

Faculty of Veterinary Medicine and Animal Sciences

# Pair-housing of dairy calves in outdoor calf hutches

- Impact on growth and play behaviour

Parhållning av kalvar i kalvhyddor utomhus

- Påverkan på tillväxt och lekbeteende

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Department of Animal Environment and Health, no 725 Degree project 30 credits Uppsala 2017

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# 1. Summary

The aim of this study was to test the effects on dairy calves (*Bos taurus*) in outdoor calf hutches of individual vs. pair-housing on behaviour, growth and health. In total 33 female dairy calves of the breeds Swedish Holstein (SH) and Swedish Red (SR) cattle were used in the study. Twenty calves were pair-housed and 13 calves were individually housed. All calves had *ad lib*. access to water, concentrate, hay and silage and were fed three litres of whole milk twice per day with buckets equipped with a rubber teat. An extra teat bucket was always available in the calf hutch. Calves were weighed at birth and then once a week. Health protocols were filled in by the farm staff every day and collected monthly. At six, seven and eight weeks of age, calves were observed in a test arena ( $3.7 \times 6.4 m$ ) with a large rubber ball, five pieces of rope tied to the gates and an alien calf. Social play, locomotor play, object play, vocalisation, walk, standing together and defecate were recorded using one-zero sampling at one minute intervals for 20 minutes per session. The study began in wintertime 2017 (January 27<sup>th</sup>) and ended in early summer the same year (June 2<sup>nd</sup>).

Data from the behaviour observations was analysed in SAS using a generalised linear model that tested for the effect of housing treatment, age at testing, observation period (first 10 vs. last 10 minutes in test arena) and their interactions. Diarrhoea was tested with a  $Chi^2$ -test and the relative risk to be diagnosed with diarrhoea was calculated with a Cohort. Weight gain was analysed with a t-test with the Cohran method.

The behaviour observations in the test arena showed that pair-housed calves performed more social play (p<0.05) and stood more together (p<0.001) than did individually housed calves. The expression of social play increased with age (p<0.01) and occurred the most during the second observation period (min 11-20) (p<0.0001). There was no significant effect of treatment or age on locomotor play (n.s.). However, the calves performed more locomotor play during the first observation period (min 1-10) (p<0.0001). There were no significant effects on object play of treatment or period, but the calves performed more object play when six weeks old than when seven weeks (p<0.05) or eight weeks (p<0.01) old. The amount of vocalisation also decreased with age (p<0.0001), but did not differ between treatments. No significant effects were found of treatment, period or age on the number of recordings of walk. Pair-housed calves had an average daily weight gain of 0.88 kg/d and individually housed calves had average daily weight gain of 0.81 kg, but there was no significant difference in weight gain between treatments. Twelve pair-housed and five single-housed calves got diarrhoea and one pair-housed calf got an airway infection, but there were no significant differences in occurrence of diarrhoea between pair-housed and individually housed calves.

In conclusion, pair-housed calves had more social behaviours in an arena test than individually housed calves. However, pair-housing as compared to individual housing of dairy calves did not significantly affect weight gain, health, locomotor play, object play or vocalisation. Further research may be needed to evaluate the effects of pair-housing on calf welfare.

# 2. Sammanfattning

Syftet med denna studie var att testa effekten av par- vs. individuell hållning på beteenden, tillväxt och hälsa på kalvar (*Bos taurus*) i kalvhyddor utomhus. Totalt 33 kvigkalvar av raserna svensk Holstein (SH) och svensk röd (SR) användes i försöket. Tjugo kalvar hölls i par och 13 hölls enskilt. Alla kalvar hade fri tillgång till vatten, kraftfoder, hö och silage och fick två mål om tre liter mjölk vardera om dagen i napphink med gummispene. En tom napphink med gummispene fanns alltid tillgänglig i kalvhyddorna. Kalvarna vägdes vid födseln och därefter en gång i veckan. Hälsoformulär fylldes i dagligen av stallpersonalen och samlades in månadsvis. Vid sex, sju och åtta veckors ålder observerades kalvarna med en främmande kalv i en arena (3.7 x 6.4 m) med en stor gummiboll och fem snören fastknutna i metallgrindar. Social lek, rörelselek, objektlek, vokalisering, stå tillsammans och gödsla observerades med ettnoll registrering under sessioner om 20 minuter. Studien påbörjades vintern 2017 (27 januari) och avslutades försommaren samma år (2 juni).

Insamlade data från beteendeobservationerna analyserades i SAS med en generaliserad linjär modell som testade för effekten av inhysning, testvecka, observationsperiod och deras interaktioner. Förekomst av diarré testades med ett Chi<sup>2</sup>-test och den relativa risken att diagnosticeras med diarré beräknades med ett Cohort. Viktökning analyserades med ett t-test med Cohran-metoden.

Beteendeobservationerna visade att parhållna kalvar utförde mer social lek (p<0.05) och stod mer tillsammans (p<0.001) än vad enskilt hållna kalvar gjorde. Social lek ökade med ålder (p<0.01) och uttrycktes mest under den andra observationsperioden (min 11-20) (p<0.0001). Ingen signifikant effekt hittades av behandling eller av ålder på rörelselek (n.s.), men kalvarna utförde mest rörelselek under den första observationsperioden (min 1-10) (p<0.0001). Ingen signifikant effekt hittades av behandling eller observationsperiod (min 1-10) (p<0.0001). Ingen signifikant effekt hittades av behandling eller observationsperiod på objektlek, men kalvarna utförde mer objektlek när de var sex veckor gamla än sju veckor (p<0.05) och åtta veckor (p<0.01). Även vokalisering minskade med ökande ålder (p<0.0001). Ingen signifikant effekt hittades av behandling, ålder eller observationsperiod på beteendet gå. Parhållna kalvar hade en genomsnittlig daglig tillväxt på 0.88 kg medan enskilt hållna kalvar ökade i snitt 0.81 kg/dag, men ingen signifikant skillnad fanns mellan behandlingarna gällande daglig tillväxt. Det fanns heller inte någon signifikant skillnad i hälsa mellan behandlingarna.

Slutsatsen är att parhållning av kalvar inte hade någon signifikant effekt på tillväxt, hälsa, rörelselek, objektlek eller vokalisering. Däremot utförde parhållna kalvar fler sociala beteenden under arenatest än enskilt hållna kalvar. Mer forskning kan behövas inom områdena för kalvhållning och kalvars välfärd.

# **3. Introduction**

Cattle are social animals, capable of forming strong social bonds to each other (MacLennan, 2013; Moran & Doyle, 2015). Despite this, calves are commonly kept individually in crates or hutches until they are weaned, which occurs when they are approximately six to eight weeks old (Gaillard *et al.*, 2014; Stull & Reynolds, 2008). Disadvantages with this housing system are, among others, impaired learning, impaired ability to cope with the environment, poor social skills and a more negative reaction to weaning (Chua *et al.*, 2002; Duve & Jensen, 2012; Gaillard *et al.*, 2014; Jensen & Larsen, 2014; Rault, 2012; Stull & Reynolds, 2008).

In the absence of their dam, calves commonly perform cross-sucking (if peers are present) and licking of inanimate objects (Margerison *et al.*, 2003). However, there are cases when calves have been pair-housed with a very low incidence of cross-sucking (Chua *et al.*, 2002). Other abnormal behaviours that have been observed in individually and group-housed calves are tongue rolling and stereotyped chewing activities (Babu *et al.*, 2004; Wilson *et al.*, 1999).

It is difficult to assess farm animal welfare because of its interdisciplinary nature and the difficulty in defining welfare (Carenzi & Verga, 2009). Stull and Reynolds (2008) have proposed a number of factors that the welfare of commercially kept calves depends upon. These factors are housing, environment, nutritional and health programs, handling, herd dynamics and management practices (transportation, euthanasia, dehorning and teat removal). It is important to keep in mind that welfare is not only related to the absence of negative emotional states, but also the presence of positive emotional states (Ohl & van der Staay, 2012). Social bonds affect how well animals are able to cope with their environment and are a very important part of the animals' welfare (MacLennan, 2013). Calves raised individually are not allowed to form this kind of bonds and thus their welfare is impaired. Play behaviour has been used to assess calf welfare and arena tests have been proven useful in studying play behaviour (Mintline *et al.*, 2013).

The public is concerned about farm animal welfare, which is made visible in a survey study from USA in which 63 % of the respondents answered that they were concerned about dairy cattle welfare (Wolf *et al.*, 2016). The majority also agreed that welfare is more important than low milk prices. In a Swedish study, 83 % of the participants answered with the two most positive responses (4 and 5 out of 5) on the question of "Importance of animal welfare for Swedish consumers" (MacGregor *et al.*, 2011). This shows that it is of financial interest for farmers to adopt and promote high animal welfare standards.

# 4. Literature review

# 4.1. Social support

Social support can be defined as benefits that accompany having a partner and it makes it possible to cope with big challenges (Rault, 2012). Social support can both have direct positive effects and act as a stress-buffer (Cohen & Wills, 1985). The positive effects of providing social supporters to animals are evident in the endocrinal responses to stress and social support.

The hypothalamic-pituitary-adrenal axis (HPA axis) is a major endocrine system responding to stress, resulting in the release of several hormones, e.g. the stress hormone cortisol (Stephens & Wand, 2012). Studies have shown that by providing a partner to an animal, the HPA axis activation following stressful stimuli can be lowered (reviewed by Rault, 2012).

Oxytocin and its correlation with social support have been much studied. Researchers have shown that positive physical contact, such as a calf suckling its dam, stimulates oxytocin release

(Lupoli *et al.*, 2001), that increased oxytocin levels cause reduced cortisol levels and thus reduced stress (Cook, 1997) and that oxytocin increases the stress buffering effect of social support (Heinrichs *et al.*, 2003). This means that if allowed to have positive physical contact with a social supporter, an animal may tolerate more stress. It also means that the released oxytocin enhances the positive aspects of social support. Additionally, endogenous opioids are released during positive physical contact, such as social play and grooming (Loseth *et al.*, 2014). These substances, e.g. endorphins, reduce pain and the feelings of anxiety following exposure to a stressor (Koneru *et al.*, 2009). Consequently, social contact with a social supporter can make an animal less negatively affected by stress. Lastly, social support positively affects the immune system of animals receiving the support and can reduce heart rate, blood pressure and adrenalin levels in stressed animals (reviewed by Rault, 2012).

Of course, social support is not a suitable coping strategy for all types of stressors (Rault, 2012). If a peer is not required to cope with the stressor faced, then a peer cannot alleviate the stress. Neither are all individuals suitable as social support providers. Firstly, a social support provider should not be stressed itself. Secondly, familiarity is required in some species, but not in others, such as cattle. Thirdly, different species require different numbers of social support providers in order to experience the positive effects of the support. In cattle, it has been shown that more than one provider is optimal; Takeda *et al.* (2003) found that four peers had a more calming effect than one. Lastly, the effectiveness of social support depends much upon the natural social organisation of the species (Henessy *et al.*, 2006). Domesticated farm animals, that in natural settings form stable social groups, can be expected to benefit from social support (Rault, 2012).

Affiliative relationships are important for having a stable social structure and to reduce the occurrence of aggressive behaviours within a herd (Bouissou *et al.*, 2001). Affiliative relations in cattle can be observed as individuals standing closer to each other, less aggressive and more positive encounters and increased tolerance in competing situations. According to Bouissou *et al.* (2001), affiliative relations are preferably created during calves' first six months. This is in accordance with MacLennan's (2013) findings, that cattle form social bonds early if allowed. Furthermore, being able to form social bonds when young increases the strength of the social bonds formed when the cow is adult. In her study, MacLennan (2013) concluded that the stress response, seen as increased heart rate, of an isolated cow was smaller if a preferred partner was present than if an unfamiliar cow was present.

Other research has shown that calves form stronger social bonds to peers that they have had full social contact with, either from birth or from three weeks of age, than with peers they have only had contact with through bars (Duve & Jensen, 2011). In this study, calves preferred to socialise with a familiar companion calf rather than an unfamiliar calf. Furthermore, when put in an arena with another calf, the calves raised with limited contact did more pushing and mounting than calves raised with full social contact. The calves raised with limited social contact were thus "pushier" (more mounting and pushing) than calves raised with full social contact with a peer and socialised less with peers than did calves raised with full social contact in social preference tests. Broom and Leaver (1978) have also observed that individually raised calves spend more time alone when put in a group than group reared calves do.

The age when calves meet matters for the relation that will be formed. According to Raussi *et al.* (2010), calves approach faster and stay in closer proximity to a calf that they have met early in life (0.5 and 3.5 months of age) than a calf that they have met later (5.25 months of age). Raussi *et al.* (2010) also concluded that calves form relationships already when two weeks old,

that these relationships lasted at least for 1.5 year and that it is beneficial for calves to have social relationships.

# 4.2. Pair-housing, social behaviour and play

Cattle are gregarious animals and their behaviours are highly synchronised (Šárová *et al.*, 2007). In semi-natural conditions, calves mainly stay in proximity (<5 m) to another calf after reaching the age of one week (Ylipekkala and Woivalin, 1991) or approximately three weeks, and sometimes close to one adult guarding cow (Bouissou *et al.*, 2001). There are many possible reasons for this behaviour, and one is that it facilitates socialisation of the calves. Social interactions between the calves increase with their age and play fighting usually begins at two weeks of age. Locomotor play, playful mountings and vocalisation is also performed. Keeping calves in individual pens affects the behaviour of the calves in several ways. Locomotor, exploratory and social behaviours are reduced and if there is insufficient space (0.65-0.70 m wide and 1,70 m long crates in the study by de Wilt, 1985), sleeping postures are affected, which affects sleeping patterns (de Wilt, 1985). Additionally, calves have been found to be more motivated to get full social contact with another calf than only head contact through metal bars (Holm *et al.*, 2002).

Keeping calves in pairs instead of individually has several positive effects on the social development of calves. Pair-housed calves sniff and lick an unfamiliar calf earlier and more than do individually housed calves and individually housed calves have been described as more fearful of unfamiliar calves (Duve & Jensen, 2012; Jensen & Larsen, 2014). In the study by Jensen and Larsen (2014) calves put in a social test sniffed the other calf sooner if they had been reared in pairs during their whole or a part of their lives than if they had been individually housed during their whole lives. It was also seen that calves that had been reared with physical contact with another calf sniffed the other calf for longer than calves reared with no physical contact with other calves. The individually housed calves were described as more fearful of unfamiliar calves than pair-housed calves were. Group reared calves have also been seen to be dominant over individually raised calves when they are all put together in one pen (Broom & Leaver, 1978). The previously group reared calves also initiated more contacts than did previously individually housed calves and mainly stayed in proximity to calves from their previous group, indicating the presence of a social bond. The previously individually housed calves also seemed to be incompetent in social encounters and spent more time alone than did previously group reared calves.

# 4.3. Arena tests

Arena tests can be used to test how different treatments affect certain behaviours of animals. Mintline *et al.* (2012) used arena tests to evaluate how different types of housing affected the motivation for locomotor play in calves and to investigate how suitable arena tests are for this sort of research. The results showed that play in an arena does reflect play in the home pen, that if the tests are done on alternate or consecutive days does not affect play behaviour and that calves show more running in long, large pens and more jumping in small pens. The authors concluded that arena tests are good tools for behavioural research, but one must interpret the results carefully.

# 4.4. Abnormal behaviour

Because animal welfare has to do with, among other things, animals showing a minimum of abnormal behaviours, it is important to define what abnormal behaviours are (Wiepkema *et al.*, 1983). In collaboration with 32 researchers, Wiepkema *et al.* (1983) defined five categories of abnormal behaviour: injurious behaviours, stereotyped behaviours, abnormal body movements,

redirected behaviours and apathetic behaviour. A behaviour is not restricted to only one of these categories, but may appear in several. Veal calves are calves that receive large amounts of milk but no concentrate and no or small amounts of hay. Behaviours stated to be performed by veal calves in crates are coat licking (injurious behaviour), tongue rolling (stereotyped behaviour) and licking pen and objects and sham ruminating (redirected behaviour) (Wiepkema *et al.*, 1983). Behaviours stated to be performed by veal calves in group housing are sucking on pen mates and urine sucking (injurious behaviour) and tongue rolling (stereotyped behaviour). Abnormal lying down or standing up can be performed by all cattle, often seen in housing systems with slatted floor (see review by Lidfors, 1989).

Cross-sucking, i.e. a calf sucking on any body parts of another calf, is an abnormal behaviour causing welfare and health problems, such as disease transmission and inflammation in body parts exposed to sucking (de Passillé, 2001; Jung & Lidfors, 2001; Wiepkema et al., 1983). Milk ingestion stimulates sucking behaviour, and when a dam or teat is not available the sucking motivation is unsatisfied and sucking can be directed towards other calves, occurring mostly directly after milk intake (de Passillé, 2001; Jung & Lidfors, 2001). Cross-sucking is a problem in artificially reared calves, receiving small amounts of milk/milk replacer that is ingested quickly (Jung & Lidfors, 2001). Furthermore, cross-sucking in calves is linked to intersucking in dairy cows and heifers, which causes milk loss and poor udder health (Keil et al., 2000). Studies have shown that cross-sucking can be reduced by increasing milk intake time by decreasing the milk flow rate (Loberg & Lidfors, 2001), by providing enough milk to satisfy the calf (51 in the study by Jung & Lidfors, 2001) and by leaving a teat bucket with the calves after milk feeding (Jung & Lidfors, 2001). It is also possible to reduce cross-sucking with technical solutions, such as automatic milk feeders with closed stalls, which makes the calves stay longer after milk ingestion and perform non-nutritional sucking on the teat (Weber and Wechsler, 2001). However, this only caused a reduction in cross-sucking in the first 15 minutes after milk feeding.

#### 4.5. Pair-housing and performance

Housing calves in pairs or groups has been shown to give several positive effects on the performance of the calf (i.e. feed consumption ratio and growth). In a study from 2015, it was found that calves that were pair-housed from an early age (3-9 days old) had a higher daily weight gain and a higher intake of calf starter throughout the study than calves kept individually or paired at an older age (40-46 days old) (Costa et al., 2015). Similar results were achieved in another study, in which pair-housed bull calves had higher concentrate intake than individually housed bull calves both pre-weaning and during weaning (Miller-Cushon & DeVries, 2016). However, there were no differences in milk intake between treatments in that study. Chua et al. (2002) have found that both before and after weaning, individually kept and pairwise kept calves gained weight at the same rate. However, the individually raised calves had a halted growth at weaning while the pair-housed calves kept growing in the same rate as before, which demonstrates a benefit of pair-housing calves. Chua et al. (2002) did not find any differences in consumption of milk, starter or hay between individually and pair-kept calves. However, these recordings were not made after mixing, but while calves were individually housed or pairhoused. Neither were any significant differences found in concentrate intake or growth between pair-housed and individually housed calves in a more recent study (Bolt et al., 2017).

In another study, single-housed calves were found to drink more milk per calf than grouphoused calves (Babu *et al.*, 2004). However, group-housed calves learned to eat solid feed earlier, thus consuming more solid feed than single-housed calves did, and they also ruminated earlier and more than did single-housed calves. Similar differences between individually housed and pair-housed calves have been found in a study by De Paula Vieira *et al.* (2010). In that study, pair-housed calves consumed more starter pre-weaning than did single-housed calves and after mixing calves in groups with new calves, the previously pair-housed calves ate more starter and started eating it sooner than did previously single-housed calves. Thus, the pair-housed calves had a higher weight gain after mixing. In accordance with the results of Chua *et al.* (2002), the pair-housed calves in this study showed less response to weaning, in form of halted weight gain, than individually housed calves did.

In an older study, no association between type of housing (individual or group housing) and weight change was found (Broom & Leaver, 1978). However, a positive correlation was found between weight change and rank change and group reared calves were found to have higher ranks than individually reared calves when all calves were put in one group.

#### 4.6. Pair-housing and health

Researchers have tried raising calves with different levels of social contact (visual, auditory or tactile contact, combinations of these or full contact) and found that none of the treatments had an effect on calf health (Jensen & Larsen, 2014). Health parameters measured were pathogen level in faeces, clinical scores and serum antibody development. Similarly, when comparing the health of pair-housed and single-housed calves, measured as rectal temperature, respiration or pulse rate (Babu et al., 2004), faecal score or respiration score (Bolt et al., 2017) or incidence of diarrhoea (Chua et al., 2002) no differences were found. Chua et al. (2002) also argued that a proper management with good hygienic routines can have a better effect on reducing diarrhoea than choosing individual housing. Considering these results, it would seem as if pair-housing has no adverse effect on calf health. However, the incidence of diarrhoea was higher in individually housed male dairy calves during their first twelve weeks than if the calves were housed in groups of four (indoors or outdoors with unheated shelters) (Hänninen et al., 2003). In that study, it was also found that the duration of diarrhoea was the longest in group-housed calves (outdoors with unheated shelters). In another study, it was found that housing calves in groups of maximum nine calves reduces the incidence of respiratory disease and increases growth rate compared to larger groups (Svensson & Liberg, 2006). However, there was no effect of group size on diarrhoea incidence or serum haptoglobin concentration.

In a smaller study on 21 dairy calves kept outdoors in hutches during winter time the occurrence of diarrhoea was higher in pair-housed than in single-housed calves (Alvegard, 2016). Another study on a large number of calves (3081 calves) found that the risk for respiratory disease was higher in large-group pens (6-30 calves) than in single pens and small-group pens (3-8 calves) (Svensson *et al.*, 2003). The results also showed that diarrhoea occurred the most in large-group pens, but the researchers are not sure whether or not that was caused by difficulties in diagnosing individual calves in large groups. However, in that study pair-housing was not investigated.

#### 4.7. Welfare

What constitutes animal welfare has been explained in several ways by different researchers. Nordenfelt (2008) presents a number of theories in his report, including those focusing on the biology, evolution and physiological functioning of animals, those mainly concentrating on the emotions of animals, those arguing that welfare is about offering animals what they need and prefer, those focused on the possibility to behave naturally as well as being healthy, and lastly some argue that animals' welfare is dependent on all these features.

There are many methods available to assess farm animal welfare (Johnsen *et al.*, 2001). The suitability of a method in a certain case depends much on the goal of the assessment (e.g. promote animal welfare at the farm or compare animal welfare at several farms) and the practicability of the method. There are two types of welfare parameters, animal-based (the state of the animal, e.g. pain) and resource-based (e.g. space allowance). It is preferable to include both types in welfare assessment to get a result that reflects the actual welfare as perceived by the animals.

According to Dawkins (2004), there are two key questions that must be answered in animal welfare assessment. These questions are "Are the animals healthy?" and "Do the animals have what they want?". By observing the behaviour of animals, we can answer these questions, according to Dawkins (2004). Behavioural observation is a good method to use because it is non-invasive and non-intrusive. To answer the first question, one looks for behaviour related to pain and sickness. To answer the second question, one can e.g. look at animals' choices (directly or retrospectively), observe their behaviour in wanted and unwanted situations or detect signals, such as vocalisations. Play has been used as an indicator of positive welfare, for example by Duve *et al.* (2012). In that study single-housed calves with a low milk allowance were found to play less than pair-housed calves with a low milk allowance and also less than both single-housed and pair-housed calves with a high milk allowance. This result can be interpreted as a lower welfare in the single-housed calves.

Gaillard *et al.* (2014) did in an experiment test the effect of social housing on the cognitive abilities of calves. Pair-housed and individually housed calves were tested in reversal learning and object recognition. In the reversal learning experiment, calves were put in a Y-maze with a milk bottle in a white box in one arm and a milk bottle in a black box in the other arm. Calves were alternately trained to learn that there was milk in the black or in the white box. In the object recognition experiment, calves were put in a pen with a novel object and the time calves spent interacting with the object was measured. The pair-housed calves performed better than the individually housed in both test types, learning a reversed task and habituating to an object faster. However, there was no difference in the initial learning to discriminate two colours. Only when the previously rewarded colour became unrewarded and vice versa did the pair-housed calves perform better in learning this new, reversed relationship between colour and reward. This suggests that raising calves individually causes impaired learning ability, makes calves less adaptable to a changing environment and results e.g. in calves taking longer before starting to eat when first put in a group.

Studying positive welfare, such as play, of dairy calves can provide further information about the welfare of calves in the pre-weaning period.

# 4.8. Aim and questions

The aim of this report is to investigate how pair-housing of calves in outdoor calf hutches affects growth, health and play behaviour in an arena with objects and another calf compared to individual housing in calf hutches. Questions to be answered are:

1. Does social, locomotor and object play behaviour as well as vocalisation in an arena with objects differ between pair-housed and individually housed dairy calves in outdoor calf hutches?

2. Does growth differ between pair-housed and individually housed dairy calves in outdoor calf hutches?

3. Does health differ between pair-housed and individually housed dairy calves in outdoor calf hutches?

The hypotheses were that the amount of performed social, locomotor and object play in an arena test would be significantly greater in pair-housed calves than individually housed calves, while the amount of vocalisation in an arena test would be significantly smaller in pair-housed than individually housed calves. It was also hypothesised that calf growth and calf health would not be significantly different between pair-housed and individually housed calves.

# 5. Material and methods

This study was carried out at The Swedish Livestock Research Centre at SLU Lövsta, a facility located in Funbo Lövsta in the county of Uppsala. At Lövsta there are stables with cattle, pigs and fowl, used for education and research. The cattle stable has about 280 cows of the breeds Swedish Holstein (SH) and Swedish Red (SR). There are 39 covered outdoor hutches, in which calves are kept individually until weaning. An animal ethics approval was decided before the study was conducted (Dnr. C89/15).

#### 5.1. Animals

Heifer dairy calves of the breeds SH and SR cattle were used. In total 33 calves participated in the experiment. Calves that were paired together had an age difference of maximally six days. The calves used were born between January 27<sup>th</sup> and April 7<sup>th</sup> 2017. Calves entered the study no earlier than directly after ingesting their last colostrum meal and no later than at an age of eleven days. Calves had their last, of in total six, colostrum meal at an age of three days. The study lasted for 127 days and ended on June 2<sup>nd</sup> 2017.

#### 5.2. Housing and management

All calves were kept in outdoor calf hutches (Holm & Laue, Gårdsby Iglu AB, USA) interior measures 208 x 107 x 122 cm), with a fenced area in connection to the hutch of approximately 2 m<sup>2</sup> (1.3 x 1.55 m) (fences from Gårdsby Iglu AB, Germany). Thirteen calves were kept individually in the hutches (Fig. 1) and twenty calves were kept in ten pairs in the hutches (Fig. 2). Water was provided *ad lib*. in buckets, hay and silage were provided *ad lib*. in hay racks and buckets, respectively. Concentrate (Idol, Lantmännen Lantbruk) was provided *ad lib*. in buckets inside the hutch. Three litres of whole milk were provided twice a day (approximately at 7.00 and 18.00) in teat buckets (Granngården, Albert Kerbl, Poland) with rubber teats (Gårdsby Iglu AB, Germany). Straw was provided as bedding material and refilled daily. An empty teat bucket was always available in the hutches. This was because of its proven effect as a partial compensation for lack of sucking possibility in calves raised without their dam (Veissier *et al.*, 2002). All calves were weaned off from milk after they had finished their participation in the study.



Figure 1. Calf kept individually in a hutch



Figure 2. Calves pair-housed in a hutch

# 5.3. Experimental design

Behaviour observations were performed on pairs of calves with one focal animal (test calf) and one companion calf. Out of all calves used in this study, 23 were test calves and 10 were companion calves in the behaviour observations. The same calf was used as a companion calf in behaviour observations for a maximum of four times. At approximately six, seven and eight weeks of age, calves were observed in a test arena (further description below) with objects and a companion calf. Behaviour observations were done at the Tuesday or Friday when the calves were closest to being six weeks, seven weeks and eight weeks old. At its first behaviour observation, the test calf had never met the companion calf before. A test calf always had the same companion calf for all three behaviour observation sessions.

The arena where play behaviour observations were carried out was built by 145 cm high metal gates and was 6.4 x 3.7 m. In the arena, there were an exercise ball (approximately 55 cm in diameter) and five pieces of blue and white 16-strand polypropylene rope (diameter 9 mm) tied to the metal gates, evenly distributed, 90-100 cm above the ground and each piece 100 cm long. Figure 3 and Figure 4 illustrate the arena.



Figure 3. Arena for behavioural observations.

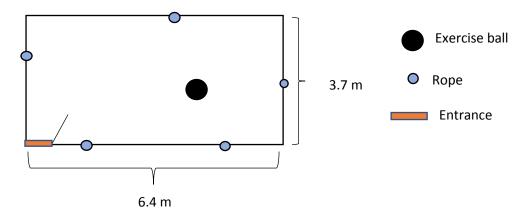


Figure 4. Arena for behavioural observations

Before each observation session, the ball was placed in start position in the centre of the arena. The ball was washed when it got too dirty and the ropes were washed after every second observation day. In most cases, the calves were led in a halter, one by one, to the arena. A small wagon was sometimes used to transport calves, one by one, between the arena and calf hutches. The companion calf always arrived first, and was left alone in the arena for maximum 5 minutes before the test calf arrived.

#### **5.4. Behaviour observations**

For behaviour observations, one-zero sampling was used to record behaviours (Martin & Bateson, 2010). Recordings were made on one of the two calves in the arena during 20 minutes. For each minute recordings were made on if a behaviour occurred or not. The behaviour observation session was divided into two periods, period one (1-10 min) and period two (11-20 min). Paper check sheets were used as recording medium. Recorded behaviours were categorised as locomotor play, social play, object play and other behaviours (see Table 1). The behaviours high bellow, low bellow and baaock were grouped to form a vocalisation variable (see Table 1).

Behaviour	Description	Source
Social play		
Sniff calf	Muzzle on other calf for $>2$ seconds	
Lick calf	Tongue visible on other calf	Adapted from Zipp <i>et al.</i> , 2016
Mount	Front legs lifted from the ground and forepart of the body is placed on another calf's head or body from front, side or back	Jensen & Kyhn, 2000
Frontal pushing	<ul> <li>Two calves standing front to front, butting head against head/neck in a <i>playful manner</i>:</li> <li>the behaviour is not accompanied by displays of dominance, such as stares or head swings.</li> <li>there is no flight/withdrawal of the losing animal.</li> <li>there is no apparent cause for fighting, such as limited amount of food/water/space that both animals seem to want.</li> </ul>	Jensen <i>et al.</i> , 1998; Somers, 2012
Locomotor		
play		
Jump	Two forelegs lifted off ground, forepart of body elevated, movement upwards and/or forwards. Hind legs may lift off ground in last phase of the movement.	Adapted from Jensen <i>et al.</i> , 1998
Buck	Body ascends from front to back with hoofs momentarily off ground	Adapted from Jensen <i>et al.</i> , 1998
Kick	One hind leg is lifted off ground and extended to the rear or side	Mintline <i>et al.</i> , 2012
Head-shake	Head shaken or rotated	Jensen <i>et al.</i> , 1998
Run	Trot (two-beat gait, leg movements synchronized diagonally), canter (three-beat gait in between trot and gallop) and gallop (four-beat gait with a phase with all legs off the ground)	Mintline <i>et al.</i> , 2012
Turn	Two forelegs lifted from the ground and stretched forward, as the forepart of the body is elevated and turned to one side, the calf moves sidewards. Movement upwards and sidewards.	Jensen & Kyhn, 2000
<b>Object play</b> Sniff/lick	Muzzle and/or tongue on object for >2 seconds	
object Butt object	Butting object with head in a playful manner, standing up	Adapted from Jensen <i>et al.</i> , 1998
Other behaviours Defecate Low bellow	Calf defecating Calf vocalising with mouth almost closed Calf vocalising with mouth open	
High bellow Baaock	Short, barking vocalisation	
Duuter	Shore, burking vocunsulon	

Table 1. Ethogram of the behaviours recorded in the calves in the play arena

Walk	Four-beat, symmetrical, slow gait. Takes $\geq 2$ steps with all four legs	Broom & Fraser, 2007
Stand together	Calves standing <1 m away from each other for >4 seconds	,

#### 5.5. Weighing

A mobile scale was used to weigh the calves at birth and then once every week.

#### 5.6. Health recordings

The personnel at Lövsta daily registered in individual calf health protocols whether or not there were any signs of disease and if so, what signs. Diarrhoea were given scores from 0 (no diarrhoea) to 3 (severe diarrhoea) (see Table 2). A calf with diarrhoea score 1, 2 or 3 was registered as having diarrhoea. Rectal temperature was measured if the calf was sick and registered in the protocol. The protocols were collected during the study period.

Table 2. Definitions used for diarrhoea scoring of calves

Score	Definition
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.

#### **5.7. Statistical analysis**

Data management was performed using Microsoft Excel version Office home and student 2016 (© 2016 Microsoft Corporation version 16.0.8431.2046). Analysis was done in SAS (SAS Institute Inc., Cary, USA, version 9.4). Data for behaviours in the arena tests were analysed using a generalised linear model (PROC GENMOD) with the fixed effects of housing treatment (individual or pair), observation period (1=1-10 min and 2=11-20 min) and age (week 6, 7 and 8) and the repeated subject was the individual calf. The grouped behaviours social play, locomotor play, object play, standing together and vocalisation were tested with PROC GENMOD. Significant level was set to 0.05. Data are shown as medians with interquartile range 1 (Q1) and interquartile range 3 (Q3).

Number of calves being diagnosed to have diarrhoea was compared to number of calves not having diarrhoea in pair- vs. individual housing with a Chi<sup>2</sup>-test. The relative risk to be diagnosed with diarrhoea was calculated with a Cohort.

Weight gain from birth to 7-8 weeks of age (min=45, max=58 days) was calculated and divided with number of days at last weighing to get average daily weight gain. The average weight gain of pair-housed and individually housed calves was analysed with a t-test with the Cohran method. Mean data and standard deviations (SD) are shown.

#### 6. Results

#### 6.1. Behaviour at arena test

Each behaviour that was recorded when calves were put in pairs in the play arena are found in the table below (Table 3). Of the social play behaviours, sniff calf was the most common (Table

3). Run, jump, kick and head-shake were the most common locomotor play behaviours and the object play behaviour sniff/lick object was more common than butt object (Table 3). Defecation and all three types of vocalisation were quite low (Table 3). The behaviour stand together will presented in more detail below (Table 3).

Behaviour group	Behaviour	Individual	Pair
Social play	Sniff calf	0.41 (0.33, 0.48)	0.53 (0.38, 0.58)
	Lick calf	0.01 (0, 0.03)	0.03 (0, 0.10)
	Mount	0 (0,0)	0 (0, 0.17)
	Frontal pushing	0.11 (0.07, 0.13)	0.09 (0.02, 0.18)
Locomotor play	Jump	0.33 (0.28, 0.42)	0.24 (0.18, 0.40)
	Buck	0.18 (0.10, 0.23)	0.18 (0.08, 0.28)
	Kick	0.23 (0.16, 0.33)	0.28 (0.25, 0.30)
	Head-shake	0.33 (0.29, 0.44)	0.23 (0.20, 0.30)
	Run	0.58 (0.53, 0.62)	0.61 (0.52, 0.65)
	Turn	0.11 (0.07, 0.18)	0.12 (0.05, 0.20)
Object play	Sniff/lick object	0.44 (0.42, 0.52)	0.60 (0.38, 0.65)
	Butt object	0.06 (0, 0.13)	0.03 (0, 0.12)
Vocalisation	Low bellow	0 (0, 0.02)	0 (0, 0.02)
	High bellow	0 (0, 0)	0.02 (0, 0.02)
	Baaock	0.03 (0, 0.05)	0 (0, 0.03)
Other behaviours	Defecate	0 (0, 0.02)	0.01 (0, 0.03)
	Walk	0.78 (0.75, 0.85)	0.78 (0.72, 0.87)
	Stand together	0.18 (0.12, 0.28)	0.35 (0.28, 0.50)

Table 3. Median number (Q1, Q3) of recorded behaviours per minute at 20 minutes of testing in a play arena in individually housed (n=10) and pair housed (n=12) calves

# 6.1.1. Social play

There were significant effects in social play of treatment (p<0.05,  $Chi^2 = 6.33$ ), age (p<0.01,  $Chi^2 = 11.77$ ) and period (p<0.0001,  $Chi^2 = 32.32$ ). Pair-housed calves performed more social play than did single-housed calves (Fig. 5). There were significantly higher number of recordings of social play when calves were 7 compared to 6 weeks old (p<0.05), and 8 compared to 6 weeks old (p<0.01), but no difference between 7 and 8 weeks of age (Fig. 5). There were also significant interactions between treatment and age (p<0.05,  $Chi^2 = 7.05$ ). There were significantly higher number of recordings of social play in pair-housed calves compared to individually housed calves both at 6 weeks (p<0.01), 7 weeks (p<0.05) and 8 weeks (p<0.05) (Fig. 5). All calves performed more social play during period 2 compared to period 1 (Fig. 9).

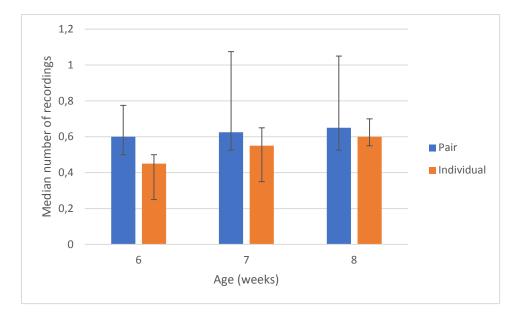


Figure 5. Median number of recordings per minute (Q1, Q3) of social play at six, seven and eight weeks of age when testing calves during 20 minutes in a play arena when being pair-housed (n=12) and individually housed (n=10)

#### 6.1.2. Locomotor play

There were no significant effects in locomotor play of treatment (n.s.,  $\text{Chi}^2 = 1.33$ ). There were significant effects on locomotor play of period (p<0.0001,  $\text{Chi}^2 = 18.48$ ) and a tendency of an effect of age (p<0.1,  $\text{Chi}^2 = 4.64$ ). There was a higher number of recordings of locomotor play when calves were 6 weeks compared to 8 weeks old (p<0.05), but there were no differences between 6 and 7 vs. 7 and 8 weeks old calves (Fig. 6). All calves had higher levels of locomotor play during observation period 1 compared to period 2 (Fig. 9). There was a significant interaction between age and period (p<0.0001,  $\text{Chi}^2 = 21.87$ ). At an age of 7 weeks (p<0.05) and 8 weeks (p<0.001), but not 6 weeks, calves had a higher number of locomotor play during period 1 than period 2. There was also a significant interaction between treatment and period (p<0.05,  $\text{Chi}^2 = 3.87$ ). During period 2, the individually housed calves did more locomotor play than individually housed calves did.

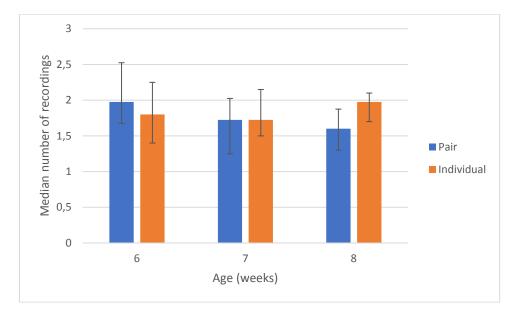


Figure 6. Median number of recordings per minute (Q1, Q3) of locomotor play at six, seven and eight weeks of age when testing calves during 20 minutes in a play arena when being pair-housed (n=12) and individually housed (n=10)

#### 6.1.3. Object play

There were no significant effects on object play of treatment (n.s.,  $\text{Chi}^2 = 0.76$ ) or period (n.s.,  $\text{Chi}^2 = 1.12$ ), but there was a significant effect on object play of age (p<0.05,  $\text{Chi}^2 = 6.97$ ). There were significantly higher number of recordings in object play when calves were 6 weeks compared to 7 weeks old (p<0.05) and 6 compared to 8 weeks old (p<0.01) (Fig. 7). There were no differences between 7 and 8 weeks old calves. There was also a significant interaction between age and period (p<0.0001,  $\text{Chi}^2 = 18.92$ ). There were significantly higher number of recordings of object play in period 2 than in period 1 in calves at an age of 6 weeks (p<0.01) and tendency of higher number of recordings in period 2 than in period 1 in calves at an age of 7 weeks (p<0.1). On the contrary, there was significantly higher number of recordings of object play in period 2 when calves were 8 weeks old (p<0.05).

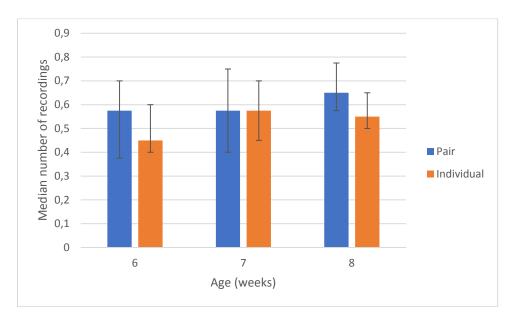


Figure 7. Median number of recordings per minute (Q1, Q3) of object play at six, seven and eight weeks of age when testing calves during 20 minutes in a play arena when being pair-housed (n=12) and individually housed (n=10)

#### 6.1.4. Vocalisation

There was no significant effect on vocalisation of treatment (n.s.,  $\text{Chi}^2 = 0.12$ ) or of period (n.s.,  $\text{Chi}^2 = 0.80$ ). There was a significant effect on vocalisation of age (p<0.0001,  $\text{Chi}^2 = 27.30$ ). There was a higher number of recordings of vocalisation in calves that were 6 weeks old (median=0.05) compared to calves that were 7 weeks old (median=0) (p<0.001) and 8 weeks old (median=0) (p<0.001). There was no significant difference in the number of recorded vocalisation between calves at an age of 7 and 8 weeks. There was also a significant interaction between age and period (p<0.001,  $\text{Chi}^2 = 16.61$ ) and a tendency for an interaction between treatment and age (p<0.1,  $\text{Chi}^2 = 4.79$ ). There was a higher number of recordings of vocalisation during period 1 in a play arena than during period 2 when calves were 8 weeks old (p<0.005), but there were no significant differences in number of vocalisations between period 1 and period 2 when calves were 6 weeks old and 7 weeks old.

### **6.1.5.** Standing together

There were significant effects on standing together of treatment (p<0.001, Chi<sup>2</sup>=11.48) and period (p<0.001, Chi<sup>2</sup>=11.32), and a tendency of an effect of age (p<0.1, Chi<sup>2</sup>=4.96). Pair-housed calves had a higher number of recordings of standing together than individually housed calves (Table 3). During period 1, calves were staying less together (median=0.2, Q1=0.1, Q3=0.3) than during period 2 (median=0.32, Q1=0.2, Q3=0.5). There was a significant difference between calves at age 6 and 7 weeks (p<0.05) and a tendency of a difference between calves at age 6 and 7 weeks (p<0.05) and a tendency of a difference between calves were staying more together as they became older whereas individually housed calves had the lowest number of recordings of standing together when they were 8 weeks old (Fig. 8). There were no significant interactions between treatment and age, treatment and period or age and period.

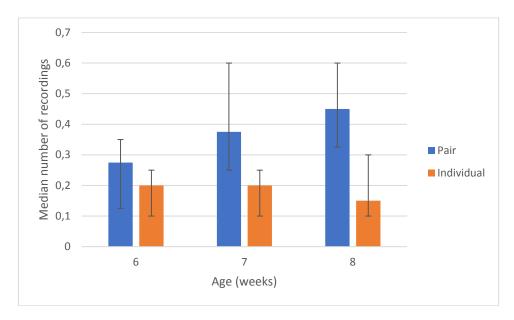


Figure 8. Median number of recordings (Q1, Q3) of standing together per 20 minutes at six, seven and eight weeks of testing in a play arena in pair housed (n=12) and individually housed (n=10) calves

How social play, locomotor play and object play differed between period 1 and period 2 is shown in Figure 9.

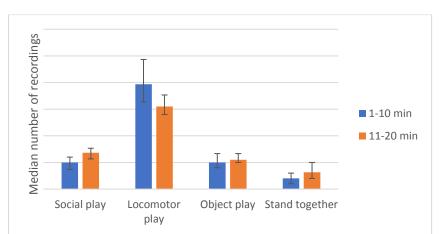


Figure 9. Median number of recordings of social play, locomotor play and object play during period 1 and period 2 of behaviour observations in a play arena (n=22 calves)

#### 6.1.6. Walk

There were no significant effects of treatment (n.s.,  $Chi^2 = 0.39$ ), age (n.s.,  $Chi^2 = 0.56$ ) or period (n.s.,  $Chi^2 = 0.90$ ) on walk. There were no significant interactions between treatment and age or between age and period. There was, however, a tendency for an interaction between treatment and period (p<0.1).

# 6.2. Growth

There were no significant differences in daily growth rate from birth until approximately seven weeks of age between treatments, but a tendency for higher daily growth in pair-housed calves compared to individually housed calves (p=0.10, t=-1.76). The average daily weight gain in pair-housed calves was 0.88 kg ( $\pm 0.07$  SD) and 0.81 kg in individually housed calves ( $\pm 0.15$  SD). The weight gain of pair- and individually housed calves over seven weeks is shown in Figure 10.

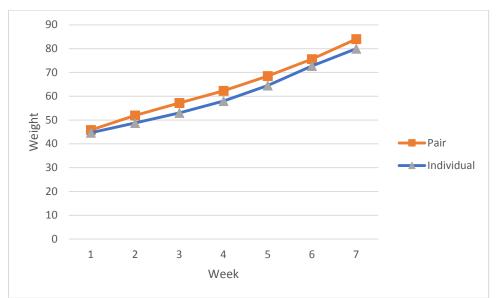


Figure 10. Weight (kg) of pair-housed and individually housed calves when 1-7 weeks old. Calves defined as 1 week are 1-7 days old, calves defined as 2 weeks are 8-14 days old and so on (n = 20 pair, n = 10 individual)

# 6.3. Health

No calf had to be taken out of the study and no pair had to be separated for health reasons. One pair, however, had to wear an anti-sucking device in hard plastic with spikes (Albert Kerbl, Germany) on their muzzles during the milk period (no recording of exact dates) to prevent cross-sucking. In total, twelve pair-housed and five single-housed calves got diarrhoea at least once and stayed sick for at least one day (Table 4). One pair-housed calf got an airway infection. Drugs used for diarrhoea were Diakur and Effydral, two rehydration agents with electrolytes. The calf with the airway infection was treated with Metacam®, a non-steroidal anti-inflammatory drug (NSAID), and Penovet®, an antibiotic.

Age	Individually		Pair-housed	
(weeks)	housed			
	Calves	Days	Calves	Days
1	0	0	0	0
2	3	1-9	6	1-4
3	2	5-6	2	10
4	0	0	4	2
5	1	1	3	1-3
6	0	0	5	1-3
7	0	0	1	3
8	1	2	5	1-4

Table 4. Number of calves diagnosed with diarrhoea and number of days (min - max) being recorded with diarrhoea when being kept in outdoor hutches either individually housed (n=13) or pair-housed (n=20) at 1-8 weeks of age. If the same calf was diagnosed again it is counted as a new case

#### 7. Discussion

The results from this study show that when tested in a play arena with an alien calf at 6, 7 and 8 weeks of age, calves that were pair-housed in hutches perform more social play and standing close together than calves that were housed individually in hutches. There were no differences in amount of locomotor play, object play, walking and vocalisation between pair-housed and individually housed calves. The amount of social play increased with age, while object play and vocalisation decreased with age. Social play, object play and standing together were performed most during observation period 2, while locomotor play was performed most during observation period 1. There were no significant differences in calf growth per day from birth until seven weeks of age or in diarrhoea and airway infections between pair-housed and individually housed calves.

#### 7.1. Behaviour

In the current study, pair-housed calves were observed to perform more social play and stood more together than individually housed calves did. This is in accordance with the results from the study by Broom and Leaver (1978), where individually housed calves spent more time alone when put in a group at eight months of age than did group reared calves. It is also in agreement with Jensen and Larsen (2014), who found that calves that had been reared with physical contact with another calf sniffed the other calf for longer and sooner than calves reared with no physical contact with other calves when tested at six weeks of age. Furthermore, it has been seen that calves that were single-housed with a low milk allowance played less than single-housed calves with a high milk allowance (Duve *et al*, 2012). In that study play was recorded in the home pen for 20 minutes once a week during week 2-6 after provision of straw. The shorter duration of play was explained by the combination of individual housing and low milk allowance (Duve *et al*, 2012).

Calves in this study performed more social play as they became older. This is in agreement with the observation that the expression of play behaviours in free-ranging dairy calves increased until the calves were six months old (Woivalin, 1990). Furthermore, the results of this study showed that more social play was performed during the last ten minutes of the observation than the first ten minutes. This latency to socially interact with the companion calf can be explained by fearfulness, because avoidance of fear stimuli is regarded as an indicator of fear (Jensen *et al.*, 1999). In the current study, the test calf was put in the test arena with an alien calf, which the test calf might be motivated to observe before encountering. However, it may also merely

illustrate that the motivation to perform social play was lower than the motivation to explore the arena and the objects and perform other behaviours.

That pair-housed calves in this study had the highest number of recordings of the behaviour standing together is suggested to be caused by the fact that pair-housing offers a higher degree of socialisation than what is possible in individual housing. This resembles the results of the study by Jensen and Larsen (2014), in which pair-housed calves sniffed an alien calf sooner and for longer than calves housed individually with auditory and/or visual contact with other calves. This socialisation can be advantageous later in life, when heifers are integrated in the milk herd, as seen by for example Wagner *et al.* (2012). In their study, calves were socialised with their mother and with other calves.

The results in this study show that all calves performed more locomotor play during the first 10 minutes of observation than during the following 10 minutes. A possible reason for this is the rebound effect, as described by Held & Špinka (2011). The larger than usual space is a new resource. Thus, the calves may perform extraordinary amounts of space requiring locomotor play to compensate for the previous absence of this resource (Held & Špinka, 2011). If this rebound effect occurs in a burst of locomotor play and then fades away, it would be seen as a larger number of recordings of locomotor play in period 1 than period 2. There was no difference in the amount of locomotor play between pair-housed and individually housed calves, as opposed to the results of earlier studies (Dellmeier et al., 1985; Valníčková et al., 2015). In those studies, individually housed calves showed a greater rebound effect than pairhoused calves did. This has been interpreted as an indication of play behaviour deprivation in single-housed calves (Valníčková et al., 2015). In the study by Dellmeier et al. (1985) bull calves were used, while in the current study and in the study by Valníčková et al. (2015), heifer calves were used. Valníčková et al. (2015) housed the calves individually or in groups of four. Possibly, the difference in level of social contact between housing treatments is not as big in the current study as in the study by Valníčková et al. (2015), leading to the difference in rebound effect between treatments not being visible. Another reason for the differing results could be the age of the calves at testing. Dellmeier et al. (1985) tested the calves at 6.5 weeks of age, Valníčková et al. (2015) tested the calves at 2, 5 and 12 weeks of age and in the current study the calves were tested at 6, 7 and 8 weeks of age.

The amount of recorded object play in this study was the highest when the calves were six weeks old, i.e. the lowest age for behaviour observation. In a study by Duve *et al.* (2012), it was found that calves had a peak in play performance (social, locomotor and object play) when they were three weeks old. After that the amount of play performed declined until there was essentially no play in week six (Duve *et al.* 2012). This is in agreement with the effect of age on object play in the current study. However, because each calf was only observed at three occasions, one cannot be sure that an increase in amount of play behaviour for each observation session was not simply a coincidence or an effect of the daily condition of the calves, but an effect of increasing age.

Calves in the current study vocalised the most at an age of 6 weeks and similarly to a study by Jensen *et al.* (1999), there was no effect of housing treatment on amount of vocalisation. Jensen *et al.* (1999) compared calves housed in open single pens, closed single pens, calf groups and groups of cows and calves and found that housing treatment did not affect number of vocalisations. Bolt *et al.* (2017) found that the pair-housed calves in their study vocalised less pre- and post-weaning than individually housed calves. This was explained to be due to social buffering or a stress buffering effect, meaning that the presence of another calf reduced the

stress experienced by the pair-housed calves, which thus vocalised less (assuming vocalisation is a sign of stress. In modern calf management, there are several routines that can be stressful for the calves, e.g. dehorning taking the temperature of a calf. Having a companion that acts as a stress buffer in these situations, and other situations both pre- and post-weaning, is a valuable resource.

One confounding factor to consider is that when the behaviour observations were done, the calves were not in their home environment, but in a play arena that they had never visited before their first observation session. However, it has been found that play behaviour in an arena does reflect play behaviour in the home pen of calves (Mintline *et al.*, 2012).

Furthermore, the shape and size of the play arena (relatively large and oblong space) may affect the type of locomotor play performed by the calves. Mintline *et al.* (2012) found that this type of arena caused calves to perform mainly running. The calves in this study did perform much running and perhaps this was stimulated by the shape and size of the arena, but whether or not this affected the performance of other behaviours (play behaviours and other behaviours) is unknown.

Season can affect the behaviour of calves. In this study, the births of pair-housed and individually housed calves have been spread over the study period to avoid having the two groups of calves born and observed in the play arena in two different seasons. There may also be diurnal patterns of play. Calves have been observed to reach a peak in play between 8.30 and 9.30 in the morning and between 18.00 and 19.30 in the evening (Ryan, 2009). Studies of semi-wild *Bos primigenius taurus* have shown similar patterns, calves playing mostly in the mid-morning and between 15.00 and 16.00 in the afternoon (Vitale *et al.*, 1986). In this study, calves were observed in the play arena at different times of the day. An alternative way to conduct the current study would have been to observe calves for 24 hours to find out during which hours the performance of play peaks and then observe all calves during these "peak hours". By not focusing the observations during these previously observed peak hours, the data obtained has not been maximised. That could be regarded as a disadvantage because more data makes better statistical analyses.

It has earlier been concluded that individual housing of calves is negative for calf development and welfare (Costa *et al.*, 2016a) and that the expression of play indicates good animal welfare (Babu *et al.*, 2004; Jensen *et al.*, 1998). Based on the results of the current study, it can be concluded that pair-housing has a positive effect on play behaviour.

# 7.2. Growth

The results of this study showed no significant difference in growth between treatments. This is in accordance with the results of the studies by Bolt *et al.* (2017), Broom & Leaver (1978), Chua *et al.* (2002) and de Paula Vieira *et al.* (2010). Bolt *et al.* (2017) found that calves housed individually in pens, or pair-housed from day five and from day 28 had an average growth rate of  $1.06 (\pm 0.14)$  % for the period 5-55 days old. The authors did also find that pair-housed calves had a higher concentrate intake than individually housed calves, which was explained by social facilitation. In that study, calves were fed pellets from day four, had *ad lib.* access to water from day one and were fed milk replacer twice daily (4 1/d on day 1-21 and 6 1/d on day 21-48). In the study by Chua *et al.* (2002), individually and pair-housed calves gained approximately 1 kg/d when 1-8 weeks old, except during week six (weaning) when pair-housed calves in this study had *ad lib.* access to water, hay, barley-based starter and milk. Weaning started on day

37 of the experiment (Chua *et al.*, 2002). De Paula Vieira *et al.* (2010) compared individually housed calves with calves that were pair-housed from four days of age. All calves had *ad lib.* access to water, calf starter, hay and whole milk (fed twice a day for two hours until calves were 36 days old). Weaning occurred between day 37-49. When eight weeks old, calves were mixed and then observed for 15 days. Pre-weaning and during weaning, there was no significant difference in weight gain between individually and pair-housed calves and for the whole pre-mixing period the average daily weight gain was 1.6 kg/d. After mixing, previously pair-housed calves had a higher weight gain than previously individually housed calves at day two (paired: 0.5 kg/calf and individual: -0.4 kg/calf) and day three (paired: 0.9 kg/calf and individual: -0.9 kg/calf). During the rest of the mixed period (i.e. 59-71 days of age), there was no significant effect of housing type on weight gain and the average gain was  $0.5 \pm 0.21$  kg/d (de Paula Vieira *et al.*, 2010). In the study by Alvegard (2016), calves were individually or pair-housed and observed until eight weeks old. Calves were fed three litres of whole milk twice daily, and had *ad lib.* access to silage, hay, pelleted concentrate and water. Weight gain from birth to week six was  $5.6\pm 2.1$  kg for both individually and pair-housed calves (Alvegard, 2016).

Costa *et al.* (2015) found that pair-housed calves had a higher growth rate than individually housed calves. The results of the study by de Paula Vieira *et al.* (2010) agrees with this, showing higher intakes of starter before weaning in pair-housed than individually housed calves, even though there was no significant difference in growth. Social facilitation, which is only possible for pair- or group-housed calves, can play an important role when calves learn to eat solid feed (de Paula Vieira *et al.*, 2010) and when heifers learn to graze (Costa *et al.*, 2016b). Thus, it can be advantageous to raise calves in pairs or groups.

During seven weeks, pair-housed calves in the current study had a slightly higher daily growth than individually housed calves. All calves had similar birth weights, but pair-housed calves were heavier than individually housed calves after seven weeks. It is possible that there would have been significant differences in growth rates between pair-housed and individually housed calves later in life, but this was not measured in the current study. It is therefore of interest to perform studies such as this, but that continue after weaning.

# 7.3. Health

The results of this study showed no significant difference in health between treatments, neither in number of sick days nor in number of times calves were re-diagnosed. This is in agreement with the results of Babu *et al.* (2004), Bolt *et al.* (2017), Chua *et al.* (2002) and Jensen and Larsen (2014). A potentially important factor to consider is that in this study, the number of calves analysed for health might have been too low to obtain significant results.

The Swedish climate can be harsh during winter. Calves that are housed in hutches outdoors may therefore be negatively affected by the weather in terms of health and growth. Young calves are more affected by thermal stress than adult cattle (Stull & Reynolds, 2008). The current study was done during spring and early summer, and the warmer climate during this time of the year may have affected the health of the calves positively. The overall number of sick calves might have been higher and the difference in health between housing treatments might have been bigger if the study was conducted in wintertime. The study by Alvegard (2016) was done in Sweden during winter and the results showed a significantly greater number of diarrhoea cases in pair-housed calves than in individually housed calves.

On the farm Lövsta, where this study was conducted, calf hutches stand close to each other, allowing older calves to reach the roughage of the neighbouring hutch and even the

neighbouring calf. Contamination by aerosol is also a possibility when hutches are so close to each other. Thus, pair-housing the calves was not as big a step as it would have been if calves normally had no contact with each other whatsoever. That fact may have contributed to the current health results showing no significant effect of treatment. Another possibility is that the hygienic routine and management is good enough at Lövsta to ensure good health in individual housed calves as well as pair-housed calves (Chua *et al.*, 2002). Although, the feeding routine at Lövsta may present risks for cross-contamination between calves in different hutches. Teat buckets for milk feeding are currently being moved between hutches without being cleaned in between. With one bucket per calf, hygiene, overall health and the starting-point for potential pair-housing might be improved.

#### 7.4. Future research

Future research could include studies of long-term effects of social isolation vs. social housing on dairy cow and heifer performance to see whether or not social housing is beneficial, regarding both the financial aspect and animal welfare. In the future, it might also be advantageous to perform more studies on how to add social and environmental enrichments to calf hutches and other types of calf housing. Perhaps alternatives to a live companion can be found, such as olfactory stimuli or dummies. Perhaps such an alternative would appeal more to farmers who are unsure of the health aspect of social housing of dairy calves. To assess and compare calf health in further detail, future studies could include blood samples (e.g. levels of oxytocin and endorphins) and immunological parameters. Further investigations of on-farm management of different types of individual and pair-housing are necessary to adapt calf management according to research in a plausible manner. The opinions of the farmer him-/herself are important to consider.

#### 8. Conclusion

The results of this study show that pair-housed calves perform more social play and stand together than individually housed calves do when tested in an arena. Other behaviours observed were not affected by treatment. However, object play and vocalisation were seen to decrease with age, as opposed to social play, which increased with age. Locomotor play occurred the most in period 1, while social play and stand together occurred the most in period 2. It was also concluded that weight gain and health was not significantly affected by type of housing (pair-or individual housing). Because of the social aspect, it is concluded that the welfare of pair-housed calves was higher than for individually housed calves.

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