70.

A. Physiological stress responses

	No contact	Partial contact		Full contact			
	S[1]	BW[1]	AW[1]	FS[1]	NF[1]	F-value	P-value
Blood							
Δ IgG (mg/mL)	4.82 ± 3.27	7.19 ± 5.04	4.77 ± 0.71	1.80 ± 2.52	6.80 ± 1.57	0.76	0.58
Δ Cortisol (ng/mL)	0.68 ± 0.45	0.76 ± 0.48	0.85 ± 0.35	-1.52 ± 1.18	-1.10 ± 0.63	1.18	0.40
Rabies IgG (IU/mL)	0.89 ± 0.24	1.52 ± 0.95	2.15 ± 0.71	1.74 ± 0.54	1.05 ± 0.30	1.07	0.44
Hair							
Δ Cortisol (ng/g)	0.68 ± 1.12	-3.88 ± 6.16	0.23 ± 1.47	-0.14 ± 1.92	1.16 ± 2.55	0.90	0.50

B. Health variables

	No contact	Partial contact		Full contact	
	S	BW	AW	FS	NF
Diarrhea[2] (%)	50.0^{ab}	33.3^{a}	58.3^{ab}	90.0^{b}	90.0^{b}
Respiratory issues[3] (%)	10	50	50	50	40

Different subscript letters within a row indicate significant differences ($P < 0.05$) between treatment groups.

[1] S: standard procedure, early separation after birth and weaning at d56; BW: reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall; AW: reducing contact after weaning by moving partial contact calves from the wall at day 63; FS: reducing contact (at day 49) by placing full contact calves behind the fence-line; NF: reducing contact at weaning by fitting full contact calves a nose-flap at day 56.

[2] For each treatment: (Number of calves with at least once feces score ≥ 2 (based on a 0-3 scale) divided by total number of calves) times 100

[3] For each treatment: (Number of calves with at least once lung score ≥ 4 (i.e. sum of scores for ocular discharge, nasal discharge, and cough based on a 0-3 scale) total number of calves) times 100

A)

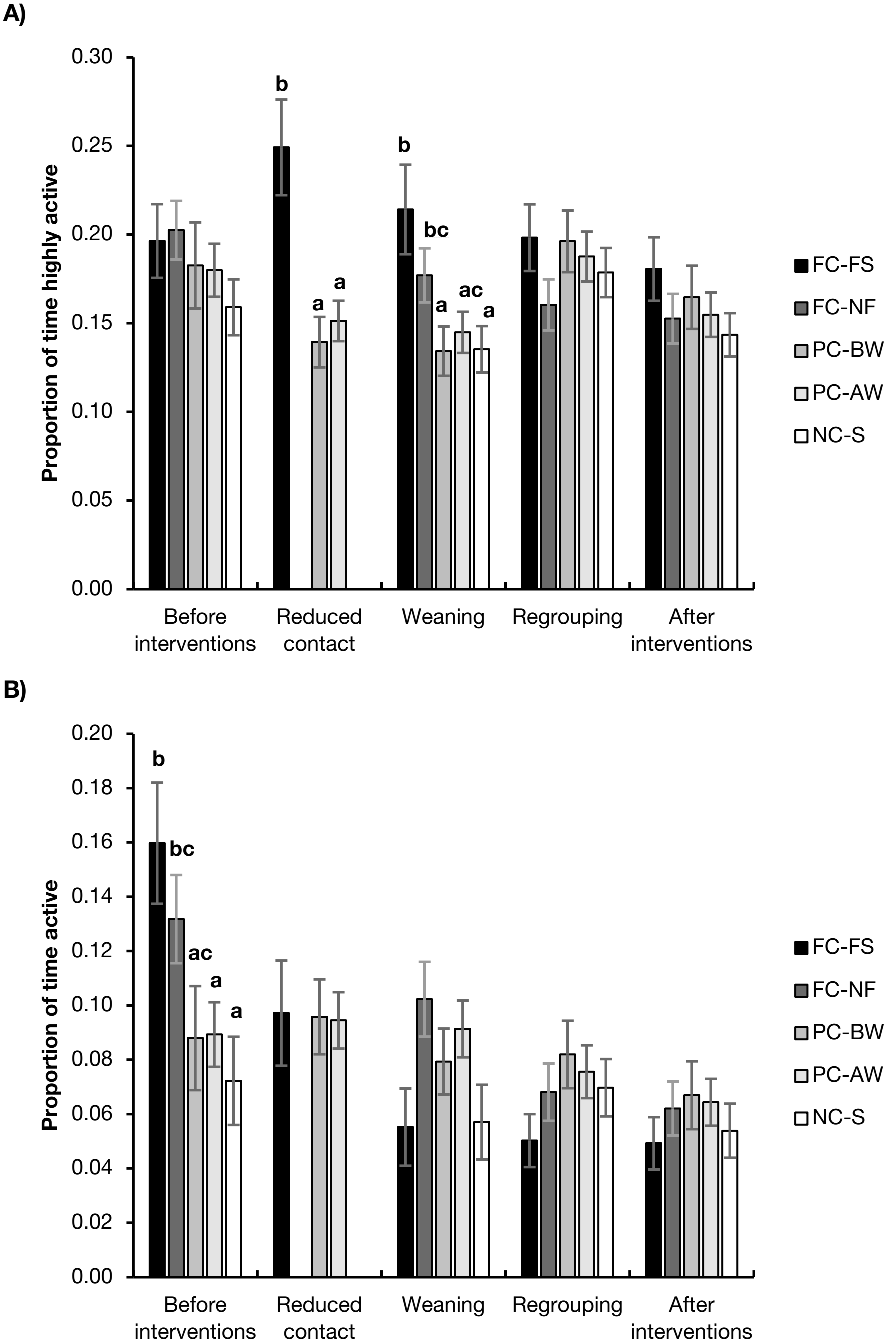

Figure 3. Interaction between treatment and time period for calf behaviour in three types of cow-calf contact systems with different debonding strategies for **A)** proportion of time spent highly active (LS means), **B)** proportion of time spent active (LS means ± SEM). Different subscript letters within a time period indicate significant differences (P < 0.05) between treatment groups.

C)

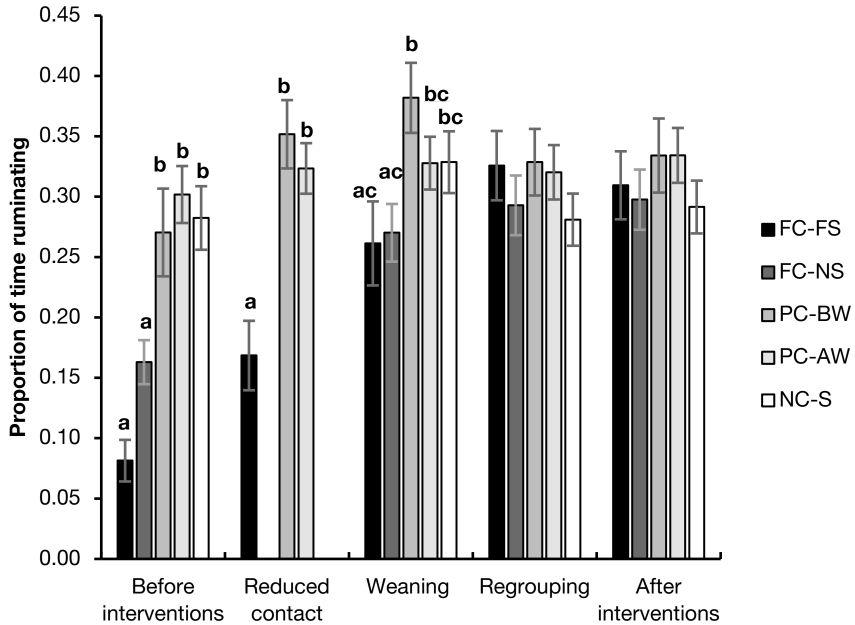

Figure 3 continued. Interaction between treatment and time period for calf behaviour in three types of cow-calf contact systems with different debonding strategies for **C)** proportion of time spent ruminating (LS means ± SEM). NC-S: no contact, standard weaning; PC-BW: partial contact, reducing contact before weaning; PC-AW: partial contact, reducing contact after weaning; FC-FS: full contact, reducing contact before weaning via fence-line separation; FC-NF: full contact, reducing contact at weaning via nose-flap insertion. Different subscript letters within a time period indicate significant differences ($P < 0.05$) between treatment groups.

3.4 Calf behaviour in behavioural tests

In the OFT, the proportion of time spent in contact with the wall or floor tended to be lower in PC-BW calves compared to FC-FS and FC-NF calves ($P = 0.06$), although the proportion of time spent walking, running, standing, or vigilant did not differ significantly between treatments. In addition, no significant differences between treatment groups were found for the frequency of solitary play and vocalizations during the OFT (Table 5).

In the NOT, NC-S calves showed a larger frequency of solitary play compared to FC-FS, FC-NF, PC-BW, and PC-AW calves ($P = 0.001$) (Table 5). No significant differences among treatment groups were found regarding the latency to first approach or first contact with the object, the proportion of time spent vigilant, and vocalizations during the NOT (Table 5).

During the voluntary approach phase in the HAT, FC-FS calves had a higher latency to first contact with the human compared to PC-AW and NC-S calves ($P = 0.04$), although no significant differences among treatment groups were found regarding the proportion of time spent in contact with the human (Table 5). During the involuntary approach phase, latency to first contact with the human was greater for FC-FS calves compared to NC-S and PC-AW calves (P = 0.04), although the proportion of time spent in contact with the human did not significantly differ among treatment groups (Table 5).

During the HT, treatment groups did not significantly differ in the number of restraint attempts or the latency to secure the halter (Table 5).

4. Discussion

Weaning and separation after prolonged CCC in farm settings is often imposed at younger ages and more abruptly than in natural settings (Sirovnik et al., 2020; Weary et al., 2008; Whalin et al., 2021). Although gradual debonding strategies like fence-line separation or nose-flap insertion contain elements of natural weaning (i.e. the calf can no longer suckle milk although other forms of physical contact still occur), the ear-tag accelerometer system exhibited that FC-FS calves spent a larger proportion of time in high activity compared to PC-BW, PC-AW, and NC-S calves during the 4-day period after reducing contact and weaning. In the same way, FC-NF calves spent more time highly active throughout the 4-day period after weaning. This high activity may be related to distress behaviours found in previous studies, such as high-pitched vocalizations, suckling attempts, fence-line pacing, or placing the head outside the fence (Enríquez et al., 2010; Johnsen et al., 2015a). The majority of those behaviours are thought to reflect the desire to reunite with the cow and/or to suckle milk (Enríquez et al., 2010; Loberg et al., 2007). In contrast, PC-BW and PC-AW calves showed no explicit distress responses when debonding interventions took place, as their behaviour was similar to NC-S calves. For PC calves, the loss of contact with the dam was not linked to loss of milk, and possibly the fixed feeding schedule with gradual milk reduction minimized PC-BW and PC-AW calves' weaning distress (Khan et al., 2011). Moreover, PC calves could not freely initiate contact from their calf boxes, as the current partial CCC system was cow-driven. Given that our previous work showed that partial contact reduced cows' affiliative behaviour towards their calves in the weeks following parturition, the PC calves might have been more socially independent from the dam (Wenker et al., 2021). Hence, it could be argued that a different mother-young bond is established when partial contact without suckling is allowed, which eventually mitigated the debonding process. This argument is supported by findings of Johnsen et al. (2018) that reported a smaller amount of vocalizations in response to separation for calves in a partial CCC system where suckling was prevented by using udder nets compared to calves with full CCC. Nevertheless, in the present study overall the behavioural responses of cows to debonding after prolonged CCC were less distinctive, as the effect of treatment depended on parity where primiparous animals subjected to the FC-NF and PC-AW treatment behaved differently compared to the other cows in weeks 7 to 10. In beef cattle, higher parity cows were also found

Table 5. Calf behaviour during the behavioural tests at 70 days of age in ascending order (i.e. A-E). Definitions of behavioural measures can be found in Appendix 5.

	No contact	Partial contact		Full contact			
	S[1] (n = 7)	BW[1] (n = 6)	AW[1] (n = 10)	FS[1] (n = 8)	NF[1] (n = 8)	F-value	P-value
A. *Open field test (4 min)*							
Walking (% of time)	30.79 ± 3.27	24.92 ± 4.79	31.04 ± 2.80	46.9 ± 5.59	35.28 ± 6.56	1.54	0.31
Running (% of time)	15.22 ± 7.18	17.02 ± 6.49	10.49 ± 3.02	0.07 ± 0.07	0.32 ± 0.26	1.67	0.27
Standing (% of time)	52.62 ± 6.92	57.57 ± 7.29	57.88 ± 3.61	49.91 ± 5.59	63.14 ± 6.61	0.21	0.92
Contact with wall/floor (% of time)	20.04 ± 3.40	16.35 ± 3.19	21.05 ± 2.71	37.28 ± 5.36	33.50 ± 5.02	5.99	0.06
Vigilant (% of time)	13.01 ± 2.52	20.80 ± 3.11	15.03 ± 2.70	11.25 ± 3.47	13.24 ± 3.81	0.99	0.49
Vocalizations (frequency)	23.26 ± 18.20	10.25 ± 8.48	21.42 ± 10.12	0.17 ± 0.17	6.25 ± 3.36	0.89	0.51
Solitary play (frequency)	11.40 ± 4.34	10.67 ± 3.84	6.30 ± 1.71	0.17 ± 0.17	0.13 ± 0.13	2.57	0.16
B. *Novel object test (4 min)*							
Latency first close approach (s)	3.50 ± 0.87	75.50 ± 35.44	141.11 ± 32.77	71.17 ± 24.45	129.40 ± 45.69	2.01	0.13
Latency first contact with object (s)	144.40 ± 56.72	231.50 ± 1.73	193.5 ± 28.19	179.83 ± 23.12	134.60 ± 43.15	0.55	0.70
Contact with object (% of time)	6.55 ± 4.33	0.42 ± 0.42	0.60 ± 0.47	4.44 ± 1.58	4.96 ± 2.39	1.75	0.17
Vigilant (% of time)	19.43 ± 3.72	25.12 ± 2.61	29.09 ± 5.34	24.49 ± 6.29	19.06 ± 4.69	1.17	0.35
Vocalizations (frequency)	8.25 ± 4.23	3.00 ± 1.55	4.75 ± 1.52	0.83 ± 0.48	8.67 ± 3.33	0.78	0.60
Solitary play (frequency)	4.40 ± 1.91[b]	1.00 ± 0.82[a]	0.50 ± 0.27[a]	0.17 ± 0.17[a]	0.00 ± 0.00[ab]	9.34	0.001
C. *Voluntary phase human approach test (2 min)*							
Latency first close approach (s)	27.25 ± 13.52	86.60 ± 20.30	56.00 ± 18.94	94.33 ± 16.81	75.40 ± 24.14	1.16	0.43
Latency first contact with human (s)	24.80 ± 11.18	74.60 ± 23.73	62.50 ± 17.88	103.67 ± 15.36	99.50 ± 17.90	0.96	0.50
Contact with human (% of time)	67.12 ± 11.52	23.21 ± 12.78	23.37 ± 10.56	1.39 ± 1.39	1.23 ± 1.23	0.62	0.67

Table 5 continued. Calf behaviour during the behavioural tests at 70 days of age in ascending order (i.e. A-E). Definitions of behavioural measures can be found in Appendix 5.

	No contact	Partial contact		Full contact			
	S[1] (n = 7)	BW[1] (n = 6)	AW[1] (n = 10)	FS[1] (n = 8)	NF[1] (n = 8)	F-value	P-value
D. *Involuntary phase human approach test (2 min)*							
Latency first contact with human (s)	3.25 ± 1.31^{a}	8.33 ± 3.14^{ab}	8.20 ± 1.78^{a}	23.20 ± 1.85^{b}	14.17 ± 5.91^{ab}	3.08	0.04
Contact with human while walking (% of time)	11.33 ± 3.23	18.61 ± 3.41	13.83 ± 2.62	8.89 ± 2.13	6.90 ± 2.76	1.65	0.20
Contact with human while standing (% of time)	46.00 ± 16.23	42.92 ± 11.52	63.83 ± 4.15	45.00 ± 12.81	45.87 ± 13.64	1.02	0.42
E. *Handling test (2 min)*							
Restraint attempts (frequency)	1.00 ± 0.00	1.00 ± 0.00	1.30 ± 0.15	1.50 ± 0.22	1.67 ± 0.67	1.23	0.41
Latency halter fitted (s)	36.5 ± 2.96	36.17 ± 2.56	36.60 ± 4.80	33.33 ± 2.96	39.17 ± 7.88	0.07	0.99

Different subscript letters within a row indicate significant differences ($P < 0.05$) between treatment groups.

[1] S: standard procedure, early separation after birth and weaning at d56; BW: reducing contact (at day 49) before weaning (at day 56) by moving partial contact calves from the wall; AW: reducing contact after weaning by moving partial contact calves from the wall at day 63; FS: reducing contact (at day 49) by placing full contact calves behind the fence-line; NF: reducing contact at weaning by fitting full contact calves a nose-flap at day 56

to move less after abrupt weaning, which was suggested to be a consequence of the younger dams being generally more agile irrespective of the weaning period (Stěhulová et al., 2017). Given that the general activity patterns of FC-FS dams were similar to NC-S dams suggests that the fence-line separation method could mitigate distress responses of dams during debonding in full CCC systems. Future research is required to enhance our understanding of stress responses of cows to debonding strategies after prolonged CCC in relation to parity.

Results from the behavioural tests indicate that type of CCC or debonding strategy did not affect calves' level of fearfulness. This finding is in line with previous studies that also reported no differences in fearfulness between calves reared without CCC or with full CCC during an OFT and NOT at 14 days of age (Santo et al., 2020) or 65 days of age (Buchli et al., 2017). Individual calves differed substantially in their behaviour responses during the different behavioural tests, which is in agreement with other studies that reported large individual differences in fearfulness that were stable over time, related to personality and sociality traits (Lecorps et al., 2018; van Reenen et al., 2005, 2004), and were linked to mood-states (Lecorps et al., 2018). Notably, we found no behavioural differences between treatment groups during the HAT or HT, which suggests that calves reared with prolonged CCC may not always be more "wild" or difficult to handle as has been described by farmers (Neave et al., 2021; Vaarst et al., 2020). This might be explained by the fact that in the present study all calves were frequently handled during weekly health and growth assessments, although other factors such as calf manager behaviour (Calderón-Amor et al., 2020) and calf personality also affect animal's reactivity to humans (Waiblinger et al., 2006). Interestingly, NC-S calves showed more solitary play during the NOT compared to the other treatment groups. This finding corresponds to work from Wagner et al. (2013) in which calves reared without CCC exhibited more solitary play during an isolation test compared to calves with full CCC. The increased solitary play may reflect activity rebound due to the increased space allowance in the test arena compared to the confined home pen (Jensen, 1999). Calves with full CCC are known to perform locomotor play in the alleys of the cow barn (Wagner et al., 2013; Waiblinger et al., 2020), so they may have been less motivated for locomotor play in the test arena compared to calves that had less space in their home pen (Jensen and Kyhn, 2000; Wagner et al., 2013). However, all PC-calves were housed in similar calf boxes as NC-S calves but did not show this rebound activity during the NOT. This finding suggests that merely maternal contact in the first weeks of life affects calves' activity but, given that we did not document play behaviour in the home pens, we recommend further studies to assess play behaviour in different CCC systems to further understand the role of CCC for calf development.

Exposing animals to stressors could also reduce animals' immune competence and increase susceptibility to diseases (Blecha, 2000; Griffin, 1989). Previous studies have compared two-step debonding strategies with abrupt weaning. These studies demonstrated lower plasma cortisol levels (Loberg et al., 2008), greater humoral antibody titer responses to a viral vaccination (Lippolis et al., 2016), and reduced morbidity (Boyles et al., 2007) indicating reduced stress responses and thus enhanced immune functioning when debonding occurs. Yet, we found no differences between two-step debonding strategies for calves' immune functioning, health status, plasma cortisol concentrations or hair cortisol levels, as inter-individual variability for the various parameters was large. Some individuals showed mild physiological stress responses during debonding, whereas others seemed to have experienced more severe stress. Given that individual animals may profoundly differ in stress responsiveness (e.g. Nogues et al., 2020; van Reenen et al., 2005), we encourage the development of tailored debonding strategies that can be adapted to individual cow-calf pairs. For instance, the use of computer-automated access gates that facilitate access to either the calf or the cow could gradually reduce contact for specific individuals over a longer period of time. One possible confounder in our work is the fact that the different debonding strategies differed in timing, intensity, and duration, which made it difficult to control the stressor severity (Sapolsky, 2015). Hence, future work should aim to standardize debonding strategies and this could be facilitated by increasing the size of relevant groups.

Interestingly, FC cows' machine-harvested milk yield seemed to recover to some extent once calves were weaned, as FC-FS and FC-NF cows no longer differed statistically significant in their daily milk yield two weeks after calves were weaned compared to PC-BW, PC-AW, NC-S cows. However, the non-significant numerical difference may still have economic consequences for farmers (de Andrade Ferrazza et al., 2020), and more research is needed on milk production effects of CCC systems over time with larger numbers of cows. The decreased volume of milk yielded in the parlor during the period when calves suckle freely is well reported, although there is no consistent evidence of reduced milk production beyond the suckling period (see review by Meagher et al., 2019). Recent work reported, however, that machine-harvested milk yields were negatively impacted throughout the whole lactation period, perhaps because the frequency of milk removal went down from several times per day to twice daily machine milking after the calves were weaned (Barth, 2020). Other studies found that cow performance recovers in full CCC systems once calves were weaned (de Passillé et al., 2008; Johnsen et al., 2015c).

In terms of calf performance, weaning negatively affected the weight gain of FC-NF calves compared to FC-FS, PC-BW, PC-AW, or NC-S calves. This finding is in line with Enríquez et al. (2010), who also reported a reduced growth in beef calves weaned with a nose-flap compared to fence-line separation. The small proportion of time spent ruminating in FC-FS and FC-NF calves before weaning accompanied by a relatively high prevalence of liquid manure between weeks 7 to 10 indicates a suboptimal adaptation to solid feed prior to weaning (de Passillé et al., 2011), which in combination with the abrupt cessation of milk supply after insertion of the nose-flap may have enhanced the weaning stress in FC-NF calves. Moreover, previous work reported heavy nasal abrasions 7 days after fitting the nose-flaps in beef calves

(Lambertz et al., 2014), and, although not documented, we also observed injuries to the calves' nostrils that may have been caused by the pressure of the nose-flaps. We also suspect that the nose-flaps might have caused some pain or irritation that affected calves' activity levels.

Overall, it appears that partial CCC minimized calves' debonding distress. In dairy calves with full CCC, nose-flaps seem less effective at reducing weaning stress compared with fence-line separation. Given that stress responses may be affected by the duration of fence-line separation and the design of the fence, we strongly recommend to further explore methods that can gradually reduce contact prior to or after weaning for full CCC systems. Alternatively, we would argue that reducing contact before weaning more gradually in full CCC systems could be accompanied by delayed weaning, which might result in less distress given that calves may then be even more socially and nutritionally independent from the dam.

5. Conclusion

Calves with partial cow-calf contact (CCC) showed minimal signs of distress during weaning and separation compared to calves with full CCC. Our results imply that debonding by reducing contact via nose-flaps was more stressful for full CCC calves compared to debonding via fence-line separation. Milk production of full CCC cows was only significantly negatively affected before weaning, and seemed to recover to some extent after calves no longer suckled. However, besides efforts that investigate the effect of parity on cows' distress responses to debonding after prolonged contact, more strategies to mitigate stress responses in calves with full CCC need to be explored.

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Appendix 1. Experimental events and measurements

A)

Experimental events in sequence of time	**N**o **c**ontact with **s**tandard weaning **(NC-S)**	**P**artial **c**ontact with reduced contact **b**efore **w**eaning **(PC-BW)**	**P**artial **c**ontact with reduced contact **a**fter **w**eaning **(PC-AW)**	**F**ull **c**ontact with **f**ence-line **s**eparation **(FC-FS)**	**F**ull **c**ontact with **n**ose-**f**lap insertion **(FC-NF)**
Move to home-pen	day 0	day 3	day 3	day 3	day 3
Pair housing	day 14	day 14	day 14	NA	NA
Reducing contact	NA	**day 49**	**day 63**	**day 49**	NA
Calf fully weaned	**day 56**	**day 56**	**day 56**	**day 56**	**day 56**
Regrouping	**day 70**	**day 70**	**day 70**	**day 70**	**day 70**
Cow leaves experiment	day 84	day 84	day 84	day 84	day 84
Calf leaves experiment	6 months	6 months	6 months	6 months	6 months

Figure 1. Trial overview with **A)** experimental events in sequence of time per treatment group.

B)

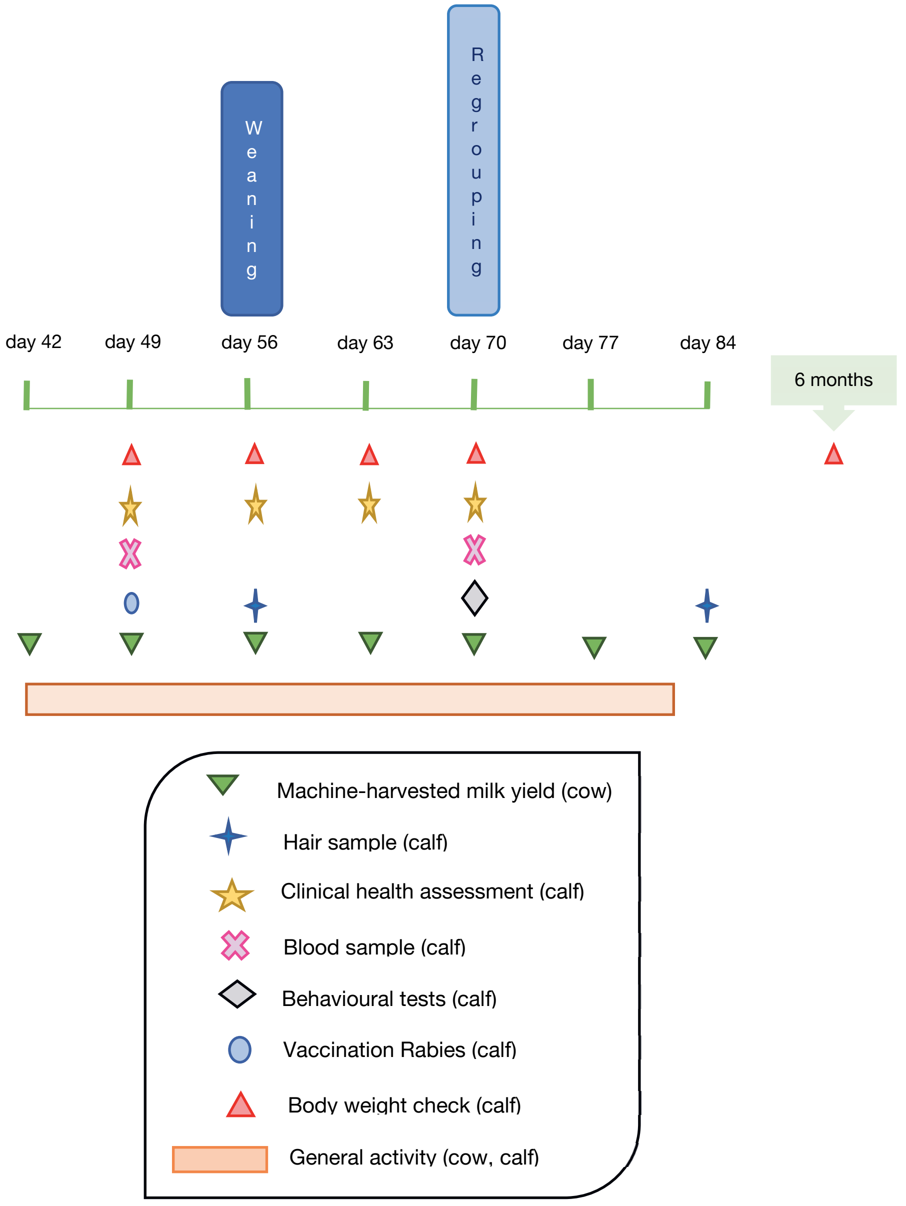

Figure 1 continued. Trial overview, **B)** animal-based measures collected during the experiment.

Appendix 2. Illustration of debonding strategies

A)

B)

C)

Figure 1. Illustration of the debonding strategies implemented to gradually reduce contact in cow-calf contact systems up until permanent separation at 70 days of age. **A)** Calf boxes were moved 0.5 m away from the wall to prevent physical contact while still allowing for visual, auditory, and olfactory contact in the partial contact group either before weaning at day 49 (i.e. PC-BW) or after weaning at day 63 (i.e. PC-AW). **B)** Fence-line separation for the full contact group (i.e. FC-FS) by using a fence through which the calf could suckle in the week prior to weaning (on initiative of the dam). At 56 days of age an extra metal bar was placed in between the lowest two bars to prevent udder access and wean the calf off milk. Subsequently, the fence-line allowed for visual, auditory, olfactory, and tactile contact (other than suckling) to take place. **C)** Nose-flap inserted in the nose of calves with full contact (i.e. FC-NF) at 56 days of age to prevent suckling.

Appendix 3. Clinical health score definitions

Table 1. Description of health parameters scored on a weekly basis between 49 to 70 days of age (adapted from Renaud et al., 2017a)

Variable	Score			
	0	1	2	3
Nasal discharge	Normal serous discharge	Small amount of unilateral discharge	Moderate amount of bilateral discharge	Copious, bilateral mucopurulent discharge
Ocular discharge	Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
Cough	No cough	Induced single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous cough
Fecal consistency	Normal consistence	Pasty, semi-formed	Pasty with large amounts of water, content adhered in the perineum and tail	Liquid with fecal content adhered in the perineum and tail
Navel inflammation	Normal	Slightly enlarged, not warm or painful	Slightly enlarged with slight pain or moisture	Enlarged with heat, pain or malodorous discharge
Rectal temperature (°C)[1]	37.8 - 38.2	38.3 - 38.8	38.9 - 39.4	> 39.4

[1] Variable was not further included in the analysis, as those variables were rarely scored as abnormal

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Appendix 4. Illustration of behavioural test arena

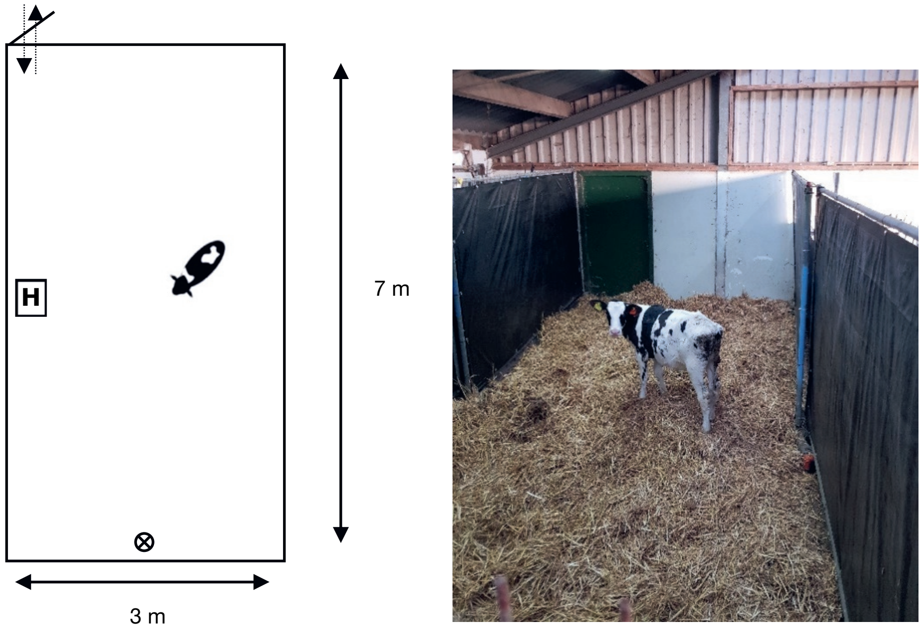

Figure 1. Test arena (3 m x 7 m) for the behavioural tests performed at day 70 (consisting of an open field test followed by a novel object test (i.e. umbrella), a human approach test (i.e. voluntary and involuntary approach) and a handling test (i.e. securing a halter). ⊗ = position of umbrella in the novel object test, H = position of immobile human during the voluntary phase of the human approach test, arrows indicate the location of the door to enter and exit the arena.

Appendix 5. Ethogram behavioural tests

Table 1. Definitions of behavioral measures and events recorded in calves during the open field (OFT), novel object (NOT), human approach (HAT), and handling (HT) tests.

Behaviour	Definition	Recorded as	Test	Reference
Walking	Calf moves at least two legs to change position in a forward, backward or sideways motion	Duration	OFT	Wagner et al. (2013)
Running	All four legs of the calf moving quickly in a forward motion, with two legs simultaneously off the ground	Duration	OFT	Adapted from Buchli et al. (2017)
Standing	Calf remains stationary with weight on all four legs, or on three legs with one leg raised or bent	Duration	OFT	MacKay et al. (2014)
Vigilant	Fixed head position, ears pointing forward/upright, and gaze directed straight ahead, while walking or standing	Duration	OFT	Buchli et al. (2017); Santo et al. (2020); Wagner et al. (2015, 2013)
Vocalizations	All types of vocalizations	Frequency	OFT, NOT	Buchli et al. (2017); Santo et al. (2020); van Reenen et al. (2004); Wagner et al. (2013)
Solitary play	Comprising locomotor play (i.e. leap, jump, buck, turn). Bouts of play counted every 5 seconds	Frequency	OFT, NOT	Wagner et al. (2013)
First close approach of object/human	Time until the calf enters a radius of 1 meter from the object/human with both front legs from the time the human stands motionless / object lands on the ground	Latency	NOT/HAT (voluntary phase)	van Reenen et al. (2004)
First contact with object/human	Time until the calf touches the object/human with its nose or tongue from the time the human stands motionless / object lands on the ground	Latency	NOT/HAT (voluntary, involuntary phase)	van Reenen et al. (2004)
Contact with wall/floor	Sniffing or touching (≤ 3cm) the wall/floor with the nose or tongue. 3 seconds between bouts	Duration	OFT	van Reenen et al. (2004)

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Table 1 continued. Definitions of behavioral measures and events recorded in calves during the open field (OFT), novel object (NOT), human approach (HAT), and handling (HT) tests.

Behaviour	Definition	Recorded as	Test	Reference
Contact with object/human	Sniffing, touching or rubbing object/human with the nose, tongue or head	Duration	NOT/HAT (voluntary phase)	van Reenen et al. (2004)
Contact with human while walking	Time spent in contact with human (any part of body) while the calf moves at least two legs	Duration	HAT (involuntary phase)	Adapted from van Reenen et al. (2004)
Contact with human while standing	Time spent in contact with human (any part of body) while the calf remains stationary with weight on all four legs, or on three legs with one leg raised or bent	Duration	HAT (involuntary phase)	Adapted from van Reenen et al. (2004)
Restrain attempts	The number of times human(s) must confine the calf to fit halter	Frequency	HT	
Halter fitted	Latency until the experimenter had securely fastened the halter strap, held the reins, and took one step back from the calf.	Latency	HT	

Chapter 6

General Discussion

1. Introduction

Early cow-calf separation shortly after birth is standard practice in the dairy industry. However, the increasing public opposition to early separation suggests that this practice may compromise the dairy industry's social license to produce (Boogaard et al., 2011; von Keyserlingk et al., 2013). Besides, growing evidence shows that the present early life environment may limit calves' physical, behavioural, and cognitive development (Costa et al., 2019a). Consequently, various stakeholders are interested in alternative rearing systems that allow for prolonged cow-calf contact (CCC) (Brombin et al., 2019). Yet, sufficient knowledge on how different types of CCC (i.e. full contact or partial contact) can contribute to optimization of calf rearing conditions with regards to animal welfare is currently lacking.

The aim of this thesis was to assess how type of CCC in calf rearing systems affects dairy cow and calf welfare in comparison to a rearing system without CCC. In this chapter, I will first highlight the main findings and then reflect on the welfare implications of different types of CCC systems including existing trade-offs and practical considerations when implementing such systems. Next, I provide an outlook for future research topics followed by recommendations for policy makers that I believe should be considered to enable a transition to prolonged CCC systems. Finally, the conclusions of this thesis will be given.

2. Type of cow-calf contact in relation to animal welfare

2.1 Main findings for the dairy cow

In **Chapter 2**, I found that cows that were suckled by their calf during nighttime pushed a greater maximum weight than non-suckled cows with partial calf contact during nighttime and early separated cows with no CCC. This result implies that a calf is more valuable to a cow when it suckles, perhaps because of a strengthened mother-young bond caused by the oxytocin release during suckling (Uvnäs-Moberg, 1998).

In **Chapter 3**, I found that partial contact resulted in less calf-directed affiliative behaviour compared to full CCC, except in the 48 hours following parturition. This finding may be explained by a different mother-young bond due to the absence of suckling, but could also be the result of the partial CCC design that limited calf accessibility.

In **Chapter 4**, I showed that dairy cow health was not affected by prolonged CCC in terms of clinical diseases and metabolic status throughout the first seven weeks of lactation. Although, machine-harvested milk yield and milk fat content were lower in cows with full CCC compared to cows with no or partial CCC during the suckling period. In **Chapter 5**, I demonstrated that cows' machine-harvested milk yield seemed to recover once calves were weaned in the full CCC system. These findings imply that prolonged CCC does not benefit nor threaten cow health, and that machine-harvested milk yields might be only negatively affected during the suckling period.

In **Chapter 5**, I also found that behavioural responses of cows during debonding after prolonged CCC were not distinctive. The effect of treatment depended on parity, as primiparous dams subjected to full CCC with nose-flap or partial CCC with reduced contact after weaning seemed to behave differently compared to the multiparous dams. However, dams subjected to full CCC with fence-line separation or partial CCC with reduced contact before weaning behaved similar to dams with no CCC. These findings suggest that parity plays an important role in dams' distress to debonding.

Overall, dairy cows seem to benefit most from full CCC with regard to the possibilities to express species-specific behaviour. Fence-line separation appeared to be an effective strategy to reduce dams' distress to debonding. Cow health is not affected by prolonged CCC, but farmers face reduced machine-harvested milk yields with a lower fat content in full CCC systems during the suckling period.

2.2 Main findings for the dairy calf

In **Chapter 4**, I assessed the effect of different types of CCC on calves' clinical health status, structural growth, fecal microbiota development, and immunological/hematological blood parameters indicative of biological functioning. Full CCC impaired calf health, as reflected by more health issues and a tendency for more antibiotic's usage accompanied by deviant hematological parameters indicative of disease compared to calves with no CCC. Presumably, the barn climate and housing conditions for calves in the full CCC pen were suboptimal and posed a risk for calf health. Nevertheless, calves with full CCC had a greater daily weight gain than calves with no or partial CCC during the milk feeding period, likely due to ad libitum milk consumption. Additionally, calves reared with full CCC seemed to have a different microbiota composition including a higher abundance of *Lactobacillus* during the milk feeding period than calves with no or partial CCC, which suggests that maternal factors, such as reciprocal licking, suckling, and direct exposure to the dam's environment, play an important role in the colonization of calves' microbiota (Wiley et al., 2017).

In **Chapter 5**, I assessed calves' stress responses to different two-step debonding strategies (i.e. fence-line separation vs. nose-flap insertion following full CCC; reducing contact before vs. after weaning following partial CCC; standard weaning following no CCC). Beneficial effects of full CCC for calf's body weight were only present during the suckling period, given that both after weaning and at six months of age calves' absolute body weight did not longer differ among treatment groups irrespective of the debonding strategy. Calves with full CCC spent a larger proportion of time highly active during debonding regardless the strategy, whereas calves with partial CCC were minimally impacted by weaning given their general activity patterns that were similar to calves with no CCC. Moreover, debonding via nose-flaps in full CCC caused nasal abrasions and negatively impacted calves' growth after weaning. These findings imply that partial CCC minimized distress during debonding regardless the strategy, and that for full CCC fence-line separation seems more effective to mitigate distress in dairy calves compared to a nose-flap.

Given that calves with full CCC are not fed by farm personnel, they usually have less contact with humans. Hence, a frequently addressed concern by dairy farmers is the handling of mother-reared heifers that become wilder and are more afraid of humans (Neave et al., 2021). During the human approach and handling test, to which calves were subjected prior to moving to the young stock barn at 70 days of age, calves' responsiveness to humans seemed not affected by type of CCC (**Chapter 5**). In order to understand the effects of prolonged CCC in early life on the human-animal relationship later in life, I also performed an active human approach test (previously described by Bokkers et al. (2009)) at six months of age to measure the behavioural response of calves to an active approach by an unfamiliar person. The active human approach test consisted of four stages: i) experimenter made eye contact with a calf standing with its head oriented towards him at a distance of approximately 1.5 m; ii) experimenter took one step towards the calf with one arm stretched out and stood still with two feet next to each other for one second; iii) experimenter took a second step and stood still again for one second, and iv) experimenter touched the calf's snout. The test was ended whenever the calf moved one of its forelegs backwards. For each successful stage, one point was awarded (0- to 4-pointscale), with 0 points when the experimenter was unable to make eye contact (maximally three attempts per calf). Generally, 6-month-old calves reared with full CCC in early life appeared to be less approachable than calves with no or partial CCC, as only 20% of the calves reared with full CCC got a score 3 or 4 (i.e. close approach or touching) compared to relatively 56% and 89% of the calves reared with no or partial CCC (Figure 1). Surprisingly, calves reared with partial CCC seemed even less fearful of humans compared to calves reared with no CCC. These findings suggest that partial CCC in early life positively affected calves' reactivity to humans later in life, whereas the results in calves with full CCC confirmed dairy farmers' worries that calves can be "wilder" and more difficult to handle later in life (Neave et al., 2021; Vaarst et al., 2020). Even though all calves were frequently handled during weekly health and growth assessments in the first ten weeks of life, those interactions were forced and likely perceived as negative by the calves (Waiblinger, 2017). Given that the human-animal relationship is important for animal welfare (Rault et al., 2020), those outcomes stress the importance of socializing dam-reared calves to humans using positive interactions (Lürzel et al., 2016).

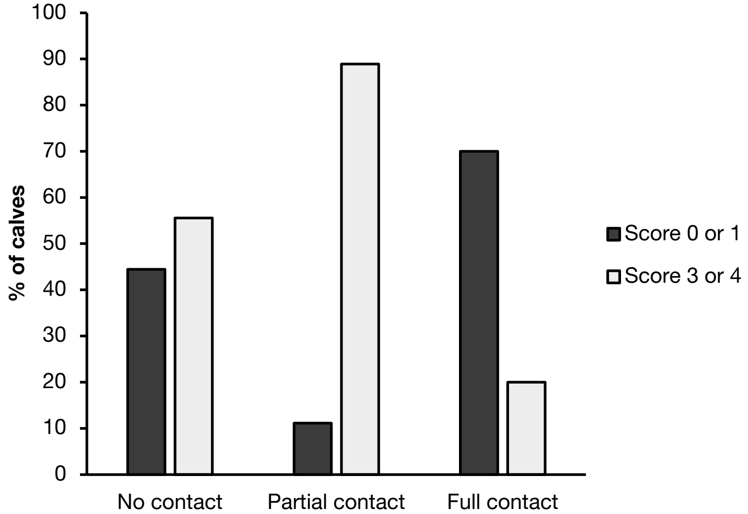

Figure 1. Percentage of calves that could be closely approached or touched (score 3 or 4) or not at all (score 0 or 1) by an unfamiliar person in an active human approach test at six months of age reflecting calves' responsiveness to humans following rearing conditions with different types of cow-calf contact in the ten first weeks of life.

Overall, in the current study the welfare implications of different types of CCC for dairy calves were not straightforward. Although full CCC systems offer calves opportunities to express their natural behaviour (Whalin et al., 2021), this system did not provide health benefits but instead compromised calves' health. Moreover, full contact induced behavioural stress responses to debonding and increased the risk for a poor-human animal relationship later in life. In contrast, partial CCC did not impair calf health, did not induce distress during debonding, and did not increase calves' responsiveness to humans.

2.3 Understanding the consequences of prolonged contact for animal welfare

The concept of animal welfare that is generally adhered to in the scientific community consists of three key elements: 1) natural living (i.e. the animal is able to express its species-specific behaviour), 2) biological functioning (i.e. the animal has a satisfactory health status, growth, and normal functioning of physiological systems), 3) affective states (i.e. the animal is free from negative emotional feelings and facing mainly positive experiences) (Duncan, 2005; Fraser et al., 1997; Hemsworth et al., 2015; Mellor, 2016, 2012). The best solutions for solving animal welfare problems are those that address all three elements (von Keyserlingk et al., 2009). In this respect, solely proposing strategies to enhance calf welfare within the boundaries of standard management practices with early cow-calf separation (e.g. via high-plane feeding and pair/group housing) appears to be insufficient, as such solutions do not provide welfare benefits to the dam (e.g. opportunities to express maternal behaviour). Prolonged CCC systems more closely resemble the social and/or nutritional environments known under natural settings (Cantor et al., 2019; Whalin et al., 2021), yet in both the partial and full CCC systems (as presented in this thesis) trade-offs for animal welfare emerged. In this section, I will reflect on those trade-offs and suggest practical considerations for each system that could perhaps mitigate drawbacks.

2.3.1 Benefits and drawbacks in a full contact system

A unique beneficial aspect of full contact is the possibility to suckle. From an evolutionary perspective for mammalian species, suckling is considered an essential and common maternal behaviour (Lévy, 2016; von Keyserlingk and Weary, 2007). This appears to be reflected by the increased motivation of suckled cows to reunite with their calf (**Chapter 2**). Moreover, suckling allows calves to nourish themselves according to their needs in a natural way (Whalin et al., 2021), given that suckling from the dam allows for consumption of high milk volumes, fulfillment of the sucking reflex, and increased frequency of milk meals compared to conventional strategies (Beaver et al., 2019b). Even though automated milk feeders could provide similar nutritional benefits, previous work suggested that calves seem to prefer to suckle an udder over an automated milk feeder (Johnsen et al., 2015c). Johnsen and colleagues investigated the behaviour of cow-calf pairs in a part-time CCC system (contact during the night), in which calves had different levels of nutritional dependency on the dam. One group of calves had partial contact (i.e. dam was fitted with an udder net) overnight and were fed with an automated milk feeder (offering 12 L/d of fresh, warm, pasteurized whole cow's milk), one group of calves could suckle their dam overnight, and one group of calves could suckle overnight but also had 24/7 access to the automated milk feeder. In the latter group, 6 of 10 calves drank very little (<1.5 L/day) from the automatic milk feeder, despite the training to use the feeder, and instead waited for 12 hours to be able to suckle milk from their dam. No calves had the opposite preference (Johnsen et al., 2015c). Moreover, cross-sucking has hardly ever been observed in calves reared by their dam or a foster cow (Krohn et al., 1999; Lidfors et al., 1994; Roth et al., 2009). This abnormal oral behaviour can still occur among calves fed ad libitum milk via automated feeders (Margerison et al., 2003). Possibly, the occurrence in cross-suckling is the result of the competition for the feeder in group housed calves, which allows little time for non-nutritive following a meal (de Passillé, 2001). Overall, full contact promotes the expression of natural behaviour in both cow and calf.

In addition, we found a first indication that suckling results in a different microbiota colonization in calves (**Chapter 4**), which is in line with previous exploratory work (Beaver et al., 2021). However, there is a lack of understanding about the clinical and biological relevance of those outcomes for calf health. Yet, there is growing evidence in human literature that a major source for colonization of the infant gut is through bacteria in the mother's milk (Arrieta et al., 2014; Wiley et al., 2017). Mother's milk contains not only a range of bacteria species but also antimicrobial compounds, immunoglobulins, cytokines, growth factors, and leukocytes that transfer passive immunity to the infant (Arrieta et al., 2014; Fernández et al., 2013; Wiley et al., 2017).

Those maternal compounds are not only expected to limit the overgrowth of potentially pathogenic compounds, but are also believed to play an important role in health status and priming of biological systems during childhood that can provide life-long benefits (Ratsika et al., 2021; Wiley et al., 2017). Hence, more research into maternal influences on fecal microbiota development in dairy calves and its long-term implications for calf health is warranted.

Furthermore, compared to partial contact, full contact allows for unobstructed cow-calf interactions (**Chapter 3**). It allows not only dams, but also calves to freely initiate interactions, such as allogrooming and resting together. This could be considered as more natural to calves, given that dairy calves usually increase their social behaviour towards their mothers from two weeks of age (Jensen, 2011; Tucker, 2009). Although this social cohesion may be initially driven by the calf's need for milk, cow-calf pairs have been reported to spend as much as 30% of their time together not suckling in an indoor farm setting (Johnsen et al., 2015c). Given that allogrooming is an important affiliative behaviour that is thought to play a key role in reinforcing social bonds and is associated with positive emotions (Boissy et al., 2007), allowing full contact is likely to positively contribute to both the expression of natural behaviour and affective states.

Despite the beneficial aspects, the results of this thesis underlined also drawbacks of full CCC, as reflected by more health issues and a tendency for more antibiotics usage in calves (**Chapter 4**), the probable negative affective states due to distress during debonding (**Chapter 5**), and an increased (negative) responsiveness of calves to humans (Figure 1). Adequate calf health in prolonged CCC systems remains a controversial topic, given the inconsistent findings in numerous studies in favor or against prolonged CCC (Beaver et al., 2019a; Neave et al., 2021; Sumner and von Keyserlingk, 2018). The housing conditions and barn climate that calves may be exposed to in a full CCC system can be challenging for calf health, given that calves are kept in a barn designed for adult animals (Johnsen et al., 2016). Furthermore, stockmanship (i.e. attention to details in calf rearing) is referred to as the most important aspect for successful calf rearing, which is dependent on the skills, time, and interests of the stockperson, as well as the available facilities (Palczynski et al., 2021). In prolonged CCC systems, farmers may need to observe and interact differently with their animals than when rearing calves separately from the cows, besides the additional infrastructure changes (Neave et al., 2021; Vaarst et al., 2020). Hence, the transition to prolonged CCC systems requires both adaptations in facilities and stockmanship, which can be challenging and take time (Hansen and Jervell, 2015).

In addition, debonding after full CCC using two-step strategies can still cause distress responses in cow-calf pairs (**Chapter 5**), which is in line with previous work in dairy cattle (Johnsen et al., 2018; Loberg et al., 2008). Moreover, maternal separation after prolonged CCC is known to cause a pessimistic judgement bias in calves, which is considered an indicator of a negative affective state (Daros et al., 2014). The negative affective states in cow-calf pairs following debonding are a major welfare concern. Yet, under natural conditions (at older ages) weaning is not necessarily stress-free, as it is also accompanied by agonistic behaviour (Trivers, 1974). Naturally, the dam gradually weans her calf around 8 months of age by preventing it from suckling, although she continues to associate with her offspring long after weaning by choosing them as grooming and grazing partner for many years (Reinhardt and Reinhardt, 1981a). The dam initiates the weaning process shortly before her next calving, which results in a distress response from the calf (e.g. vocalizations). From a functional perspective, the natural distress response at weaning can be viewed as an adaptation designed to signal need for resources, such as milk (Weary et al., 2008). In fact, it is the result of a parent-offspring conflict that emerges around weaning. As described by Trivers (1974), both dam and her young benefit from maternal investment when the calf is still fully dependent on maternal care to survive. However, conflict about the level of maternal investment increases as offspring age: the calf still benefits from a high level of investment, but the dam may better start to invest in new offspring and leave older offspring to increasingly forage for themselves. Consequently, a so-called "weaning conflict" emerges in which the calf's behaviour is designed to request additional investment, whereas the dam attempts to repulse this request (Trivers, 1974). Accordingly, weaning can be accompanied by agonistic behaviour of the dam, such as moving away and kicking or butting the calf, to terminate a suckling attempt (Weary et al., 2008). As stated by Weary et al. (2008), the calf's behavioural response at weaning is not necessarily an artefact of rearing practices as it is also a natural response to request maternal care.

Yet, the increased responsiveness to an unfamiliar human in 6-month old calves reared with full CCC can be a long-term drawback, as the poor human-animal relationship may result in dangerous situations during routine care and management activities (Neave et al., 2021; Vaarst et al., 2020). Hence, besides workers wellbeing, full contact can have negative consequences for the animals' affective state and biological functioning later in life (Rault et al., 2020; Waiblinger et al., 2006).

2.3.2 Practical considerations for full contact systems

Based on scientific literature and personal experiences throughout the on-farm experiments, I propose some prerequisites in terms of housing and management that could potentially mitigate the drawbacks of a full CCC system for the animals' welfare.

- **Newborn management**: A maternity pen should provide a cow with a clean, quiet, comfortable, secluded area to give birth (Proudfoot, 2019). Individual maternity pens promote bonding between mother and young in the first days postpartum without interference from other animals, and avoid suckling of colostrum among alien cow-calf pairs (Edwards, 1983; Illmann and Špinka, 1993). A thick layer of fresh bedding should be added daily, and maternity pens should be cleaned and disinfected after each birth to reduce disease transmission

(Proudfoot, 2019). Nonetheless, a prolonged stay in the maternity pen can be challenging in terms of cleanliness throughout this stay, especially because cows are usually moved into this pen (and thus contaminate it) already several hours to days before calving. It might be worthwhile to consider a so-called bonding pen for cow-calf pairs in the days following parturition. By placing the new-born calf with the dam in a dry and clean bonding pen after birth, the farmer removes them from the contaminated calving area while still providing an secured area to bond that also allows for close monitoring of the cow-calf pair. Especially close monitoring of colostrum intake is strongly recommended, as previous studies indicated that calves left with their dams after birth are at a higher risk for failure of passive transfer (Besser et al., 1991; Trotz-Williams et al., 2008). Although freely suckling colostrum from the dam can result in higher levels of serum IgG concentrations (**Chapter 4**; Quigley et al., 1995; Selman et al., 1971; Stott et al., 1979), two intervention procedures can be suggested to secure colostrum ingestion in full contact calves: early assistance to reach the udder and suckle (Franklin et al., 2003; Quigley et al., 1995), or feeding additional colostrum by bottle to the calf (Logan et al., 1981; Michanek et al., 1990). Moreover, providing heating lamps can be desirable in cold seasons to create a micro climate for new-born calves (Borderas et al., 2009).

- **Calf monitoring and interactions**: Full CCC systems appear to require a different perception in terms of calf care including a shift from controlling the animals to observing the animals (Vaarst et al., 2020; Wagenaar and Langhout, 2007). Yet, close monitoring of young calves' appearance to evaluate their health and milk consumption remains essential in full CCC systems. Preferably, the daily checks also involve some positive human-animal contact (e.g. provision of calf starter, stroking) to habituate the calves to humans (Rault et al., 2020).

- **Housing conditions**: Housing animals in an environment that allows for more expressions of natural behaviour can lead to increased incidence of diseases when poorly managed (von Keyserlingk et al., 2009). Creating a calf-creep area in the cow pen could provide calves with a calf-friendly micro climate, easy access to water and solid feed, and the opportunity to distance themselves from the cows as seen in nature (van Dixhoorn et al., 2010). Furthermore, I recommend to check the cow pen for risk factors that can harm young calves (and make adjustments accordingly), such as barn climate (e.g. prevent draught or high wind speed indoors, especially in the preferred resting areas of calves), bedding (e.g. avoid moisty or dusty materials), fencing (e.g. limit potential escape routes), as well as flooring in free stall barns (e.g. barricade openings to the manure pit to prevent accidents, spacing between slats to prevent the hoofs of calves getting stuck) (Neave et al., 2021; Roland et al., 2016; Vaarst et al., 2020). Given that keeping cattle in mixed age groups may be challenging for the control of transmissible or contagious diseases (Johnsen et al., 2016), I recommend to only allow for prolonged CCC in dairy herds that are free from paratuberculosis, bovine viral diarrhea virus, and salmonella, as especially those specific pathogens spread rapidly on herd level (van Dixhoorn et al., 2010).

- **Debonding strategies**: The relatively young calf age at weaning in farm settings can play an important role regarding stress responses in cow-calf pairs during debonding (Stěhulová et al., 2017). By initiating debonding at an older age, distress responses could be mitigated, as calves may then be even more socially and nutritionally independent from their dam (Newberry and Swanson, 2008). Besides loss of the dam, debonding involves a variety of other changes in the calf's social and physical environment as a result of regrouping (Weary et al., 2008), hence I recommend to wean and separate calves in pairs or small groups. Moreover, given the individual differences observed in the animals' behaviour (e.g. calves' solid feed intake, cows' motivational strength, responsiveness to debonding strategies, cow-calf interactions), tailored two-step debonding strategies that meet the needs of individual animals may mitigate weaning distress as well (Neave et al., 2018). Fence-line separation as two-step debonding strategy in full CCC system shows potential to alleviate stress responses in both cow and calf. Yet, improving the debonding process of cow-calf pairs in a farm setting requires workable systems that not only benefit the animals but are also practical for farmers (Weary et al., 2008). Especially now that transponder-controlled technologies are increasingly used on farms, the use of computer-automated access gates (as alternative to fence-line separation) that facilitate access to either the calf or the cow has potential to gradually reduce contact for each specific individual over a longer period of time. Additionally, (automated) measuring individual early solid feed intake may help to predict an appropriate weaning age for calves (Neave et al., 2019).

Nevertheless, it remains open to what extent these suggestions substantially reduce negative affective states during debonding and effectively improve calves' health status and responsiveness to humans later in life (see also section 3.3). In addition, for a successful implementation of managerial changes, such as full CCC, also the perceptions, experience, and strategies of individual farmers and their advisors play an important role (Hansen and Jervell, 2015) (see section 4.1 and 4.2).

2.3.3 Benefits and drawbacks in a partial contact system

Partial contact seems to mitigate some of the drawbacks of full CCC systems regarding animal welfare, as the clinical health of young calves seemed not to be impaired (**Chapter 4**), minimal distress responses during debonding were observed (**Chapter 5**), and calves seemed to have a positive human-animal relationship based on their responsiveness to an unfamiliar human at six months of age (Figure 1). Moreover, partial contact offers opportunities to express affiliative behaviour among cow-calf pairs, and most dams used the opportunity to interact with their calf in a partial CCC system (yet to a lesser extent than dams with full contact) (**Chapter 3**). In contrast to early separation shortly after birth (and similar to full contact), partial contact also allows the dam to extensively perform postpartum maternal licking that is known to stimulate the calves' breathing, digestive, and circulation system, besides cleaning of the calves' coat (von Keyserlingk and Weary, 2007). All in all, partial contact shows potential to positively contribute to expression of species-specific behaviour, while maintaining adequate biological functioning and avoiding negative emotional states.

Nevertheless, it can be questioned how valuable the cow-calf interactions in a partial CCC system are to the animals, as partial contact reduces the quantity (**Chapter 3**) and possibly also the quality of the cow-calf interactions. First, calves were pair-housed until 10 weeks of age and fed limited amounts of milk by animal caretakers according to standard feeding practices. In addition, the design of partial contact in the current study provided calves with limited opportunities to initiate contact with their dam. Dairy calves are known to become the initiator of contact around two weeks of age (Jensen, 2011). Calves with partial CCC could possibly still have used vocalizations to initiate contact (Padilla de la Torre et al., 2016), but this remains unknown as no auditory recordings were made in my studies. Overall, the opportunities for calves to express their natural behaviour (e.g. suckling and social behaviour (Whalin et al., 2021) were limited. Secondly, the limited calf-accessibility in the current design may have hampered dams to effectively interact with their calf whenever they desired (i.e. dam could not always reach her calf). The restricted contact may potentially have caused frustration or stress in the dam or her calf, which would negatively affect their affective state. Partial contact by means of udder nets would allow easy calf access, as well as calves to be initiator of contact. Previous work revealed similar affiliative behaviours among cow-calf pairs in a part-time CCC system with either full or partial CCC using udder nets (Johnsen et al., 2015). Yet, it remains unknown how udder nets affect the quality of cow-calf interactions, also considering the non-suckled dams' reduced motivation to reunite (**Chapter 2**).

2.3.4 Practical considerations

Partial CCC could be considered as alternative to a full CCC system, however I suggest to take into account the following prerequisites that may potentially reduce some of the drawbacks to improve animal welfare.

- **Newborn management**: Similarly to a full CCC system, the risk of mismothering and disease transmission could be diminished by permitting a prolonged stay of cow-calf pairs in an individual maternity or bonding pen that is well-bedded, dry, and cleaned regularly (Proudfoot, 2019). Besides, I recommend to use a calf box that provides the cow with easy calf-access to reduce frustration or stress in dams (Green et al., 2020). Preferably, the calf box should provide a heating lamp during cold seasons and sufficient space allowance for the calf to initiate its postpartum standing.
- **Housing conditions**: I encourage designs that provide the dams with more easy calf-access to promote the expression of natural behaviour. Partial CCC systems that also allow calves to be initiator of contact would contribute the expression of maternal-filial behaviour. Besides, the partial CCC system should allow for early

social housing of calves given the welfare concerns related to single housing (Costa et al., 2019). Furthermore, I recommend to provide calves with more space allowances than the minimum legal standards used in my study. As calved aged, the calf boxes appeared rather small as calves sometimes hit pen fixtures when they expressed play behaviour. Play and opportunities to play have been convincingly argued to have positive effects on animal welfare (see review by Ahloy-Dallaire et al., 2018). Greater space allowance is known to promote the expression of play behaviour (Jensen et al., 1998; Jensen and Kyhn, 2000), and larger pens may reduce environmental obstruction and thus give a better opportunity to play.

- **Feeding management**: Dairy calves need to be fed according to their biological needs (i.e. free choice when to eat and how much to eat) accompanied by gradual weaning programs, while having the opportunity to suckle (i.e. teat) and early access to water and solid feed (Costa et al., 2019). Furthermore, the provision of hay and milk feeding methods that allow for adequate nutritive and non-nutritive suckling can help to diminish cross-suckling behaviour reported in group housed calves (de Passillé, 2001; Margerison et al., 2003). Especially, ad libitum access to automated milk feeders show potential to tailor milk feeding management to the individual's needs (Costa et al., 2019).

2.3.5 Additional considerations for prolonged cow-calf contact systems

In addition to the considerations for both systems mentioned above, I would like to address a few additional notions that I believe are relevant when considering prolonged CCC systems.

- **Breed**: The Holstein Friesian breed is the predominant dairy breed in the world, which can be largely attributed to the substantial genetic progress in milk production (Buchanan, 2002; McGuffey and Shirley, 2011). However, this classic dairy cow might be less suitable for suckling calves (Selman et al., 1970). First, the traditional Holstein Friesian breed has been thought to have lower colostral immunoglobulins concentrations than other dairy breeds (see review by Puppel et al., 2019), and particularly in high yielding dairy cows there can be a dilutional effect (Godden, 2008). This dilutional effect may also be present in milk composition, as Holstein Friesian cows have relative lower percentages of milk fat and protein compared to other dairy breeds (Buchanan, 2002). Therefore, it has been speculated that whole milk of this breed may perhaps no longer meet the biological needs of calves (Antonis et al., 2017). Secondly, the genetic selection for milk production and machine milking contributed to changes in udder shapes of Holstein Friesian cows, in particular udder size and teat confirmation (Oltenacu and Broom, 2010). The relatively large pendulous udders can result in an increased time spent teat seeking and greater latency to suckle of new-born calves, causing a delay in the ingestion of colostrum (Selman et al., 1970; Ventorp and Michanek, 1992). As described by Selman and colleagues (1970), udder morphology of dairy heifers and most beef cows is thought to resemble wild ungulates where the udder is the highest part of the female's underbelly, whereas dairy cows are known to have larger pendulous udders. In cases where the udder was the highest point of the dam's underbelly, calves were reported to quickly find the udder and suckling usually occurred soon after, whereas calves carried out misdirected teat seeking around the forelegs or high above the teats in dams with “poor shaped” udders (Selman et al., 1970).

Overall, rather than the pure-bred Holstein Friesian cows, other dairy breeds or crossbreds with a more suitable udder morphology and lower milk production should perhaps be considered for full CCC systems. This might possibly also result in more vigorous calves that potentially thrive better under the challenges of modern dairy operations. Dairy cows are lactating for most of their pregnancy, meaning that the fetus is competing for nutrients with the requirements for milk production (i.e. cows producing more milk likely provide less nutrients to the fetus) (Abuelo, 2020). Previous work found that heifer calves born to dams with high milk production levels during gestation had a lower survivability, reduced milk production later in life, and were metabolically less efficient than those born to dams with lower milk yields (Berry et al., 2008; González-Recio et al., 2012), although other management factors that affect the dam’s feed intake may also affect fetal development (Abuelo, 2020). Additionally, it has been suggested that through artificial genetic selection for milk yields we may have selected against some aspects of maternal behaviour (e.g. cows must let down milk in the absence of her calf and be amenable to early handling of the calf and separation from it) (Edwards and Broom, 1982). Although the Holstein Friesian cows included in this study showed no mismothering behaviours, in view of the literature, they might not fit best to rearing systems with prolonged CCC.

- **Future outlook**: Welfare of dairy cattle will continue to receive societal attention, and it is foreseen that dairy farm facilities will be modified to improve the animals’ welfare (Britt et al., 2018). Considering the ethical concerns regarding early cow-calf separation, it is important to address the question where dairy systems should aim to be in 20 years. Transitioning to full contact may require more systemic changes (e.g. initiatives to label dairy products; see section 4.2) considering the economic viability and practical feasibility on conventional farms, whereas partial contact may potentially be easier to implement. Both systems offer opportunities to improve animal welfare, yet each system has also multifaceted drawbacks for animal welfare. It remains open what prolonged CCC system would suit best in existing dairy production systems, especially because people place different weights on the three elements of welfare (von Keyserlingk et al., 2009; Weary and Robbins, 2019). For instance, people working in the dairy industry often define animal welfare mainly based on the animals’ biological functioning, and argue that high levels of milk production and good health are clear evidence of high standards of welfare (von Keyserlingk et al., 2009). Additionally, producers often view naturalness in animal husbandry systems as a luxury or as a welfare risk (e.g. harsh weather conditions for outdoor-housed animals) (Spooner et al., 2012). Prolonged

CCC systems seem to conflict with the perceived best practice (i.e. early separation) of the dairy industry, which may partly be attributed to the emphasis on natural behaviour in such alternative systems (Beaver et al., 2019b). On the contrary, citizens usually define animal welfare based on the animal's ability to live a relatively natural life, and argue that high levels of naturalness (e.g. outdoor access, leaving cows and calves together) reflect good welfare (Beaver et al., 2020; Cardoso et al., 2016). Even though there are farmers that acknowledge the importance of natural behaviour alongside affective states for good animal welfare (Hansson and Lagerkvist, 2016) and citizens that also maintain an awareness on animal health and affective states (Cardoso et al., 2016), there seems to be an overall misalignment between perceptions of what constitutes good animal welfare (Weary and von Keyserlingk, 2017). Given that full contact allows for more natural behaviour to occur, this system could actually be preferred by the public. Nevertheless, a question that arises is whether the life of a domesticated animal in a farm environment can even be called natural or whether a natural life can be equated with well-being (Placzek et al., 2020). For example, farm animals kept in barns are protected against predators and unfavorable weather conditions, which is unnatural but beneficial for the well-being of the animals (Špinka, 2006).

Despite its complexity, animal welfare itself can no longer be ignored as public value with regards to food production in animal agriculture (Weary and von Keyserlingk, 2017). Future dairy facilities should in my view foster an environment conducive to the expression of species-specific behaviour that animals are motivated for and reduce negative experiences whilst safe-guarding animal health as well (Beaver et al., 2019b; Britt et al., 2018). In addition, I would like to highlight that the results of this thesis reflect considerable inter-individual variability, given that certain cows expressed a greater interest in being near or interacting with their calf (**Chapter 2** and **3**), certain individuals experienced more stress during debonding (**Chapter 5**), and certain individuals coped better (e.g. immunologically and physically) in a CCC system (**Chapter 4** and **5**) than others. It can be questioned whether our current animal husbandry approach sufficiently considers the individual nature of animal welfare (Winckler, 2019), as ultimately animal welfare refers to the quality of life from the perspective of the individual animal (Broom, 2008). Despite the opportunities for enhanced animal welfare, it should be acknowledged that re-design of dairy farms in such way to transform them into tailored CCC production systems without high-cost and time consuming structural changes is a big challenge (Brombin et al., 2019).

3. Perspective for future research on cow-calf contact

Given the limited scope of this study and the existing tradeoffs regarding the three key elements of animal welfare in partial and full CCC systems, I recommend four future research topics in this section that in my opinion can, on the one hand, contribute to a broader understanding of the welfare implications of prolonged CCC systems and, on the other hand, contribute to the development of future-proof designs of such systems.

3.1 Affective states

Given that the experimental designs used throughout this study were designed to examine effects on behaviour and biological functioning, I was not able to extensively investigate the effects of prolonged CCC on affective states of cow-calf pairs. It remains currently unknown if prolonged CCC results in positive emotional states or moods (Meagher et al., 2019). Furthermore, there is still a lack of understanding regarding the negative emotional impact following early or late separation. Even though dairy calves showed a negative bias indicative of low mood following separation after prolonged contact (Daros et al., 2014), no work investigated whether cows show a similar pessimistic bias. It also remains unknown how long such a low mood would last after either early or late separation. Consequently, there is a lack of understanding as to what extent the stress during debonding is worth the welfare benefits that prolonged full CCC can potentially offer prior to that. For a good quality of life, the frequency of pleasant experiences should outweigh the frequency of unpleasant experiences throughout an animal's life (Green and Mellor, 2011). Therefore, it is important to enhance our understanding of the balance between positive and negative emotions in various calf rearing systems.

3.2 Housing conditions

This thesis showed that full contact did not guarantee adequate calf health, despite its potential to promote positive animal welfare (Meagher et al., 2019). In this experimental context, the system was implemented in an existing dairy facility without major barn adaptations or managerial changes. Perhaps, efforts that identify strategies to optimize current housing conditions when full CCC is considered can help to create an environment conducive to the young calves' health. In the same way, refining the design of partial CCC systems could encourage more cow-calf interactions. Nonetheless, the practicability of prolonged CCC in a farm setting is still questioned by dairy farmers (Neave et al., 2021; Vaarst et al., 2020), and such systems should not only benefit the animals but also be workable for farm staff. Hence, I recommend future studies to design cow-calf housing systems that provide the animals the opportunity to express species-specific behaviour while safe-guarding animal health and farmers' work pleasure. This effort may well be accompanied by a socio-economic evaluation of such systems (e.g. using the framework of Knierim et al. (2020) for full cost accountings with monetary and non-monetary factors).

3.3 Duration of contact

Currently, the optimal duration of prolonged CCC, and with that weaning age, remains unknown. An increased understanding of what defines a beneficial time period for CCC will not only impact the animals' welfare (e.g. reducing stress by initiating debonding when calves are more ready from a social and nutritional perspective), but also the economic viability of farms (e.g. estimating machine-harvested milk losses).

Moreover, this study included only heifer calves that were reared for the future dairy herd. Many surplus calves (all males and a part of the females) are sent to veal farms at a rather young age, as nowadays the veal industry only accepts calves younger than 35 days of age. Those animals should not be forgotten when considering alternative calf rearing strategies, given the welfare concerns (similar to replacement calves) during their stay at the dairy farm that can even affect their wellbeing on veal farms later in life (Renaud et al., 2017b). Hence, I encourage future studies that investigate the welfare implications for surplus calves (and their dams) alongside the economic effects in scenarios with various durations and types of prolonged CCC.

3.4 Public acceptability

To allow a viable future for the dairy industry, it is crucial that dairy farming practices are acceptable to the majority of the general public (Barkema et al., 2015; von Keyserlingk et al., 2013). Given that the public is especially concerned about the natural living aspect of animal welfare (von Keyserlingk and Weary, 2017; Beaver et al., 2019b), it is important to investigate the societal attitude towards partial contact between cow and calf. It is currently unknown to what extent the public supports partial CCC as alternative to full CCC or the standard calf rearing practices.

4. Recommendations for policy makers to enable change

Nowadays, animal welfare is an important ethical and social concern that is integrated into the concept of sustainable agriculture (Buller et al., 2018; Keeling et al., 2019). Prolonged CCC systems provide several opportunities to enhance the welfare of both cow and calf on dairy farms, bearing in mind the considerations that I provided in section 2.3 and the knowledge gaps presented in the previous section. Successfully adopting a new farm management system is not easy and the transformation process takes time, as it requires not only changes on-farm in terms of management and infrastructure, but it also involves changes on a personal level and on a sector level (Hansen and Jervell, 2015; Ritter et al., 2017). In this section, I will provide recommendations to the dairy sector and policy makers that I believe can guide managerial changes on dairy farms to effectively adopt prolonged CCC in the future.

4.1 Understanding behavioural change on farm level

Farmers can face several conflicts and difficulties when allowing for prolonged CCC, either as animal care-taker, given the alternations in the nature of daily work and farmer–animal interactions, but also regarding the economic and practical realities accompanied by the social context in which they operate (Hansen and Jervell, 2015; Vaarst et al., 2020). Those factors can affect whether a transition will be successfully implemented. So, if I would be asked to recommend a type of CCC to the dairy sector, I would respond that it depends on the farm-specific circumstances (e.g. herd size/health status, barn design) and the attitude of the farmer (e.g. skills and inner motivation to change). To explain this reasoning, I will use the Capability, Opportunity, and Motivation for effective Behavioural changes (COM-B) model that describes what conditions internal to an individual and in their social and physical environment need to be in place for a specific behavioural change to occur (Michie et al., 2011) (Figure 2).

The success of managerial changes (i.e. behavioural change) on farm level relies on three factors, namely a strong intention to perform the managerial changes (i.e. motivation), external factors that make it possible to perform the managerial changes (i.e. opportunity), and knowledge and skills necessary to perform the managerial changes (i.e. capability). Opportunities can influence motivation as can capabilities, and on the other hand, a change in behaviour can alter the individual's motivation, capabilities, and opportunities (Michie et al., 2011) (Figure 2). Motivation not only involves emotions and impulses based on personal goals and desires, but also includes reflective processes, such as making plans and evaluating things that have already happened. Personally, I believe inner motivation is the key to successfully transform to a prolonged CCC system. As stated by Hansen and Jervell (2015), intrinsic interest and motivation is necessary to be persistent enough to solve all the demanding tasks involved in managerial changes. Changing farm management is usually accompanied by unforeseen problems that require problem-solving skills, but the person has to be motivated to use them. Motivated farmers are more proactive to seek information and experience more control over problems than less-motivated farmers (Hansen and Jervell, 2015). Besides, farmers should also have the opportunities to adopt managerial changes based on external factors, such as time,

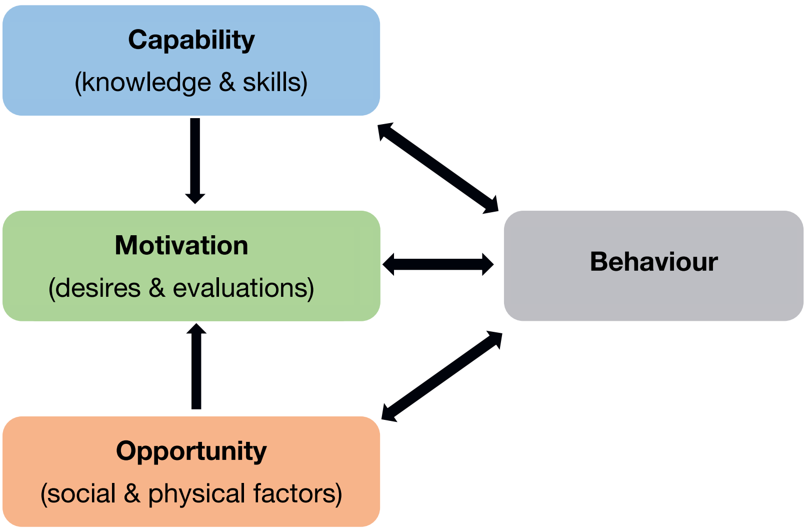

Figure 2. Three underlying factors that affect behavioural change in terms of farm management according to the COM-B model (redrawn from Michie et al., 2011).

barn infrastructure, and social support. Lastly, farmers with standard calf rearing practices may think they do not have what they consider to be the appropriate skills or knowledge to change their management system, given that full CCC systems appear to mandate a different perception of calf monitoring (Vaarst et al., 2020). A transformation in calf management entails a change in routine care, hence it may no longer be sufficient to rely solely on existing competences and skills (Hansen and Jervell, 2015). The farm in my study was unexperienced and the time boundaries of the experiments may have not allowed all involved animal care takers to acquire adequate opportunities, capabilities and motivation to optimize the alternative rearing systems accordingly. Therefore, the outcomes may reflect challenges, such as impaired calf health, that farmers could face during the transition to prolonged CCC.

4.2 Interventions and policy that can enable transitions to alternative systems

Based on the Behavioural Change Wheel framework, as defined by Michie and colleagues (2011), I will propose interventions (i.e. activities targeted to one or more of the three components, mentioned in the previous paragraph, to enable behavioural change) that can support farmers to successfully change their management and can support choices in policies to create effective and long-standing managerial changes. This particular framework was chosen, as it specifically provides policies and intervention functions that are expected to increase the capabilities, opportunities, and motivation of farmers to make the necessary managerial changes (Table 1).

Given that farmers are the gatekeepers of the welfare of the animals under their care, they are usually seen as the stakeholder responsible for implementing changes that benefit animal welfare (Albernaz-Gonçalves et al., 2021). However, implementing changes in farm management also demands for support (and with that also a change) from numerous other stakeholders in the dairy sector, as they provide farmers with resources and knowledge for certain management practices (Hansen and Jervell, 2015; Ritter et al., 2017). Behavioural change on farm level is predominantly influenced by external parties, such as colleagues, veterinarians, agribusiness consultants, dairy producer companies, farmer organizations, and agricultural policy institutions (Hansen and Jervell, 2015). Given the innovative character of CCC systems, acquiring new skills and knowledge is also necessary for vets and agribusiness consultants for them to be able to support farmers in transition. Therefore, I recommend policy makers to create *educational/networking platforms* that involve a collaboration with researchers (e.g. both social and animal welfare scientists), veterinarians, and farmers that have experience in prolonged CCC to provide unexperienced dairy farmers, veterinarians, consultants with know-how and know-why. This action would not only target the intervention functions (Table 1) for education and training (i.e. to increase capabilities and motivation), but also those regarding enablement (i.e. reducing barriers or fears that farmers perceive to increase capabilities and opportunities) and modelling (i.e. providing an example for people to aspire to or imitate that can increase motivation) (Michie et al., 2011). The platforms

Table 1. Definitions of interventions and policies to guide behavioural change (adapted from Michie et al., 2011).

Interventions	**Definition**
Education	Increasing knowledge or understanding
Persuasion	Using communication to induce positive or negative feelings or stimulate action
Incentivization	Creating expectation of reward
Coercion	Creating expectation of punishment or cost of consumption
Training	Imparting skills
Environmental restructuring	Changing the physical or social context
Modelling	Providing an example for people to aspire to or imitate
Enablement	Increasing means/reducing barriers to increase capability or opportunity
Restriction	Using rules to increase the target behaviour by reducing the opportunity to engage in the competing behaviour
Policies	
Communication/marketing	Using print, electronic, telephonic or broadcast media
Guidelines	Creating documents that recommend or mandate practices
Fiscal measures	Using the tax system to reduce or increase the financial cost
Regulation	Establishing rules or principles of behaviour or practice
Legislation	Making or changing laws
Environmental/social planning	Designing and/or controlling the physical or social environment
Service provision	Delivering a service or establishing support services in communities

should enable participants to discuss all aspects of farm management in CCC systems in detail, including farmer's goals, perceived constraints, and expectations, as well as economics and daily routines (Hansen and Jervell, 2015). For instance, organizing study groups, workshops/seminars, excursions, or online forums that offer videos and other teaching material to all kinds of stakeholders can be used to share (scientific and practical) knowledge and create room for dialogues about solutions and challenges related to the managerial changes in CCC systems. Perhaps, such efforts should not only be accessible to farmers and those who advise farmers, but also at times to the people who buy dairy products (as consumers) and reflect public expectations for dairy farming (as citizens). Understanding the shared and divergent values of different stakeholders and their attitudes towards welfare issues could harmonize industry practices with societal expectations (Weary and von Keyserlingk, 2017).

Additionally, successful implementation of full CCC systems requires a change in the economic system of dairy production, which may be partly achieved through *incentivization* (e.g. monetary reward to increase motivation) (Michie et al., 2011). Nowadays, low cost production to increase quantity of sold milk is a main driver for farm management and income (Barkema et al., 2015). Suckling will have a significant impact in the economic viability of farms, unless premium prices or another economic compensation is offered to farmers that adopted full CCC as calf rearing management practice. Correspondingly, policy makers may consider subsidies or fiscal advantages to enable adaptations in barn facilities or infrastructure (Michie et al., 2011).

In line with incentivization, I believe a change in certification of dairy products is needed. There are currently no *national certification schemes* that certify (or include strict regulation for) farmers that produce milk with CCC, although in some European countries private and organic labels proclaim forms of prolonged CCC (Placzek et al., 2020; Vaarst et al., 2020). Having such a certification scheme could allow dairy producers/retail companies to offer these dairy products to consumers for a higher price and receive monetary compensation (Knierim et al., 2020). However, extensive *marketing strategies and communication*, for instance by NGO's, retail companies, and dairy producers, will be necessary to inform both consumers and farmers about such a certification scheme. Consumers play a crucial role as well, as the demand for higher welfare standards by citizens requires a willingness to pay higher prices for food purchases (Harvey et al., 2013).

Lastly, I encourage policy makers to *fund research* that emphasizes collaborations amongst natural and social sciences, as not only animals should be studied but also the technical, human, economic, and social factors relating to animal welfare and changes in farm practices. Natural sciences can help to enhance our understanding of certain farm practices for animal welfare (e.g. this thesis) and develop science-based solutions for animal welfare problems, whereas social sciences are needed to not only understand the perceived barriers to implement proven welfare solutions on farms but also to build consensus between stakeholders for practices that resonate with societal values and benefit animal welfare (von Keyserlingk and Weary, 2017).

Overall, besides technical innovations, guidelines to best-practices, and social platforms, we need to envision new economic arrangements to provide a context that facilitates on-farm change. For that, collaboration among stakeholders, assisted by scientists and communication specialists, is necessary.

5. Conclusions

This dissertation showed that calf rearing systems with full CCC enhanced the expression of maternal-filial behaviour and increased the motivation of cows to reunite with their calf. The improved weight gains in calves reared with full CCC during the milk feeding period were no longer visible at six months of age, and in dams with full CCC the reduced machine-harvested milk yield during the suckling period seemed to recover the week after weaning. During debonding of cow-calf pairs with full CCC, the two-step debonding strategy with fence-line separation seemed more effective to reduce distress compared to nose-flaps. Nevertheless, the drawbacks of full CCC for calf welfare are reflected by a compromised health status, the increased distress during weaning and separation, and the risk of a poor human-animal relationship later in life. Even though partial CCC limited the expression of maternal-filial behaviour to some extent, it showed to mitigate the drawbacks of full CCC and could therefore be considered a feasible alternative to enhance animal welfare during the rearing period of dairy calves.

More research is needed to i) identify suitable cow-calf housing systems including the necessary managerial changes, ii) understand the effects of various types of CCC on the animals' affective states, iii) evaluate the optimal duration of daily cow-calf contact and the minimum calf age to initiate weaning and separation, and iv) investigate the public acceptability of partial contact as alternative to other CCC systems.

Given that successful managerial changes depend on the motivation, capabilities, and opportunities of farmers, interventions and policies on sector level (e.g. educational platforms and monetary incentives) are recommended to enable a transition on dairy farms. To support the on-farm changes that are needed for a successful implementation of prolonged CCC systems, participation of various stakeholders is required.

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Summary

It is standard practice on dairy farms to remove calves from the dam shortly after birth, after which they are individually housed for the first days or weeks of life and fed limited milk allowances. This practice has become a topic of public and scientific discussion. First, there is growing evidence showing that the current early life environment in standard rearing practices may limit calves' physical, behavioural, and cognitive development. Second, early cow-calf separation itself deprives much of cows' natural maternal behaviour. Alternative calf rearing systems that re-introduce (prolonged) maternal contact into dairy production systems are currently receiving increasing interest from various stakeholders. Allowing for prolonged cow-calf contact (CCC) with suckling has been proposed to enhance animal welfare, as such rearing conditions more closely resemble the social and nutritional environments known under natural settings. Yet, little is known about how different types of prolonged CCC (i.e. full contact or partial contact) can contribute to optimization of calf rearing conditions in a farm setting with respect to animal welfare. Partial contact (PC) that allows for limited cow-calf interactions without suckling may meet dairy farmers' concerns, although full contact (FC) that includes suckling allows for more natural behaviour to occur. The aim of this thesis was to assess how type of CCC in calf rearing systems affects dairy cow and calf welfare in comparison to a rearing system without CCC.

Motivation tests can be used to understand the relative importance of species-specific behaviours to the animal by determining how hard animals are willing to work for a given resource that allows them to express certain behaviours. Hence, I investigated how different types of CCC affected cows' motivation for calf contact (**Chapter 2**) by training cows to push a weighted gate to reunite with their calf. Testing occurred once daily after the afternoon milking. Weight on the gate gradually increased. Cows that were suckled by their calf during nighttime pushed a greater maximum weight (45.8 ± 7.8 kg) than non-suckled cows with partial CCC during nighttime (24.3 ± 4.5 kg) and early separated cows with no CCC (21.6 ± 6.7 kg). This result implies that cows are willing to invest physical effort to reunite with their calf even when separated at birth, but that cow motivation to reunite with the calf is greatest when cows are suckled.

To further understand the welfare implications of a partial CCC system that may possibly better suit the current dairy production system than full CCC, I investigated cows' affiliative behaviour towards their calf in an indoor-farm setting with partial CCC (i.e. calves were housed in a pen adjacent to the cow pen allowing limited physical contact on initiative of the dam but no suckling) or full CCC (i.e. calves were housed together with the dams in the cow pen and could freely suckle the dam) in the first five weeks postpartum (**Chapter 3**). Partial CCC resulted in less calf-directed affiliative behaviour (i.e. close proximity, allogrooming) compared to full CCC, except in the 48 hours following parturition. This finding suggests a

different mother-young bond, but could also be the result of the partial CCC design that limited calf accessibility compared to full CCC where calves roamed freely and could also initiate contact with the dam.

Given the contradictory literature and existing knowledge gaps regarding the effect of maternal contact on the animals' biological functioning, I evaluated the effect of different types of CCC on the health and performance of dairy cow and calf in the first seven weeks postpartum (**Chapter 4**). Cow-calf pairs had either no CCC (early separation, NC), partial CCC, or full CCC. For calves, data were collected regarding their clinical health status, fecal microbiota, hematological profile, immune and hormonal parameters, and growth rates, whereas for dams the clinical health status, metabolic responses, and milk performance was assessed. Overall, cow health was not affected by the type of CCC, but FC cows had a lower milk fat content alongside reduced machine-harvested milk yields during the milk feeding period compared to NC or PC cows. FC calves had an impaired health status compared to NC calves, as reflected by more health issues, elevated hematological parameters indicative of infections, and a tendency for higher antibiotic usage. Yet, full CCC resulted in a greater average daily gain in calf's body weight and different calf fecal microbiota composition in contrast to no or partial CCC. These results suggest that full CCC posed a risk for calf health, perhaps because the barn climate and housing conditions in this experimental context were suboptimal, although partial CCC did not compromise calf health.

One major welfare challenge in prolonged CCC systems is breaking the mother-young bond for weaning and separation (i.e. debonding). Therefore, I examined the effect of different two-step debonding strategies on the health, performance, and stress responses of cow-calf pairs with either partial or full CCC compared to cow-calf pairs with no CCC (**Chapter 5**). Between week 7 to 10, cow-calf pairs with full CCC were either subjected to reduced contact prior to weaning via fence-line separation or to reduced contact at weaning by inserting a nose-flap in the calf's nose, whereas cow-calf pairs with partial CCC were either subjected to reduced contact before or reduced contact after weaning by spatially preventing physical contact. Behavioural responses of cows during debonding after prolonged CCC were not distinctive, as the effect of treatment depended on parity where primiparous animals subjected to the full CCC with the nose-flap strategy or the partial CCC with the reduced contact after weaning strategy seemed to behave differently compared to higher parity dams. Machine-harvested milk yields of dams with full CCC seemed to recover once calves no longer suckled. Calves with full CCC spent a larger proportion of time highly active during debonding regardless the strategy, whereas calves with partial CCC were minimally impacted by weaning given their general activity patterns that were similar to calves with no CCC. Moreover, debonding via nose-flaps in full CCC caused nasal abrasions and negatively impacted calves' growth after weaning. These findings imply that in calves partial CCC reduced distress during debonding, and that for full CCC fence-line separation seems more effective to mitigate distress in cow-calf pairs compared to nose-flaps.

To conclude, trade-offs for animal welfare were identified in both partial and full CCC systems (**Chapter 6**). Calf rearing systems with full CCC enhanced cows' motivation to reunite with their calf and increased the expression of maternal-filial behaviour, although it compromised calf health, caused distress in calves during debonding, and increased the risk for a poor human-animal relationship later in life. Partial CCC limited the expression of species-specific behaviour, but seemed to mitigate the drawbacks of full CCC. Strategies that could potentially mitigate the downsides of both systems relate to improved cow-calf housing, calf feeding and monitoring, and newborn management. Overall, I suggest that we need to initiate, develop and implement new economic arrangements (e.g. monetary incentives), technical innovations (e.g. with regard to climate control in the barn), and improved guidelines for best-practices (e.g. housing and management practices). In addition, social platforms should be established (e.g. farmer study groups) to exchange knowledge and experiences, thereby facilitating on-farm changes that are beneficial for prolonged CCC. For that, collaboration among various stakeholders, assisted by scientists and communication specialists, is necessary.

Samenvatting

Op melkveebedrijven is het gebruikelijk om kalveren kort na de geboorte bij de moederkoe weg te halen, waarna kalveren de eerste dagen of weken individueel gehuisvest worden en een beperkt (kunst)melkrantsoen krijgen. Er is een maatschappelijke en wetenschappelijke discussie gaande over de vroege scheiding van koe en kalf. Ten eerste tonen steeds meer wetenschappelijke onderzoeken aan dat de huidige leefomstandigheden van pasgeboren kalveren in de gangbare melkveehouderij mogelijk een negatief effect kunnen hebben op de fysieke, cognitieve, en gedragsontwikkeling van kalveren. Ten tweede, de vroegtijdige koe-kalf scheiding ontneemt koeien de mogelijkheid tot het uiten van natuurlijk maternaal gedrag. Alternatieve vormen van kalveropfok, waarbij koe en kalf (langdurig) contact met elkaar hebben, krijgen momenteel steeds meer aandacht van verschillende stakeholders. Langdurig koe-kalf contact (KKC) waarbij het kalf ook zoogt zou mogelijk het dierenwelzijn in de melkveehouderij kunnen verbeteren, aangezien deze vorm van kalveropfok meer overeenkomt met het natuurlijke gedrag van de dieren vanuit nutritioneel en sociaal oogpunt. Er is echter weinig bekend over hoe verschillende soorten KKC (d.w.z. volledig contact of gedeeltelijk contact) kunnen bijdragen aan het verbeteren van de kalveropfok omstandigheden in relatie tot dierenwelzijn. Gedeeltelijk contact, waarbij beperkte interacties tussen koe en kalf mogelijk zijn zonder dat het kalf zoogt, zou aan praktische bezwaren tegemoet kunnen komen, hoewel volledig contact, waarbij het kalf ook zoogt, meer mogelijkheden biedt wat betreft het uiten van natuurlijk gedrag. In dit proefschrift zijn de gevolgen van deze vormen van KKC gedurende de opfokperiode voor het welzijn van koe en kalf onderzocht en vergeleken met een gangbaar opfoksysteem zonder KKC.

Motivatie testen kunnen worden gebruikt om meer inzicht te krijgen in het belang van soorteigen gedrag voor het dier. Hierbij wordt onderzocht hoe hard dieren bereid zijn te werken voor een bepaalde hulpbron die hen in staat stelt om bepaalde gedragingen te uiten. Daarom heb ik het effect onderzocht van verschillende soorten KKC op de motivatie van koeien om zich te herenigen met hun kalf (**Hoofdstuk 2**) door koeien te trainen om een verzwaard hek open te duwen om bij hun eigen kalf te komen. Het testen vond eenmaal daags plaats na het middag melken. Het gewicht aan het hek nam geleidelijk toe. Koeien die 's nachts door hun kalf werden gezoogd, duwden een hoger maximumgewicht (45,8 ± 7,8 kg) dan niet-gezoogde koeien met gedeeltelijk KKC tijdens de nacht (24,3 ± 4,5 kg) en vroeg gescheiden koeien zonder KKC (21,6 ± 6,7 kg). Dit resultaat suggereert dat koeien bereid zijn fysieke inspanningen te leveren om zich te herenigen met hun kalf, zelfs na de vroege koe-kalf scheiding, maar dat de motivatie van de koe om zich te herenigen met het kalf het grootst is wanneer de koeien worden gezoogd.

Om meer inzicht te krijgen in de gevolgen van een gedeeltelijk KKC-systeem (dat mogelijk beter past bij het huidige zuivelproductiesysteem dan volledig KKC) voor het dierenwelzijn, heb ik het sociaal gedrag van koeien richting hun kalf onderzocht (**Hoofdstuk 3**) in een stal met gedeeltelijk KKC (d.w.z. kalveren werden apart gehuisvest naast de koeien waardoor beperkt fysiek contact zonder zogen mogelijk was op initiatief van de koe) of volledig KKC (d.w.z. kalveren werden samen met de koeien in de ligboxenstal gehuisvest en konden zogen) gedurende de eerste vijf weken na afkalven. Gedeeltelijk KKC resulteerde in minder sociaal gedrag richting het kalf (d.w.z. nabijheid, likken) vergeleken met volledig KKC, afgezien van de eerste 48 uur na afkalven. Deze bevinding suggereert dat gedeeltelijk contact kan leiden tot een andere band tussen moeder en jong, maar zou ook het resultaat kunnen zijn van het huisvestingsontwerp dat koeien maar beperkte toegankelijkheid tot hun kalveren verschafte in vergelijking met volledig KKC waarbij kalveren vrij rondliepen en zelf ook contact met de moeder konden initiëren.

Gezien de tegenstrijdige literatuur en bestaande kennishiaten met betrekking tot het effect van maternaal contact op het biologisch functioneren van de dieren, onderzocht ik het effect van verschillende soorten KKC op de gezondheid en prestaties van koe en kalf in de eerste zeven weken na afkalven (**Hoofdstuk 4**). Koe en kalf hadden ofwel geen contact (vroege scheiding), gedeeltelijk contact, of volledig contact. Bij kalveren werden de klinische gezondheidsstatus, de samenstelling van het microbioom in de mest, het hematologisch bloedbeeld, immuun- en hormonale parameters, en de groeisnelheid bepaald. Bij de koeien werden de klinische gezondheidsstatus, het metabole bloedbeeld, en de melkproductie onderzocht. Koegezondheid bleek niet beïnvloed door het soort KKC, maar koeien met volledig contact hadden een lager melkvetgehalte naast lagere melkgiften in de melkstal vergeleken met koeien die geen of gedeeltelijk contact hadden. Kalveren met volledig contact hadden een lagere gezondheidsstatus vergeleken met kalveren zonder contact, wat tot uiting kwam in meer ziekteverschijnselen, een verhoging van hematologische parameters die indicatief waren voor infecties, en een tendens tot een verhoogd gebruik van antibiotica. Niettemin resulteerde volledig KKC in een grotere dagelijkse toename van het gewicht van de kalveren en een andere samenstelling van het microbioom in de mest vergeleken met geen of gedeeltelijk KKC. De resultaten suggereren dat volledig KKC mogelijk een risico vormt voor de gezondheid van kalveren, wellicht omdat het stalklimaat en de huisvestingsomstandigheden in deze experimentele context suboptimaal waren, maar dat gedeeltelijk KKC geen negatieve gevolgen had voor de kalvergezondheid.

Een grote uitdaging op het gebied van dierenwelzijn in langdurige KKC-systemen is het verbreken van de band tussen moeder en kalf tijdens het spenen en scheiden (d.w.z. de onthechting). Daarom heb ik het effect van verschillende geleidelijke speen- en scheidingsstrategieën onderzocht op de gezondheid, prestaties en stressreacties van de koeien en kalveren met gedeeltelijk of volledig KKC, in vergelijking met dieren zonder KKC (**Hoofdstuk 5**). Tussen week 7 en 10 werden koeien en hun kalveren met volledig KKC onderworpen hetzij aan verminderd contact voorafgaand aan het spenen door middel van scheiding via een 'fence-line', hetzij aan verminderd contact bij het spenen door een neusflap in de neus van het kalf. In diezelfde periode werden koeien en kalveren met gedeeltelijk KKC onderworpen hetzij aan verminderd sociaal contact vóór, hetzij aan verminderd sociaal contact na het spenen, door het verhinderen van fysiek contact tussen koe en kalf. Het gedrag van koeien als reactie op deze strategieën was niet onderscheidend, aangezien het effect van de behandelingsgroep afhing van de pariteit van de koe. Eerste kalfskoeien met volledig KKC en de neusflapstrategie, of met gedeeltelijk KKC en verminderd contact na spenen, leken zich anders te gedragen vergeleken met hogere pariteit koeien. De lage melkgift in de melkstal van koeien met volledig KKC leek te herstellen zodra de kalveren niet meer zoogden. Kalveren met volledig KKC gedroegen zich vaker zeer actief tijdens het spenen en scheiden, ongeacht de strategie, terwijl het gedrag van kalveren met gedeeltelijk KKC minimaal werd beïnvloed tijdens het onthechten omdat hun algemene activiteitenpatroon vergelijkbaar was met dat van kalveren zonder KKC. Daarnaast had de neusflapstrategie een negatieve invloed op de groei van de kalveren na het spenen en veroorzaakte de neusflap nasale drukwonden bij een deel van de kalveren. Deze bevindingen suggereren dat gedeeltelijk KKC de stress rondom spenen en scheiden in kalveren verminderd, en dat bij volledig KKC het spenen en scheiden via de 'fence-line' strategie effectiever lijkt om stress bij koe en kalf te verminderen in vergelijking met de neusflap.

Alles overziend zijn er in dit proefschrift in KKC-systemen met zowel gedeeltelijk als volledig contact trade-offs voor dierenwelzijn aan het licht gekomen (**Hoofdstuk 6**). Volledig contact versterkte de motivatie van koeien om zich te herenigen met hun kalf en leidde tot meer uitingen van soorteigen gedrag, hoewel het negatieve gevolgen voor de gezondheid van de kalveren had, stress in kalveren veroorzaakte tijdens het spenen en scheiden, en het risico op een verslechterde mens-dierrelatie in de toekomst vergrootte. Gedeeltelijk contact beperkte het uiten van soorteigen gedrag, maar leek de nadelen van volledig contact teniet te doen. Strategieën die de nadelen van beide systemen mogelijk kunnen beperken hebben betrekking op het verbeteren van niet alleen de huisvesting, maar ook van het management rondom afkalven, het voeren van kalveren, en het controleren van de algehele staat van kalveren. Om de benodigde veranderingen voor langduriger KKC op melkveebedrijven mogelijk te maken lijken nieuwe economische initiatieven (bijv. een financiële beloning), naast technische innovaties (bijv. op het gebied van stalklimaat) en richtlijnen voor 'best practices' (bijv. qua huisvesting en management), noodzakelijk. Ook stel ik voor om sociale platforms in het leven te roepen (bijv. in de vorm van studiegroepen) waarbinnen kennis van en ervaringen met dit innovatieve opfoksysteem gedeeld kunnen worden. Hiervoor is samenwerking tussen verschillende stakeholders, bijgestaan door wetenschappers en communicatiespecialisten, onontbeerlijk.

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6088, 6093, 6097 en hun kalveren: 30, 114, 1015, 1127, 3013, 3104, 5013, 5019, 6007, 6035, 6036, 6037, 6040, 7177, 8092, 8093, 8095, 8097, 8098, 8102, 8104, 8109, 8110, 8112, 8113, 8116, 8117, 8119, 8120, 8123, 8127, 8128, 8129 en 9010. In het bijzonder, koe 28 met haar buitengewone zorg voor en waakzaamheid over haar premature kalf 8103; zij hebben een speciaal plekje in mijn hart. Ook dank aan de koeien in het experiment op KTC: 480, 483, 1277, 1323,1323, 1330, 1330, 1362, 1379, 1737, 1739, 2554, 2698, 2703, 2714, 2730, 2732, 2732, 2734, 2786, 2793, 2881, 2882, 2885,2887, 2894, 3362, 3968, 4322, 4323, 4324, 4325, 4327, 4332, 4646, 5483, 5888, 5890, 5911, 6547, 7205, 7207, 7209, 7209, 7264, 8061, 8132, 8147, 8167, 8173, 8182, 8196, 8556, 8564, 8628, 8741, 8754, 9149, 9154, 9154, 9505, 9505, 9506, 9511, 9540, 9674, 9687, 9692, 9694, 9694 en hun kalveren: 2508, 2510, 2511, 2513, 2518, 2519, 2520, 2522, 2523, 2526, 2527, 2528, 529, 2530, 2539, 2541, 2547, 2549, 2550, 2555, 2561, 2565, 2568, 2572, 2573, 2575, 2580, 2582, 2583, 2584, 2585, 2586, 2588, 2589, 2590, 2591, 2592, 2593, 2594, 2595, 2596, 2597, 2598, 2599, 2600, 2601, 2603, 2604, 2606, 2607, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2623, 2624, 2625, 2626, 2628, 2629, 2631 en 2632. Opdat zij niet vergeten worden.

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About the author

Margret Wenker was born in Hoogwoud, the Netherlands, in 1992. She obtained her BSc degree in Animal Sciences (cum laude) from Wageningen University in 2013. During her thesis, commissioned by Stichting Milieukeur, Margret compared criteria of a certification system for sustainable housing of dairy cattle with animal related indicators for dairy cow welfare. In 2016 she obtained her MSc degree in Animal Sciences from Wageningen University. For her thesis, conducted at ETH Zurich (Switzerland), she assessed whether an automated grooming brush and/or a mirror could reduce stress in dairy cows kept in social isolation. In her internship, commissioned by Stichting Demeter, Margret explored the current dairy calf rearing practices and the possible support for keeping calves together with the dam among bio-dynamic dairy farmers.

From 2017 onwards, Margret continued in this research domain with her PhD at the Animal Production Systems group of Wageningen University & Research, that assessed the welfare implications of different types of prolonged cow-calf contact in dairy farming. This research was part of the PPS-project ‘Kansen voor het Kalf in de Keten’, which was funded by ZuivelNL and the Dutch Ministry of Agriculture, Nature and Food Quality. Findings of this thesis were presented at international conferences, graduate school days as well as invited lectures and published in peer-reviewed scientific journals.

Currently, Margret is working as a postdoctoral researcher at the Institute for Animal Welfare and Animal Husbandry of the Friedrich-Loeffler-Institute (Germany).

Publications

Refereed scientific journals

Wenker, M.L., Bokkers, E.A.M., Lecorps, B., von Keyserlingk, M.A.G., van Reenen, C.G., Verwer, C.M., Weary, D.M. 2020. Effect of cow-calf contact on cow motivation to reunite with their calf. Sci. Rep. 10, 14233. https://doi.org/10.1038/s41598-020-70927-w

Wenker, M.L., van Reenen, C.G., de Oliveira, D., McCrea, K., Verwer, C.M., Bokkers, E.A.M. 2021. Calf-directed affiliative behaviour of dairy cows in two types of cow-calf contact systems. Appl. Anim. Behav. Sci. 105461. https://doi.org/10.1016/j.applanim.2021.105461

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. 2022. Effect of different types of cow-calf contact on health, blood parameters, and performance of dairy cow and calf. Front.Vet.Sci. 9, 855086. https://doi.org/10.3389/fvets.2022.855086

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. 2022. Comparing gradual debonding strategies after prolonged cow-calf contact: Stress responses, performance, and health of dairy cow and calf. *Submitted to Applied Animal Behaviour Science.*

Conference proceedings

Wenker, M.L., Verwer, C.M., van Reenen, C.G., de Boer, I.J.M., Bokkers, E.A.M. 2018. Implications of cow-calf contact for dairy calf health and welfare. Abstracts of the WIAS Science Day 2018: Impact in Science and Society, 5th of February, Wageningen, NL - p. 42.

Wenker, M.L., von Keyserlingk, M.A.G., Bokkers, E.A.M., Lecorps, B., van Reenen, C.G., Verwer, C.M., Weary, D.M. 2019. Effect of cow-calf contact on motivation of dairy cows to access their calf. Proceedings of the 53rd Congress of International Society for Applied Ethology: Animal lives worth living, 5th-9th August, Bergen, NO - p. 259.

Wenker, M.L., Bokkers, E.A.M., Verwer, C.M., van Reenen, C.G., von Keyserlingk, M.A.G., Weary, D.M. 2019. Effect of cow-calf contact on motivation of dairy cows to access their calf. Abstracts of the WIAS Science Day 2019: Trade-Offs in Science - Keeping the balance, 18th of March, Lunteren, NL - p. 47.

Wenker, M.L., Verwer, C.M., Bokkers, E.A.M., van Reenen, C.G. 2021. Do different types of prolonged cow-calf contact affect dairy calves' health and growth? Proceedings of Annual Meeting American Dairy Science Association, 11th of July, Online COVID-friendly edition - p. 168.

Wenker, M.L., van Reenen, C.G., Verwer, C.M., Bokkers, E.A.M. 2021. Maternal bonding in dairy cattle: does type of cow-calf contact matter? Abstracts of the WIAS Annual Conference 2021: Resilience, 28th-29th of April, Online COVID-friendly edition - p. 53.

Wenker, M.L., van Reenen, C.G., Verwer, C.M., de Oliveira, D., Bokkers, E.A.M. 2021. Affiliative behaviour of dairy cows in two types of cow-calf contact systems. Proceedings of the 8th International Conference on the Assessment of Animal Welfare at Farm and Group level, 16th-19th of August, Online COVID-friendly edition - p. 128.

Education certificate

Completed training and supervision plan[1]

The Basic Package	3 ECTS
WIAS Introduction Day	2017
WIAS course Essential skills	2017
WGS course Research Integrity & Ethics in Animal Sciences	2019
Disciplinary Competences	**14 ECTS**
Writing a Research Proposal	2017
Statistics Course Design of Experiments	2017
Species-specific Laboratory Animals Course on Ruminants	2019
The Fundamentals of Animal Emotions	2019
Scientific Analysis of Transition and Change Processes Related to Animal Agriculture	2020
How Mothers Influence their Offspring	2021
Professional Competences	**6 ECTS**
Research Data Management	2018
Project and Time Management	2020
The Choice: take charge of your own performance	2020
Reviewing a Research Proposal	2021
The Final Touch: writing the general introduction and discussion	2021
Career Orientation	2021
Presentation Skills	**4 ECTS**
Poster, WIAS Science Day	2018-2019
Oral, 53rd Congress of International Society for Applied Ethology	2019
Oral, 8th Congress of Assessment of Animal Welfare at Farm and Group Level	2021
Oral, Annual Meeting American Dairy Science Association	2021
Oral, WIAS Annual Conference	2021
Teaching Competences	**6 ECTS**
Course Supervising BSc and MSc Students	2019
Course Start to Teach	2020
Thesis supervisor, 2 MSc theses	2019-2021
Guest lecturer, Summer School Global Interconnectivity for Animal Production	2021
Education and Training Total	**33 ECTS**

[1] With the activities listed the PhD candidate has complied with the educational requirements set by the Graduate School of Wageningen Institute of animal Sciences (WIAS). One ECTS credit equals a study load of 28 hours.

Colophon

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