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Play behaviour in preweaned Norwegian Red calves housed in different social settings

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Animal Science – Ethology

Preface

The process of writing this master thesis has truly been a journey through all kinds of emotions, from enjoyable to frustrating. Primarily, it has been inspiring, and I am grateful for getting the opportunity to work with a part of the CalfComfort project. I have learned so much.

I would like to thank my supervisors Sabine Ferneborg (NMBU), Laura Katherine Whalin (Norwegian Veterinary institute) and Julie Føske Johnsen (Norwegian Veterinary institute) for putting up with me and all my questions, for professional advice, good discussions and help with practical work. I appreciate all comforting words and your knowledge, keeping me grounded and helping me forward.

Great thanks to my favorites; family, boyfriend, friends, and dog, for all the support, fun times, and good conversation throughout my education. An extra shout-out to my dog Bella for not letting me forget that I need breaks and fresh air.

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Abstract

The aim of this study was to describe play behaviours of calves in three different management systems during a play test using straw as a play-object. Thirty-six Norwegian Red calves were divided into two batches, with one group of six calves from each of three treatments included in each batch: Minimal: Housed individually until 26 ± 3 d of age and then moved to a group pen with 5 other calves, fed 7L milk/d; Cow-calf-contact: Housed with the dam and 5 other cow-calf pairs, no restriction to milk; Enriched: Housed in a group of 6 calves and no restriction to milk (consumed on average 12 L milk/d). Group allocation was based on birthdate to minimize age differences within a group. At 52 ± 4 d of age, pairs of calves from the same treatment were habituated to an arena for 30 min. The following day, the same pairs were subjected to the arena for a straw play test. Play behaviour was recorded for 10 min after provision of straw. All calves played, but low duration of straw play was recorded. Enriched and minimal calves appeared to be more similar in locomotor play behaviour and appeared to play more than cow-calf-calves. Cow-calf-calves and enriched calves appeared to explore the environment more. In conclusion, positive welfare in CCC, EC and MC systems should be further investigated.

Sammendrag

Målet med denne studien var å beskrive lekatferd hos kalver i ulike oppstallingsmåter under en lek-test med halm som lekeobjekt. Trettiseks Norsk rødt fe (NRF) kalver ble delt inn i to runder, med en gruppe på seks kalver fra hver av tre behandlinger inkludert i hver runde: Minimal: Holdt individuelt til 26 ± 3 dager og deretter flyttet til en gruppebinge med 5 andre kalver, fôret 7L melk/d; Ku-kalv-kontakt: Holdt sammen med mor og 5 andre ku-kalv-par uten begrensning på melk; Beriket: Holdt i en gruppe på 6 kalver uten begrensning på melk (inntok i gjennomsnitt ca. 12 L melk/d). Gruppefordelingen var basert på fødselsdato for å minimere aldersforskjeller innen en gruppe. Ved 52 ± 4 dagers alder ble par av kalver fra samme behandling forsiktig ledet til en arena og tilvent i 30 minutter. Dagen etter ble de samme parene ledet til arenaen for lek-testen. Lekatferd ble registrert i totalt 10 minutter etter tilførsel av halm. Alle kalvene lekte, men det ble registrert lav varighet av lek med halm. Berikede og minimale kalver så ut til å være mer like i bevegelseslek og lekte mer enn ku-kalv-kalver. Ku-kalv-kalver og berikede kalver så ut til å utforske miljøet mer. Avslutningsvis, positiv velferd i CCC-, EC- og MC-systemer burde undersøkes videre.

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1. Introduction

The first definition of animal welfare was perhaps the Brambell committee's five freedoms in 1965: Freedom from hunger, thirst and malnutrition, freedom from discomfort and exposure, freedom from fear and distress, freedom from pain, injury and disease, and freedom to express normal behaviour (Mellor, 2016; Ot.prp. nr. 15 (2008-2009), 2008). Since this early definition, scientists have provided nuance and different perspectives in a variety of different definitions. The definition of Broom (1991) is about the state of an individual in relation to its environment and defines how environmental effects can lead to failing to cope with the environment, or having difficulty in coping, which can compromise life quality and cause the animals' welfare to deteriorate (Broom, 1986, 1991). Even though Broom acknowledges pain, it says little about affective states or natural living. Fraser et al. (1997) acknowledges the biological functioning of the animal, affective states (i.e., the subjective experience and feeling that motivates the animal to achieve a goal), for example pain or pleasure, and natural living. The five domains model developed by Mellor and Reid (1994) divides into physical and functional states related to internal states and external circumstances such as nutrition, environment, health and behaviour, and the fifth domain mental state. Models and definitions are made to make it easier to come to an agreement, and to define animal welfare in scientific work; however, the nuances and different understanding of animal welfare can make it complicated to interpret.

Animal welfare research has used these definitions to formulate questions regarding the experiences of animals; however, much of the early animal welfare work has focused on negative events such as fear, stress, disease and pain instead of positive emotions (Mellor, 2016). In more recent years, there has been a search for an agreement on how to assess positive experiences, and it has been acknowledged that animals can feel pain and suffering. It has become accepted that good welfare is not only the absence of negative experiences, but also the presence of positive experiences (Boissy et al., 2007; Mellor, 2015). There has been an increasing interest in measuring positive welfare in animals, such as the current gold standard play behaviour. Positive emotions can have an ethical and practical importance for animal welfare, and the absence of positive affect can indicate discomfort in the animal, though its basic needs (e.g., food and water) are met (Boissy et al., 2007).

More available information about the farming industry around the world has led to increased animal welfare concerns from the public. These concerns are ethically based (Hyland et al., 2022), thus stakeholders may approach these concerns differently. For example, the public may expect that dairy cows live together with their calves, while they are in fact separated early (Placzek et al., 2021). The early separation has gotten attention as a welfare concern, and often addressed as unnatural by the public (i.e., breaks with what is believed to be natural) (Placzek et al., 2021; Ventura et al., 2013). In Norway, calves are routinely separated from their mothers and housed in individual pens for a median of 2 weeks, while it is allowed to use single housing up to 8 weeks of age. Calves are usually given a restricted amount of milk at approximately 7L/d (Johnsen et al., 2021a; Landbruks- og matdepartementet, 2004). There is increasing evidence that individual housing and restricted milk allowance can lead to less exploration and locomotor activity. For example, individual housing early in life could delay exploratory behaviour (Jensen et al., 1997). Less exploration and locomotor activity may be effects of isolation (Færevik et al., 2006), insufficient feed being available (Costa et al., 2019), and limited social contact which can compromise growth, social learning, and stress coping (Costa et al., 2016; Costa et al., 2019; Gaillard et al., 2014; Meagher et al., 2015), compared to pair or group housed calves. There are fewer studies that have explored the effects of keeping cows and calves together, but there may be benefits such as better growth, health, and social behaviour of the calf, and can also reduce the risk of mastitis in cows (Beaver et al., 2019; Flower & Weary, 2001; Hansen et al., 2023). Also, play and social behaviour of the calf with and without access to their dam and other cows have been studied, and found higher welfare in calves that had contact with their dam and other cows (Waiblinger et al., 2020).

Play behaviour can indicate positive emotions and is often expressed when the animals' primary needs are fulfilled (Boissy et al., 2007; Held & Špinka, 2011). Play can be reinforcing and is associated with positive emotions (Jensen et al., 1998; Krachun et al., 2010; Yeates & Main, 2008). The motivation to play may therefore indicate good welfare (Boissy et al., 2007; Held & Špinka, 2011). Even though the animal is motivated to play, they may not be able to express their motivation due to low space, no play partners, or other objects to play with (Jensen et al., 1998). For example, calves are highly social animals and individual housing can lead to impaired behavioural development and cognitive ability (Costa et al., 2019). Insufficient feed intake, especially milk, and painful procedures may also suppress play behaviour in calves (Boissy et al., 2007; Krachun et al., 2010; Mintline et al., 2013). One play-object of interest for calves can be straw, and Jensen et al. (1998) found a peak in play behaviour when they added straw to the pen, though Duve et al. (2012) saw a low duration on play behaviour when straw was provided. Straw play tests have usually been performed in the calves home pen, and perhaps space allowance can reflect in a rebound (i.e., increase in motivation to perform a behaviour due to the inability to perform the behaviour (Jensen et al., 1999)) if tested in an arena, as calves moved from a small space to bigger space have shown increases in play behaviour (Jensen, 2001; Jensen & Kyhn, 2000; Valnickova et al., 2015). For example, group housed calves may have access to more space which increases the occurrence and quality of locomotor play in calves (Jensen & Kyhn, 2000; Jensen et al., 1998), and group housed calves tend to show more play behaviour (Jensen et al., 1998). To my knowledge, similar studies have not been done on Norwegian Red calves.

As stated, there have been studies on play behaviour in calves, but straw as play-object has not been tested in an arena. The aim of this thesis is to describe play behaviours of calves in different management systems during a play test using straw as a play-object. The main prediction for this study is: 1. The test will stimulate play in all calves. Since behaviour in a play test can depend on space and milk allowance, and social housing, the next predictions are: 2. Calves with the lowest space allowances may express more locomotor play and be more reactive and aggressive, 3. Calves given the lowest milk allowance would play less and be less active, 4. Calves reared in a more complex social environment and higher milk allowances may use less time to explore straw and explore the environment more.

2. Literature

2.1 Defining animal welfare

Animal welfare discussions emerged as farming transitioned to more industrialized and intensive methods (Fraser, 2003). There are differing perspectives for which ethical considerations need attention (Fraser, 2008). For example, one person may place importance on an animal's health and growth, while another may value the space available to the animal. Furthermore, people with a more "romantic" view of the world may value the emotions and a natural life, while those with an industrial view may see good welfare when the animals are healthy, and value the scientific basis of the system (Fraser, 2008). Perhaps the first definition developed was the Brambell committee's five freedoms (Ot.prp. nr. 15 (2008-2009), 2008). These freedoms focus more on the lack of suffering, and early animal welfare assessment has therefore focused most on negative welfare indicators, e.g., lameness and abnormal behaviour (Mattiello et al., 2019). The use of these indicators has therefore indicated that if the presence of them is low, then the welfare is good (Mattiello et al., 2019). More recently, the five freedoms have been criticized for not having enough focus on good welfare and positive experiences, since a lack of suffering is not the same as a positive welfare state (Mattiello et al., 2019; Mellor, 2016). The five freedoms have also been criticized for not capturing the increased knowledge of biological processes (Mellor, 2016).

Another definition of animal welfare is three overlapping ethical concepts: biological function, affective states and natural living (Fraser et al., 1997). Biological functioning focuses on the health, growth and productivity of an animal (Fraser, 2003; Fraser et al., 1997). The biological function based concern may be more common among farmers, veterinarians and those with practical responsibility for animals (Fraser et al., 1997). The second concern emphasizes the affective states, i.e., the subjective experience and feeling that motivates the animal to achieve a goal, for example pain or pleasure (Fraser, 2003, 2008). Positive affective states, or the value of the feelings of animals, have been more emphasized in science (Reviewed by Yeates & Main, 2008). The third concern emphasizes the natural living of the animal, i.e., the animal's ability to express their natural behaviours, with performance of natural behaviours and natural elements in their environment (Fraser, 2003, 2008; Fraser et al., 1997). For example, Lidfors et al. (2005) and Whalin et al. (2021) explored natural living in more detail regarding cows and calves, and how we can accommodate natural living to reduce unnecessarily stress and disease.

The term welfare can refer to the state of an individual in relation to their environment (Broom, 1991). The motivational state is affected when the animal has a need. Those states can be measured, and is affected when the animal has a need, so behavioural and physiological responses can be made. Poor welfare can indicate that the animal fails to cope with environmental effects, or has difficulties in coping, which can refer to how much the animal needs to do to cope and how their coping attempts succeed (Broom, 1986, 1991). An animal can succeed in its attempts to cope in their environment and adapt to the conditions. Welfare can be assessed by external indicators, immune functions and behavioural changes (Broom, 1991). Welfare can be poor without suffering, therefore, measurement of welfare should be done on each individual rather than a group, since each individual may have different experiences of their environment due to social situations (Broom, 1986). The ethology group at Norwegian University of Life Science (NMBU) has defined animal welfare as an individual's physiological, mental and physical state in its attempt to master their environment (Ot.prp. nr. 15 (2008-2009), 2008).

Yet another definition is the five domains model developed by Mellor and Reid (1994), expanding from the five freedoms. The domains were made as domains of potential compromise to better emphasize the extent of welfare compromises rather than the absence. The domains are divided into physical and functional states related to internal states and external circumstances such as nutrition, environment, health and behaviour, and the fifth domain mental state (Mellor, 2017).

One last definition is Spruijt et al. (2001) that defines welfare as the balance between positive (reward) and negative (stress) experiences or affective states, that ranges from good welfare to poor welfare. The positive balance may result in adapting to the environment. The affective states may be momentary or transient states that occur against the background of and are integrated with the state of the balance. Affective states have developed through evolution as a motivational system that can reduce or strengthen the animal's fitness (i.e., a genotype, an individual or a population, broadly the abilities of an animal to survive and reproduce in their environment (Barker, 2009)). Acute stressors can be balanced by a reward, and poor welfare may therefore be the presence of chronic stressors rather than acute (Spruijt et al., 2001).

There are numerous approaches to animal welfare, yet there are some overlaps. Critique about not having enough focus on good welfare and positive experiences have been made, since a lack of suffering is not the same as a positive welfare state (Mattiello et al., 2019; Mellor, 2016). This has led to more awareness around the animals' feelings. For example, the five domains model by Mellor and Reid (1994) expanding from the five freedoms, but with more focus to the extent of welfare compromises instead of absence. Therefore, including more physiological, mental and physical states in relation to their environment is now emphasized. Animal welfare assessment has moved further away from the five freedoms, to the discussion about positive welfare indicators and consideration of the positive aspects of animal welfare (Reviewed by Yeates & Main, 2008).

2.2 Public concern

Animal welfare science developed due to public concerns for animals (Hyland et al., 2022), and animal welfare stem from public engagement (Fernandes et al., 2019; Sweeney et al., 2022). Even though consumers have been more aware of how farm animals are managed, the public perceives insufficient knowledge regarding farming and practices (Sweeney et al., 2022). Just as there are differing views for how to define animal welfare, different stakeholders value aspects of animal welfare differently (Verbeke, 2009). For example due to demographic differences, and the animals' use and value (FAO, 2015). Van Poucke et al. (2006) found that men and people from rural areas perceived animal welfare as being better than what the women perceived. A person's experience with animals can influence their perception of animal welfare. Pet owners seemed to be more negative to the welfare of farm animals, and people with farm experience were more positive to the life quality of farm animals than people without experience (Boogaard et al., 2006). The dairy industry has had a positive reputation over several years in European countries, but there are differences between countries (Placzek et al., 2021). For example, in a study of Boogaard et al. (2010) between Norwegians and Dutch people, the Norwegians seemed to be less concerned about the conflict between modern production and naturalness.

There are many practices that the public is unaware of, such as early cow-calf separation (Placzek et al., 2021). More attention has been paid to cow-calf separation, and the emotions of the calf, in recent years. The practice of early separation is often rejected because it is considered unnatural and associated with stress for the animals. Studies from Germany, USA and Brazil have found that most of their participants were uninformed about the separation (Cardoso et al., 2017; Hötzel et al., 2017; Ventura et al., 2013). Many of the citizens rejected the practice when they were informed, while some supported it (Busch et al., 2017; Cardoso et al., 2017; Hötzel et al., 2017). There can be both positive and negative sides of weaning age, and even though Busch et al. (2017) presented them to the citizens, most did not change their opinion. For example, arguments such as that separation is emotionally stressful, health, unnaturalness and the industry can be against early separation, while arguments for early separation can be that it is less stressful to separate earlier than later, that it can promote health and limitations in the industry (Ventura et al., 2013).

2.3 Calf management

In Norway, dairy calves are routinely separated from their mother shortly after birth (Hansen et al., 2023), then placed in individual pens for a median of 2 weeks, while it is allowed to use single housing up to 8 weeks of age (Johnsen et al., 2021a; Landbruks- og matdepartementet, 2004). Arguments for early separation includes economics, ethics, pathogens, diseases (e.g., diarrhea and respiratory diseases) and to ensure high-quality colostrum (Beaver et al., 2019; Costa et al., 2019; Gorden & Plummer, 2010; Svensson et al., 2006). However, one study found that calves had fever bouts of diarrhea when they were separated later (Weary & Chua, 2000), another concluded that there was no consistent evidence in support of early separation when it comes to cow and calf health (Beaver et al., 2019). Allowing the calves to perform natural behaviours can be more efficient than restricting them and give the calves positive experiences, as incidences of stress and disease can be reduced (Lidfors et al., 2005; Špinka, 2006).

Calves are often fed approximately 7L milk/d divided by two meals (Johnsen et al., 2021a), complying to the minimum standard of milk meal frequency in Norway (Landbruks- og matdepartementet, 2004). In contrast, calves have been reported to perform 8 to 12 suckling bouts/d during their first month of age when raised with their mother (Kour et al., 2021; Lidfors & Jensen, 1988; Vitale et al., 1986), and calves can drink up to 15 L/d when given *ad libitum* milk (Borderas et al., 2009; De Passillé & Rushen, 2006). Current literature argues that 7 L/d is insufficient for calves; restrictive milk allowance can lead to poor growth and hunger (Costa et al., 2019; Jasper & Weary, 2002), and calves tend to perform more unrewarded (receiving no milk) visits per day to the milk feeder when given a low milk allowance (De Paula Vieira et al., 2008). This in turn can lead to abnormal oral behaviours such as cross-sucking (i.e., non-nutritive sucking) other calves or objects (Jensen, 2003), and competition at the feeder (De Paula Vieira et al., 2008).

The early social environment affects the behavioural development of calves and especially how they engage in social play (Reviewed by Cantor et al., 2019). Also, the early life environment can influence the calf's physical, behavioural and cognitive development (Costa et al., 2019). Group housing appears to be viable in terms of the calf's health, performance and behaviour, perhaps because it provides opportunity for socialization, access to space and therefore results in higher levels of activity and play (Chua et al., 2002). Social deprivation can lead to abnormal behaviour and other developmental problems (Costa et al., 2019), such as less exploration and locomotor activity (Færevik et al., 2006). Dairy calves are highly motivated to have social contact and engage in social contact on average 2% of their time the first 8 weeks of life (Chua et al., 2002). Indeed, a study found that calves will work harder to have full social contact than limited contact across a barrier (Holm et al., 2002). Numerous studies have found benefits to rearing calves with social companions (Reviewed by Cantor et al., 2019; Costa et al., 2016). For example, calves raised individually appear to be more reactive to novel environments (De Paula Vieira et al., 2012; Jensen et al., 1997), novel foods (Costa et al., 2014), and novel calves (Jensen & Larsen, 2014) than pair or group housed calves. This fearful behaviour may affect their willingness to try concentrates in their home pen as evidenced by a study that found that group housed calves ate more concentrate than individually housed calves (De Paula Vieira et al., 2010; Jensen et al., 2015). Other studies have found that individually housed calves struggle to complete reversal learning tasks, unlike socially housed calves (Gaillard et al., 2014;

Meagher et al., 2015). Though there have been several studies detailing the benefits of raising calves in pairs or small groups, less is known about the added effects of raising calves with their mothers. A few studies have found that, beyond suckling, cows and calves will perform allogrooming (Johnsen et al., 2015; Johnsen et al., 2021b; Wenker et al., 2021), and spatial proximity and synchronized behaviour. However, future work is needed to understand the experiences of calves in CCC systems. For example, in assessing their health and using welfare indicators such as play.

2.4 Positive welfare

Animal welfare science has historically studied negative feelings such as abnormal behaviour, excessive aggressiveness, hunger, pain and fear (Fraser & Duncan, 1998). It is accepted that animals can feel pain and suffering, and methods such as behavioural (e.g., anhedonia), or endocrine (e.g., blood pressure (cortisol)) measurements have been developed to assess these states (Boissy et al., 2007). There is increasing interest in understanding positive states such as pleasure, play, curiosity, and agency (Mellor, 2011), as good welfare is more than the absence of negative experiences (Boissy et al., 2007). However, there are few validated methods to assess these states (Boissy et al., 2007; Mellor, 2011).

Behavioural markers can be useful for assessing positive emotions, as they may occur when animals are in a positive affective state (Mendl et al., 2010; Yeates & Main, 2008). Mendl et al. (2010) stated that "Emotional states occur in response to stimuli or situations that are actually, or potentially, rewarding or punishing.". Rewarding or punishing stimuli can enhance fitness, for example food and water, or threaten fitness, for example thermal damage. Positive behaviours can be a result of pleasure, happiness, liking or wanting (Reviewed by Yeates & Main, 2008), and occur when the animal is able to express engagement, positive or active, with the environment and other animals (Mattiello et al., 2019). If an animal is motivated to work for something, it might tell us that it could help the animal "feel better", and hence are important for the animal (Reviewed by Yeates & Main, 2008). Liking can come from a reward, which can make the animal feel positive feelings. Wanting can be associated with the motivation to gain a resource. For example, self-grooming, licking or rubbing the body, can be performed during a stressful situation as a form of self-comfort (Boissy et al., 2007; Herskin et al., 2004) but also as a positive behaviour when animals are motivated to scratch (Rushen & Passillé, 1992; Westerath et al., 2014). However, there are some promising behaviours that may indicate positive welfare. For example, animals perform exploratory behaviour when they are gathering information (Mattiello et al., 2019) and want control over their environment (Westerath et al., 2009). Affiliative behaviours, such as synchronization (i.e., members of a group perform the same type of behaviour (Green, 1992)), and allogrooming (licking directed toward another calf's body or head) (Bouissou et al., 2001; Horvath & Miller-Cushon, 2019); may allow animals to reinforce social bonds (Boissy et al., 2007; Bouissou et al., 2001; Val-Laillet et al., 2009).

Perhaps the gold standard for assessing positive states is play behaviour. Play behaviour is reinforcing and can therefore indicate the presence of good welfare and positive feelings (Napolitano et al., 2009). Play is thought to help animals physically (motor training) and mentally (decision making, and emotion moderation) train for unexpected situations (Špinka et al., 2001). Play may occur more when there is sufficient nutrition and under favorable conditions such that the animal's primary needs are met (Boissy et al., 2007; Held & Špinka, 2011; Špinka et al., 2001). Play may also spread between individuals and thus promotes social behaviours (Held & Špinka, 2011).

The most common types of play in calves are social and locomotor play (Boissy et al., 2007). Locomotor play can include galloping, jump, different types of bucking, turn, leap and head shake (Jensen et al., 1998; Valnickova et al., 2015). Social play can include contact play such as butting and mounting (Valnickova et al., 2015). Locomotor play is usually expressed individually, but can be seen as parallel play, i.e., several calves express it at the same time (Jensen, 2011).

In calves, play increases during the first 2 weeks of age (Jensen, 2011), and can be affected by several factors. For example, increased space allowance may promote play behaviour reflecting a rebound (Jensen & Kyhn, 2000; Jensen et al., 1998). Calves that have been moved from a small space to a larger space showed increased motivation to perform play, and thus increased play behaviour (Jensen, 2001; Jensen & Kyhn, 2000; Valnickova et al., 2015). Fresh straw and milk allowance can also affect play behaviour (Duve et al., 2012; Jensen et al., 1998), such that calves with lower milk and a low space allowance play less (Jensen et al., 1998; Krachun et al., 2010).

Play can be assessed in the home pen, or in an arena test. An arena test takes place by releasing animals individually into a barren open area for a period and observing their behaviour (Jensen, 2001; Mintline et al., 2012). Arena tests have been used to assess fear, exploration, and play in calves (Forkman et al., 2007; Mintline et al., 2012; Perals et al., 2017), and can be modified to investigate play behaviour by adding straw (Zhang et al., 2021). These types of tests are useful but have a number of challenges. For example, the shape and size of the enclosure, and the time of testing may affect the behaviour of the animal (Mattiello et al., 2019). Additionally, most studies have conducted arena tests with isolated animals (e.g., (Jensen et al., 1999; Mintline et al., 2012; Sutherland et al., 2014)). This may cause the animals to behave differently than they would in their home pen, if raised in groups. As calves are social animals, testing calves in pairs may enable us to understand their behaviour more accurately.

3. Method

3.1 Research approach

The data used in this thesis is from a larger study, CalfComfort. The Norwegian Veterinary Institute designed, conducted, and received funding from NFR, project nr. 325663, for CalfComfort (Veterinærinstituttet, n.d). The data used for this thesis were collected from October 2022 to March 2023 at the Norwegian University of Life Sciences (NMBU) Livestock Production Research Centre in Ås, Norway. The study received ethics approval (ID 29664) from the Norwegian Animal Research Authority (part of the Norwegian Food Safety Authority).

The observer who did the data analysis approached this study with a small amount of on-farm experience and a theoretical background of animal science and ethology (scientific study of animal behaviour). The observer also has an appreciation for caring for and learning from each individual animal to understand them better. In this study the observer had no familiarity with either the calves or cows used in the study (i.e., blinded). The analysis and interpretation are influenced by the observer's values and interest in the natural living of animal welfare (Fraser et al., 1997).

Before the data collection, literature research was conducted to build knowledge of the theme and give relevant background information. ScienceDirect, Oria and Google Scholar were used to search for literature with the use of key words such as "play behaviour", "calf", "play test", "animal welfare" and "positive behaviour". Relevant references of used literature were also looked at to find more information. Criteria used to choose the literature was relevance, language: Norwegian, English, Swedish or Danish was accepted, and the availability. All literature used was open to the public or accessed through NMBU's database.

Play behaviours in response to the straw were analyzed through video recordings and Behavioural Observation Research Interactive Software (BORIS) (Friard & Gamba, 2016).

3.2 Research design

My contribution was to make an ethogram relevant for the straw play test and develop it for this thesis, and to analyze video recordings from September 2022 and March 2023. Other articles methods were used to build this method by critically examining what they had done, and to decide what should be included and not.

3.2.1 Animals, housing and feeding

Thirty-six Norwegian Red calves (birth weight: 39.1 ± 4.8 kg) were used in a larger experiment from birth to 12 weeks of age. The data for this thesis was collected when the calves were 53 ± 4 d of age, and all calves were healthy for the data collection in this thesis. The study included two batches, and each batch included one group of six calves from each of three treatments: minimal housing (6 females, 6 males), cow-calf-contact (8 females, 4 males), and enriched (7 females, 5 males). Each group was filled in chronological order according to calf birthdate to minimize age differences within a group. Fresh straw was provided to the home pens on Mondays and Thursdays. An overview of the pen designs is outlined in figure 1.

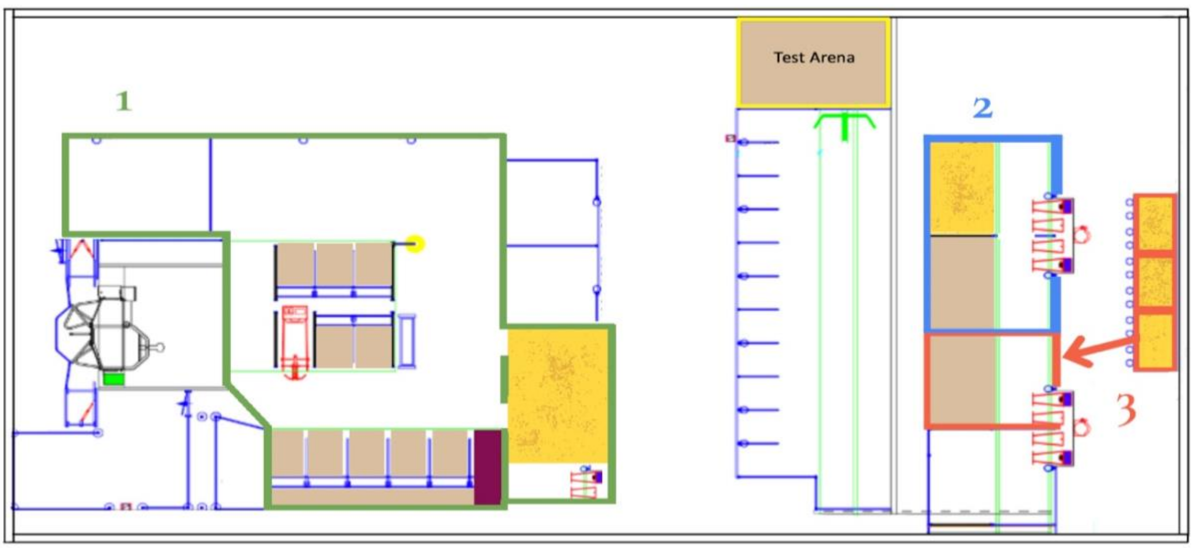

Figure 1. *CalfComfort pen design seen from above. 1: Cow-calf contact (outlined in green) – 6 pairs of cows and calves had access to 133.5 m^2; calves could freely visit a straw bedded creep (21.3 m^2) , 2: Enriched calves*

(outlined in blue) – 6 calves had access to 23.1 m^2 including a straw bedded creep, 3: Minimal housing (outlined in red) – calves were housed individually in straw bedded pens (2.4 m^2) until approximately 26 d of age, at which point they were moved to a group of 6 calves (11.55 m^2). Yellow substance indicates straw bedding and red cone-shaped figures indicate feeders. Test arena is marked as "Test Arena".

All calves were bottle fed or tubed 4L colostrum from their mothers within 6h of birth, and had *ad libitum* access to hay, water, concentrates, and silage. When they were 24-48h old, the calves underwent a health check using the Wisconsin Calf Health Scoring App. Calves that had diarrhea, poor appetite or an attitude score above 1 were excluded from the experiment. Twins and calves from a difficult birth were not included in the experiment. Ten calves were not enrolled as they failed to meet inclusion criteria (7), difficult birth (2), or dangerous cow behaviour (1). A trained observer examined the calves twice per week using the Wisconsin Calf Health Scorer App. Calves experiencing diarrhea were given oral electrolytes once per day following farm protocols. All calves with horns were disbudded at 4 weeks of age by veterinarians.

Minimal calves (MC) (n = 12) were separated from their mothers within 30 min of birth and moved to individual pens (2.4 m^2) bedded with straw. Fresh straw was added to the individual pens on Mondays and Thursdays, but they did not receive straw in the group pens (i.e., the 3 weeks leading up to the test). There were solid walls between each individual pen, but calves could have visual and tactile contact with neighboring calves by reaching out the front gate of the pen. Calves remained in the individual pens until 26 ± 3 d of age, at which point they moved to a group pen (11.55 m^2) with a total of 6 calves. The flooring consisted of rubber mattresses lightly bedded with sawdust and rubber slatted floors. The walls around the rubber mattress were solid, metal gating over the rubber slatted floors divided into group pens. Thus, calves in group pens had full contact with their penmates, and could see and sniff neighboring calves through the gating. The calves were fed 7 L of milk/d divided into 3 feedings at approximately 06:00, 12:00 and 19:00. They were fed from a teat bucket in individual pens, and two milk bars with 5 teats each in the group pen. This means that calves who drank milk slower might have had slightly less than 7 L/d when they moved to the group pen. In the individual pen, each calf had a hay rack filled with hay and silage, a water bowl and a concentrate bowl. When calves moved to the group pen, hay and silage were provided on a feeding table at the front of the pen. In the group pen calves had access to a water bowl, and an automatic concentrate feeder.

Cow-calf-contact (CCC) calves remained in the maternity pen (2.9 x 3.2 m, deep bedded with straw) with their mothers for the first 3 d of life. The maternity pen was outfitted with a water bowl, and a feeding table where the cow and calf had access to silage. The calves and their mothers were then moved to a group pen with a total of 6 cow-calf pairs (n = 12) for the duration of data collection. The calves had free-access to a deep-bedded straw creep and cow-contact area. The creep (21.3 m^2) was separated from the cow-area by a concrete wall with exits that only a calf could fit. In the creep, calves had access to 2 stationary brushes, 2 water bowls, concentrates from an automatic calf feeder, and hay and silage on a feeding table. Fresh straw was added to the creep on Mondays and Thursdays. The cow area (133.5 m^2) only housed cows and calves included in the project. The cow area had 1 water trough, 4 water bowls, 11 free stalls with rubber mattresses lightly bedded with sawdust, a feed alley with 10 headlocks, and rubber slatted floors with 32 mm opening. Calves had free access to their mothers all the time, except when the cow was in the milking robot or waiting to be milked.

Enriched calves (EC) (n = 12) were separated from their mothers within 30 min of birth and moved to groups of 6 calves. Group pens (23.1 m^2) included a deep-bedded straw pack, 2 stationary brushes attached to the side walls, a rubber mattress lightly bedded with sawdust, and rubber slatted floors. Fresh straw was added on Mondays and Thursdays. Calves were fed *ad libitum* concentrates from automatic feeders, hay and silage on a feeding table, and water from a water bowl. They were given *ad libitum* milk from 2 milk bars with 5 teats 4 hours per day at approximately 06:00 – 07:00, 10:00 – 11:00, 14:00 – 15:00 and 19:00 – 20:00. Milk feeders were checked throughout the hour, and milk was added such that the milk bar was never empty. The calves consumed on average approximately 12 L milk/d.

3.2.2 Experimental design

At 52 ± 4 d of age, pairs of calves from the same treatment were gently guided from a weight scale to a novel arena (4.90 x 3.39 m) with rubber mats lightly bedded with sawdust. Calves had no visual access to the outside environment in the arena. The pair-mates, and order of testing were randomized within treatment. Pairs were habituated to the arena for 30 min before they were once again guided to a weight scale. The following day (at approximately 09:00 – 11:00), pairs underwent the same process, but this time, to record play behaviour. A GoPro camera (GoPro Hero8, GoPro Inc.) was positioned 3.62 m above the arena, and recorded the behaviours for the duration of the 12 min test. The GoPro camera failed to record the behaviours of one pair (Enriched, 1 male, 1 female), thus a total of n = 17 pairs were observed and analyzed in this thesis. After the pair spent 2 min in the arena, approximately 7.5 kg of straw (divided into two bags) was emptied into the pen by 2 experimenters (see Figure 2). The location was picked to easily deposit the straw into the pen so that the calves were not disturbed by sounds, or mechanical apparatuses entering the center of the pen. Straw has been used to stimulate play in calves in other studies (Duve & Jensen, 2012; Duve et al., 2012; Jensen et al., 1998), thus an ethogram focusing on play and exploratory behaviours was developed (see Table 1). After 12 min, the calves were guided from the testing arena to the weight scale.

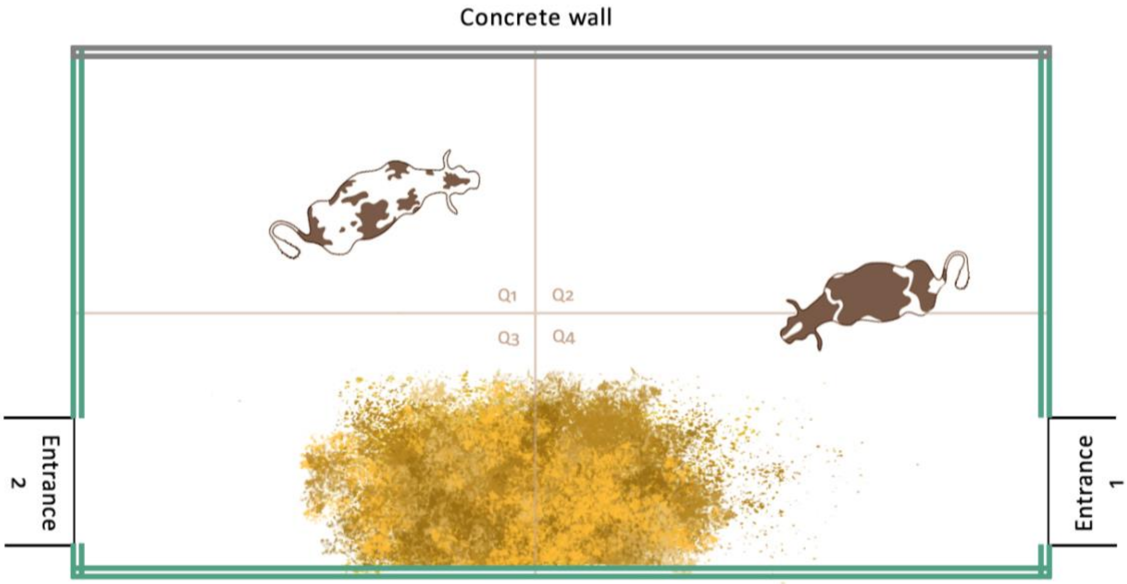

***Figure 2**. Test arena design for the straw play test seen from above. Green lines show fence walls covered with tarp. Yellow substance shows straw. The entrances are where the calves were guided in. MC and EC pairs used entrance 1, and CCC pairs entrance 2. Entrance 1 was closed with a garage door, and entrance 2 was closed with fences. Q1 is quadrant 1, Q2 is quadrant 2, Q3 is quadrant 3 and Q4 is quadrant 4 for the active behaviour in the ethogram (table 1).*

***Table 1.** Ethogram describing play behaviours recorded in a straw play test. Locomotor play was scored as separate behaviours (i.e., jumping, kicking, bucking, and running). Durations of behaviours were terminated when the break was >3sec.*

Behaviour	Description
Straw play	Time spent butting (head in contact with straw), mock butting (head oriented downward and toward but not in contact with straw) or rubbing head, throat, or neck in the straw playfully while kneeling on both forelegs, or standing.[1,2,3]
Butting fixture	Time spent standing and pushing the head against any fixture in the pen in a playful manner. [1,3]
Exploring straw	Time spent with muzzle or tongue in contact with, or less than one muzzle length from the straw while moving or stationary. [1,3,4]

Latency to touch straw	Time until calf touches the straw (muzzle contacts the straw).
Exploring environment	Time spent with muzzle or tongue in contact with, or less than one muzzle length from the walls or floor (excluding straw) while moving or stationary. [1,3,4]
Active	Total number of squares crossed with more than half the body (at least 2 feet). Test arena is divided into 4 equal quadrants. [4]
Inactive/standing	Time spent standing still, 3 to 4 hooves in contact with the floor supporting the weight of the body, without interacting with walls or floor. [2,4]
Locomotor play	*Jumping:* Frequency of lifting both forelegs from the ground, elevating the forepart of the body. May be followed by lifting the hindlegs from the ground. [1,3] *Kicking*: Frequency of one leg lifted off the ground and extended backwards. [5] *Bucking*: Frequency of body ascending from front legs to hind legs, raising the hooves and stretching both hind legs backwards. [1,3] *Running*: Frequency of calf trotting (2 beats) or galloping (3 beats) across or around the enclosure. [4]
Contact with other calves	Time spent allogrooming (licking head, neck, or body of other calves), or sniffing (muzzle in contact or close proximity with other calves). [2]
Butting calf	Time spent pushing the head against the head or body of another calf with force in a playful manner, or mock butting where head is oriented downward and toward but not in contact with the other calf. [1,3]
Mounting	Duration of lifting the forelegs from the ground while jumping onto the back, side, or head of another calf. [1]
Withdrawal	Frequency of sudden movement backwards or sideways.

[1] Duve et al. (2012)
[2] Duve and Jensen (2012)
[3] Jensen et al. (1998)
[4] Neave et al. (2018)
[5] Sutherland et al. (2014)

3.3 Data collection and analysis

One observer, blind to treatments, was trained to score behavioural videos by a trained supervisor. The observer first scored a video unrelated to the study using the established ethogram (Table 1; adapted from (Duve & Jensen, 2012; Duve et al., 2012; Jensen et al., 1998; Neave et al., 2018; Sutherland et al., 2014)). Once the observer was confident with the definitions, they proceeded to score a play-test video. Their observations were compared to the supervisor's observations. Agreement was not reached, and so the observer re-scored the video. After this second scoring, the supervisor and observer were well-aligned. The observer scored the video a third time and achieved a sufficient intra-observer reliability (Cohen's kappa = 0.811). Intra-observer reliability test, i.e., agreement between successive observations of the same individual (Battini et al., 2014), was conducted for every 10th calf (Cohen's kappa > 0.8).

Video recordings were analyzed in BORIS (v.7.13.9) using the ethogram describing the different behaviours (Table 1). BORIS is a software that offers a method to quantify data for behavioural observations with the help of ethograms (Friard & Gamba, 2016). For this thesis, the behaviours of 34 calves during the 10 min test were analyzed.

The behaviour data were downloaded from BORIS, and analyzed in R-studio (version 2022.07.1+554 (R Core Team, 2022)) with tidyverse (Wickham et al., 2019). This study was exploratory, thus descriptive statistics (mean ± SD, and ranges) were calculated for each behaviour. Data was not transformed to fit normality. The duration and frequency of each behaviour for each calf was calculated in R Studio. The behaviours running, kicking, jumping and bucking occurred infrequently, and thus were summed in a single behaviour "locomotor play". As the behaviours of each pair were related, behaviours were first averaged by pair (n = 17), and then descriptive statistics were conducted on each treatment.

4. Results

The results from video scoring after straw was added are presented in Table 2. All calves expressed play behaviour, though not straw play. EC (18.5 ± 11.5 events) and MC (15.6 ± 7.7 events) calves may have expressed more locomotor play (figure 2) than CCC (10.2 ± 6.4 events). Most calves spent less than 15 sec playing with straw, and the majority (20 out of 34) did not engage in straw play.

Table 2. *Behavioural response (raw arithmetic mean ± SD) of pairs (n=17) in a test arena after straw was added (10 min observation time). CCC = Calf-cow pair, EC = Enriched calves, MC = Minimal calves. min = minutes, sec = seconds.*

Behaviour	Treatment	Mean	SD	Min/max
Straw play (sec)	CCC	1.8	2.2	0 – 5.4
	EC	5.3	5.5	0 – 12.7
	MC	14.1	11.6	0 – 29.4
Butting fixture (sec)	CCC	30.9	20.3	4.5 – 58.2
	EC	11.3	9.0	0 – 21.7
	MC	6.5	10.8	0 – 27.7
Exploring straw (min)	CCC	2.9	2.0	1.0 – 5.4
	EC	2.1	1.0	1.1 – 3.6
	MC	4.8	2.8	1.2 – 8.5
Latency to touch straw (sec)	CCC	10.9	2.6	6.9 – 14.9
	EC	11.0	6.9	6.2 – 22.9
	MC	16.4	9.5	6.4 – 34.0
Exploring environment (min)	CCC	3.8	1.4	1.9 – 6.1
	EC	3.3	1.1	2.1 – 4.6
	MC	0.9	0.7	0.2 – 1.9
Active (quadrants crossed)	CCC	32.4	12.5	13.5 – 49.0
	EC	42.2	14.1	27.0 – 59.5
	MC	33.3	15.5	8.0 – 49.5
Inactive/standing (min)	CCC	1.1	0.5	0.5 - 1.9
	EC	2.5	0.9	1.6 – 3.6
	MC	2.6	1.8	0.7 – 5.0
Contact with other calves (sec)	CCC	0.0	0.0	0.0
	EC	0.0	0.0	0.0
	MC	0.0	0.0	0.0
Locomotor play (frequency)	CCC	10.2	6.4	4.0 – 20.0
	EC	18.5	11.5	6.5 – 29.5
	MC	15.6	7.7	5.0 – 26.0
Butting calf (sec)	CCC	8.0	7.0	0 – 15.1
	EC	1.9	3.7	0 – 8.4

	MC	2.8	4.4	0 – 11.3
Mounting (sec)	CCC	0.0	0.0	0.0
	EC	0.0	0.0	0.0
	MC	0.0	0.0	0.0
Withdrawal (frequency)	CCC	1.3	0.9	0 – 2.5
	EC	3.1	2.2	0 – 6.0
	MC	2.1	1.7	0 – 4.0

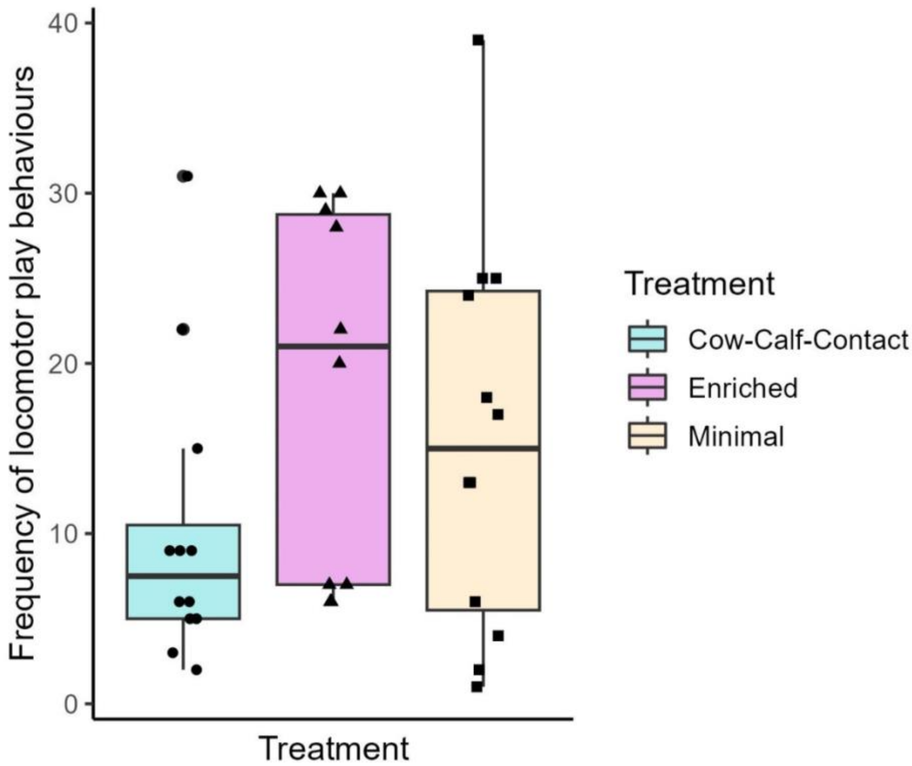

Figure 2. *Frequency (raw data) each calf (n = 34) spent performing locomotor play (i.e., running, jumping, bucking, kicking) during 10 min after straw was added to a test arena. Calves were tested in pairs, and had been habituated to the arena 30 min the day before the test. Box plots show medians (horizontal line) and 25th and 75th percentiles (boundaries of the box or hinges; first and third quartiles). The upper whisker extends from the hinge to the largest value no further than 1.5 times the interquartile range. The lower whisker extends from the hinge to the smallest value, at most 1.5 times the interquartile range. Round dots indicate CCC treatment, triangles represent EC treatment, and MC treatment are represented by square points.*

On average and with a large standard deviation, CCC calves may have spent more seconds butting fixtures in the pen than calves in the other treatments. Withdrawals happened infrequently (CCC: 1.3 ± 0.9 events, EC: 3.1 ± 2.2 events, MC: 2.1 ± 1.7 events). Calves in all treatments spent on average < 10 seconds head butting other calves. No behaviours of mounting and contact with other calves were seen in this test. Cow-calf calves (CCC) may have been less active (32.4 ± 12.5 quadrants crossed) than enriched calves (EC: 42.2 ± 14.1 quadrants crossed) while similar to minimal calves (MC: 33.3 ± 15.5 quadrants crossed), but could have spent their time on behaviours other than inactivity (1.1 ± 0.5 min spent inactive). For example, CCC calves appeared to engage in exploratory behaviour (Figure 3). MC calves may have spent less time exploring the environment than both CCC and EC (CCC: 3.8 ± 1.4 min, EC: 3.3 ± 1.1 min, MC: 0.9 ± 0.7 min).

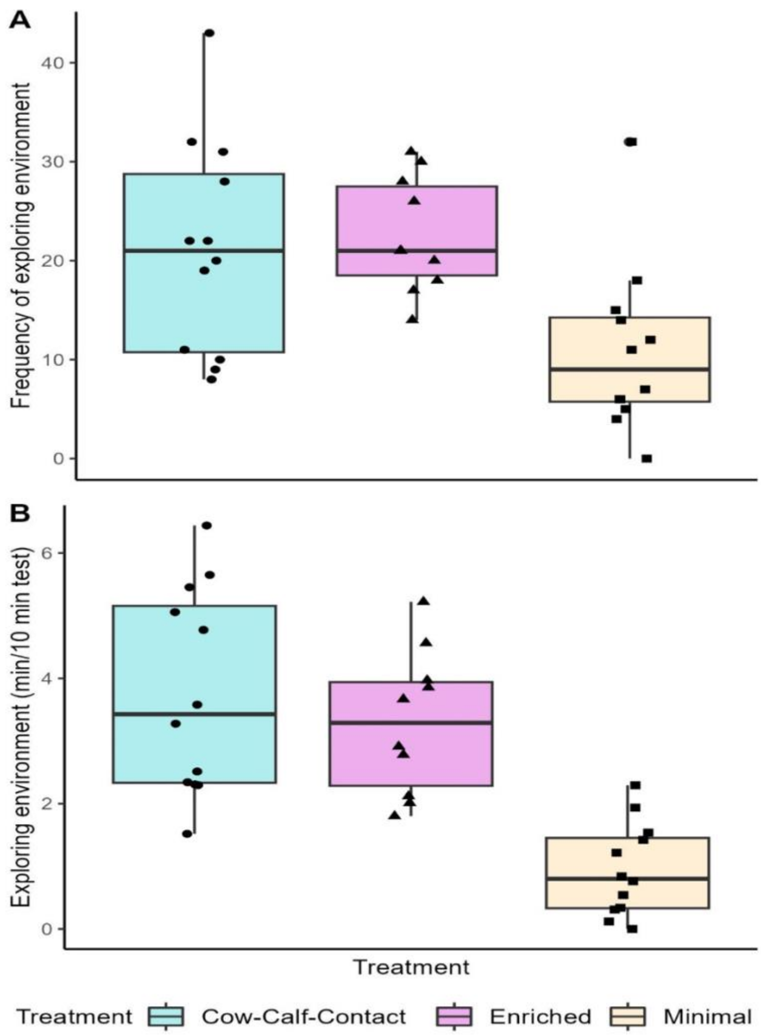

Figure 3. *A: Frequency (raw data) each calf (n = 34) explored the environment during 10 min after straw was added to a test arena. B: Duration (raw data; minutes) each calf (n = 34) spent exploring the environment during 10 min after straw was added to a test arena. Calves were tested in pairs, and had been habituated to the arena 30 min the day before the test. Box plots show medians (horizontal line) and 25th and 75th percentiles (boundaries of the box or hinges; first and third quartiles). The upper whisker extends from the hinge to the largest value no further than 1.5 times the interquartile range. The lower whisker extends from the hinge to the smallest value, at most 1.5 times the interquartile range. Data points indicate each individual calf. Round dots indicate calves from the CCC treatment, triangles represent calves from the EC treatment, and MC treatment are represented by square points.*

5. Discussion

The aim of this thesis was to describe play behaviours of calves reared in different management systems during a play test using straw as a play-object. The main prediction was that our play test would stimulate play in calves. All calves expressed locomotor play behaviour, and most calves spent less than 15 sec playing with straw. The majority (20 out of 34) did not engage in straw play. Earlier studies have suggested that play can be used as an indicator of positive welfare (Boissy et al., 2007) because it is a "surplus" behaviour expressed under favorable conditions. The motivation to play can indicate that the primary needs are met (Held & Špinka, 2011), and play in a test arena has been though to reflect play in the home environment (Mintline et al., 2012). Because all calves played, perhaps this arena-straw-playtest could be a tool in future studies to stimulate play. Though a small sample size with only descriptive data, this test could be useful to explore management differences in dairy calves. For example, descriptively the CCC calves may have expressed lower frequencies of locomotor play behaviours than calves in the other treatments. This is similar to other studies where CCC calves seemed less motivated to perform locomotor play in an arena (Wagner et al., 2013; Waiblinger et al., 2020). Waiblinger et al. (2020) reported that their artificially reared calves, housed in a dynamic group with more space per calf but lower milk allowance than in our study, showed more locomotor play in a test situation than their CCC calves. However, they saw more play behaviour in their CCC than artificial treatment in the home pen, which may indicate that although the artificially reared calves had companions, their space allowance may have limited their ability to express locomotor behaviour in the home pen, causing a rebound in the test arena. Play behaviour was not recorded in the home pens of our test, but similarly, CCC had bigger space (133.5 m^2 + creep 21.3 m^2) than both EC and MC.

This project was designed to find new indicators of positive welfare, by assuming that CCC, EC and MC calves have the potential, by design, to perform a decreasing degree of natural behaviours. Perhaps, less play in the test arena indicates that the space in CCC calves home pen was sufficient and that the motivation to perform locomotion was fulfilled. Thus, space allowance in the home pen of our study might have had an impact on play behaviour in the test arena, and recording play in both home pen and the arena may have clarified any potential treatment differences in the straw play test. In a different trial, one could investigate if CCC calves play more than calves kept without the dam but otherwise kept under similar conditions (space, milk allowance etc.). The current play test design (small testing arena for only 10 min) may not be a stand-alone indicator for the calves' welfare, but could be a useful tool to elicit play. If one assumes that CCC calves would have higher welfare because they were housed with their mother and satisfied their motivation in the home pen, perhaps limited space resulting in a rebound can tell us that EC and MC calves had lower welfare due to being deprived of some of their motivated behaviours. Perhaps play can be an indicator of positive emotions and mood in a specific environment rather than an indicator of positive welfare in general, as play could be more prominent if the animal feels well (Held & Špinka, 2011).

Pair-housed calves tend to express more locomotor play than individually housed calves in their home pen (Duve et al., 2012; Zhang et al., 2021). However, similar to Waiblinger et al. (2020) and Wagner et al. (2013), our calves had companions, though with differences in duration and space allowance. EC and MC calves appeared to have similar results regarding locomotor play, indicating that there may not have been a substantial difference in welfare between the two groups. Our play test may not be suitable to indicate positive welfare differences that were implicated in these two treatments, given that play would be a positive welfare indicator. For example, although EC calves had more space (MC: 11.5 m^2, EC: 23.1 m^2), the space might still be insufficient to fulfil their motivation for locomotor play, thus they may play as a form of a rebound behaviour when moved to a new space or given more space. Even though EC calves had bigger space in their home pen than the test arena (23.1 m^2 vs. 16.6 m^2), they still got more space per calf in the test arena due to fewer calves (group pen: 3,85 m^2/calf, test arena: 8.3 m^2/calf). Play has been reported to occasionally increase in stressful situations as a displacement behaviour, such as when parental care is reduced (Held & Špinka, 2011). Our CCC calves did not appear to play more than EC and MC calves. Perhaps, the CCC calves in our study experienced some stress from their separation from their mother, and were more focused on returning to their mother in the home pen than engaging in the play test. This might be expressed in other behaviours such as exploring as they try to gain information about the environment.

Our study did not show any big differences in straw play between the treatments due to large variation. However, it appeared that the MC calves may have played more than CCC and EC calves. There also appeared to be a large variation between MC calves (0 – 29.4 sec), while CCC (0 – 5.4 sec) and EC (0 – 12.7 sec) calves had a lower variation within the group. Perhaps the deprivation of straw in MC calves made some calves motivated to play with the straw as there can be individual differences, or maybe the straw itself is of no interest, but can still stimulate play. Maybe a higher number of calves in our test could have made the difference more prominent, and considering that the results are in seconds; perhaps the difference is small and overlapping. Jensen et al. (1998) saw a peak in play behaviour in response to straw. However, Jensen et al. (1998) conducted their test in the calves' home pen with different space allowances, whilst our test added straw both in the home pen (not recorded behaviour) and in a test arena (recorded behaviour). The MC calves in our study did not receive straw after they were moved to the group pen at approximately 26 d of age and the lack of straw in the home pen may have contributed to their time spent exploring the straw (MC: 4.8 ± 2.8 min, EC: 2.1 ± 1.0 min, CCC: 2.9 ± 2.0 min), and playing with it (MC: 14.1 ± 11.6 sec, EC: 5.3 ± 5.5 sec, CCC: 1.8 ± 2.2 sec). Thus, MC appeared to have more interaction with straw in total (mean: 5 min) than EC (mean: 2.2 min) and CCC calves (mean: 2.93 min). Other studies saw no effect of adding physical enrichment to the home pen on locomotor or social play (Zhang et al., 2021), or saw a very low duration of play behaviour when straw was added to the home pen (Duve et al., 2012). Perhaps other studies results would have differed if they tested the calves in a test arena, which could have given the calves more space to express their motivation. They tested calves individually in their home pen, whilst ours were group housed and tested in pairs in an arena, as group housing may have welfare benefits as it provides opportunity for socialization and access to more space (Chua et al., 2002).

My next prediction was that calves with lowest space allowance (i.e., MC) may express more locomotor play due to less space in the home pen and be more reactive and aggressive. It is suggested that play deprivation can increase fear and uncertainty, and aggressive behaviour towards conspecifics (Špinka et al., 2001). Our results show that MC appeared to not differ substantially from EC treatments in play or aggressiveness (for example, butting calves). This is similar to Jensen (2001) who did not observe aggression in the presence of another calf in a test arena. It appeared that CCC calves used more time butting calves and fixtures, although with individual differences. Butting might be considered an agonistic behaviour, but social play can include contact play such as butting and mounting (Jensen et al., 1998; Valnickova et al., 2015; Waiblinger et al., 2020; Zhang et al., 2021). Perhaps CCC calves engaged in more social play than EC and MC because they were housed with their mothers and have been exposed to subordinate positions, and that they were more interested in other types of behaviour than locomotor play. Other studies have seen a bigger rebound in play during open-field and social tests, when given access to more space (Jensen et al., 1999; Rushen & de Passillé, 2014; Valnickova et al., 2015). MC calves in our study did not appear to show more locomotor play than EC calves, but appeared to express more than CCC calves, which may indicate that both groups experienced more motivation to perform locomotor play in an arena. Play is often associated with a more relaxed and secure state, but exploration can be associated with fear, and exploration may also be associated with play; thus, moderate amounts of fear may through exploration lead to play (Špinka et al., 2001). However, MC calves appeared to use less time exploring the environment, and did not appear to withdraw more than EC calves. Both EC and MC calves appeared to withdraw more than CCC calves, and perhaps they were more affected by fear and observed more from afar. Other studies on rebound did not habituate their calves to their test arena. All calves in our study were habituated to the test arena with their companion, which may have made the environment less novel and the calves more comfortable in the environment. Also, the habituation time (30 min), may have given the calves an opportunity to rebound as the first exposure may elicit more play than subsequent others. Thus, the results might have been different if the calves were not habituated the day before. In addition, behaviours such as running can have great variation from day to day (Mintline et al., 2012), and age or familiarity with the arena (Rushen & de Passillé, 2014), or longer confinement (Jensen, 2001) can increase the motivation to perform locomotor play and trotting.

Our calves were paired randomly within treatment. Testing in pairs means that the calves can influence each other's behaviour. For example, if one of the calves is more confident in their environment while the other is not, the less confident calf might express behaviour such as withdrawal or inactivity, in response to the behaviour of the other calf. To overcome this concern the behaviours for each pair was averaged. Other ways to handle this could be to test the calves individually, or test each calf several times with different partners. Calves may be more active and express more locomotor play when tested in pairs than individually (Jensen, 2001), and multiple testing may affect the locomotor play itself because of the familiarity to the arena or lower motivation. Perhaps a play partner can stimulate locomotor play, although it may alter the other calf's behaviour in other ways; indeed, Bertelsen and Jensen (2019) saw that calves deprived of arena access performed more parallel locomotor play. It has been suggested that parallel locomotor play can be contagious (Held & Špinka, 2011), represent a social interaction, and therefore is a form of social play (Gomendio, 1988). How much habituation time that should be provided, and perhaps finding an equilibrium between too much and too little habituation, can be a focus of further research, since it can affect the level of novelty and motivation to play.

My third prediction was that calves given the lowest milk allowance (i.e., MC) would use less energy on play and active behaviour. Our results suggest that there appeared to be a small difference between treatments, but MC calves (milk allowance 7L/d) did not appear to play less or cross fewer quadrants (active behaviour) than EC calves (milk intake average 12L/d). This result differs from several other studies (Duve et al., 2012; Jensen et al., 2015; Krachun et al., 2010) who saw that calves with low milk allowance spent less time playing, but similar to Rushen and de Passillé (2014) that did not see any effects of feed level (3L milk/d until week 8, then 8L/d vs. 2.25 L/d, then 6L/d) on running and jumping when the calves were approximately 10 weeks old. Other studies have used a lower milk allowance (5-6L milk/d) than our test (7L milk/d) and although it is still a lower amount of milk, perhaps the primary needs were more fulfilled. Though, we don't know if each calf in MC consumed 7L/d, because they were given pooled milk from two milk bars in the group pen. Thus, the calves who drank milk more slowly might have had less than 7 L/d when they moved to the group pen. Perhaps a 10 min test is insufficient to capture differences in surplus energy when calves are fed at least 7 L/d and *ad libitum* concentrates, hay and silage. Maybe they would have expressed less play and active behaviour due to less energy over time if tested for longer. In addition, the calves had no restriction on solid feed. Thus, they may not have been energy deprived depending on whether they ingested more solid feed compared to the other treatments or not. Perhaps their willingness to eat was confounded if they found the food novel. If that was the case, the effect could have been negated due to the gaining of pen mates, since social housing can stimulate concentrate and solid feed intake (Costa et al., 2015; Jensen et al., 2015). Though, Jensen et al. (2015) saw that social housing stimulated concentrate and solid feed intake for their enhanced-fed calves (first 9L milk/d, then reduced to 5L/day) and not with standard milk allowance (5L/d). Calves fed a lower amount of milk can show increased motivation to ingest solid feed due to hunger. How much effect social housing can have may vary, and the critical time is between 3 – 6 weeks of age (Costa et al., 2015). Thus, our MC calves might have benefitted from social housing as they were group housed at approximately 4 weeks of age. Our calves were tested at approximately 8 weeks of age, perhaps earlier testing would have made the milk restriction more prominent as the calves would have been more dependent on milk, or that they had less time with pen mates and therefore given less benefit of social housing.

My last prediction was that calves reared in a more complex social environment and higher milk allowances (EC and CCC) may use less time to explore straw and spend more time exploring the environment. Our results show that there appeared to be no difference between the treatments regarding exploring the straw. However, it appeared that both EC and CCC spent more time exploring the environment than the MC calves. When changing the environment, the calves' curiosity may elicit an exploratory behaviour toward objects. Perhaps access to straw in the home pen made other items in the test arena (e.g., tarp) attract the calves' attention. The more complex social environment of EC and CCC may have stimulated their curiosity in the test arena more than for MC calves. Other studies (De Paula Vieira et al., 2012; Lv et al., 2021; Zhang et al., 2021) have reported that group housed calves expressed more exploratory behaviour than individually housed calves, and Jensen et al. (1997) suggested that individual housing early in life could delay exploratory behaviour. However, Santo et al. (2020) saw no effect on exploratory behaviour based on whether the calves were raised with their mother or not. All our calves were group housed before being tested in the arena and had additional straw, even though MC calves were individually housed first, they had olfactory, visual and limited tactile contact with other calves, and maybe this eliminated some of the effect of individual housing. For example, Zhang et al. (2022) saw no difference in housing treatment on their calves' exploratory behaviour, maybe because even though their calves were individually housed, they could have olfactory, visual and limited tactile interactions with other calves. Exploratory behaviour has been thought to be linked with play behaviour. Rushen and de Passillé (2014) saw that the calves that explored the environment more in their test, showed more play behaviour such as jumping and running. Our results differ from this, since MC calves appeared to express more locomotor play than CCC, and CCC calves appeared to explore the environment more than MC, while EC calves may support this link more since they appeared to both express locomotor play and exploratory behaviour toward the environment.

Though we tried to control for different concerns, there were limitations in this study. First, the sample size (n = 17) of this project might not be representative for the population, and minimal was a descriptive term which might not be representative of other farms, regions, or countries. Although similar research has had approximately the same sample size on average. The arena size (4.90 x 3.39 m) and design (squared) could have had an impact, as long-shaped/elongated areas make the calves run more (locomotor play) than quadratic ones (Mintline et al., 2012). While one of the entrances was closed with a garage door (used for EC and MC calves), the other was closed off by moving and binding the fence in place (used for CCC calves). Perhaps calves behaved differently depending on which entrance was used, thus affecting the time spent exploring and butting fixtures, or inactive behaviour. There was a gap in the fence where some of the calves put their heads through and seemed to want out of the arena. Perhaps as an attempt to get back to the dam, and closing that gap could have limited the possibility to put their heads out. The garage door could have been novel as it moved and made noise, some of the calves were inactive and watched it close, while some tried to exit the arena before it closed. Working with individuals also means that there will be individual differences, and outliers within each treatment may have an impact on the total result. Our MC calves were individually housed first, but moved to group pens later. Perhaps they would have shown more of other behaviours, and differences may have been more pronounced, if they were housed individually until the test.

The ethogram was based on other published papers and was based on, and connected to, positive behaviours. Thus, chewing and sucking at the tarp in the test arena was not considered a sign of hunger, but rather as exploring the environment or butting fixture. It looked like some of the calves were more interested in the tarp than the straw, and maybe they thought it was more fun with something that moved and made sound than the straw on the floor. Some calves withdrew because of the tarp. Some approached and explored the tarp again, or were inactive while looking at either tarp or the other calf playing with it. Future studies should consider using arenas with solid walls, or walls of the same material.

6. Conclusion

Play behaviours of calves in different management systems during a play test using straw as a play-object has been described. In conclusion, our play test stimulated play in Norwegian Red calves. There appeared to be slight differences between the treatments. EC and MC calves appeared to be more similar in locomotor play behaviour and appeared to play more than CCC calves. It did not appear to be a difference in reactive or aggressive behaviour as withdrawal happened infrequently and all calves spent <10 seconds head butting other calves. EC and CCC calves appeared to explore the environment more than MC calves. Positive welfare in CCC, EC and MC systems should be further investigated.

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