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Uppsala 2016

Animal Science – Master's programme

Studentarbete
Sveriges lantbruksuniversitet
Institutionen för husdjurens miljö och hälsa

Nr. 674

Student report
Swedish University of Agricultural Sciences
Department of Animal Environment and Health

No. 674

ISSN 1652-280X

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Student report 674, Uppsala 2016

Animal Science - Master degree project, EX0567, 30 ECTS, A2E
Animal Science – Master's programme

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Keywords: dairy calves, calf hutches, redirected suckling, cross-sucking, growth rate
Nyckelord: kalvar, kalvhyddor, omriktat sugbeteende, tillväxt

Series: Student report / Swedish University of Agricultural Sciences
Department of Animal Environment and Health
no. 674, ISSN 1652-280X

Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
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1. Summary

Cows are social animals but today dairy calves are generally housed in single-hutches or -pens instead of together with other calves during the first period of their lives. Concerns are that housing calves in pairs or groups increases the risk of health problems such as diarrhoea and respiratory diseases and also increases the occurrence of redirected suckling behaviours including cross-sucking. Especially cross-sucking is problematic as it can lead to injuries to the receiving calf and calves performing the behaviour can continue to perform the behaviour when older.

The aim of this study was to test the effects on dairy calves (*Bos taurus*) of single vs. pair-housing in outdoor calf hutches on growth, general health and redirected suckling behaviour. In total 21 calves of Swedish Red and Swedish Holstein cattle were used whereof 14 calves were housed in pairs and 7 were single-housed. Both treatments were given six litres of whole milk per calf divided into two meals per day in teat-buckets. They also had access to an empty, clean teat-bucket at all hours of the day except during feeding. Calves were kept individually in calf hutches until they joined the study at day ten. Cases of diarrhoea and other diseases were recorded daily and the calves were weighed weekly. Temperature was recorded in four hour intervals. Behavioural observations were done on day 14 and then twice per week for each hutch until eight weeks of age. Recordings from behavioural observations during week 2-6 were included in the statistical analysis. Behavioural observations were made by continuous recording ten minutes before milk feeding and 20 minutes after the calves received their milk in the morning and afternoon. Non-nutritive sucking and cross-sucking were considered redirected suckling total and were grouped to test separately from only cross-sucking. Both single- and pair-housed calves were able to perform cross-sucking.

All data except diarrhoea were tested for normality and homogeneity assumptions with the Shapiro-Wilk test and Bartlett's test, respectively, and all variables were square root transformed to provide normal residuals. Not normally distributed after data transformation was tested with a non-parametric Wilcoxon rank-sum test with continuity correction. REML analysis was performed on weight and behaviour, and ANOVA was also used on weight gain. Diarrhoea was tested with a Chi^2-test.

Weight gain was not affected by treatment or temperature (N.S.). Pair-housed calves had more diarrhoea than single-housed calves ($P<0.05$). Pair-housed calves had a higher frequency of drinking milk ($P<0.001$) while single-housed calves licked fixtures more often than pair-housed calves before receiving milk ($P<0.05$). Pair-housed calves performed all registered cross-sucking both before and after receiving milk ($P<0.01$). There was no significant difference in redirected suckling total (non-nutritive sucking and cross-sucking) between treatments before receiving milk (N.S.), but after milk delivery pair-housed calves performed more redirected suckling total than single-housed calves ($P<0.01$). There were no sucking induced injuries found on any of the calves and no coughing was recorded nor any antibiotics prescribed to the calves.

In conclusion, as diarrhoea and cross-sucking have negative impacts on calves' welfare more research is needed in how to be able to house dairy calves in a way where they are able to perform social behaviours without a negative impact on welfare in terms of an increase of health issues and subjection to cross-sucking.

2. Sammanfattning

Kor är sociala djur men idag hålls kalvar generellt enskilt i boxar eller kalvhyddor under den första perioden av sitt liv. Att hålla kalvar i par eller grupp anses öka risken för diarré och luftvägssjukdomar samt för omriktade sugbeteenden både mot omgivningen och andra kalvar. Speciellt sugandet på andra kalvar är problematiskt då det kan leda till skador på den utsatta kalven och kalvar som suger på andra kalvar fortsätter ofta med beteendet även i vuxen ålder.

Syftet med denna studie var att se hur parhållning jämfört med individuell hållning av mjölkraskalvar (*Bos taurus*) utomhus i kalvhyddor påverkar tillväxt, hälsa och omriktat sugbeteenden hos kalvarna. Totalt 21 kalvar av SRB och Svensk Holstein deltog i studien varav 14 kalvar hölls i par och 7 hölls individuellt. Båda grupperna utfodrades med tre liter helmjölk två gånger om dagen i napphinkar. En tom, ren napphink var också fäst vid kalvhyddan hela tiden förutom under utfodring. Fall av diarré och andra sjukdomar registrerades dagligen och kalvarna vägdes en gång i veckan. Temperaturen registrerades var fjärde timme. Varje grupp gick in i försöket dag tio och hade fyra dagars tillvänjning före beteendeobservationer som genomfördes dag 14 och därefter två gånger i veckan till åtta veckors ålder för varje hydda. Data från beteendeobservationer under vecka 2-6 var inkluderade i den statistiska analysen. Beteendeobservationerna genomfördes som en kontinuerlig registrering tio minuter före mjölkutfodring och 20 minuter från det att kalvarna fått sin mjölk i samband med morgon- och kvällsutfodring. Sugbeteenden riktat mot omgivningen och mot andra kalvar sågs som totala mängden omriktade sugbeteenden och analyserades separat från omriktat sugbeteende enbart mot andra kalvar. Både kalvar som hölls enskilt och i par kunde suga på andra kalvar.

All data förutom diarré testades med Shapiro-Wilk test och Bartlett's test och alla variabler var kvadratrot-transformerade för att generera normala fördelningar av data. Data som trots det inte var normalfördelad testades med Wilcoxon rangsumme-test med kontinuitetskorrigering. REML-analys utfördes på vikt och beteenden och på viktökning utfördes även ANOVA. Diarré testades med ett Chi^2-test.

Tillväxten påverkades inte signifikant av behandling eller temperatur (N.S.). Parhållna kalvar hade diarré oftare än individuellt hållna kalvar under hela studien ($P<0,05$). Parhållna kalvar hade en högre frekvens av beteendet "dricka mjölk" är individuellt hållna kalvar ($P<0,001$) medan individuellt hållna kalvar slickade mer på omgivningen än parhållna kalvar ($P<0,05$). Parhållna kalvar utförde alla registrerade sugbeteendena riktade mot andra kalvar både före och efter att de utfodrats mjölk ($P<0,01$). Ingen skillnad i totala mängden omriktade sugbeteenden sågs innan kalvarna utfodrats mjölk (N.S.) men däremot efter där parhållna kalvar utförde totalt fler omriktade sugbeteenden än individuellt hållna kalvar ($P<0,01$). Inga skador på grund av omriktade sugbeteenden eller hosta registrerades och ingen antibiotika användes under studien.

Slutsatsen är att då diarré och omriktat sugbeteende mot andra kalvar har en negativ inverkan på kalvars välfärd behövs det mer forskning inom området för att få fram en hållningsform där kalvar har möjlighet att utföra och utveckla sociala beteenden utan en ökad risk för sjukdomar eller att utsättas för fler omriktade sugbeteenden.

3. Introduction

Cows are social animals that under natural or semi-natural conditions live in groups with their calves (Chua *et al*., 2002). Today dairy calves kept for replacement in dairy farms are generally separated from their dam directly after birth and housed separately from their dam in single-pens or calf hutches to minimize the risk of transmissions of diseases (Loberg & Lidfors, 2001; Phillips, 2010). The calves are moved from their pen or hutch into group-pens before or after weaning and later on integrated in production where they are ultimately housed in groups (Marcé *et al*., 2010). This gives the calf a need to learn certain social skills to successfully interact with future group mates. Jensen *et al.* (1997) suggested that at three months of age single-reared calves are more fearful than group-housed calves when meeting an unfamiliar calf. The lack of social stimuli experienced by single-reared calves may also affect calves' responses to a new social situation or when isolated in a novel environment. de Paula Vieira *et al.* (2009) has also seen that pair-housed calves shows a higher behavioural flexibility than single-reared calves as pair-housed calves visited a feeder more often, spent more time at the feeder, and started eating concentrate more rapidly after moving to group-housing. A risk of keeping calves in pairs is cross-sucking; Lidfors (1993) defined cross-sucking as an abnormal behaviour of non-nutritive sucking directed toward another calf's head or body. Cross-sucking may lead to health and welfare problems for the exposed calf and cause inflammation of penis, navel or scrotum, or may lead to hairless body parts (Jung & Lidfors, 2001). But if the calves have unlimited access to a teat-bucket the calves might direct non-nutritive sucking from other calves towards the teat (de Passillé, 2001; Jensen & Budde, 2006). In pairing calves, the calves' get the opportunity for social interactions and it can lead to a higher feed intake due to social facilitation (Bøe & Færevik, 2003; de Paula Vieira *et al*., 2009). Keeping calves in pairs or groups is therefore of interest as it hopefully leads to a higher growth rate and more social adaptable animals.

4. Literature review

4.1. Calf hutches

Outdoor calf hutches have become more and more popular during recent years, one of the reasons being that calf hutches usually are a less expensive system than conventional barns. They are less expensive to build and maintain and do not require any mechanical ventilation (Sanders, 1985). Calf hutches are either a four sided structure (Stull & Reynolds, 2008) or are igloo shaped (Marcé *et al*., 2010) and should be placed on solid concrete (Mohler *et al*., 2008) or on well-drained soil (Stull & Reynolds, 2008). They are commonly constructed of fiberglass, polyethylene or wood and usually have an outdoor pen attached to it or the calf is tethered to the hutch (Stull & Reynolds, 2008). Calf hutches can be either for holding one calf or for group housing. When constructed for one or two calves the calf hutch has holders for water and roughage on the outside of the pen and a holder for concentrate inside the hutch. During January and February in Hokkaido, Japan, with an average mean outdoor air temperature between -7.1 °C and -10.1 °C Okamoto *et al*. (1993) saw that the air temperature in the calf hutch was +2 to +6 °C higher than the outdoor temperature.

4.2. Seasonal differences

Because the hutches are kept outdoors, calves are more exposed to the weather and the climate in the hutches is affected much more by outdoor weather than is the climate inside a barn. During summer it can become extremely hot inside the hutches (Mohler *et al*., 2008) and the position of the hutches plays an important role (Spain & Spiers, 1996). If the hutches are placed in the sun during the warmest time of the day, Spain and Spiers (1996) observed that the calves were more heat stressed with higher skin temperature and respiration rates than those in calf hutches kept in the shade. Placing the hutches in the shade reduces the risk of heat stress for the calves as the hutch air temperature was lower when in the shade (Spain & Spiers, 1996). Cold or heat stress has a larger impact on young, sick or injured animals than on mature and healthy animals (Stull & Reynolds, 2008). According to Spain and Spiers (1996) exposure of dairy cows to ambient temperatures outside their thermal comfort zone can have an adverse effect on cow performance. Thermal comfort is the animals' thermal neutral zone and this means according to Webster (1978) the temperature span in which the animal maintains body temperature by contraction or dilation of blood vessels, changing behaviours or postures to conserve or dissipate heat, sweating and panting, and changes in the hair coat insulation properties. For young calves the thermal neutral zone range from 15 °C to 25 °C (Phillips, 2010). If the temperature drops below the lower critical temperature, for calves 15 °C, the calf needs to divert energy to maintain its core body temperature (Phillips, 2010). However Okamoto *et al*. (1993) saw that it is possible to maintain calf growth at temperatures as low as -20 °C in sheds or hutches as long as the calves had access to enough bedding material for thermal comfort and enough feed. Cold exposure can lead to increased feed intake because of a higher energy demand due to thermoregulation (Phillips, 2010).

4.3. Redirected suckling behaviour

Survival of the young mammal depends mostly on suckling success and according to de Passillé (2001) this means that a strong suckling motivation in the calf is needed to reassure the desired milk intake. Sucking deprivation could therefore result in frustration which can have a negative impact on calf welfare (de Passillé, 2001). Non-nutritive sucking leads to an increase of cholecystokinin in the blood (de Passillé *et al*., 1993) which is involved in satiety and can be a short-term hunger inhibitor (Rushen & de Passillé, 1995). Redirected suckling behaviour is non-nutritive sucking on the surrounding or other calves and is a redirection of natural suckling behaviour. de Passillé *et al*. (1992) saw that the ingestion of milk stimulates sucking and bunting at a teat-bucket, which according to the authors can mean that the milk is a stimulus that induces redirected suckling behaviour. Lidfors (1993) develops this further and suggests that redirected suckling behaviour could be a natural redirected response from the calf since calves suckling their mother changes teat when the milk flow decreases and the behaviour can mostly be seen when the milk is finished. Bunting is the response to a low milk flow were bunting serves to stimulate the milk let down in the beginning of a meal and to empty the udder at the end (Lidfors *et al*., 1994). Drinking water stimulates little sucking even when the cues associated with milk feeding, such as hearing other calves drink, the smell of milk or feeding at the milk feeding time, was present (de Passillé *et al*., 1992) but even ingestion of a small amount of milk can trigger redirected suckling behaviour (Rushen & de Passillé, 1995). Redirected suckling behaviour generally starts directly after the milk is finished and decreases in a similar way to when calves suckle their dam (Lidfors, 1993; Lidfors, 1994). A sucking bout from the cow udder takes 8-12 minutes (Lidfors *et al*., 1994) and ingesting three litres of milk from a teat-bucket only takes a few minutes (Loberg & Lidfors, 2001). The calves' motivation to suckle is then redirected

towards either the empty teat (non-nutritive sucking), its surroundings or towards other calves ear, mouth, scrotum, prepuce, tail, udder area or navel (cross-sucking) (Lidfors, 1993). Factors that affect the time for milk intake from a teat-bucket is among others age of the calf and the size of the hole in the teat (Loberg & Lidfors, 2001). In this paper redirected suckling towards the empty teat or the surroundings will be referred to as non-nutritive sucking and sucking directed towards another calf as cross-sucking. Redirected suckling will be used as a collective term for both behaviours in the text. Most redirected suckling occurs during the first 10-15 min following milk feeding (Lidfors, 1993). If the empty teat-bucket is removed after milk intake it will increase the occurrence of cross-sucking, so leaving an empty teat-bucket after milk intake will reduce cross-sucking since the calves then can continue to suckle the teat instead of redirect the sucking towards other calves (Jung & Lidfors, 2001). Feeding by teat-bucket also allows calves to express their natural sucking reflex during feeding and the calves can keep on sucking the empty teat-bucket after the milk is ingested (Jung & Lidfors, 2001). According to Loberg and Lidfors (2001) it seems that the motivation for sucking is reduced with the possibility to perform the behaviour and the time spent ingesting milk. This is mostly in line with what Rushen and de Passillé (1995) concluded; the motivation for sucking is reduced more by performing the non-nutritive sucking behaviour itself than by ingesting milk. They also saw that reinforcement, in form of a teat, is not necessary to maintain a non-nutritive sucking as it is stimulated by the taste of milk (Rushen & de Passillé, 1995).

4.3.1. Cross-sucking

A risk factor with pair-housed calves is the occurrence of cross-sucking (Jensen, 2003) and a calf which cross-sucks before weaning is likely to continue after weaning (Keil & Langhans, 2001). Intersucking in dairy cows, i.e., when cows are sucking on the udder of heifers or other cows, can lead to udder damage, mastitis, milk loss and culling of breeding animals (Keil *et al.*, 2001). Cross-sucking is seen in artificially reared calves but not in suckler calves (Lidfors, 1994: Jung & Lidfors, 2001). The behaviour stems from a redirection of the natural sucking behaviour (Jensen, 2003). Keil and Langhans (2001) concluded that cross-sucking was most frequent around milk feeding but it was observed nearly all hours of the day. Lidfors (1993) found that the frequency of cross-sucking decreased with time after milk feeding and it ceased within 15 minutes after milk feeding. Also single-housed calves have be seen cross-sucking on the neighbouring calves' ears and mouth (Chua *et al*., 2002). Cross-sucking may be reduced by using an artificial nipple or teat-bucket at milk feeding rather than a regular bucket, or by providing a non-nutritive artificial teat following milk feeding (de Passillé, 2001; Jensen & Budde, 2006). Leaving the teat-bucket or providing an artificial teat for the calves at all hours could reduce redirected suckling, because the possibility for non-nutritive sucking on a teat after intake of a small quantity of milk has been seen to reduce the duration of redirected suckling later on (Rushen & de Passillé, 1995). The amount of milk fed has an impact on performance of cross-sucking: when calves received either five litres or only 0.1 litres there was almost no cross-sucking compared to when the calves received one or 2.5 litres during weaning (Jung & Lidfors, 2001). According to Jung and Lidfors (2001) this was because the higher amount of milk satisfied the calves and therefor reduced cross-sucking whereas some drops of milk were too little amount to induce the behaviour. Jensen (2003) summarizes that the best way to prevent cross-sucking is to offer the opportunity for the calves to fulfil their need to suck in connection with the ingestion of milk, preferably by teat-buckets which are left for a period of time after feeding.

4.4. Pair versus single housing

According to EU-legislation calves above eight weeks must be group housed (Council Directive 97/2/EC, 1997) and for calves in organic farming group housing is a must from one week of age (Council Regulation 1804/1999/EC, 1999). In Sweden calves in conventional farms are generally single-housed for two weeks after they are born and then either stays in single-housing (60% to 65% of the calves) or moving to group-housing (Marcé *et al*., 2010). Stull and Reynolds (2008) conclude that the general objectives for housing of dairy calves are to provide an environment where the calves are protected from thermal and climatic extremes. This is achieved by providing proper feed that are accessible to each calf, to ensure that the calves are safe from environmental factors that can injure them and to provide an environment where the calves health and welfare can be monitored (Stull & Reynolds, 2008). According to Stull and Reynolds (2008) both single and group-housing systems can be designed to meet these demands. During single-housing calves must be allowed to have visual and tactile contact with other calves (Council Directive 97/2/EC, 1997). In single-housing the calves' opportunities for locomotion and social behaviours are restricted and are therefore not recommendable from an animal welfare point of view (Broom, 1990).

4.4.1. Feed intake and weight gain

Replacement calves are generally weaned from milk at 8 weeks of age in Sweden (Marcé *et al* 2010), while in feral cattle suckling can continue until the cow has her next calf, which may be one year or even more after the birth of the first calf (Phillips, 2010). In response to weaning from milk, calves generally show increased vocalizations and activity in combination with a period of growth check (Weary *et al.,* 2008). In contrast, Chua *et al*. (2002) observed that pair-housed calves continued to gain weight at twice the rate at week six during the weaning period compared to single-housed calves. de Paula Vieira *et al*. (2009) found that single-housed calves vocalized three times more than paired calves did after weaning when receiving only water in their teat-buckets. During the milk feeding period, pair-housed calves had a higher intake of concentrate than the single-housed calves which could be because of social facilitation (de Paula Vieira *et al*., 2009). This was also seen in the calves in the same study after the calves was weaned and moved to group-housing. After weaning and moving to group-housing, previously paired calves had a shorter latency to start feeding, spent more time at the feeder, visited the feeder more often and consumed more starter than previously single-housed calves (de Paula Vieira *et al*., 2009). de Paula Vieira *et al*. (2009) suggested that pair-housing of calves during the milk feeding period reduces the calves' negative responses to weaning and improves their performance after weaning in a group-housing system. One reason for not holding calves in groups before weaning is the concern that it could have a negative effect on calf health and performance (Radostits, 1974; Kung *et al.,* 1997). Chua *et al*. (2002) observed no difference in growth rate between group-housed calves and single-housed calves before five weeks of age and de Paula Vieira *et al*. (2009) found no difference in growth rate before or during weaning. On the other hand, Terre *et al.* (2006) found that group-housed calves had a lower growth rate than single-housed calves. Because the management practice and experimental designs varies between the studies caution should be taken while comparing them. Weight gain during early life can have an effect on future milk yield. Van Amburgh (2008) found that heifers that had a daily weight gain of 0.91 kg/day produced 612 kg more milk during their first lactation compared to calves that had a lower weight gain. Weight gain is therefore of importance for dairy producers as it has an impact on the future production and economics of the farm.

4.4.2. Social interactions

Under semi-natural conditions calves spend most of their first week alone hiding or together with their dam, isolated from the herd (Bøe & Færevik, 2003). After their first week they spend more time with other calves and at around two weeks of age their social interactions with other calves' intensifies and social interactions, excluding suckling behaviour, with other calves exceeds those with their dam (Bøe & Færevik, 2003).

In dairy production systems, when the calves are weaned, they are moved from their pen or hutch into group pens and later on integrated in the dairy farm where they are ultimately housed in groups (Marcé *et al*., 2010). Placing the calves in group-housing early in life gives the calves' access to social contact with other calves, which can facilitate the development of appropriate social and herd behaviours (Bøe & Færevik, 2003). According to Holm *et al.* (2002) prefers calves to have full social contact, which is the case in group-housing, rather than social contact through bars as it is in single-housing. Jensen *et al*. (1997) showed that calves kept single-housed showed less exploratory behaviour and were more fearful of unfamiliar calves than calves that had previous social experience, suggesting that lack of social experience can have an effect on how the calf responds to isolation in a novel environment. This difference in exploratory and fearful behaviour between single-housed calves and group-housed calves was not found at six months of age after all calves had been tethered for three months after the study (Jensen *et al*., 1997). But in farm conditions calves are seldom first reared in groups and then single-tethered so the practical implementation of these results should be interpreted with care. The difference in exploratory and fearful behaviour can indicate that group-housed calves may have a higher behavioural flexibility than single-housed calves, giving them the ability to modify their behaviour in unfamiliar situations such as mixing with unfamiliar calves in a new environment (de Paula Vieira *et al*., 2009). Single-housed calves must learn to cope with conspecifics as well as for example learn to use the feeder at mixing. Pair-housed calves can use previous social experience to observe and imitate the behaviour of the other calves in the group and thus learn to use the feeder much faster (de Paula Vieira *et al*., 2009).

4.4.3. Health

The most common health disorders in calves are enteric and respiratory diseases; diarrhoea and respiratory diseases are most common in calves during their first three months of life (Marcé *et al*., 2010). They are associated with lower growth rate and/or death and have an economic impact for the farmer in form of treatment costs, lower growth rates and replacement capacity of the herd (Marcé *et al*., 2010). Enteric and respiratory pathogens can be transmitted through contact between calves, depending on the management and calf housing system (Macé *et al.*, 2010). A risk factor that increases when keeping calves in outdoor calf hutches is the impact of the weather; a wet and cold calf in windy conditions can deplete its energy reserves rapidly. If the calf also is sick it can easily succumb to the disease (Mohler *et al*., 2008). It is therefore crucial that the bedding in the calf hutch is dry and in sufficient amount depending on the season.

Diarrhoea has a negative impact on the calves' welfare and some of the microorganisms causing diarrhoea have also been shown to be zoonotic (Ortman & Svensson, 2004). The diseases can infect the calf caretakers and some have been linked with foodborne diseases. Rotavirus is the most common reason for diarrhoea in neonatal calves in Sweden and it is recommend using oral electrolyte solutions as treatments (Ortman & Svensson, 2004). If a test concludes it to be bacteria causing the diarrhoea instead of a virus antibiotics can be used (Ortman & Svensson,

2004). The use of antibiotics can enhance the antibiotic resistance in bacteria which is a large problem facing the world today and so the use of antibiotics should be kept at a minimum (Ortman & Svensson, 2004).

Young calves, before weaning, are more susceptible to pathogens than older animals (Phillips, 2010) and single-housing may minimize the spread of disease (Radostits, 1974). By keeping the calves in single-housing there is minimal animal-to-animal contact which reduces the transmissions of pathogens between the calves (Mohler *et al*., 2008). It also provides the caretaker with an easy overview of the health and feed intake of each calf and permits easy access to each calf for management or medical treatments (Radostits, 1974; Kung *et al.*, 1997). In a study by Chua *et al*. (2002), calves kept single and in pairs were compared and the occurrence of diarrhoea was recognized as a health problem but there were no differences between single or pair-housed calves. However, according to Marcé *et al*. (2008), the risk of both enteric and respiratory diseases increases in group-housing of calves compared with single housing. Chua *et al*. (2002) argue that the lack of difference in occurrence of diarrhoea between single- and pair-housed calves in their study was not surprising as it is viral pathogens that often cause enteric disease which means that the type of housing will have little effect of these organisms. In addition, oral and nasal contact between the calves still occurred between the pens or hutches, independent of the housing-type, which allow faecal-oral transmissions of organisms (Chua *et al*., 2002). According to Chua *et al*. (2002) proper management of pens and hutches, such as cleaning, adequate ventilation and feeding, as well as calf immunity is of greater importance to disease susceptibility than housing system.

A health risk with cross-sucking is frostbites occurring in cold weather because of the wet area on the calf that is left after being sucked on (Ekesbo, 2011). If an ear is sucked on it can for example lead to necrosis arising and part of the ear falling off (Ekesbo, 2011). Single-housing could prevent most cross-sucking but not the underlying motivation to perform the behaviour.

4.5. Aim and questions

The aim of this study was to compare pair-housing to single-housing of calves in outdoor calf hutches and the effects on growth, general health and redirected suckling behaviour. The questions to be answered are if pair-housed calves have a higher growth rate than single-housed calves during their first six weeks after birth and if the surrounding temperature during the cold season has a smaller negative impact on growth on pair-housed compared to single-housed calves. Also if there is a difference in health in terms of diarrhoea, cough, sniffles and an increase in sucking related injuries when the calves are pair-housed compared to single-housed? Another question to be answered is if there is a higher frequency of redirected suckling behaviour including cross-sucking in pair-housed compared to single-housed calves?

Calves are social animals and therefore it is predicted that there will be an increased feed intake due to social facilitation in pair-housed calves, leading to a higher growth rate compared to single-housed calves. As the temperature inside a calf hutch is higher than the surrounding temperature when calves are kept single-housed it should be higher still when calves are pair-housed as two calves should generate more radiant heath. Calves kept in pairs in hutches can also benefit from each other's body heat to keep warm during the cold season; therefore, less of the calf's energy should be used for keeping the body temperature. If so, more energy can then be used to support growth and the immune system leading to reduced health problems. Temperature

should therefore have less of an impact on pair-housed calves' growth than on single-housed calves.

When the calves' moves into a pair-hutch it is expected that there will be a negative effect on health in terms of diarrhoea during their first week from introduction as a result of a possible higher pathogen load as the two calves now share hutch. This effect should disappear when the calves' immune system adapts to each other and no difference between pair-housed and single-housed calves should be seen after the first period together. No other difference in health is predicted.

As the calves have access to a teat-bucket at all hours of the day the amount of sucking related injuries on the calves should not increase when the calves are pair-housed compared with single-housed. As the possibility to cross-suck on another calf increases when kept in pairs that behaviour probably will increase. But the occurrence of non-nutritive sucking should be lower in pair-housed calves since they have an increased possibility to cross-suck instead.

5. Material and methods

Data collection was conducted at Swedish Livestock Research Centre at Lövsta in Uppsala between November 2015 and March 2016. The experimental procedure, including the animal handling, was within the boundaries of Lövstas ethical approval dnr: C332/12.

5.1. Animals

A total of 24 heifers of Swedish Red and Swedish Holstein calves entered the study whereof 21 heifers were included in the statistical analysis (single-housing n=7 calves, pair-housing n=7 pairs, Tab. 1). Calves excluded from entering the study were twins, bulls and if the calf had diarrhoea or general bad health when supposed to enter the study. No calves were excluded after entering the study.

Table 1 *Number of calves of each breed in single vs. pair-housing in calf-hutches in the statistical analysis*

Breed	**Single-housed**	**Pair-housed**
Swedish Red	2	8
Swedish Holstein	5	6

5.2. Housing and management

The calves were separated from their dams directly after calving and fed colostrum with a bottle (SHOOF Easy Feeder 2.5 litres) within four hours after calving. The amount suckled as the first meal was at least 2.5 litres, but calves were allowed to suckle more if they wanted. If the calves did not want to drink the first meal it was given through tube feeding (BOVIVET Calf Drencher 2 litres with rigid probe[1]). All calves were marked with ear identity tags with individual numbers before moved out to a calf hutch according to farm practise. Calves were fed three litres of colostrum in a teat-bucket twice a day during their first three days (a total of six meals). From day four until day 56 the calves were fed whole milk. Three litres of whole milk were fed in teat-

[1] http://www.kruuse.com/sv-SE/ecom/Hest_produktionsdyr/Drenching/prod_230545.aspx

buckets twice per day at around 7:00 and 18:30 o'clock. Silage, hay, pelleted concentrate (IDOL, Lantmännen[2]) and water were available *ad libitum*. Water was available in open buckets and concentrate was given inside the calf hutch in a container measuring 0.28 x 0.36 x 0.21 m (0.1 m^2). The calves in the study were managed by the personnel of the farm according to regular routines of the farm, except during milk feeding. Regular routines included scraping the outdoor pen once a day, replenish silage, hay and concentrate, giving fresh water and checking if needing to replenish with fresh straw inside the calf hutch. Calves were dehorned before 30 days of age and before the procedure they were sedated and given pain-relief by injection.

5.2.1. Outdoor hutches

After the calves were fed their first meal of colostrum indoors they were immediately moved to outdoor calf hutches (Calf-tel PRO 2[3]) where they were housed individually until selected into single or pair-housing (see below). The calf hutches measured 2.12 x 1.14 x 1.22 (~2.4 m^2) on the inside with an outside pen attached measuring 1.36 x 1.26 m (~1.7 m^2, Fig. 1). All calves were housed in the same area and all calves were able to hear and see other calves and also engage in muzzle contact with the calves in adjacent calf hutches. This allowed single calves to perform cross-sucking on the calves muzzle in the adjacent calf hutches and also to perform social grooming. The calf hutch area consisted of a concrete floor measuring 36 x 14 m with a metal roof and open sides (Fig. 1). The calf hutches were placed under the roof in two rows of 18 (n=36) with an alley separating the rows. Bedding material used in the hutches was straw.

Figure 1 *Calf-hutches used in the study and the platform where they all were placed (Alvegard, 2016)*

5.3. Design of the experiment

Calves were single-housed during the period that they received colostrum (six meals, three days). After meal six the calves could be sorted into either single or pair-housing depending on if there was another suitable calf available. Pairing was done when one of the calves was ten days old; the other calf was then from four to ten days of age. In pairing calves, the calves' weight and age was taken into account; if it differed more than 10 kg or more than one week of age between the calves they were not paired. If sorted into single-housing the calf stayed in the same calf hutch while if sorted into pairs the two calves would be moved to a new calf hutch.

[2] http://www.lantmannenlantbruk.se/sv/produktkatalog/foder/kalv-fardigfoder/idol/
[3] http://www.calftel.com/products/pro

The calf hutches were marked with letters in the order that they were included into the study (A, B, C, etc.). The calf hutch was equipped with an empty teat-bucket during all times except during milk feeding (Fig. 2). At milk feeding the personnel was not allowed to be at the calf hutch platform 20 minutes before feeding. The calves in this study were to receive milk first and afterwards the other calves at the platform could be fed. The personnel had to make sure that the calves all started to drink the milk before moving on to another hutch. After the last calf in this study had received milk the personnel was not allowed to interfere with the calves for 20 minutes and had to leave the platform if done feeding the other calves before the 20 minutes had passed. After the 20 minutes and within one hour the milk buckets were taken down and the empty teat-bucket were put back up again. The teat-buckets used for milk was taken apart and washed with water and soap. The teats were placed in Virkon S 1% (DuPont Animal Health[4]) for 5-10 minutes and the buckets and the teats were then assembled and hung up for drying. The teats were controlled regularly to ensure teat quality.

Figure 2 *Calf-hutches included in the study with the clean teat-bucket hanging on the outside (Alvegard, 2016)*

The weaning-period started at six weeks of age with reduction from three litres of milk per feeding to two litres and to one litre at seven weeks of age and the calves were considered weaned at eight weeks of age. Management of calves kept in either single or pair-housing during the study is described in Tab. 2.

Table 2 *Management of calves kept in either single or pair-housing during the study*

Age	**Treatment**
Birth	Received colostrum within 4 hours of birth Weighed Moved out to single-hutch after having received colostrum
1-3 days	Received colostrum during first 6 meals
4 days	Weighed
4-10 days	Paired with suitable calf of 10 days of age
7 days	Weighed
10 days	Weighed and if no suitable calf had been found calf was selected to single-housing
14 days	Based on oldest calf first behavioural observation. Weighed
3-8 weeks	Weighed once a week and behavioural observation twice a week
6 weeks	Weaning started and daily milk amount decreased from 6 to 4 litres
7 weeks	Daily milk amount decreased from 4 to 2 litres
8 weeks	Calves considered weaned and were weighed. Last behavioural observation

[4] www.virkons.com

5.4. Behavioural observations

Behavioural observations were done on each hutch twice a week between 2-8 weeks of age, where week was based on the age of the oldest calf in the hutch. Each calf hutch was observed in conjunction with milk feeding at 7:00 and 18:30 o'clock. The observer took place in front of the calf hutch 20 minutes before feeding (6:40 or 18:10 o'clock), giving 10 minutes for the calves to habituate to the observer and then started observing for 10 minutes before feeding. After the calves had received milk the observation continued for 20 minutes. During each observation both calves in the calf hutch, if kept in pairs, were observed.

Continuous recording in one minute intervals was done. Behaviours recorded were redirected suckling behaviours, bunting, ingesting milk, sucking on empty teat, grooming and inactivity (Tab. 3). If other behaviours were performed that was not defined they were noted under "Other activity".

Table 3 *Ethogram adapted from Jensen and Budde (2006)*

Behaviour	**Definition**
Ingesting milk	The calf is ingesting milk by sucking a teat.
Sucking empty bucket or teat	The calf is sucking on an empty teat or an empty bucket, but no milk is ingested. Sucking movements are performed with part of bucket or teat in the mouth
Cross-sucking head or neck	The calf is sucking on the head (muzzle, ear or skin) or on the skin of the neck of another calf. The sucking movements are performed with the body part in the mouth
Cross-sucking under belly	The calf is sucking under the belly of another calf, mainly on navel, scrotum or udder base. The sucking movements are performed with the body part in the mouth
Bunting calf	The calf is pushing its forehead with a rapid and forceful movement against another calf's head, neck or body
Bunting bucket or teat	The calf is pushing its forehead with a rapid and forceful movement against a bucket or a teat
Licking fixtures	The calf's tongue is out of its mouth and in contact with any fixtures of the pen, except teat or bucket
Social grooming	The calf's tongue is out of its mouth and in contact with the head, neck or body of another calf
Self-grooming	The calf's tongue is out of its mouth and in contact with its own body
Inactive	The calf is standing or lying still
Other activity	The calf is performing any activity not described above

5.5. Diseases and treatments

All cases of health disturbances, such as diarrhoea and coughing, and treatments were recorded daily for each calf by the calf personnel in a health protocol (APPENDIX 1). Occurrence of diarrhoea was divided in to four categories (Tab. 4) which was used to score the diarrhoea. If the calves were diagnosed as ill they were treated according to the current veterinary practice adapted on the farm. If diagnosed with diarrhoea (score 1-3) by the calf personnel the calf hutch was

marked and the calf/calves were given Diakur (Protect Diakur Super, Lantmännen[5]) in their milk. If considered necessary Effydral (Zoetis Effydral Animal Electrolyte Tablets) was also given in their water. Diarrhoea scored for each week was seen as one case of diarrhoea and diarrhoea stretching over several weeks was considered the same case.

Table 4 *Diarrhoea was scored according to four categories where calves without diarrhoea were scored 0 (Eriksson, 2013: adapted from Silverlås et al. 2010)*

Diarrhoea score	Definitions
0	Normal faeces. Firm consistency. Brown colour. Clean and dry tail and perineum
1	Faeces with a paste-like consistency without shape.
2	Watery consistency (flowing out)
3	Watery consistency (flowing out) with blood

5.6. Other recordings

Live weight was measured every week after morning milk feeding and also at birth, after last meal of colostrum and at day 10. Weighing was done with a scale that was brought to the hutches and the calves were moved into the scale at each weighing (Maréchalle Pesage Weighing Crate PM 120[6]). Each calf was examined as an extra health control in conjunction with the weekly weighing. Daily air temperature (°C) and relative humidity (RH %) was recorded in four hour intervals 24 hours a day during the study period (HOBO U12-013 data logger[7]).

5.7. Statistical analysis

Data management was performed using Microsoft Excel version Office home and student 2010 (© 2010 Microsoft Corporation version 14.0.7166.5000). Analysis was done in R (R version 3.3.1 2016-06-21), using packages lme4 (version 1.1-12) for mixed models and LmerTest (version 1.0) to obtain P values on all data except for diarrhoea. Significant level was set to 0.05.

Hutch values (7 single-housed calves and 7 pairs of pair-housed calves) were used to generate means for behaviour, cough, fever, diarrhoea, and weights for statistical analysis. Behaviour data analysed included week 2-6; week one was excluded when analysing the behaviours since the first behavioural observations were done at two weeks of age and all calves included were then in their hutch. Week 1-6 was included when analysing weight and diarrhoea. Week 7-8 was excluded from the statistical analysis since not enough calves was included in the study for that period. The average temperature of the day was categorized in three categories: above +5 °C (1), between +5 °C and -5 °C (2), and under -5 °C (3).

Data are presented as weekly means ± standard error (SE) of the weekly mean. All data except diarrhoea were checked for normality of the residuals (Shapiro-Wilk test) and homogeneity of variance with Bartlett's test and all variables were square root transformed to provide normal residuals. Redirected suckling behaviours performed before receiving milk and sucking on empty

[5]https://c4produktkatalog.lantmannen.se/index.php/component/virtuemart/?page=shop.product_details&flypage=flypage.tpl_mol&product_id=9138&category_id=9730

[6] http://www.marechalle-pesage.fr/fiche/weighing-crate-pm-120/

[7] http://www.onsetcomp.com/products/data-loggers/u12-013

bucket after receiving milk had normal residuals but were square root transformed to provide a better pass of the Shapiro-Wilk test. The result was not back-transformed. If data was not normally distributed after data transformation (before milk: sucking on empty bucket or teat, bunting on bucket or teat, cross-sucking or social grooming; after milk: drinking milk, redirected suckling total, cross-sucking and social grooming) a non-parametric Wilcoxon rank-sum test with continuity correction was performed where the sum of the different behaviours performed before and after receiving milk was analysed separately. Differences in weight as a response to treatment over time were tested by the Residual Maximum Likelihood (REML) analysis, with a treatment x day interaction, temperature and breed as fixed effects, hutch identity as a random effect and birth weight as a covariate. An analysis of variance (ANOVA) was done to assess the effects of treatment on weight gain between week 2 and 6, with treatment as a fixed effect and birth weight as a covariate.

For behaviour the weekly means are the average of the two observations per calf hutch per week. The mean value of each behaviour performed per observation before and after milk feeding was used and analysed separately. Behaviours bunting calf, cross-sucking under belly, cross-sucking on head or neck and licking fixtures was compiled to categorise all redirected suckling behaviours (redirected suckling total). Bunting calf, cross-sucking under belly and cross-sucking on head or neck was compiled to categorise all behaviours redirected towards another calf (cross-sucking) excluding licking fixtures. Differences in behaviour as a response to treatment over time were tested by REML analysis, with a treatment x day interaction, temperature, and breed as fixed effects and hutch identity as a random effect.

For diarrhoea age in weeks was compiled into period 1 (week 1-3) and period 2 (week 4-6) and significance of treatment on cases of diarrhoea during each period was tested with a Chi^2-test.

6. Results

6.1. Calf performance

6.1.1. Health

No coughing or fever was recorded and no antibiotics had to be prescribed to the calves included in the study. No sucking related injuries were found. Diarrhoea was recorded 42 times and the longest period for a calf to continuously have diarrhoea was eight days and the shortest one day. The occurrence of diarrhoea was most common at three weeks of age for pair-housed (8 calves) and least common in week six (0 calves). Only one hutch of the single-housed calves had diarrhoea and that was during week one (Tab. 5). All pair-hutches had at least one case of diarrhoea; two hutches had two cases each. There was a significant difference between the treatments in amount of cases of diarrhoea during both period 1 and period 2 (Tab. 5).

Table 5 *The effect of housing treatment on number of hutches with diarrhoea (score 1-3) in period 1 and 2 for dairy calves kept single or in pairs in calf hutches between 1-6 weeks of age*

Period	Single	Pair	Chi^2	P-value
1 (week 1-3)	1	5	4.7	0.03
2 (week 4-6)	0	4	5.6	0.02

6.1.2. Weight and impact of temperature

The mean birth weight was 40.6±15.4 kg for single housed calves and 39.9±15.1 kg for pair-housed calves. There was no significant difference in birth weights between treatments (P>0.1). No significant effect of treatment on weight between single and pair-housed calves was found (Fig. 3a) but there were significant main effects of week ($F_{(5,58)}$=58, P<0.001) and birth weight ($F_{(1,11)}$=75.1, P<0.001). Weight gain from birth to week six (5.6±2.1 kg vs 5.6±2.1 kg/week) or from week two to week six (5.0±1.9 kg vs 5.1±1.9 kg/week) were also not affected by treatment. Individual differences between the calves in pair-hutches could be seen where one calf in general had slightly higher weight gain than the other calf in the hutch (Fig. 3b).

The average daily mean outdoor temperature from November to March was 0.7±0.3 °C (Tab. 6). January hade the lowest average temperature and also the lowest daily temperature with -21.6 °C (2016-01-15). Temperature had no significant effect on weight and there was no difference on treatments as an effect of temperature (Tab. 7).

Table 6 *Mean temperatures (°C) during November 2015 to March 2016*

Month	**Mean temperature**	**Max**	**Min**
November	3.8±1.4 °C	16.8 °C	-10.2 °C
December	2.2±0.8 °C	12.2 °C	-12.7 °C
January	-5.2±2.0 °C	6.3 °C	-21.6 °C
February	-0.3±0.1 °C	15.1 °C	-14.0 °C
Mars	3.0±1.2 °C	14.6 °C	-6.8 °C

Table 7 *Mean weight (±SE) at temperature categories 1-3 (1: above +5 °C, 2: between +5 °C and -5 °C 3: under -5 °C) during week 1-6 for dairy calves kept single or in pairs in calf hutches. – means no weight was registred*

Week	**Temperature category 1**		**Temperature category 2**		**Temperature category 3**	
	Single	Pair	Single	Pair	Single	Pair
1	47.45±17.93	-	48.74±18.42	47.62±18.00	-	47.15±17.82
2	53.23±20.12	-	54.10±20.45	53.08±20.06	55.45±20.96	51.55±19.48
3	60.70±22.94	-	57.24±21.63	55.85±21.11	-	59.63±22.54
4	-	-	64.72±24.46	63.73±24.09	58.30±22.04	59.10±22.34
5	68.60±25.93	-	69.46±26.25	69.04±26.10	63.60±24.04	62.50±23.62
6	74.53±28.17	78.55±29.69	77.45±29.27	73.47±27.77	66.10±24.98	66.35±25.08

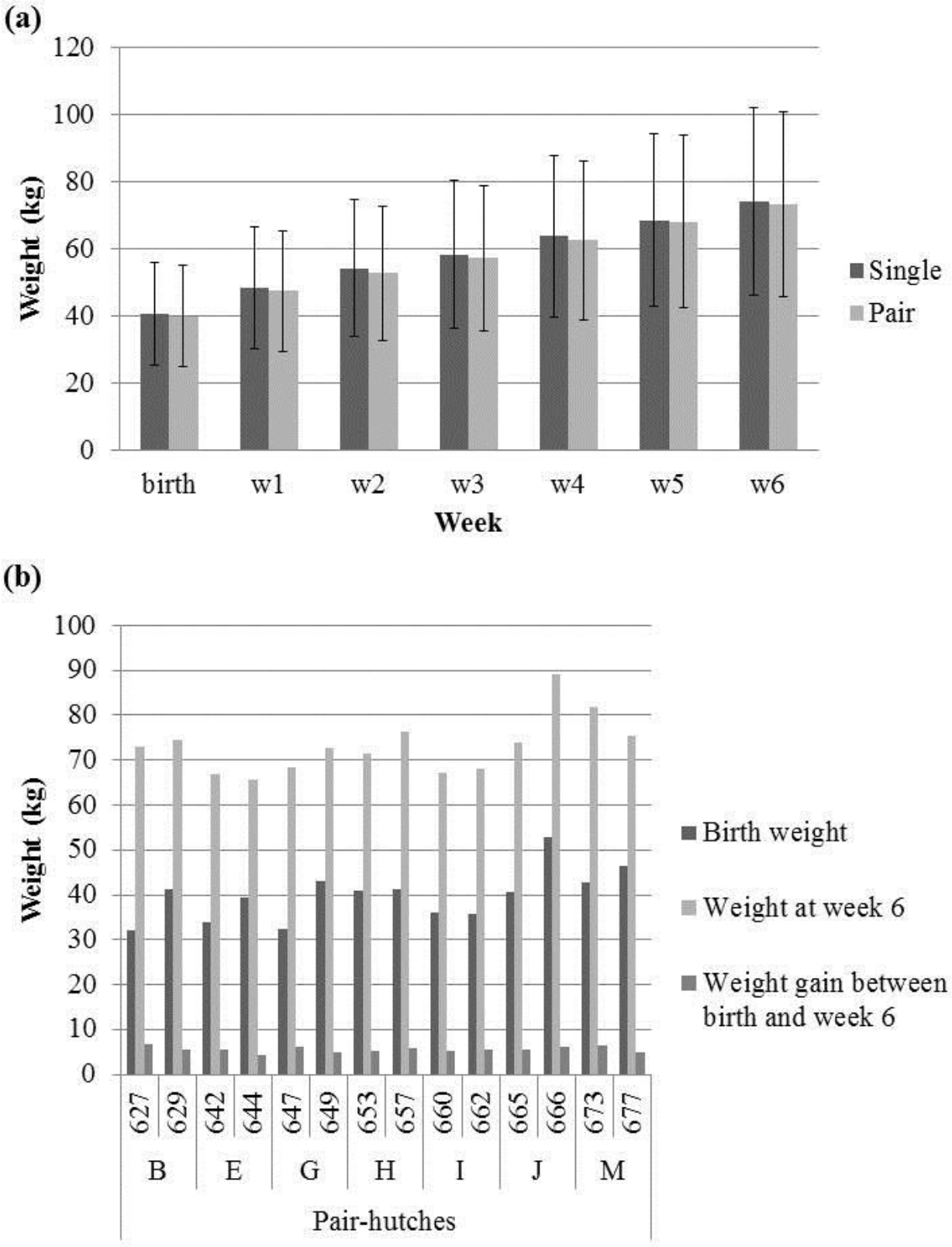

Figure 3. *The effects of housing treatment on mean (± SE) weight (a) and birth weight, weight at 6 weeks of age and weight gain from birth to 6 weeks of age (b) for pair-housed calves.*

6.2. Behaviours

Effect of treatment, treatment-day interaction and day on behaviours can be seen in Table 9. There was no effect of breed on any of the tested behaviours.

6.2.1. Redirected suckling total

There was no significant effect of treatment on redirected suckling total before receiving milk but after receiving milk there was a significant effect of treatment ($P<0.05$) where pair-housed calves performed the behaviour more than single-housed calves (Fig.4).

Single housed calves had a higher frequency of licking fixtures than pair-housed calves before receiving milk ($P<0.05$) but there was no significant effect of treatment after receiving milk (Fig. 5).

No significant effect of treatment on bunting bucket or teat before receiving milk was found. However, there was an effect of treatment ($P<0.001$), day ($P<0.05$) and temperature ($F_{(2,108)}=5.2$, $P<0.01$) on bunting bucket or teat after receiving milk where single-housed calves performed the behaviours more frequently (Fig. 6). During temperature category 2 (+5 to -5 °C) bunting bucket or teat had a higher frequency then during category 1 (above +5°C) or category 3 (below -5°C) (Tab. 8).

Table 8 *Mean ±SE of bunting bucket or teat at temperature category 1-3 (1: above +5 °C, 2: between +5 °C and -5 °C and 3: under -5 °C) after receiving milk for dairy calves kept single or in pairs in calf hutches between 2-6 weeks of age*

	1	**2**	**3**
Single	23.43±8.86	25.75±9.73	19.20±7.26
Pair	5.60±2.12	9.94±3.76	6.62±2.50

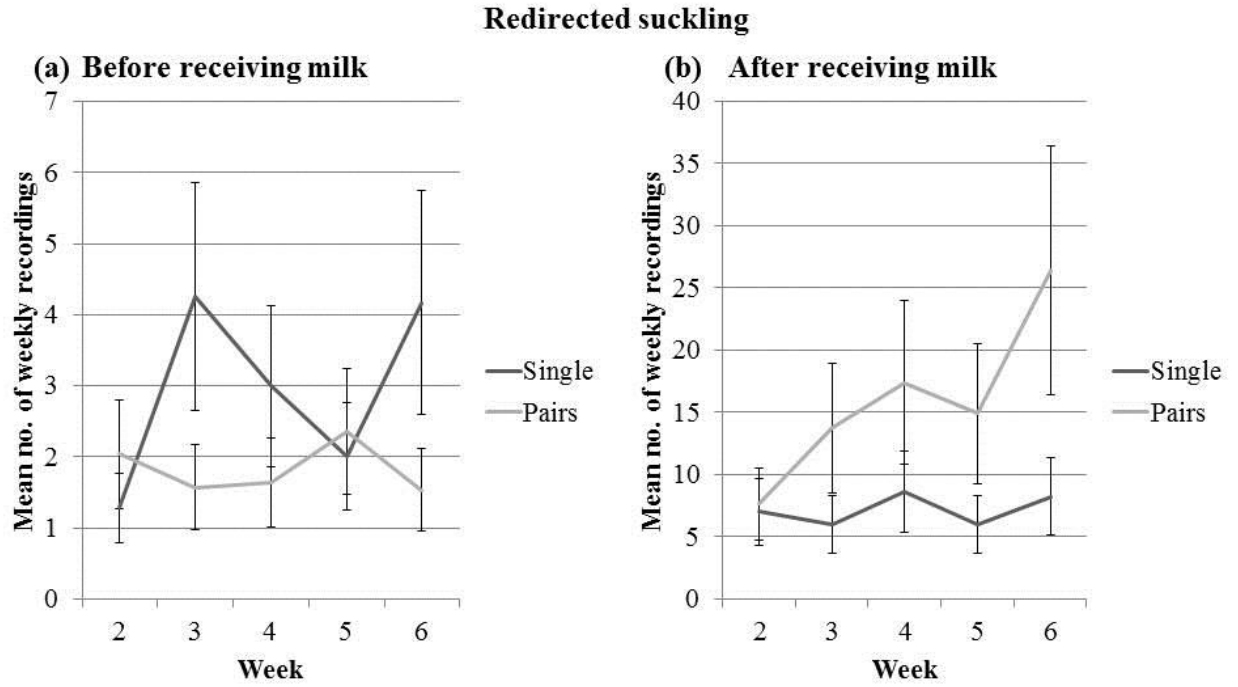

Figure 4. *The effects of housing treatment on redirected suckling before (a) and after (b) receiving milk mean (± SE) for dairy calves kept single or in pairs in calf hutches between 2-6 weeks of age.*

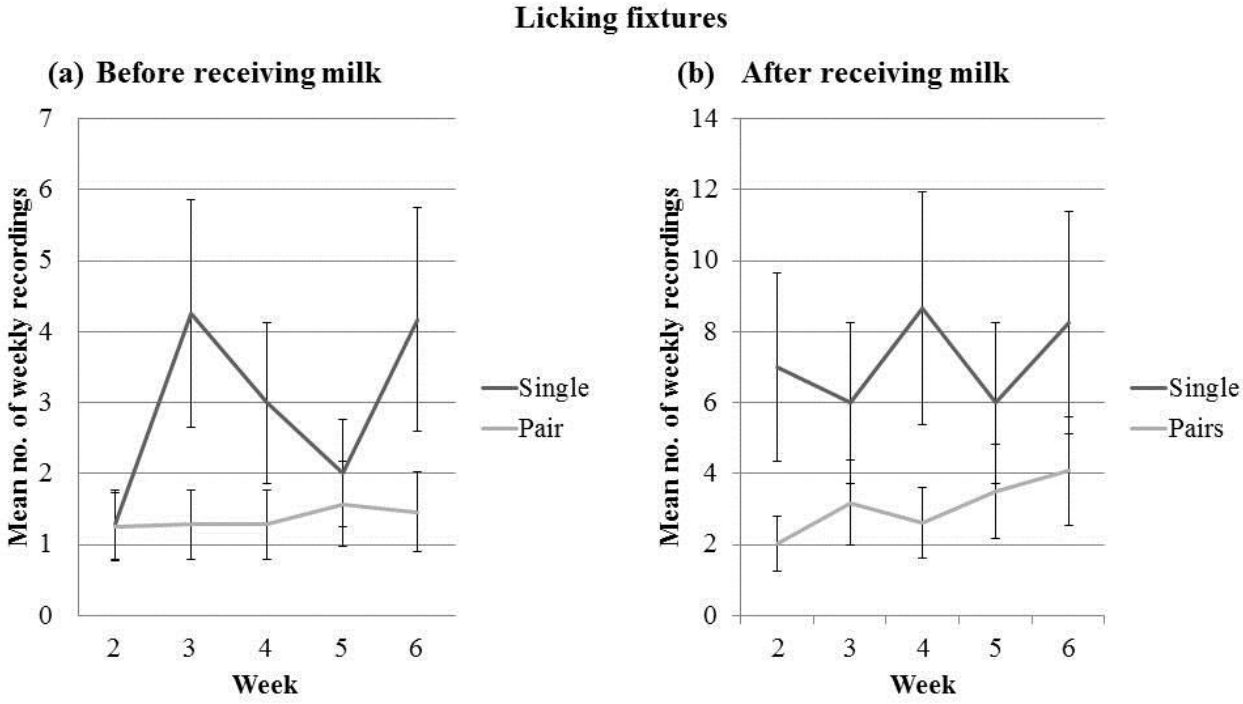

Figure 5. *The effects of housing treatment on licking fixtures before (a) and after (b) receiving milk mean (± SE) for dairy calves kept single or in pairs in calf hutches between 2-6 weeks of age.*

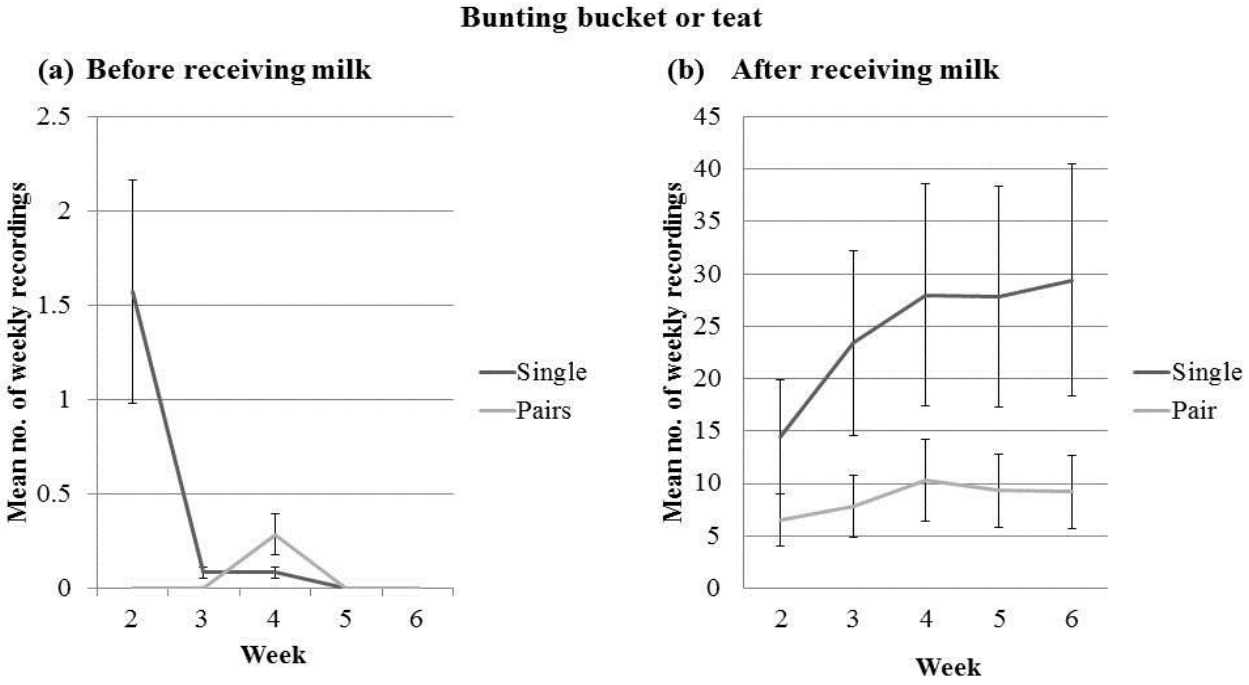

Figure 6. *The effects of housing treatment on bunting bucket or teat before (a) and after (b) receiving milk mean (± SE) for dairy calves kept single or in pairs in calf hutches between 2-6 weeks of age.*

6.2.2. Cross-sucking

There was a significant effect of treatment on cross-sucking both before ($P<0.01$) and after receiving milk ($P<0.001$). Pair-housed calves performed all the behaviours recorded during the behavioural observations and single-housed calves performed none of the behaviours (Fig. 7).

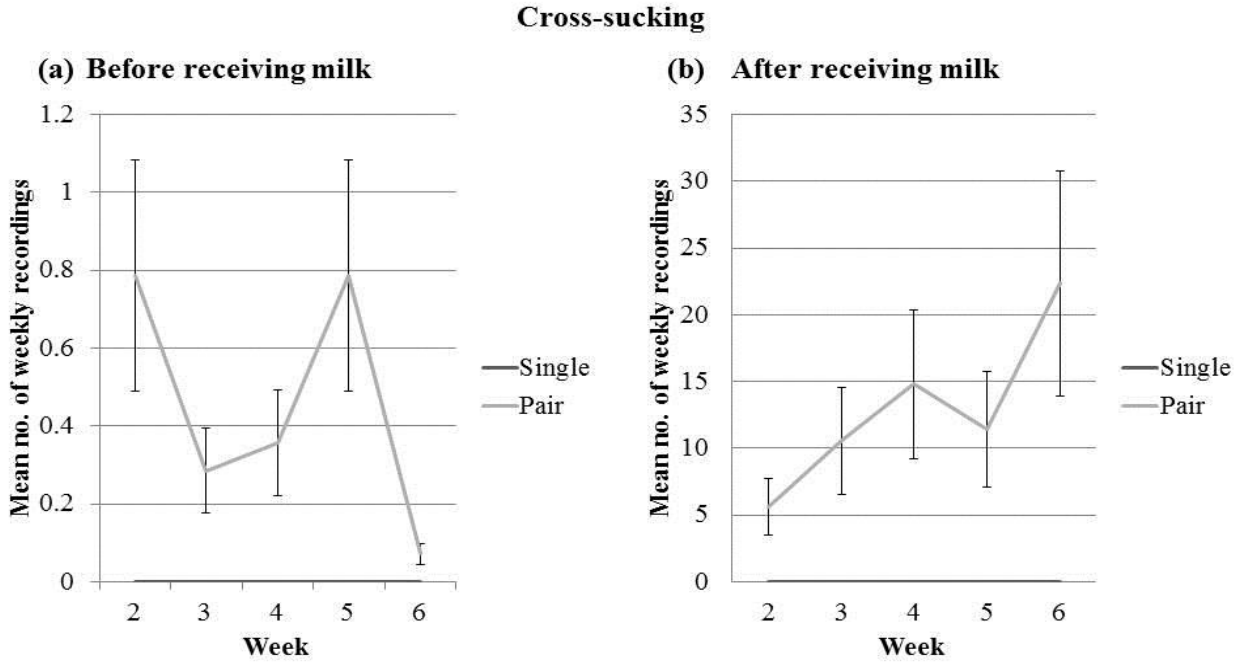

Figure 7. *The effects of housing treatment on redirected suckling (a) and on cross-sucking (b) after receiving milk mean (± SE) for dairy calves kept single or in pairs in calf hutches between 2-6 weeks of age.*

There was a difference between the calves in the pair-hutches were one calf generally cross-sucked more than the other (Fig. 8a). Cross-sucking was mostly performed on the other calves head or neck both before and after receiving milk (52.5% total or 2.1±1 and 9.3±3 respectively) or as bunting at the other calf (46.5% or 1.0±0 and 8.1±3 respectively). Sucking under belly was performed least of the three behaviours (1.0% or 1.0±0 and 1.8±1 respectively) (Fig. 8b).

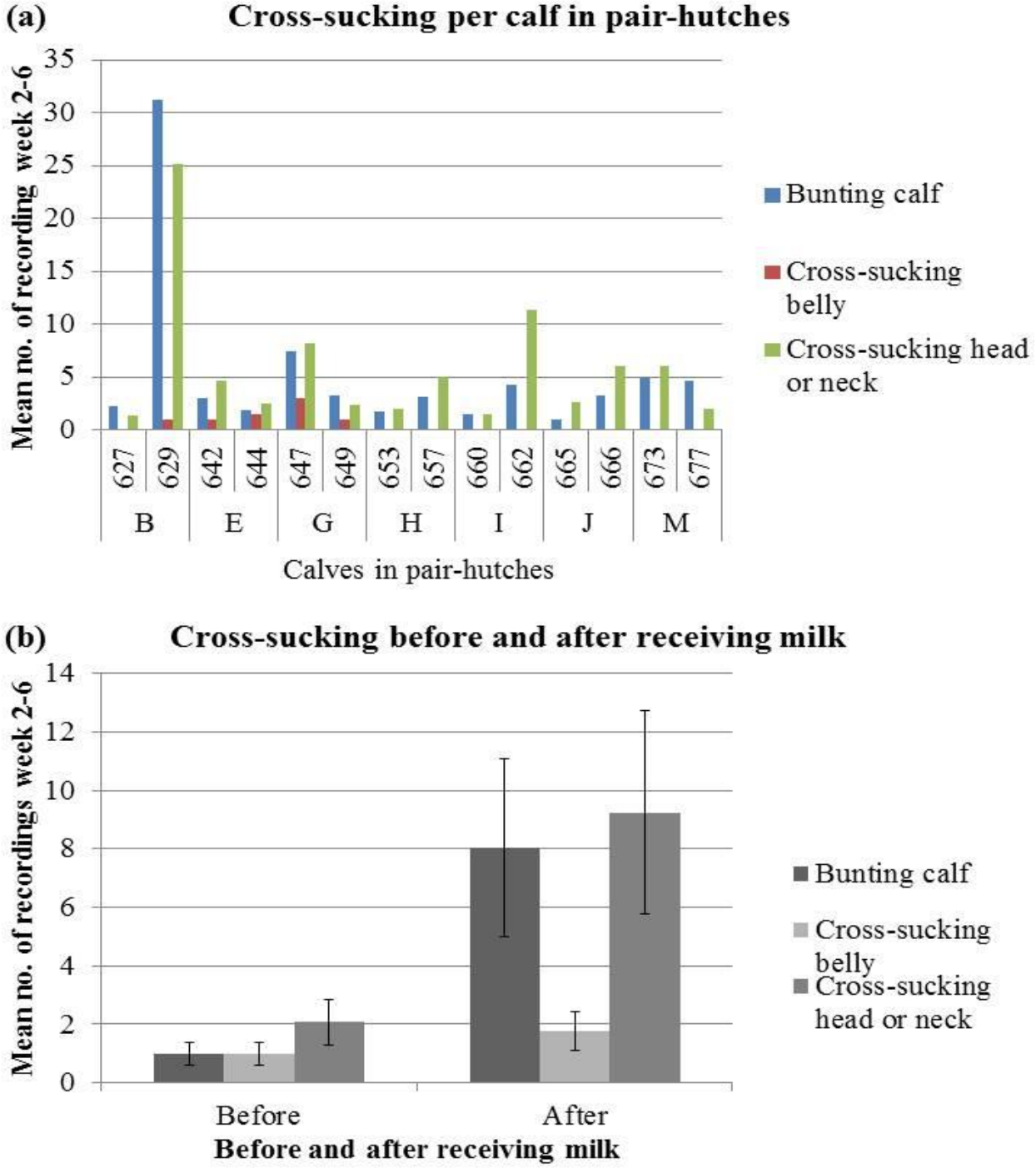

Figure 8. *Cross-sucking per calf in pair-hutches (a) and cross-sucking before and after receiving milk (b) mean (± SE) for dairy calves kept in pairs in calf hutches between 2-6 weeks of age.*

6.2.3. Other behaviours

Pair-housed calves had a higher frequency of drinking milk than single-housed calves ($P<0.001$, Tab. 9) but there was no significant effect of treatment on sucking on empty bucket or teat neither before nor after receiving milk (Tab. 9).

Self-grooming was significantly affected by a treatment-day interaction both before ($P<0.001$) and after ($P<0.05$) receiving milk (Tab. 9). There was also a main effect of day on self-grooming before receiving milk ($P<0.05$, Tab. 9). Social grooming was significantly affected by treatment where pair-housed calves performed all the behaviours recorded during the behavioural observations both before ($P<0.01$) and after ($P<0.01$) receiving milk whereas single-housed calves performed none of the behaviours (Tab. 9).

Table 9 *Mean values (±SE) week 2-6 of behaviours before and after receiving milk for single vs. pair housed dairy calves kept in outdoor calf hutches and results from statistical testing on the effects of housing treatment, observation day and treatment-day interaction for normally distributed behaviours tested with an REML model and for not normally distributed data tested with Wilcoxon rank-sum test. Significant results are marked with bold and – means that it was not tested due to the behaviour was not performed or it being tested with Wilcoxon rank-sum test.*

Behaviour	**Before receiving milk**					**After receiving milk**				
	Single mean	**Pairs mean**	**Effect of treatment**	**Treatment and day interaction**	**Main effect of day**	**Single mean**	**Pairs mean**	**Effect of treatment**	**Treatment and day interaction**	**Main effect of day**
Redirected suckling total	2.86±1.08	1.83±0.69	$F_{(1,11)}$=1.6	$F_{(9,101)}$=1.5	$F_{(9,101)}$=0.89	7.14±2.70	16.03±6.06	**W=6**	-	-
			P=0.23	P=0.16	P=0.23			**P=0.02**	-	-
Licking fixtures	2.86±1.08	1.37±0.52	**$F_{(1,12)}$=5.1**	$F_{(1,102)}$=1.4	$F_{(9,102)}$=1.5	7.14±2.70	3.08±1.16	$F_{(1,10)}$=1.74	$F_{(9,100)}$=0.48	$F_{(9,101)}$=1.25
			P=0.04	P=0.19	P=0.17			P=0.22	P=0.89	0.28
Bunting bucket or teat	0.38±0.14	0.06±0.02	W=33.5	-	-	24.41±9.22	8.64±3.26	**$F_{(1,10)}$=11.5**	$F_{(9,101)}$=0.56	**$F_{(9,101)}$=2.1**
			P=0.23	-	-			**P=0.006**	P=0.83	**P=0.05**
Cross-sucking	0.±0.	0.46±0.17	**W=3.5**	-	-	0.±0.	12.95±4.89	**W=0**	-	-
			P=0.004	-	-			**P=0.001**	-	-
Drinking milk	-	-	-	-	-	3.91±1.48	6.51±2.46	**W=0**	-	-
			-	-	-			**P=0.0006**	-	-
Sucking on empty bucket	2.28±0.86	1.79±0.67	W=20	-	-	30.22±11.42	30.49±11.53	$F_{(1,10)}$=0.13	$F_{(9,100)}$=0.48	$F_{(9,100)}$=0.58
			P=0.62	-	-			P=0.73	P=0.89	P=0.81
Self-grooming	1.97±0.74	1.86±0.70	$F_{(1,110)}$=0.06	**$F_{(9,110)}$=4.4**	**$F_{(9,110)}$=2.5**	4.17±1.58	3.16±1.19	$F_{(1,11)}$=0.94	**$F_{(9,101)}$=2.1**	$F_{(9,101)}$=0.86
			P=0.81	**P=0.00006**	**P=0.014**			P=0.36	**P=0.03**	P=0.54
Social grooming	0.±0.	0.27±0.10	**W=3.5**	-	-	0.±0.	0.26±0.10	**W=3.5**	-	-
			P=0.004	-	-			**P=0.004**	-	-

7. Discussion

The general objective in calf-housing must be to provide a housing system that protects the calves from thermal discomfort, to provide proper nutrition that is accessible to each calf and to be able to monitor the health and welfare of the calf (Stull & Reynolds, 2008). Both single- and pair-housing can be designed to meet these needs but all housing systems have their strengths and weaknesses. Therefore, changing to a different housing system can result in switching from one set of welfare problems to another. It is therefore crucial to understand the pros and cons of the housing system that is to be implemented and to be aware of any welfare issues that may arise.

The aim of this study was to find out how pair-housing of calves in outdoor calf hutches affected growth, general health and redirected suckling behaviour in comparison to single-housing of calves. There is a concern among producers that keeping calves in pairs or group at young age can have a negative impact on weight gain and general health (Phillips, 2010). There was no difference in weight or weight gain between single- and pair-housed calves in this study even though pair-housed calves had a higher occurrence of diarrhoea during the whole study period. Redirected suckling, and in particular cross-sucking, is of concern as it can give rise to welfare issues as a cross-sucking calf generally continues to cross-suck when older (Keil & Langhans, 2001). In this study pair-housed calves performed more redirected suckling total than single-housed calves after receiving milk and also performed all cross-sucking registered, but no sucking related injuries were recorded.

7.1. Calf performance

In this study no significant effect of treatment on either weight or weight gain per week from birth to six weeks of age was found. This result is consistent with the result of de Paula Vieira *et al.* (2009) who saw no difference in body weight during the pre-weaning (day 1 to 36) and weaning period (day 37-55) between pair-housed and single-housed calves. In their study the calves were fed pasteurized whole milk via teat twice a day at ad lib volumes and weaning was done gradually by diluting the milk with water at the rate of 10% per feeding during five days starting at day 37 of age (five weeks and two days) (de Paula Vieira *et al.*, 2009). In the current study, the weaning period started at week six with a reduction from three litres to two litres per feeding. Since weaning in this study started at week six and only weights from birth to week six was included in the analysis no impact of weaning can be shown as the calves only had the reduced milk amount for one day. De Paula Vieira *et al.* (2009) also weighed their calves every other day compared with once a week in this study. Weighing every other day could give a more accurate insight on the fluctuation of the calves' weight during the whole study period but there should be no significant difference in weight gain data in comparison to when measuring weight once a week. Terre *et al.* (2006) on the other hand saw that group-housed calves (five calves in each pen) had a lower growth rate than single-housed calves. In their study the calves were fed milk replacer in buckets twice daily and the daily milk allowance was four litres per day from day 1-5, five litres per day from day 6-13 and six litres per day from day 14-19. The weaning period started at 20 days of age (two weeks and six days) with a decrease in the daily milk allowance to four litres per day and the calves were completely weaned at 28 days of age (four weeks) (Terre *et al.*, 2006). Terre *et al.* (2006) weighed the calves weekly, which is the same as in this study. As there are several factors that differ between the studies, such as milk replacer instead of whole

milk, group-housing instead of pair-housing, milk fed in buckets instead of via teat and a difference in weaning age with two weeks no real comparison of the results can be made. Chua *et al.* (2002) saw that pair-housed calves' continued to gain weight at pre-weaning levels during the weaning period at six weeks of age whereas single-housed calves only gained at half the rate of the pair-housed. Since not enough data was obtained for week 7-8 in this study the effect of weaning on weight cannot be seen.

The results obtained in this study contradict our hypothesis which was that pair-housed calves should have a higher growth rate than single-housed calves due to social facilitation. Terré *et al*. (2006) hypothesized that when an animal performs a behaviour the likelihood for another animal to start performing the same behaviour should increase. In the daily health protocol the amount of milk each calf received and drank is recorded, so is also the amount of concentrate given to each hutch. But the amount of milk each calf drank in pair-hutches is just estimation; since the calves stole milk from each other it is hard to determine exactly how much milk each calf drank. The concentrate given is also per calf hutch meaning that the individual concentrate intake cannot be seen. The calf hutches used in this study measured ~2.4m^2 on the inside with an outside pen attached measuring ~1.7m^2; perhaps the size of the hutch made it harder for the calves to perform the same behaviour at the same time. The container with concentrate was at the rear end inside of the hutch with an opening measuring 0.1 m^2 which might not be sufficient for both calves to feed on concentrate at the same time. The bucket with silage was only available to one calf at the time as it was situated on the outside of the outdoor pen but hay was available in a way that both calves could eat at the same time. A higher intake of concentrate and roughage, with a reduced reliance on milk, before weaning is generally assumed to reduce the response to weaning when it takes place (Weary *et al*., 2008). As a result of lack of data, we could not compare the weight gain of single and pair-housed calves at weaning. de Paula Vieira *et al*. (2009) argued that group housed calves respond much less to weaning than single-housed calves in terms of less vocalization and higher performance after weaning in a group-housing system, and more studies in the area should be of interest to increase production and the welfare of the calves.

No impact of temperature on weight could be found. The hypothesis in this study was that when two calves are kept in the same hutch they should generate more radiant heat than a single-housed calf and the temperature inside therefor should be higher than for single-housed calves. As temperature inside the hutch was not measured this cannot be said to be true, though Okamoto *et al*. (1993) saw that the air temperature inside a calf hutch was +2 to +6 °C higher than the outdoor temperature when keeping one calf in the hutch. Pair-housed calves should also be able to benefit from each other's body heath to keep warm during the cold season and hence be able to use more energy to support growth and the immune system that otherwise would be used to keep warm. But in this study there was no difference in growth between treatments as an effect of temperature.

During the study the calves' health was recorded every day and except for diarrhoea no other health issues occurred. Neither coughing, fever nor any sucking related injuries were recorded on any of the calves' included. Cross-sucking increases the risk for frost damage on the soft tissue during cold weather as sucking on a body part makes it wet (Ekesbo, 2011). In this study no occurrence of sucking related injuries could be seen meaning that pair-housing did not increase the risk of sucking related injuries, even as the temperature dropped during the study period. This is in line with the hypothesis that there should be no increase in sucking related injuries when

calves were pair-housed compared to single-housed. That no sucking related injuries were recorded is positive especially if the aim is to keep calves in pairs/groups. If the absence of sucking related injuries is due to the teat-bucket that was available all hours of the day or due to other factors cannot be concluded in this study.

Diarrhoea has a negative impact on growth (Marcé *et al*., 2010) but this was not tested in this study. Pair-housed calves had more diarrhoea than single-housed calves during the whole study. The hypothesis stated that is should have been an increase during their first week from introduction (week two) as a result of a higher pathogen load when two calves are housed in the same hutch but that this effect should have disappeared when the calves' immune system adapted. No such adaption can be seen in the result as the occurrence of diarrhoea is similar during both periods. The higher occurrence can be due to that if one calf in the pair-hutches got diarrhoea the other calf could also got it due to sharing of space and that they suckled on the same teats.

Cold stress has a larger impact on young, sick and injured animals than it has on mature and healthy animals (Stull & Reynolds, 2008). Perhaps the pair-housed calves had a higher energy reserve due to less energy being used to maintain body temperature which could be used to maintain weight gain even during a case of diarrhoea. As no significant difference could be found in weight or weight gain between the two treatments pair-housing could be seen as a viable option during the cold season.

7.2. Behaviour

Dairy producers can be hesitant to keep heifers calves in groups because cross-sucking can have a negative impact on their health at an older age (de Passillé, 2001). To minimize the risk of developing cross-sucking one solution is to place the calves in individual hutches where they cannot reach each other but this routine do not satisfy the calves' need for social interactions.

7.2.1. Redirected suckling total

No significant difference in redirected suckling total was found before the calves received milk but after receiving milk pair-housed calves performed more redirected suckling total than single-housed calves. In this category all redirected suckling was included (bunting calf, cross-sucking under belly, cross-sucking on head or neck and licking fixtures) so that a comparison between the two treatments on redirected suckling could be done. When looking at the behaviour licking fixture separately from the other redirected suckling behaviours, a significant difference between the two treatments before receiving milk can be seen where single housed calves perform this behaviour more frequently than pair-housed calves. This is in line with the hypothesis that the occurrence of non-nutritive sucking should be less for pair-housed calves.

Redirected suckling is stimulated by the taste of milk (de Passillé *et al*., 1992) and most redirected suckling occurs during the first 10-15 minutes after milk feeding (Lidfors, 1993). But this does not answer the question why single-housed calves displayed a higher frequency of licking fixtures before receiving milk than pair-housed calves. The higher frequency of licking fixtures in single-housed calves before milk can be due to that pair-housed calves directed their non-nutritive sucking towards the other calf (cross-sucking) instead of licking the fixtures. The finding that no significant difference in licking fixtures was found after receiving milk can be due to that single-housed calves were able to satisfy their need for suckling on the teat-bucket while

pair-housed calves did not because of competition about the teats and therefore cross-sucked instead.

There were significant effects of treatment, day and temperature on bunting bucket or teat after receiving milk where single-housed calves performed the behaviour to a larger extent than pair-housed calves. Bunting has been seen to increase over time as the calf's effort to maintain milk flow from the udder and to empty the teat and udder more completely increases as a result of the growing demand for feed with age (de Passillé *et al.,* 1992). Pair-housed calves might perform less bunting on bucket or teat because of the competition for milk between the calves. The calf that did not get access to the milk bucket could have performed bunting towards the calf instead of the other empty bucket or teat.

At week six the weaning period started with a decrease from three litres of milk per feeding to two litres of milk per feeding and the increase of redirected suckling total and cross-sucking that can be seen in week six in the figures is therefore not unexpected. A slow milk flow (0.5 litre/minute) decreases the frequency of non-nutritive sucking in comparison to a fast milk flow (1 litre/minute); by reducing the diameter of the pipe that connect the teat to the bucket the milk flow can be reduced and it could reduce the frequency of non-nutritive sucking (Loberg & Lidfors, 2001). As a high amount of milk (5 litres per meal) also has been seen to reduce redirected suckling (Jung & Lidfors, 2001), a combination of a high amount of milk with a reduced milk flow should have a positive effect on reducing the redirected suckling following milk feeding that could be seen in this study.

7.2.2. Cross-sucking

Pair-housed calves performed all cross-sucking behaviours both before and after receiving milk. Cross-sucking could be performed by both treatments but was only seen in pair-housed calves. This is in line with the hypothesis that predicted an increase of cross-sucking in pair-housed calves as the possibility to perform the behaviour increased. But it is interesting that single-housed calves did not perform any cross-sucking at all even though they could cross-suck on the neighbouring calves' muzzle. This could be due to the access to the teat-bucket where the single-housed calf did not have to compete for the teat or milk with another calf.

Competition for milk depends on milk allowance where a low milk allowance increases the occurrence of cross-sucking (Jensen & Budde, 2006). Competition for the teats increases when access to the teat is reduced, but competition for the teats are seen even when the calves have one teat each (Keyserlingk *et al*., 2004). Lidfors (1993) saw an increase of cross-sucking during the weaning period but also a significant drop the first three days after weaning. The increase of cross-sucking seen by Lidfors (1993) is consistent with the findings in this study were an increase of cross-sucking can be seen in week six at the start of the weaning period.

There were individual differences between the pair-housed calves in the frequency of cross-sucking. Most of the recorded behaviours were performed by one calf in each hutch but all calves performed cross-sucking at least two times. This is in line with results obtained by Loberg and Lidfors (2001) where three calves performed most of the cross-sucking but another ten out of the 16 calves performed cross-sucking at least one time during their study.

The calves directed 52.5% of the cross-sucking towards the other calves' head or neck, 1.0% towards the belly and 46.5% on bunting the other calf. Note that all calves in this study were heifers which exclude sucking on penis and scrotum as in other studies. Jung and Lidfors (2001) studied how different milk flow affected redirected suckling and in their study the calves directed most of their cross-sucking towards other calves' belly (52.7%), which was not seen in this study. Jung and Lidfors (2001) also looked at sucking on mouth and ears separately (16.3% and 1.3% respectively) which was not done in this study, but even when combined (17.6%) it does not reach the levels of cross-sucking towards another calves' head or neck seen in this study (52.5%). In contrast Lidfors (1993) reported that ~40% of the cross-sucking in her study was directed towards other calves' mouth, ~34% towards their ears and ~3% towards their throat. This gives that 77% of the cross-sucking in their study was towards another calves head or neck which exceeds our result. Cross-sucking towards the head and around the muzzle can be due to it being smeared with milk (Jensen & Budde, 2006) but in this study also as a response to the other calf stealing the teat and that the calf started to cross-suck on that calf´s mouth as it could not move the calf from its teat (personal observation). The low frequency of cross-sucking under the belly is extra desirable as excessive cross-sucking under the belly can lead to inter-sucking in heifers and milk stealing in cows (Keil & Langhans, 2001).

7.2.3. Other behaviours

After receiving milk there was a significant effect of treatment on drinking milk. Pair-housed calves had a higher frequency of drinking milk than single-housed calves. As this behaviour recorded the number of times the individual calf took the teat in its mouth it does not give any indication on milk consumed. That pair-housed calves had a higher frequency of drinking milk than single-housed calves is not surprising since the pair-housed calves competed for the milk; when one calf had finished its milk it often tried to steal the milk from the other calf, giving a higher recorded frequency of drinking milk when the calves competed over the bucket that still contained milk. In future studies it would be recommended to add a column with the behaviour "stealing milk" to get a more correct result on which behaviours that were performed.

Self-grooming was affected by both treatment, day and treatment-day interaction. Single-housed calves generally performed more self-grooming than pair-housed calves and pair-housed calves performed all social grooming. The frequency of social grooming was low and could not explain the difference in self-grooming.

7.3. Source of error

During data collection there were problems with finding suitable calves for the study thus the number of calves included in the data analysis was lower than planned from the start. The desired amount of calves included in the study was set to 36 but only 24 calves entered the study, whereof 21 were included in the statistical analysis. A small sample size can give both Type I and Type II errors, meaning that it is a higher risk of either rejecting the null hypothesis even when there is no difference between the treatments, but also to not reject the null hypothesis even when there is a difference (Biau *et al.*, 2008). With a small sample size it is harder to reach the desired level of statistical significance and the variability of the data has a larger effect on the results (Biau *et al.*, 2008).

The study was conducted on a research farm with personnel used to changes in routines following different ongoing studies. During the study a scoring system for diarrhoea used in another

ongoing study on the calves was used. The scoring system was suitable for the data needed in this study and it was also positive that the personnel were well-rehearsed in the system. But even when using an implemented scoring system the human error can still occur; since not all personnel has the same knowledge in the different types of diarrhoea a calf could be scored for diarrhoea by one person one day but seen as healthy by the next person working. This would give scores on "one-day-diarrhoea" that would be seen as a case in the statistics but that might in fact not have been diarrhoea at all. A higher occurrence of diarrhoea than there actually were would have been the result.

The routines for this study was reviewed for the personnel at several occasions and at briefings but as there are so many different persons working at Lövsta not all of the calf personnel were versed in the feeding routines. This was observed at several occasions and at some occasions it also interfered with the observations as information about the routines had to be given or correction of the buckets set-up adjacent to the milk feeding. The movements and sounds from the observer after the ten minutes adaptation period could have had an impact on the calves' behaviour as the observer then might have been interesting again.

When the calves were included to the study pair-housed calves were moved to a new hutch while single-housed calves stayed in their old hutch. The movement to a new hutch could have an impact on the calves' behaviour, an impact that would have not been seen in single-housed calves. As the calves had four days for adaptation after being included in the study this should not have affected the results.

7.4. Future research

In this study cross-sucking was mainly directed towards the head or neck of the other calf. As the general concern is that a calf that cross-sucks will perform intersucking on its herd mates (Keil *et al*., 2001) , it would be of interest to see if the preference for cross-sucking area in calves has an impact on where the calf would direct its inter-sucking at an older age. Since especially cross-sucking under the belly is undesirable in a dairy herd cross-sucking towards the head or neck might not be perceived as having a negative impact on the cows' welfare if there is no injuries. If so it means that even if calves are kept in pairs, as long as they mainly direct the occurring cross-sucking towards the other calf's head or neck cross-sucking should not be a reason for not keeping calves in pairs or groups. It would therefore be of interest to research the risk factors for cross-sucking on head or neck in calves and how it will affect the sucking behaviours at an older age. The risk of accepting cross-sucking towards the head or neck if it doesn't have any negative implications later on is that the welfare of the receiving calf is set aside. The potential negative effect on the receiving calf must be taken into account before accepting any cross-sucking.

The higher occurrence of diarrhoea in pair-housed calves is a welfare problem as it affects the wellbeing of the calf and also its growth rate. If calves should be kept in pairs or groups' research should be done in the area to find at what age it would be optimal to introduce the calves into the pair-hutch and how the management around the pair-housed calves should be adapted for reducing the risk of diarrhoea.

Keeping calves in pairs or groups' can be of interest both for economical and practical aspects as it saves spaces, needs less area per calf and generates less hutches and pens for cleaning. But when doing research on the area it is easy to focus on one factor in the equation, which makes up

for good research, but might give results that are not always easy to implement on a farm level. If focus of the research is the calves' welfare all aspects must be taken into consideration, such as if it is possible to do on a general farm level and if it is economically viable, if the goal is that the result should be possible to implement. This is in general not aspects that are considered in scientific research and it should neither be the foundation that our research is based upon but it should be considered if the goal is to implement the results on a farm level. A multi-disciplinary approach where the practical implications of animal welfare research are taken into consideration could increase the possibility for new research in the area to reach the producers and to be implemented on a farm level hopefully leading to a higher welfare for the animals.

8. Conclusions

In this study there was a higher occurrence of diarrhoea for calves in pair-hutches than single-housed calves which is not desirable as diarrhoea has been shown to have a negative impact on growth and the calves' welfare. However, no effect of treatment could be seen on weight gain and there was no difference in weights between the treatments even though the pair-housed calves had more diarrhoea. This indicates that pair-housed calves could have had higher growth rate than single-housed calves but that it could have been compromised by the higher occurrence of diarrhoea. No other effect on health was seen and no injuries due to redirected suckling were recorded during the study even though pair-housed calves performed all the recorded cross-sucking behaviours and more redirected suckling total than single-housed calves. The occurrence of cross-sucking in calves can lead to welfare problems later on but since these calves were not followed until introduction to the milk production this is unknown. As both diarrhoea and cross-sucking are considered welfare issues, pair-housing of calves under the conditions that was implemented in this study cannot be recommended as a general on-farm routine even though weight gain can be maintained. Further studies on how to reduce the occurrence of diarrhoea and cross-sucking in pair-housed calves during on-farm conditions is needed.

9. Acknowledgments

Many thanks to Swedish Livestock Research Centre at Lövsta for allowing me to perform this study in their facility and for their help and support during the study period. Great thanks to Lena Lidfors for being my supervisor and helping me with both the design of the study and also for taking the time to comment on the report. A special thanks to Else Verbeek for being my assistant supervisor and for all those hours spent helping me with my statistical analysis and reading through my many versions of the report. Without the help of my supervisors this report would have taken a lot more time to write.

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11. Appendix

Appendix 1. Health protocol

KALVHÄLSOPROTOKOLL HYDDOR

DITT NAMN: **DATUM:**

HYDD-PLATS	KALV ID	HÄLSA/SJUKDOM				UTFODRING			
		HÄLSA	*Om* tecken på SJUKDOM, fyll i:	Diarré	Temp.	Mjölk (L)		Koncentrat (gram)	
		OK? Skriv: 0	Symtom, åtgärd, behandling, övrigt	Score: 1, 2 el 3		Mängd (skriv: 3, 2 el 1L) Fm / Em	Rester (mät m kanna) Fm / Em	Mängd (Mått = 400g)	Rester (använd våg)
18						/ /	/ /		
17						/ /	/ /		
16						/ /	/ /		
15						/ /	/ /		
14						/ /	/ /		
13						/ /	/ /		
12						/ /	/ /		
11						/ /	/ /		
10						/ /	/ /		
9						/ /	/ /		
8						/ /	/ /		
7						/ /	/ /		
6						/ /	/ /		
5						/ /	/ /		
4						/ /	/ /		
3						/ /	/ /		
2						/	/		

						/	/		
1						/	/		
						/	/		

KALVHÄLSOPROTOKOLL HYDDOR

DITT NAMN: **DATUM:**

HYDD-PLATS	KALV ID	HÄLSA OK? Skriv: 0	Om tecken på SJUKDOM fyll i: Symtom, åtgärd, behandling, övr.	Diarré 1, 2 el 3	Temp.	Mjölk (L) Mängd (3, 2 ,1L) Em / Fm	Mjölk (L) Rester (kanna) Em / Fm	Koncentrat (g) Mängd (mått)	Koncentrat (g) Rester (våg)
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