Norwegian University of Life Sciences

Master's Thesis 2022 30 ECTS
Faculty of Biosciences

Effect of access to dam on play behavior in dairy calves in a cow-calf contact system.

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Acknowledgements

The process of writing a master thesis has primarily been inspiring and challenging, though with good help and support it has been an enjoyable journey to finish my studies at NMBU.

Firstly, I will thank my supervisors, Sabine Ferneborg (NMBU) for professional advice throughout the entire process of writing a master thesis, Julie Føske Johnsen (Norwegian Veterinary institute), Stine Grønmo Kischel (TINE) and Kristian Ellingsen-Dalskau (Norwegian Veterinary institute) for good discussions and help with practical work. I appreciate your knowledge and dedication for the project, it has inspired me in the process of writing the thesis.

Great thanks to my family for supporting me throughout my education, and especially thanks to my boyfriend Ole Kristian for motivational words and support any day of the week. I appreciate all the good conversations, advice, motivation, and fun times with friends and fellow students through my years at NMBU.

I am thankful for the opportunity to write for the SUCCEED project. Being a part of a bigger project has been meaningful and educational. I thank the Agriculture and Food Industry Research Funds (FFL/JA) (project number 310728, Norwegian Research Council) for financing the study, and thank participating industry partners and research institutions for own contributions.

Faculty of Biosciences

Norwegian University of Life Sciences

Ås, May of 2022

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Abstract

The common practice of immediate separation of cow and calf in dairy production is being questioned, and there is an increasing interest from the public and from the industry to assess alternative solutions for cow-calf contact (CCC). However, after a prolonged time together, the separation can be a stressful event in the life of a calf. Therefore, finding a separation method that is gentle to the cow and calf is important. We can use play as an indicator of positive welfare to assess animal welfare on farm level. I hypothesized that reduced access to the dam would affect the behavioral response of play in calves. 30 Norwegian Red (NRF) calves in a cow-calf contact system, divided into four batches, were analyzed for play (alone or with peers), and play with cow (dam or alien cow) from video recordings. Each batch consisted of four different periods with gradually reduced access for the cow: 24h, 12h, 6h and 0h access per day. Two separation treatments were tested: long adaptation (LA) where the reduction in access from 24h/d to 0h/d lasted for 4 weeks, and short adaption (SA) where the reduction in access from 24h/d to 0h/d lasted for 10 days. Cows and calves were held in separate pens, where cows could enter a meeting area through smart gates. The first access reduction (24 to 12 h), resulted in a slight reduction in the daily duration of all play (sum of “play” and “play with cow”) from 14.1 ± 7.6 to 10.8 ± 6.4 minutes per calf. A reduction in the number of bouts per calf was observed at the 12h access: 18 ± 11 bouts of play and 3 ± 2 bouts of play with cow, with mean bout lengths (s/bout) of 32 ± 52 and 46 ± 37 seconds, respectively. The reduction of dam's access seemed to affect the amount of play behavior in calves, but the reduction only seemed evident during the first reduction of cow access. Calves in the short adaptation seemed to stagnate in the development of mean bout length, in comparison to calves in the long adaptation, which continued to increase mean bout length as cow access was further reduced and calves were weaned off milk.

Sammendrag

Dyrevelferd i norsk melkeproduksjon har fått en økende interesse fra allmennheten og fra næringen, spesielt med tanke på at kalv i vanlig praksis blir skilt fra kua kort tid etter kalving. Derfor er det et behov for å utforske løsninger for systemer som innebærer ku-kalv-kontakt gjennom dieperioden. Forlenget kontakt mellom ku og kalv fører til at separasjonen kan være en stressende tid, og det er viktig å finne den mest skånsomme separasjonsmetoden for både ku og kalv. Positive velferdsindikatorer kan brukes for å måle og evaluere dyrevelferd på gårdsnivå, f.eks. lekatferd som ble brukt i denne masteroppgaven. Jeg antok at en reduksjon i tilgang på mor ville påvirke lekatferden til kalvene. 30 NRF-kalver (norsk rødt fe) i et system for ku-kalv-kontakt, delt på fire runder, ble analysert for lek (alene eller med annen kalv) og lek med ku (mordyr eller annen ku) ved bruk av videoopptak. Hver runde bestod av fire perioder med gradvis reduksjon i tilgang til ku: 24, 12, 6 og 0 timer tilgang per dag. To separasjonsbehandlinger ble testet: lang adapsjon (LA) med gradvis reduksjon i tilgang over 4 uker og kort adapsjon (SA) med gradvis reduksjon i tilgang over 10 dager. Kyr og kalver ble holdt i separate binger, der kyrne kunne besøke fellesarealet ved å bruke datastyrte porter. Første reduksjon i tilgangen til ku (24 til 12 timer) resulterte i kortere varighet av all daglig lek (sum av "lek" og "lek med ku") fra 14.1 ± 7.6 til 10.8 ± 6.4 minutter per kalv. En reduksjon i antall tilfeller av lek ble observert: 18 ± 11 for lek og 3 ± 2 for lek med ku, med en gjennomsnittlig varighet (s/bout) på henholdsvis 32 ± 52 sekunder og 46 ± 37 sekunder. Reduksjonen i tilgang på ku synes å ha en påvirkning på lekatferd hos kalver, men effekten var tydeligst ved reduksjon til 12 timers tilgang på ku. Kalver med lang adapsjonstid virket å ha en økende varighet per tilfelle av lek frem til de ble avvent, mens kalver med kort adapsjonstid virket derimot å stagnere i varighet per tilfelle av lek ved reduksjon til 6 timers tilgang på mor.

List of Terms

CCC	Cow-calf contact
Separation	Gradual reduction in access to dam
Play	Play alone or with other calves
Play with cow	Play interaction between calf and cow
All play	Combined values of play and play with cow
Baseline	First observation (24h) with no treatment applied
24h	24 hours of access to cow per day
12h	12 hours of access to cow per day
6h	6 hours of access to cow per day
0h	0 (zero) hours of access to cow per day
LA	Long adaptation
SA	Short adaptation

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1 Introduction

The common practice of immediate separation of cow and calf in dairy production is being questioned, and there is an increasing interest from the public and from the industry to assess alternative solutions for cow-calf contact (CCC).

The separation between cow and calf in dairy production is unavoidable to an extent, due to the cow's function in the dairy production. The level of stress experienced by each cow and calf may be influenced by the strength of the cow-calf bond and time spent together. Under natural conditions the separation would happen at around 8-12 months or when the cow gives birth to the next calf (Krohn, 2001).

Consumers are demanding higher levels of animal welfare in animal production, and are viewing early separation as problematic for the wellbeing of the calves (Ventura et al., 2013). There is hence an increasing interest from farmers to test out the possibilities for implementing CCC systems to develop their farm (NORSØK, 2020; Norwegian Veterinary Institute, n.d.).

Norwegian dairy farms are undergoing a shift in production design, from tie stalls to free stalls, due to new requirements in the regulation for cattle husbandry (*Forskrift om hold av storfe*, 2004). The Norwegian Animal Welfare Act §23 specifies that the animal's living environment shall give the opportunity to "stimulating activities" and "other natural behavior" (*Dyrevelferdsloven*, 2009). Implementing a CCC system may increase calf welfare and facilitate natural behavior between cow and calf in the suckling period (Johnsen et al., 2018; Krohn, 2001; Meagher et al., 2019).

1.1 Background for the study

There is a need to study the behavior of calves in CCC systems, especially looking at ways to maintain good animal welfare in the stressing period of separation. Presence of natural behavior, and the possibilities to perform it, is an important reason for implementing CCC systems. The main function is to allow for the calf and cow to bond, suckle, groom and play. Play behavior is viewed as a "surplus behavior", that animals might perform if their basic needs are met. Also, play indicates a feeling of pleasure and good emotions (Jensen et al., 2015).

Using play as a positive indicator of welfare has been suggested (Krachun et al., 2010), but might have some limitations to the feasibility in terms of interpretation (Held & Špinka, 2011). Play behavior is mainly categorized into locomotor play, social play, and object play (Jensen et al., 1998). The duration and amount of play behavior is likely influenced by milk allowance, as piglets perform less play behavior during weaning (Donaldson et al., 2002), and calves spend less time playing when milk allowance is reduced (Jensen et al., 2015; Krachun et al., 2010). Waiblinger et al. (2020) found that dam-reared calves showed more solitary locomotor play than calves that were reared alone or with other calves. Also, calves in the same CCC system were exposed to more agonistic behavior mostly from cows other than dam and developed more complex social relations to conspecifics. Nabukalu (2021) showed that dam-reared calves were more motivated for social behavior than calves raised conventionally, at five to six months of age.

The separation between cow and calf will in most cases trigger stress responses like vocalizing and restlessness (Krohn, 2001). Johnsen et al. (2018) suggests that vocalization from calves, during separation, is associated with hunger. To assess welfare during separation, there are several indicators that can be measured either manually or automated, like vocalization, body condition score, weight gain, stress hormones, amongst others (de Oliveira et al., 2020; Johnsen et al., 2015; Leliveld & Provolo, 2020; Rushen et al., 2012). Assuming all basic needs like feed, water, comfort, space, and good health, are met, one can use positive welfare indicators to measure good quality of life (Jensen et al., 2015).

1.2 Research Aims & Hypothesis

The separation is a stressful event in the life of a calf, therefore finding a separation method that is gentle to the cow and calf is important. There are no current studies done on NRF, on calf play behavior during the milk feeding period. Several studies on play behavior in calves exist, though few studies have been done in CCC systems. Exploring the topic of calf welfare in terms of positive welfare indicators during separation in CCC systems is needed. The SUCCEED project focuses on two separation treatments: one long adaptation treatment (LA) of 4 weeks, and one short adaptation treatment (SA) of 10 days. The results of the thesis will hopefully provide more insight into dairy calves' behavioral response to separation in CCC systems.

There are two main aims for this project. One is to explore play behavior during the separation period in the SUCCEED system. The second is to research differences in play behavior by comparing the two treatments. These aims lead to my research question: How will play behavior in calves evolve when gradually reducing access to dam?

I hypothesize that reducing access to dam will affect the behavioral response of play. From this general hypothesis I predict that: 1) the calves will reduce total amount of play behavior during the separation period; 2) total amount of play will increase after complete denied access; and at last, that 3) there will be a difference between the treatments in type and amount of play behavior.

2 Methods

2.1 Research Approach

The current study was performed in collaboration with and for the SUCCEED project led by the Norwegian Veterinary Institute. In early preparation for the study, I had meetings with supervisors to inform about the project and to discuss on how to include a thesis project in the SUCCEED project. Practical work at the Livestock Production Research Centre was carried out to increase the understanding of the CCC system.

Preliminary research was conducted before the data collection, decision of parameters and analysis was conducted, to ensure that the thesis will provide new literature of importance and to build knowledge of the theme. Systematic research was needed to sort and identify relevant literature, with the use of Web of Science, PubMed, and Google Scholar. Key words used in the search were "play behavior", "dairy calves", "calf", "separation", "welfare", "CCC" and "cow-calf-contact". Criteria for choosing articles was language (Norwegian, English, or Swedish was accepted) and availability for the public or access through NMBU's database.

Play behavior was used as an indicator of good welfare. Behavioral data was collected from video recordings. Specifically, I analyzed general play behavior in calves and play interactions with adult cows, either the dam or alien cow.

From here on I will be referring to the higher-level project of SUCCEED as "SUCCEED project", to distinguish it from the thesis project. The chapter of Methods will contain information about the organizing of work between me (student) and the researchers in the SUCCEED project, the research material and research analysis that was conducted.

2.2 Research design

2.2.1 Part of Project

This master thesis project will contribute to work package 1 (WP1) in the SUCCEED project, the controlled on-farm study of CCC systems at the Livestock Production Research Centre at NMBU. A controlled clinical trial was conducted from 12th of October 2020 to 8th of March 2022, including four batches divided into two separation methods: two batches with long adaptation, and two batches with short adaptation (table 1). Each batch lasted for 10-11 weeks, due to differing calving dates.

The setup of study design; subjects, pen design, feed, ethogram, daily routines, and gate allowances was done by researchers from the SUCCEED project. My contribution to the setup was to further develop the already existing ethogram to also fit my thesis project. The preparation for data collection was time consuming, with many meetings to improve the ethogram to be as precise as possible. The work of collecting data from video material was distributed between me and the other researchers (also co-supervisors), because both parts would benefit from the data material. Modifying the collected data material in Microsoft Excel was mainly done by me. In total eleven behaviors were observed for in the SUCCEED project, in which two of the behaviors ("play" and "play with dam") were exported from the original dataset to use in this thesis.

Table 1 *Overview of batches and treatments, and visual description of each access in both treatments. Each period of the trial is described with number of hours per day (h) the cow had access to the meeting area. Dashed lines mark the trial period, in total 9 weeks.*

				Access			
Batch	**Treatment**	Calving pen	Full access 24h	Restricted 12h	Restricted 6h		No access 0h
1 4	LA	3-5 d	28 days	14 days	14 days		7 days
2 3	SA	3-5 d	46 days		5 days	5 days	7 days

2.2.2 Research Material

The subjects in the controlled clinical trial consisted of 32 Norwegian Red (NRF) cow-calf pairs (32 calves and 32 cows) divided into four batches (8 pairs per batch) run in a parallel group design. There were no upper or lower age limit for the cows included. Inclusion criteria for cows were no calving difficulties, no sign of illness, no history or frequent health issues, no dry period treatment, no sign of aggressive behavior towards own calf or staff, interest in own calf, and no earlier experience of caring for a calf over an extended period. Cows expected to join the study were put in the SUCCEED pen weeks prior to calving to let them get used to the new area. Criteria for including calves in the study were no sign of illness, purebred Norwegian Red, independent suckling behavior and born on straw bedding in designated calving pen. If illness occurred in the first two weeks, there would be a replacement by a new cow-calf pair. Two cow-calf pairs were excluded during the third batch, due to claw problems necessitating antibiotic treatment for the cows, resulting in 30 cow calf pairs in total.

Two separation treatments were trialed: 1) long adaptation (LA), and 2) short adaptation (SA). Batch 1 and 4 were assigned the LA treatment, and batch 2 and 3 were assigned the SA treatment. Each experimental trial lasted for 9 weeks in total, based on the age of the median calf (date between 4^{th} and 5^{th} calving). Both separation treatments consisted of four different periods with gradually reduced access to the meeting area for the cow; from 24h, to 12h (access 11:00-23:00), to 6 hours (access 11:00-17:00) and then 0h. Table 1 shows the setup of each batch, showing duration of each access and the differences between the two separation treatments. Median age of the calves in each batch was used to determine dates for the various levels of access restriction. The separation period started when the median age was 28 days (LA) and 46 days (SA).

The SUCCEED pen design consisted of cow area, meeting area and a calf creep (see figure 1). The cow area and meeting area were separated by custom made smart gates (DeLaval International AB, Tumba, Sweden) on one side, and a partially open hardwood fence on the other side of the pen. This allowed the cow and calf to still have auditory and physical contact while the smart gates were closed. The smart gates were driven by computer-controlled access. Cows included in the study were familiar with smart gates, though the primiparous cows included were not necessarily familiar with the smart gates. The cow area housed only

cows included in the SUCCEED project. The cows were milked in a DeLaval milking robot (VMS Classic, DeLaval International AB, Tumba, Sweden). Calves could freely pass from the calf creep to the meeting area 24/7 throughout the trial. An automated concentrate feeder and an automatic milk feeder were placed in the calf creep. These installations were connected to the RFID tag in the calves' ears to record visits and intake of each calf. The calf creep consisted of deep straw bedding with slatted wooden floor in one end to ensure permanent deck for the milk feeder and concentrate feeder. The meeting area consisted of slatted concrete flooring, with a layer of slatted rubber mats on top, making sure the spacing meets the Norwegian regulations. Two cameras were mounted in the pen; one recording the meeting area (orange star) and one for recording the calf pen (red star) (see placements in figure 1), resulting in two videos per observation.

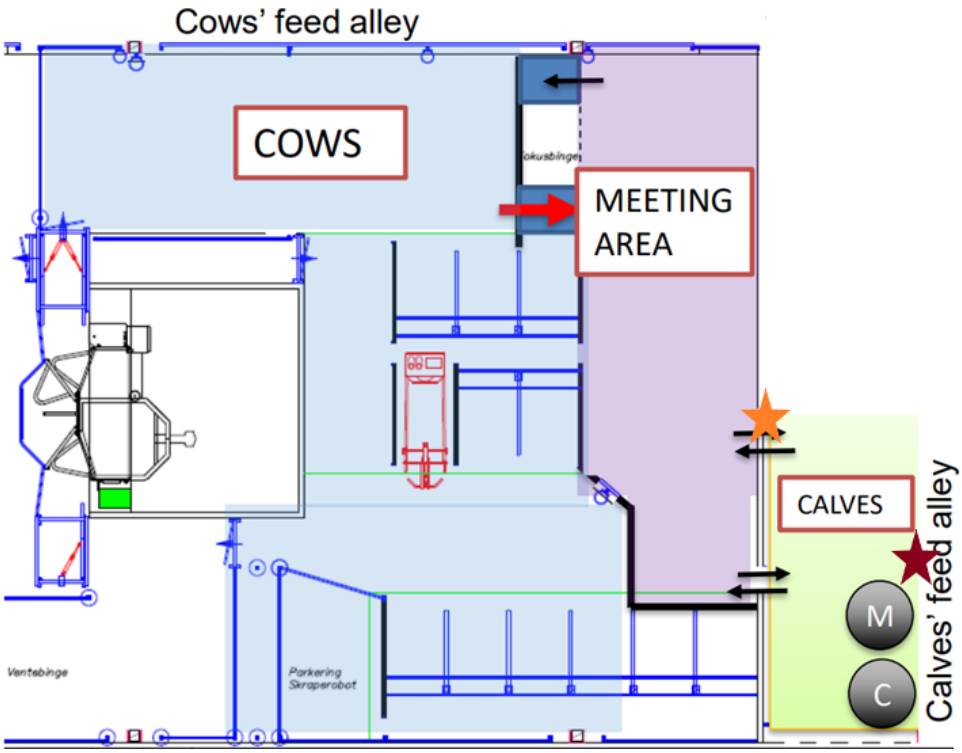

Figure 1 *SUCCEED pen design. Overview of cow area (blue), meeting area (purple) and calf creep (green). IN-Smart gate is marked with red arrow and OUT-Smart gate is marked with black arrow. M = automated milk feeder. C = automated concentrate feeder. Stars showing the location of cameras recording the pen; orange star = meeting area and dark red star = calf creep.*

Routines post calving was to give the calf some time to gather and get a chance to suckle on its own. Staff assisted the calf to suckle when dam was safely locked in the headlock if no independent suckling was observed after 30-60 minutes from birth. The calf was ensured colostrum, offered from a teat bottle, weighed, ear-tagged and the navel was sprayed with an iodine solution to prevent infection. Recordings of gender, birth weight, ID-number and amount colostrum consumed from teat bottle was done by staff. Maternal behavior was recorded by a researcher within 48 hours from parturition, and the subjects were marked using hair color. The cow-calf pair was assigned a number from 1-8 for identification on videos. The numbers were assigned consecutively, meaning the youngest calf was number 1 and the last calf was number 8. In batch 4, cow-calf pair number 5 was excluded due to mastitis. A new cow-calf pair was assigned the number 9. After 3-5 days in the calving pen, eligible cow-calf pairs were moved to the SUCCEED-pen by researchers and staff, following safety standards. During the trial regular routines included weighing the calves twice a week, calibrating the concentrate feeder, and collecting feed samples. Cows were not rushed out of the meeting area after the gates were closed. Cows were not allowed to lie down in the meeting area and were escorted out of the meeting area if it happened.

The calves were fed ad libitum hay in the calves' feed alley. At week 8, when the cows were denied access to the meeting area, a mix of silage and straw (same feed as cows) was offered to the calves. Fresh milk from the automated milk feeder was offered from the first day of separation (LA = 28 days of age and SA = 46 days of age). At the first day of separation and the following five days, the calves were trained to drink from the automated milk feeder, first by learning to drink from teat bottle, then advancing to the automated milk feeder. Positive reinforcement was the first approach for the training, but most calves needed physical handling and convincement to start drinking from the teat bottle. Maximum milk allowance for the calves were twelve liters per day. The calves had ad libitum access to the concentrate feeder throughout the trial.

2.2.3 Research Strategy

To research play behavior in calves, video material of the subjects' behaviors was analyzed using BORIS software, then the events recorded were exported to Excel® for further analyses. An ethogram was created, describing the physical structure and spatial relation of the behaviors play and play with cow (table 2). Play was described as how the subjects moved motorically (jump, kick, running, headshake, or kick) and spatially (alone, with other calves, mounting other calves play fighting with other calves, forwards, backwards and sideways). Play with cow included the same structural descriptions for locomotor play but play behavior must happen in spatial relation to an adult cow (dam or alien cow, which was recorded). Play with object was not recorded due to difficulties of seeing if the behavior was self-grooming, playful or curious and explorative.

Day five after the change in access was thought to be representative for the effect of treatment, because the acute stress was thought to have been reduced and the group would be more settled with the new conditions (reduced access).

Behavior sampling with continuous recording was used to gather information about the subjects' behaviors. Only the meeting area and calf pen was recorded. Each occurrence of play behavior was recorded and connected to the specific calf that performed it and at the specific time it occurred. Eight calves and present cows were watched continuously for 24 hours, for 1 day in each access of the trial for all four batches.

As little human interaction as possible with the subjects was desired. The Livestock Production Research Centre is frequently visited by many people and the environment could not be controlled in terms of human interaction outside the pens. Frequent human activity in the surroundings was thought be normal conditions for the calves.

Table 2 Ethogram describing the behaviors play and play with cow that was recorded and analyzed.

Behavior	Description		Modifier
Play (state)	*Calf was play fighting, mounting, kicking, headshaking, jumping, running alone or with other calves. Event was stopped if break >3 sec.*		*N/A*
	The following behaviors described were incorporated and scored as play:		
	Play fight	Two calves are butting heads front to front or against neck of other calf. Includes "standoff" positions with lowered frontal body and front legs widespread. Play fighting may be a forward, backward, or sideways movement.	
	Mount	A calf mounts another calf's body from back, front or side.	
	Jump	Upward movements with two or four legs lifted from the ground.	
	Run	Fast forward movement alone or with other calves.	
	Kick	Kicks from one or two legs in sideways or backwards direction.	
	Headshake	Head is rotated or shaken	
Play with cow (state)	*Calf, with the attention focused on (and/or in physical contact with) cow, was play fighting, kicking, headshaking, jumping, or running in a playful manner. Event was stopped if break >3 sec. Modifier cow was registered.*		*DAM or ALIEN*
	The following behaviors described were incorporated and scored as play with cow:		
	Play fight	Butting heads against each other, or calf is butting head against dam's neck or other frontal body parts. Includes "standoff" positions with lowered frontal body and front legs widespread. Play fighting may be a forward, backward, or sideways movement.	
	Jump	Upward movements with two or four legs lifted from the ground, around dam or with attention focused on dam.	
	Run	Fast forward movement around/with dam or with attention focused on dam.	
	Kick	Kicks from one or two legs in sideways or backwards direction.	
	Headshake	Head is rotated or shaken	

2.2.4 Data Collection

To analyze play behavior the video material had to be quantified for analysis. Video material was recorded and saved on a NMBU server and later exported to a hard drive. Four days (24 hours per day) for each batch was observed. All hours when cows had access to the meeting area were assigned to me (student). An additional hour at 12h and 6h access was added to ensure that all behavior was recorded in case the cows were still in the meeting area after the gates had closed. This resulted in a total of 176 hours (4*[24+13+7]h) of video material. The remaining hours were split among 3 researchers (208 hours in total). Assigning all hours with access to cow to one person made it possible to exclude inter-observer variability for all behaviors interactions between cow and calf. A Cohen's Kappa inter-rater reliability test was carried out to assess the agreement between observers. A lower limit of 0.85 Cohens Kappa was set before observations started.

Recorded videos were analyzed using BORIS v. 7.12.2 (Friard & Gamba, 2016), a software program that allows the user to analyze video material, score behaviors and export desired values. Both recordings (meeting area and calf pen) were analyzed simultaneously. Each behavior was assigned a key on the keyboard, and each calf was assigned a number key corresponding to the subject number (1-9).

2.2.5 Data analysis

Analyses were carried out in Excel for Microsoft 365 Version 2203. Play and play with cow (with cohering information) was extracted from the original data set for the SUCCEED project (containing 11 behaviors). Pivot tables were used get an overview of the result, finding relevant information for the different variables and to make descriptive diagrams. During analyses there was a need to combine the two behaviors (play and play with cow) to find overall results, which is referred to as all play in the results (chapter 3).

The numerical variable used to analyze play was "duration", which was automatically calculated by BORIS based on the start and stop time of each event for each subject. Median bout length (s/bout) and mean bout length ± SD (s/bout) were automatically calculated by Excel, based on all events recorded for all batches at all access. Bouts per calf ± SD to measure frequency and mean duration per calf ± SD (min/d) was calculated in SAS Enterprise Guide 7.1.

Results were visualized and presented by using Microsoft Excel diagrams, tables, and graphs. In graphs used to visualize the results, keep in mind that batches in the long adaptation (1 and 4) experience the separation at 4 and 6 weeks of age, but batches in the short adaptation (2 and 3) experience separation at 6,5 and 7 weeks of age (see Table 1).

Data from one of the excluded cow-calf pairs, in batch 3, occurred at the baseline observation. The subject was excluded from the dataset. This decision strengthens the dataset by using only subjects present in all observations, which contribute to results showing the true evolvement of the calves.

3 Results

3.1 Play behavior at different access restrictions

At the baseline measurement (24h access), the daily duration of all play (mean ± SD) was 14.1 ± 7.6 minutes per calf, of which 78.7% (11.1 ± 6.9 min/d) was with peers or alone and 28.4 % (3.0 ± 3.6 min/d) was with cow (table 3). 33 ± 16 bouts of play per calf were recorded, with a bout length (mean ± SD) of 20 ± 24 seconds, and a median bout length of 12 seconds. For play with cow, 5 ± 4 bouts per calf were recorded with a mean bout length of 45 ± 40 seconds, and a median bout length of 32 seconds.

The first access reduction (24 to 12h), resulted in a slight reduction in daily duration of all play to 10.8 ± 6.4 minutes per calf at 12h (figure 2). The duration of play and play with cow both decreased (mean ± SD) at 12h access to 9.5 ± 6.6 min/d (play) and 1.3 ± 1.9 min/d (play with cow). A reduction in number of bouts per calf (# bouts ± SD) was observed: 18 ± 11 for play and 3 ± 2 for play with cow, with mean bout lengths (s/bout) of 32 ± 52 and 46 ± 37 seconds, respectively.

Table 3 *Overview of values of bouts per calf ± SD, median bout length, mean bout length ± SD and mean duration per calf ± SD in each behavior recorded (play and play with cow) and for all play (combination of play and play with cow).*

Behavior	Access	Bouts per calf (#)	Bout length, median (sec/bout)	Bout length, mean ± SD (sec/bout)	Mean duration per calf ± SD (min/d)
play	**24 h**	33 ± 16	12	20 ± 24	11.1 ± 6.9
	12 h	18 ± 11	15	32 ± 52	9.5 ± 6.6
	6 h	20 ± 14	16	35 ± 56	11.7 ± 9.5
	0 h	18 ± 12	20	43 ± 63	13.0 ± 12.0
play with cow	**24 h**	5 ± 4	32	45 ± 40	3.0 ± 3.6
	12 h	3 ± 2	29	46 ± 37	1.3 ± 1.9
	6 h	2 ± 1	33	53 ± 53	1.0 ± 1.1
all play	**24 h**	37 ± 17	13	23 ± 27	14.1 ± 7.6
	12 h	20 ± 11	16	33 ± 51	10.8 ± 6.4
	6 h	21 ± 14	16	36 ± 56	12.7 ± 9.7
	0 h	18 ± 12	20	43 ± 63	13.0 ± 12.0

At the second access reduction (12 to 6h), the mean duration of all play increased slightly to 12.7 ± 0.7 minutes per calf, with 7.8% play with cow and 92.2% play alone or with peers (figure 2). Play was recorded with 20 ± 14 bouts per calf, with a mean bout length of 35 ± 56 s/bout and median bout length of 16 seconds. At 6h access, play with cow had 2 ± 1 bouts per calf, with a mean bout length of 53 ± 53 seconds, and a median bout length of 33 seconds.

When cow access was denied completely (0h access), there was no considerable difference in play, compared to the mean duration at 6h access. Mean bout length (± SD) for calves was 43 ± 63 seconds and a median bout length of 20 seconds.

Overall, there was a decrease in the number of play bouts per calf, while there was an increase in both median and mean bout length for play (table 3). The duration of play seemed to stagnate after the reduction from 6 to 0h access (figure 2).

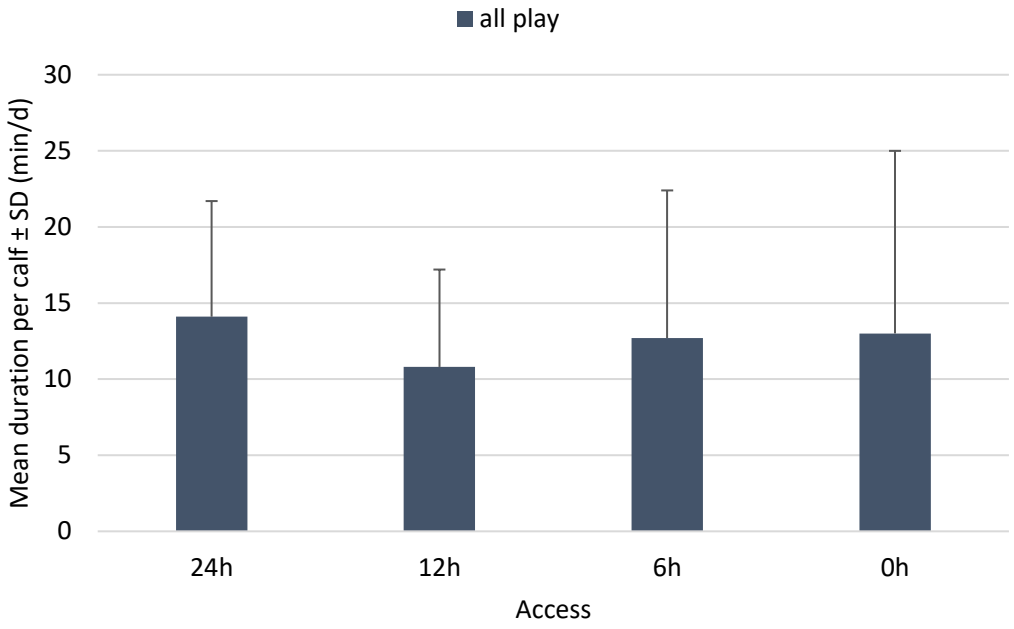

Figure 2 *Showing mean duration (mean duration ± SD) of all play per calf (min/d) at each access (24h, 12h, 6h and 0h. Note that 12h and 6h access happen at different ages between the treatment groups, while 24h and 0h occur at the same time.*

Play with cow seemed to decrease as access was further restricted, from a mean duration per calf (± SD) of 3.0 ± 3.6 minutes (at 24h) to 1.3 ± 1.9 minutes (at 12h), but it did not differ from 12h to 6h (figure 3). In contrast, play alone or with peers seemed to increase in duration at 6h and 0h in comparison to 12h. Both behaviors (play and play with cow) showed a reduction in the number of bouts per calf at each access reduction (table 3). For play with cow, 91% of the total duration was performed between calf and dam, and the remaining 9% was play interaction between calf and alien cow (supplementary table 1). Out of the 30 calves present in the study, all but one calf performed play with the dam cow and 16 calves (53.3%) interacted with an alien cow through play (supplementary table 1).

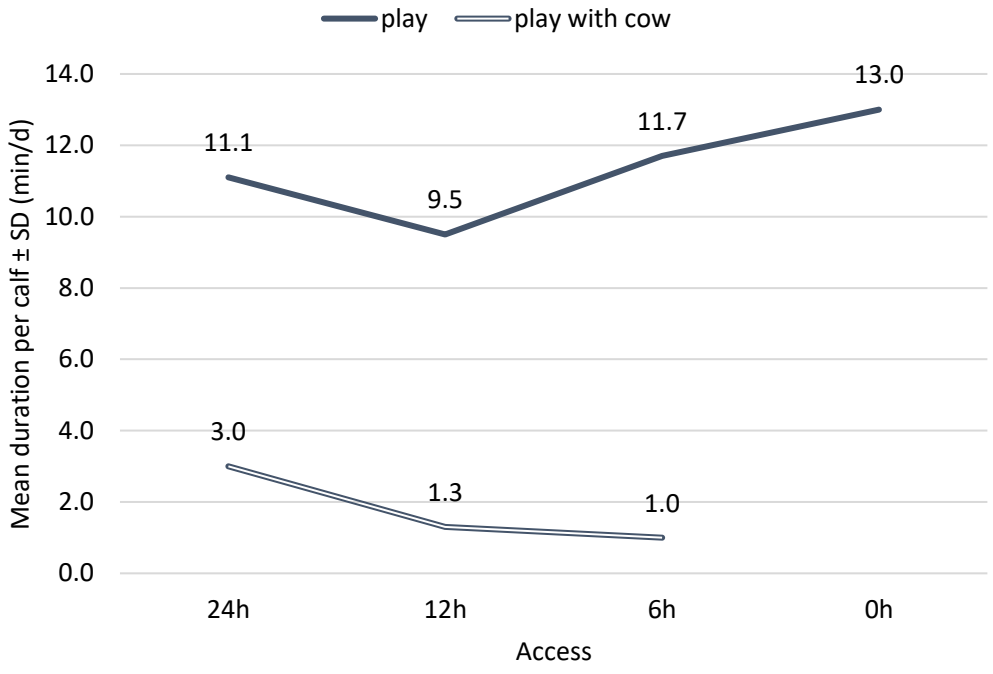

Figure 3 *Mean duration per calf (min/d) for play and play with cow at different access.*

Across all batches and treatments, the total amount of all play differed between the calves from the lowest of 0.7 minutes to 49.0 minutes at highest (figure 4). At the baseline measurement (24h), one outlier calf from batch 1 was recorded with a sum of duration of 40.0 min/d. At the 12h access, there were no extreme outliers. At the 6h access, the distribution of individuals was slightly extended by 3 calves (passing sum of duration of over 30 min/d), of which two belonging to batch 1 and one belonging to batch 2. For the 0h access, two outliers from batch 4 were recorded, with a sum of duration of 41.7 and 49.1 minutes per day, respectively. Also, an effect of batch (supplementary table 2) is indicated at the baseline measurement (24h), where there was considerable difference between the batches. Batch 1 and 4 was constantly higher than batch 2 and 3 when comparing mean duration per calf through the whole trial.

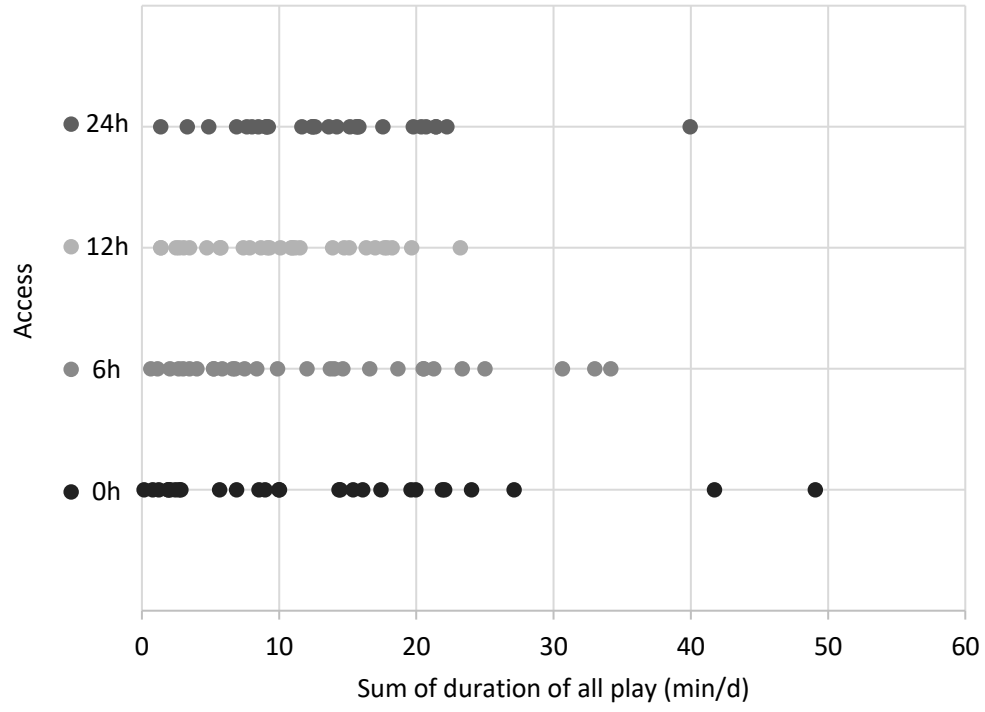

Figure 4 *Distribution of duration for all subjects at different access (24h, 12h, 6h and 0h) by total duration of all play (min/d).*

3.2 Effect of treatment

Play made up 91% of total duration of all play in the long adaptation (LA) and 87% of total duration of all play in the short adaptation (SA) (table 4). There were no considerable differences in mean bout lengths between the treatments at 24h (23 ± 29 vs. 22 ± 25 s/bout) and 12h access (34 ± 54 vs. 32 ± 44 s/bout). At the first restriction (12h), the variance across calves seemed to increase in both treatments (figure 5), though the mean and median bout length seemed unaffected by treatment. Once access was restricted to 6h, calves in the long adaptation treatment continued to increase e the mean bout length of all play. At both 6h and 0h access, the LA (long adaptation) calves seemed to engage more in play behavior than the SA (short adaptation) calves, with median bout length also with a slight increase (figure 5). Mean bout length in the short adaptation seemed to stagnate after the first separation (12h access).

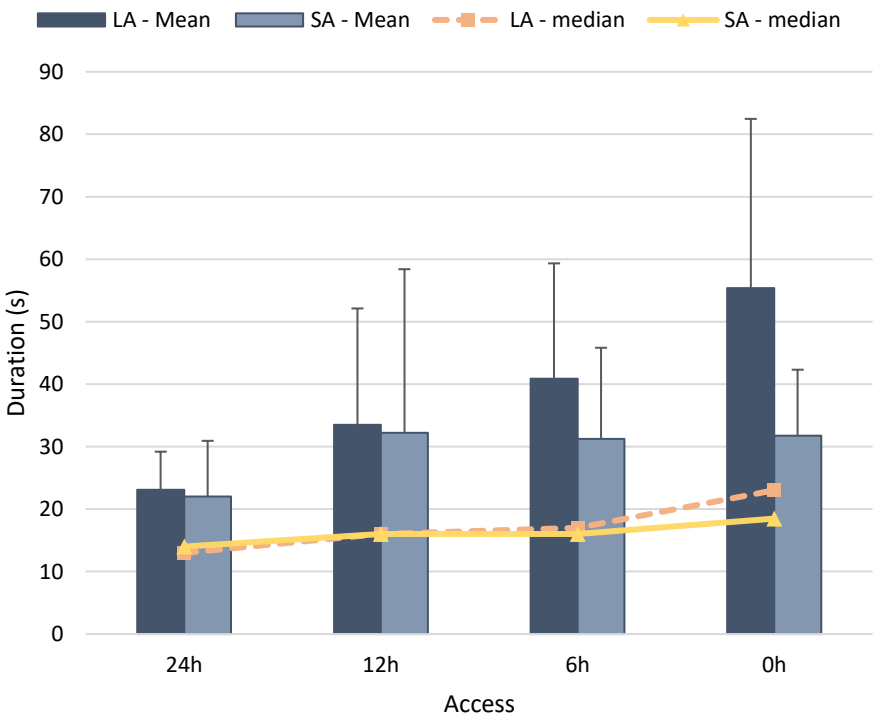

Figure 5 *Showing the change in mean bout length ± SD and median bout length of all play for both treatments (LA and SA) through different access.*

Table 4 *Overview of number of bouts ± SD, median bout length, mean bout length± SD and mean duration per calf ± SD, play with cow and all play for both treatments (LA an SA)*

		Bouts per calf, mean ± SD (#)		Bout length, median (s/bout)		Bout length, mean ± SD (s/bout)		Duration per calf, mean ± SD (min/d)	
Behavior	**Access**	**LA**	**SA**	**LA**	**SA**	**LA**	**SA**	**LA**	**SA**
play	*24h*	42 ± 14	22 ± 12	12	13	21 ± 26	19 ± 18	14.6 ± 6.8	6.8 ± 3.9
	12h	24 ± 11	11 ± 8	16	14	33 ± 54	29 ± 45	13.1 ± 6.6	5.4 ± 3.6
	6h	19 ± 10	21 ± 18	16	15	40 ± 63	30 ± 46	12.9 ± 9.6	10.4 ± 9.6
	0h	16 ± 12	21 ± 11	23	18	55 ± 79	32 ± 38	14.7 ± 14.8	10.9 ± 7.4
play with cow	*24h*	6 ± 5	4 ± 4	31	33	43 ± 39	50 ± 44	4.5 ± 3.5	3.3 ± 3.8
	12h	2 ± 1	3 ± 3	28	31	46 ± 39	45 ± 36	1.9 ± 1.3	2.6 ± 2.3
	6h	2 ± 1	2 ± 1	32	40	47 ± 45	65 ± 68	1.9 ± 1.0	2.2 ± 1.3
all play	*24h*	47 ± 14	25 ± 12	13	14	23 ± 29	22 ± 25	18.2 ± 6.7	3.1 ± 5.0
	12h	25 ± 10	14 ± 9	16	16	34 ± 54	32 ± 44	13.9 ± 6.5	7.2 ± 4.1
	6h	21 ± 9	21 ± 18	17	16	41 ± 62	31 ± 48	14.1 ± 9.9	11.1 ± 9.7
	0h	16 ± 12	21 ± 11	23	18	55 ± 79	32 ± 38	14.7 ± 14.8	10.9 ± 7.4

4 Discussion

4.1 General discussion

Across all batches and treatments, the descriptive results of the thesis showed a reduction in all play at the 12h access, which indicate an effect of reducing access to dam from 24 to 12 hours per day. The frequency of play seemed to decrease as the calves aged. Between the treatments there were no clear difference in the daily duration of all play, though calves in the short adaptation seemed to stagnate in the development of bout length as the access to dam was reduced, while calves in the long adaptation seemed to continue the increase of bout length. It needs to be taken into consideration that the results in this thesis are only descriptive, and further statistical analyses should be performed to ensure valid interpretations.

The importance of play is well discussed in literature due to the occurrence across species and especially in juveniles (Jensen et al., 1998), and the consensus is that play is often linked to states of positive emotions and suggested as a positive indicator of welfare (Jensen et al., 2015; Krachun et al., 2010). Vitale et al. (1986) found that semi-wild calves had two peaks of play per day: one in the early morning and one at mid-afternoon. As for conventional systems, Größbacher et al. (2020b) found that play peaked at milk feeding times. Even if play is well studied, it may still be difficult to predict (Gladden et al., 2020), maybe due to individual variations. Play may be affected by several factors such as stress, milk intake and space allowance, which will all be discussed later in the discussion. Reipurth et al. (2020) found that daily duration of play per calf decreased from 1 to 12 weeks of age, for group-housed Danish dairy calves with various periods of time spent with the dam. In the current thesis, no clear trend in daily duration of play as the calves aged was found. This may be due to the influence of separation treatments. However, the results indicate that the calves played less frequently per day, but with a longer bout length as they got older.

Earlier studies have shown that play can be used as an indicator of positive welfare (Boissy et al., 2007; Jensen et al., 2015; Krachun et al., 2010), due to it being a "surplus" behavior that may be performed if all basic needs are met. However, Held and Špinka (2011) suggests that play might also happen as a response to stress, maybe as a displacement activity to acute stress. The separation between cow and calf induces dietary and social changes, but also environmental changes can occur if animals are regrouped in other pens. It is highly stressful for calves to experience all these changes at the same time, and should be avoided (Jasper et al., 2008). After a period of contact, separation of cow and calf may trigger stress responses, such as vocalizing and restlessness (Johnsen et al., 2018; Krohn, 2001). It is therefore not straightforward how a change in the total amount of play or several types of play should be interpreted. In the current study, play was interpreted as a behavior associated with positive emotions because observations were conducted five days after the reduction in access, which may be the most stressful event. Play was indeed found across all batches in all observations, which may indicate that basic needs were met. However, factors such as dietary changes and separation from dam may have been the reason a reduction in duration of daily play at the first reduction in access was found. Using presence of play as an indicator of welfare is supported by this study. Though, measuring frequency and duration of play by direct observations may not be feasible for on-farm regular welfare assessments (Napolitano et al., 2009). Gladden et al. (2020) found that accelerometer can be used for measuring play behavior in newborn calves up to 48h but should further be developed for higher accuracy. Accelerometers to measure locomotor play on older calves have also been researched (Größbacher et al., 2020a), and can indicate an effect of reduced energy intake (Luu et al., 2013).

Several papers have shown examples of ethograms separating between locomotor, social and object play (Færevik et al., 2008; Jensen et al., 1998; Jensen & Kyhn, 2000). When looking at the recordings for this study, it was hard to distinguish the different types of play from each other. Therefore, the ethogram was simplified to only include “play” and "play with cow". Play was defined as "Calf was play fighting, mounting, kicking, headshaking, jumping, running alone or with other calves". Play with cow was defined as "Calf, with the attention focused on (and/or in physical contact with) cow, was play fighting, kicking, headshaking, jumping, or running in a playful manner." The use of several types of play in the ethogram would have been beneficial for the results, but I often found myself wondering about how to clearly define the differences between locomotor and social play when the calves would run after each other, turn together, or jump together. I ended up generalizing play by splitting it into whom the play engaged. Play therefore consisted of locomotor and social play for calves only, while play with cow was supposed to record the play interactions between adult cows and calves. Object play, in example play with straw, was not included due to the difficulties of knowing the differences between self-grooming, exploration and play. Interactions with straw was usually observed immediately after straw was added to the pen, but not recorded in the dataset. There was a possibility to have measured all the specific types of play, or only a specific part of play, though the amount of video recording must have been reduced. One reason for not focusing on only one part of play, in example play running or head butting, was that during the testing observations I experienced that both locomotor and social play contributed to the total play that was happening.

It is well known that space allowance affects the behaviors performed among animals (Kondo et al., 1989; Randolph et al., 1981). The calves in this study were reared in a pen that was large compared to conventional calf pens, but small compared to pasture. This limited the room for the calves to have much space on their own. Sufficient space allowance is necessary to allow calves to express play behavior (Jensen et al., 1998; Jensen & Kyhn, 2000). Not only the calves, but also the cows could have been affected by space allowance. (Kondo et al., 1989) found that agonistic behavior increased, in both cows and calves, as the space allowance was reduced. The meeting area was limited in size, which may have restricted cow movements

into, within and out of the area. As calves grew bigger and access times were limited, it may be theorized that more cows hesitated to enter, behaved differently inside, or exited the area sooner, all leading to restrictions in time and space available for play with the calf. Vitale et al. (1986) found that semi-wild Maremma calves naturally tend to increase distance to cows as the get older. If the same behavior is applied to calves kept in pens, it may be theorized that as the calves age, they may experience the pen to be less spacious, and it may affect the behavior.

Reducing the access to dam may be a stressful event to the calf, due to the forcing of separation that happens at a far quicker pace than under natural conditions (Krohn, 2001), which could result in variations in play behavior due to stress (Held & Špinka, 2011). It has been shown that milk intake may influence the amount of play being performed, where calves on enhanced milk allowance have a longer daily duration of play (Jensen et al., 2015; Krachun et al., 2010). Calves in the SUCCEED project had similar, if not higher, daily weight gain than calves in previous mentioned studies, and can therefore be classified as calves with "high milk allowance". The high milk allowance may facilitate that the calves were not malnourished at times with access to the cow. The milk intake from suckling was most likely reduced when the access to dam was reduced, which again could affect energy allocated to play. Measuring the milk intake of suckling calves may be difficult. One solution may be to measure weight gain, or to assess the number of head buttings to the milk feeder, which may indicate hunger, as proposed by de Passille (2001). Future studies of the SUCCEED project may look into the association of supplemental milk intake from the milk feeder and calf play.

Social support amongst calves may reduce behavioral reactions to social separation, especially if in companionship with familiar peers (Costa et al., 2016). Lv et al. (2021) showed that calves housed in groups performed more behaviors associated with positive emotions, such as exploration behavior and locomotor play. In this thesis, the acute stress response at the day of reducing access was not recorded. The calves were observed at day 5 after the reduction in access, a time where the acute stress of separation may have passed, and the calves started to adapt to the change. This was a deliberate choice, as we sought to investigate the effects of access restriction rather than the effects of acute stress. The importance of social support is well discussed in literature (Ozbay et al., 2007), though literature on the duration of social support after a stressor occurs or how it works when all individuals are affected is hard to find.

Bolt et al. (2017) found that pairing calves at 5 days of age increased the stress-buffering effect of social support compared to isolated calves or calves paired at 28 days of age. Even though the research by Bolt et al. (2017) was done on calves that were separated from the dam immediately after birth, the "early pairing" indicate the importance of allowing for social bonds in calves at early age. In the SUCCEED pen the calf and cow can still have physical and visual connection through a hardwood fence, separating the pens. This strategy has been found to reduce the acute stress response like vocalization and vigilant behavior of dairy calves (Johnsen et al., 2015). Dam reared cows tend to lick and nurse own calf more from 2 weeks to 2 months after calving, than non-dam reared cows (Le Neindre, 1989). None of the cows in the SUCCEED project had been dam-reared, but it could possibly have affected to amount of play with cow.

4.2 Development of play for calves in CCC systems

I predicted that the calves would reduce the amount of play during separation. Indeed, there seemed to be an effect where access to the dam influenced the amount of all play behavior in dairy calves, especially the first access reduction in the separation (12h). When limiting the time that the calf and dam have together, more essential behaviors like suckling or grooming may replace play behavior. Calves and cows were observed doing grooming interactions through the hardwood fence at times when the cows did not have access to the meeting area, which might alleviate stress and ensure calves that the dam is still in close range. No playful behavior through the hardwood fence was observed.

Amount of play alone or with peers increased when the calves no longer had access to the cows, as predicted. The prediction was based on the idea that the calves would replace the play with cow, with playing with other calves. As the results show; that play with cow decreased throughout the trial, it is not certain that the calves would continue to play with the cows as they did in the 6h access. For all calves, play with cow had a total duration of 29.7 min/d at the 6h access, and play increased with 26.2 min/d from 6h to 0h access, which can be interpreted as the play with cow turning into play with peers. Play with cow was observed to occur for a few minutes a day per calf (table 4), which may indicate that the behavior is of lower importance. However, there are a lot of uncertainties involved and the hypothesis has

not been investigated properly. Another important finding was that most of the play happening between calf and cows were with the dam. This may mean that the stress caused by the reduction in access is due to the separation from dam and not from adult cows in general, as predicted.

Amount of all play behavior at baseline was different between batches, before there were any differences in treatment and the median age in all batches was 28 ± 4 days. Unfortunately, batch 1 and 4 which belonged to the same treatment (LA), had in total a higher duration of play than batch 2 and 3 (SA) already at the baseline, despite identical management up to that time point. This gives the need for extra caution when interpreting the results, as differences between batches or individuals may be interpreted as treatment differences. Outlier calves with high duration of play may be one possible cause of the difference at baseline, because play can be contagious (Held & Špinka, 2011). The amount of play performed by the outlier calves might have led to more calves engaging in play, even if they in other circumstances might not play much at their own initiative. The spread of duration of all play per calf (figure 4), and the standard deviations from table 3 indicates a large effect of individuals. It may be theorized that the outlier calves with low duration of play may be less susceptible for the contagiousness of play, which further can lead to less activity in the group due to less calves engaging in social play. Overall, the change in access to dam seemed to affect the amount of all play, though the standard deviations indicate high individual variations, and we cannot distinguish age effects from access effects.

4.3 Differences in play behavior between treatments

I predicted that there would be a difference between treatments if the access to cow affected play behavior. As discussed in the previous chapter, it may look like access does affect play behavior. The differences at baseline, as discussed in the previous chapter, need to be taken in in consideration when interpreting differences between the treatments. For daily duration of all play per calf, there was no clear difference between the treatments. Mean bout length, median bout length and number of bouts per calf seemed to differ. For calves in the long adaptation, bout mean and bout median per calf seemed to experience a steady increase

throughout the trial at each access, the number of bouts per calf reduced at each access. For the calves with short adaptation, bout mean and bout median seemed to stagnate at around 31 ± 48 s/bout and 16 s/bout, respectively at the 6h access. SA calves only got 5 days to adapt before a new reduction in access occurred, which may be too rapid. It may be that the calves did not get enough time to recover and find new eating and suckling habits before the new stressful reduction in access occurred.

Median bout duration for both treatments were lower than the mean bout duration values, meaning that there are some large values in the upper 50th percentile of the value range, which increases the mean duration considerably. It is important to mention the difference at baseline that was discussed in the previous chapter, due to the unfortunate coincidence of batches in the same treatment had different baseline amount of play. This may unfortunately be interpreted as a treatment effect even if it may not be. But when looking at mean bout length and median bouts, there seems to be an effect of treatment. This may be considered as differences between treatments due to the change between them happens at the time where the separation treatment differs. It seemed like calves in the short adaptation stagnated in mean bout length, while calves in the long adaptation continued to increase the mean bout length. The stagnation for the SA calves may be due to the calves not having enough time to recover from the acute stress of getting reduced access to dam.

4.4 Methodological limitations

I would like to address some methodological limitations to the study. Sometimes it seemed like play behavior was initiated by human activity/interference in the pen. Either the calves were curios of the people, or the activity performed by the people (example, flushing the floor, scraping, straw) gave a burst of energy/or triggered the calves to play and run. The Livestock Production Research Center at NMBU have staff, visitors and others that might affect the calves' attention to people, conspecifics, vehicles, loud machines or other. This is unavoidable and must be considered as normal conditions for the calves in the study, though it may not be representative for the living conditions for calves reared in conventional farms. Secondly, essentials for good quality of video analyzing consist of correct video material

and the quality of observations. In batch 3, two of the calves were not present (n=6), but by presenting the results per calf that did not become a big problem, though large enough sample sizes are always wanted. For batch 2 there were some technological issues with the cameras, resulting in the loss of some video material. There was also a small issue of calves escaping the pen, only two of the escapes happened at days of observation. Now the largest affecting limitation was that the camera for the meeting area did not cover the whole area (supplementary figure 1). This became an issue because if cows or calves were positioned in the specific area, behavior was not recorded. When distributing the workload of analyzing video in BORIS, I (student) was assigned all videos where there was access to meeting area, this was done to eliminate the interobserver variation for all the behaviors (11 behaviors) that were recorded when cows had access to the meeting area. For all hours where the cow did not have access only 3 behaviors were scored, and an inter-observer reliability test was carried out to ensure high level of agreement between the four observers. Though it was a meaningful task to score all the 11 behaviors that could happen in the access period of the 24-hour observation day, it was a lot of work to keep track of 11 behaviors for 8 subjects, and I cannot be sure if all play that truly happened was registered. Although a thought of mine was that I may have had an unconscious bias to overestimate play behavior due to that being the theme of my thesis. To ensure the quality of the scorings, a precises ethogram was made, discussed, and developed through several meetings.

5 Conclusion

In conclusion, the reduction of dam's access seemed to affect the amount of play behavior in calves. Relative to the baseline period when the cow had full access to the calves, daily duration of play was reduced, but this reduction only seemed evident during the first reduction of cow access. Calves in the short adaptation treatment seemed to stagnate in the development of mean bout length, in comparison to calves in the long adaptation which continued to increase mean bout length as cow access was further reduced and calves were weaned off milk.

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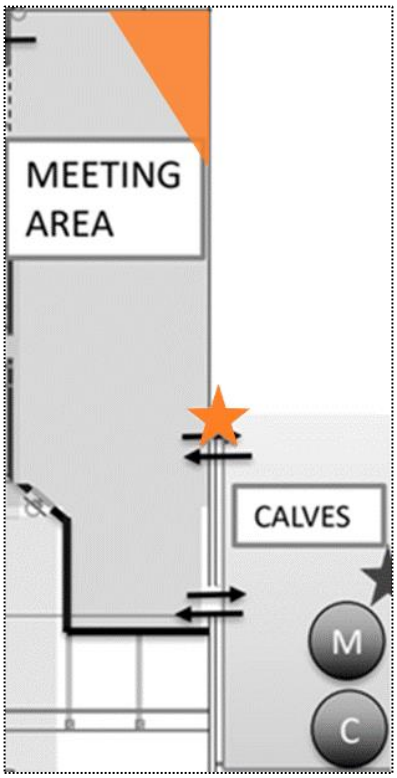
Appendix - Supplementary tables & figures

Supplementary table 1 *Total duration (min) of play with cow, first split by subject, then split by access across all batches.*

	Total duration of play with cow (min)	
Split by subject	**Alien**	**Dam**
7076	0.6	12.7
7081	0.6	5.8
7082		1.9
7084	0.2	4.6
7085		10.3
7089	2.3	7.4
7095		5.1
7096	0.4	8.5
7161		4.7
7162	2.9	8
7163	0.2	4.4
7164		4.8
7166		8.3
7167		0.5
7168		2.4
7174	0.1	3.4
7221		4.1
7225		4
7229		17.7
7233	0.3	0.4
7314	0.5	0.7
7315		3
7317		10.7
7318		6
7320	2	
7321	3.3	1.3
7326	0.1	2.5
Split by access		
24h	6.7	81.5
12h	3.5	35.3
6h	3.2	26.5
total	**13.4**	**143.3**

Supplementary table 2 *Showing daily duration of play per batch in each access.*

	Mean duration of all play per batch ± SD (min)			
Access	**1**	**2**	**3**	**4**
24h	20.6 ± 8.3	6.2 ± 3	12.5 ± 4.8	15.9 ± 4.5
12h	16.7 ± 4	6.8 ± 4.9	7.8 ± 3.2	11.2 ± 7.6
6h	17.3 ± 11.3	15.1 ± 10.6	5.9 ± 5.5	10.8 ± 7.5
0h	8.4 ± 8.9	8.3 ± 6.8	13.9 ± 7.5	21 ± 17.3



Supplementary figure 1 *Excerpt of figure 1 that shows an orange triangle that indicate the blind spot area that the meeting area camera (orange star) was not able to record.*

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