

# Observations on the effects of non-maternal adult contact on the behavioural patterns of pre-weaned dairy heifers

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## ABSTRACT

**Context.** Dairy calves are often separated from their dams following birth, despite the beneficial effects of early life adult contact on behavioural development across species. Cow-calf contact systems are rare and often difficult to implement in the modern dairy industry. The development of alternative systems offering some of the benefits of adult social contact during early life, such as contact with non-maternal adults, has been limited. Aims. This study explored the behavioural patterns of grouped pre-weaned calves reared with or without non-maternal adult contact, and is the first in a series of studies following the social and behavioural development of experimental calves from the age of 2 weeks to 2 years. Methods. Four groups of 10 calves housed at pasture were studied from 2 to 12 weeks of age. Two groups were housed with three non-maternal dry cows each (+S). Calf behaviour in these groups was compared with that of calves in the remaining two groups, housed without adult contact (-S). Observations were conducted on behaviours including grazing, locomotion and lying, using 5-min scan sampling between morning (0930 hours) and afternoon (1600 hours) milk-feeding on 1 day every second week of the experiment ( $\bar{x} = 5.9$  h of data  $\pm 0.4$  h/group.day). Key results. Few behavioural differences were found between groups. All groups performed mostly lying behaviour in the middle of the day and spent the most time grazing in the afternoon before the evening milk feed. Conclusions. Our results indicated that calves housed at pasture behave according to innate diurnal patterns previously observed in studies of calves housed with the dam, and choose to spend the majority of daylight hours lying or grazing. Being housed with non-maternal adults has few effects on these observed behavioural patterns. Implications. Our study suggests that non-maternal adult contact does not affect the immediate behavioural development of calves housed at pasture. Future research needs to explore longitudinal effects of this contact.

**Keywords:** calves, cow–calf, diurnal patterns, nanny cows, pasture-rearing, social enrichment, social models, welfare.

# Introduction

Several elements found in the dairy heifer's natural rearing environment are absent from current commercial rearing environments, including the social complexity offered by contact with the dam and with other herd members outside of the animal's age and sex. In free-ranging herds of cattle, the calf's earliest social relationship is formed with the dam within hours of birth, but calves will also form relationships with young peers and older animals in the herd from around a week of age (von Keyserlingk and Weary 2007). Within these herds, cattle will typically follow predictable daily routines (reviewed by Whalin *et al.* 2021). Vitale *et al.* (1986), for instance, observed semi-wild calves from birth to 2 months, recording peaks of play and grazing activity almost simultaneously in the early to mid-morning and late afternoon, with long bouts of lying and inactivity during the middle of the day. Many of these behaviours, such as grazing and lying, are believed to be socially facilitated, and may be modelled to calves by

older, more experienced members of the herd (Shingu *et al.* 2017; Mattiello *et al.* 2019; see review by Whalin *et al.* (2021)).

These varied social relationships have largely been ignored in the development of modern calf-rearing practices. Most Australian dairy calves, for example, are born seasonally within short calving periods, removed from their dam within 24 h of birth and raised artificially indoors in small, same-sex groups. A typical Australian replacement heifer will not be housed outdoors until weaning, while her first contact with an older animal outside of her small, same-sex peer group will be immediately prior to or following her first calving (Abuelo *et al.* 2019). Understanding how artificial calf-rearing practices alter the natural behavioural expression of livestock is necessary to inform the continued evolution of best-practice rearing systems.

The socially barren rearing environment common to modern dairies may affect both the short-term and lifelong development and welfare of replacement heifers. Infancy is a sensitive period for development across multiple species (Rodenburg and de Haas 2016). The physical and social qualities of the rearing environment can affect the developmental trajectory of neonates, through changes to neuroendocrine, immune and behavioural systems (Kanitz et al. 2009; Stiller et al. 2011). Maternal contact in mammals, birds and fishes affects the development of fearfulness, social motivation, cognition, behavioural flexibility and feeding behaviour (see, for instance, Arnold and Taborsky (2010), Edgar et al. (2016), or Latham and Mason (2008)). Current conventional calf-rearing practices may limit the opportunities for calves to experience maternal interactions necessary to achieve important developmental milestones.

Social contact and opportunities for social learning from other adults in the herd also shape calf development (reviewed by Cantor et al. 2019). Social-learning theory suggests that both the dam and other dominant adult members of the herd are the most effective social models calves can learn from (Bandura 1977, reviewed by Cantor et al. 2019). Alloparenting, in which adults other than the mother provide care to the young in the form of feeding, adopting or supervising, among other roles, has been observed across multiple species of mammals (Jensen 2001). Previous studies have observed both buffalo and beef cattle herds forming 'creche' groups, wherein one to three adults will maintain close supervision of groups of up to 32 calves (reviewed by Whalin et al. 2021). Commercial rearing practices separate the calf not only from the dam, but also from the social environment of the herd. Limited studies have explored the effects of exclusively non-maternal adult contact on the development of juveniles. It appears to improve positive social behaviour and reduce agonistic social interactions in horses (Bourjade et al. 2008). In cattle, housing juveniles with older, experienced conspecifics has been linked to an accelerated uptake of novel feeds and improved feeding behaviour (Velázquez-Martínez et al. 2010; Costa *et al.* 2016). Aside from feeding-behaviour studies, little research has explored the effects of contact with non-maternal adults on the behavioural development of cattle.

More naturalistic alternatives to current dairy calf-rearing systems may improve the early life development of heifer calves by capitalising on benefits associated with social enrichment through the presence of older animals. The system developed in this trial aimed to provide the benefits of adult contact to calves during their early development in a manner which could be more easily implemented on largerscale dairy farms than transitioning to a cow-calf retainment model. In the absence of the dam, the presence of other nonmaternal adults acting as social models may mitigate the deficiencies of a rearing environment lacking adult contact; however, little is known about the extent to which such contact may affect calf development. This study addressed the current research gap by exploring how contact with non-maternal dry cows between the ages of 2 and 12 weeks affects the behavioural patterns of replacement dairy heifer calves housed at pasture.

The results reported in the present paper are the first in a series, forming a longitudinal study following the development of the experimental calves from their second week in life to their first month of lactation, including differences in stress reactivity and their integration into a herd of adult cattle. We hypothesised that calves reared with nonmaternal adult contact would exhibit more social behaviour, begin grazing at a younger age and graze more often than would calves reared without adult contact. Due to the known diurnal behaviour patterns of calves at pasture, we hypothesised that these differences would be mostly observed during peaks of behaviour at the beginning and end of our observation days, with all calves spending time lying in the middle of the day.

## Materials and methods

This experiment was conducted between August and November 2019 at the Tasmanian Dairy Research Facility near Elliott in north-western Tasmania, Australia (TDRF; 41°08'S, 145°77'E; 155 m above mean sea level). Forty mixed-breed dairy calves and six mixed-breed dry dairy cows participated in this 10-week experiment. All animal procedures were approved by the University of Tasmania Animal Ethics Committee (A0018141) under the *Tasmanian Animal Welfare Act* (Tasmanian Government 1993).

#### Animals

Forty mixed-breed dairy heifer calves (Friesian = 23, Jersey = 3, FJ, FFFJ, FJJJ or majority  $FJ \times$  other dairy genetics including Swedish and Australian Red = 13, and a single dairy  $\times$  Angus cross) born within a 12-day period were studied. Calves were subjected to the same

management practices for the first 2 weeks after birth. Animals were born at pasture and separated from their dams within 12 h of birth. They were fed colostrum twice after being relocated from the calving paddock to wood-chip-bedded group-housing pens (three walls and a roof;  $3.5 \text{ m} \times 7 \text{ m}$ ) containing 12 calves each, after which they were fed 2 L of whole milk twice a day. Calves had free access to water and calf starter pellets during this period. They were weighed 4 days prior to entering the experiment.

The dry cows used in this experiment were selected for hoof, udder and overall health on the basis of farm records, temperament on the basis of stockperson assessment, and the fact that they were not in calf. All cows had been managed according to research farm protocol for all prior calvings; calves were removed from them within 12 h of birth, and each animal, therefore, had little to no experience with calves. None of the animals used had any previously recorded instances of dystocia.

## **Experimental design**

The 40 calves were allocated to one of four groups of 10 animals, with one of two treatments then randomly assigned to each group. Treatments were as follows: (1) hand-reared, group-housed calves (two control groups of 10 calves each, called -S), or (2) hand-reared, grouphoused calves, each group housed with three non-familial dry cows (two treatment groups of 10 calves each, called +S).

To ensure that all calves were a minimum of 14 days old at the commencement of the experiment, treatments were imposed over two time-replicates of 20 calves, one group of 10 calves per treatment, with time-replicates separated by 7 days. Groups were balanced for age at entry to the experiment (Replicate 1, 16.4  $\pm$  1.3 days old; Replicate 2, 17.9  $\pm$  2.2 days old), breed, and weight 4 days before entry to the experiment (Replicate 1:  $42.4 \pm 5.3$  kg; Replicate 2:  $45.2 \pm 5.5$  kg). All calves were reared outdoors in paddocks from approximately 16 days of age until weaning from milk at 13 weeks of age.

The six dry cows were weighed 4 days prior to entering the experiment and then randomly allocated to one of the two +S groups, balanced for age and weight (Replicate 1:  $5 \pm 1$  years old,  $516 \pm 28.6$  kg; Replicate 2:  $6 \pm 2.7$  years old, 566  $\pm$  69 kg). To balance traits of cows assigned to each replicate of +S calves, available animals were paired with the most similar other available animal and then each member of the pair was randomly allocated to one of the two replicates. Replicate 1 cows therefore comprised one 4-year-old Friesian who had previously calved twice, a 5-year-old FJ-cross who had calved three times and a 6-year-old FJ-cross who had calved four times. Replicate 2 cows comprised one 4-year-old Friesian who had previously calved twice, one 5-year-old FJ-cross who had calved three times, and one 9-year-old Friesian who had calved seven times.

## Study site

Two 1 ha paddocks were allocated for this experiment, one per replicate. Within replicates, the +S treatment was allocated to one half of the paddock, and the –S treatment to the other, and side of the paddock used was balanced for treatment in Replicate 2 (Fig. 1). Experimental groups of 10 calves (housed with or without the three dry cows) were therefore housed on approximately 0.5 ha of cultivated ryegrass pasture. Paddocks included a water trough and a large creep area that was accessible only by calves, and which contained a shelter and a milk-feeding area. Visual barriers constructed from doubled hessian at 0.8–1.0 m height, and buffer zones of 15–20 m constructed using four-string

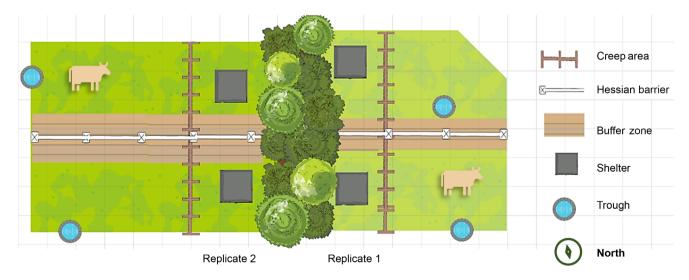


Fig. I. Experimental paddock layout (not to scale) illustrating +S and -S housing for Replicates 1 and 2. +S paddocks are denoted using the cow icon.

electric tape ensured that only auditory contact between groups was possible.

#### **Animal management**

Three days prior to the commencement of the experiment, cows were fitted with udder nets (Boerenwinkel, Netherlands) to discourage suckling by calves and observed to ensure safe adaptation. Udder nets were removed from all but one cow in Replicate 1 within the first 3 weeks of treatment. This cow retained her udder net to prevent a specific calf from performing non-nutritive suckling and causing damage to her teats.

The day prior to the commencement of the experiment, calves in the +S treatment were introduced to a small grass pen with a shelter and water trough adjoining a small paddock housing the three allocated dry cows of the group. After 24 h fenceline contact between +S calves and their allocated cows, both +S and -S calves were introduced to their respective paddocks. The three dry cows were then also introduced to the +S paddock. Pasture provision for the adult cattle was managed through strip grazing, achieved by moving the electric line of the creep area daily. When pasture available in the paddocks was visually assessed as <1500 kg DM/ha, cows were given *ad libitum* access to pasture in a neighbouring paddock for up to 3 h/day. This occurred primarily during the final 2 weeks of the experiment.

Calves were offered 3 L of whole milk each twice daily, at approximately 0930 hours and 1600 hours, on 10-teat group feeders. Calf starter concentrate was provided ad libitum in feed troughs and checked twice daily at milk-feeding. At 6-8 weeks of age (Week 4-5 of the study), all calves were sedated at the milk feeder in their home paddocks by a veterinarian, and non-polled calves (approximately 80% calves/group) were treated with long-lasting analgesic and local anaesthetic before being disbudded using hot-iron cautery. All calves also received RFID ear tags and 7-in-1 vaccinations during this period. Medical treatment was administered when necessary - this occurred only on one occasion, in an 8-week-old calf from the +S treatment group of the second replicate. The calf abruptly developed bloat and unusual stool; after overnight separation from the group and a single dose of antibiotics, the calf returned to full health and was returned to the experiment within 24 h.

Cows were removed from the paddocks into an area out of auditory and visual contact with the paddocks 1 week prior to weaning, after exactly 10 weeks in the treatment and when the youngest calf in the replicate was a minimum of 12 weeks old. Calves remained in their respective paddocks with continued access to pasture and calf starter concentrate, while weaning from milk was then undertaken over 7 days. This was achieved by gradually reducing the amount of milk offered, first by reducing the milk offered at afternoon feed by 0.5 L per day for 4 days, then removing the afternoon feed and reducing the milk offered at morning feed by 0.5 L per day for 3 days. After the calves received only 1.5 L for the morning feed, they were no longer offered milk feeds. At the completion of weaning, the youngest experimental calf was 13 weeks old. After weaning, all experimental calves received the booster dose of their 7-in-1 vaccination. They were then mixed on pasture with the remaining non-experimental replacement heifer calves born during the same calving period and were managed by the farm moving forward. All weaned heifer calves continued to be housed outdoors and rotationally grazed rainfed pasture following completion of this experiment.

#### Data collection: calf behaviour

The behaviour of calves in their paddocks was recorded by video on a single day during Weeks 1, 3, 5, 7 and 9 of the experiment; Week 1 videos were taken 3 days after calves entered the experiment and the following video data were then collected fortnightly on this day moving forward. Replicate 2 video data for Week 9 were lost after hard-drive malfunction prior to analysis and were therefore excluded. Video data were collected from approximately 30 min prior to the morning milk feed until approximately 1 h after the afternoon milk feed by using four cameras, capturing the daylight hours spanning approximately from 0830 hours to 1730 hours (Go-Pro Hero7 fitted with external battery packs; approximately 8 h of footage per paddock per observation day) erected on tripods at each corner of the paddock, covering the entirety of each paddock. An ethogram for all expected calf behaviours was developed with reference to Kerr and Wood-Gush (1987a), MacKay et al. (2014) and Webb et al. (2014; Supplementary Table I). Behaviours included lying, standing, grazing, locomotion, environmental exploration and self-grooming, as well as social behaviours such as allogrooming, cross-sucking and exploration, and were recorded to provide a full summary of time budgets across the experimental period regardless of expected treatment differences.

Behavioural data were collected by a single researcher using Behavioural Observation Research Interactive Software (BORIS) Ver. 7.8 video player (Friard and Gamba 2016). Videos were watched continuously, and behavioural data were collected using scan sampling at a group level at 5-min intervals, commencing 15 min after morning milk feed, and concluding 15 min before afternoon milk-feeding. The number of calves engaged in each behaviour described in the ethogram was therefore recorded every 5 min. In this manner, approximately 6 h of behavioural data were collected from the videos of each observation day ( $\bar{x} = 351$  min of data  $\pm$  24 min). To ensure reliability of the initial observations and ethogram, a second researcher unfamiliar with the animals or treatments was randomly allocated 15% of the videos to observe, spread across all treatment groups. Inter-observer reliability was calculated using Cohen's

kappa and agreement between observers was found to be very good ( $\kappa = 0.736$ ).

While the cameras captured each experimental paddock in its entirety, occasional camera malfunction meant that not all calves were visible at each scan sampling point. To allow for this, the number of animals in and out of view at each scan sampling point was recorded, in terms of posture (standing or lying), area, and behaviour. This data allowed the proportion of animals in view performing each behaviour to be calculated, a method previously used where not all animals are captured in the camera field of view, and where individual animal recognition is not possible (e.g. Hemsworth et al. 2016). Prior to analysis, any observations in which less than half of the calves in the paddock were in view were discarded (21.2% of total observation points). This was to ensure that once proportions were calculated, the resulting data were reflective of the majority of the animals in the group.

No data were recorded at any one sample point if an outside influence on behaviour was observed at that point, particularly the presence of the researcher checking cameras, non-experimental animals being moved around the farm, or farm vehicles such as tractors and UTVs being driven close to the experimental paddocks (2.9% of total observation points).

#### Statistical analyses

All statistical analyses were performed using R (ver. 4.1.1, R Core Team 2021). The treatment labels were either S+ or S- (n = 2 groups of 10 calves per treatment).

To best capture the diurnal patterns of behaviour exhibited by cattle at pasture, each day was divided into four equal time periods (quartiles). We based these time quartiles on the approximate 90 min following morning milk feed from approximately 0930 hours where activity remained high, 90 min as calves transitioned into peak lying behaviour commencing approximately 1100 hours, 90 min where calves transitioned out of peak lying behaviour commencing approximately 1230 hours, and 90 min prior to the afternoon milk feed where calf activity again increased, commencing approximately 1400 hours.

Behavioural observations from the 5-min scan samples were aggregated to a total count per group, per quartile. The total sum of calves in view from each scan sample was also aggregated per group, per quartile. These numbers were then used to estimate the relative frequency of each behaviour as a proportion, per quartile (i.e. total times the behaviour was observed/total number of observations). The base behavioural data analysed in the charts and tables are therefore the proportion of calves in view that were recorded displaying each behaviour, per group and quartile.

Most behaviours in our ethogram were very rarely observed and, therefore, while means of all non-social behaviours are presented, some were not analysed beyond this. Social interactions were especially rarely observed

656

during 5-min scan sampling. Due to the limited number of times social interactions, including calf–calf interactions, interactions with older cows in +S groups and proximity to +S cows, were observed, only descriptive data in the form of total counts of these behaviours across all observation days are presented. The behaviours 'calf–calf kick' and 'calf groom cow' were not observed and are therefore not included in the results.

To further account for the overdispersion of zero-count data, solitary play (a non-social behaviour observed six times across the duration of the experiment; +S = 4, -S = 2) was aggregated with running and walking observations to form the new non-social behaviour category 'Locomotion.'

After the mean proportion of calves performing each nonsocial behaviour was calculated for each observation week, all remaining behaviours except for graze, lie, locomotion and standing, which made up 91% of behavioural observations, were then removed from analysis. The means are presented.

For each quartile, differences in grazing, lying, locomotion and standing behaviour of calves were analysed using twoway ANOVA regression models. Each model included the main effects of treatment (+S vs -S), replicate (1 vs 2) and a treatment  $\times$  replicate interaction (treatment:replicate) in addition to a categorical dummy variable for behaviour during the first week (coded as 1 for the first week and 0 for all other weeks) to assess whether behaviours changed as calves adjusted to the experiment. Five weeks of data across four groups of animals, with occasional missing values due to camera errors, entailed that n = 17 or 18 for each regression. This strategy resulted in 16 different models but enabled the assessment of behavioural changes across the day, which addressed our hypothesis relating to diurnal patterns of behaviour. Analysing these data with one model for all quartiles and weeks would not have been tenable with such a low sample size, particularly after taking into account intra-week dependencies and associated non-homogeneous variance structures.

The residuals from the estimated ANOVA regressions were checked for evidence of a relationship between model errors and the four groups, with no indication of repeated patterns suggesting that a linear mixed model was not required. The formal analysis in the text includes summary statistics and a graph of results, while the full set of ANOVA results and residual plots from the interaction models are presented in the supplementary material (Supplementary tables II, III, IV, V, VI and figures VII and VIII).

#### Results

#### **Behavioural expression**

Mean proportions of animals from each treatment performing each behaviour across the experiment are presented in Table 1. These results suggest that there was little difference,

ltem	Experimental week											MEANp		SEp	
	1		3		5		7		9						
	+ <b>S</b>	<b>-S</b>	+ <b>S</b>	<b>-S</b>	+ <b>S</b>	<b>-S</b>	+ <b>S</b>	<b>-S</b>	+ <b>S</b>	<b>-S</b>	+ <b>S</b>	<b>-S</b>	+ <b>S</b>	<b>-S</b>	
Lying	0.497	0.457	0.680	0.688	0.643	0.670	0.634	0.603	0.605	0.696	0.612	0.623	0.069	0.100	
Standing	0.137	0.185	0.071	0.046	0.044	0.077	0.054	0.086	0.069	0.054	0.075	0.090	0.036	0.056	
Grazing	0.216	0.157	0.148	0.163	0.163	0.113	0.198	0.200	0.120	0.104	0.169	0.147	0.038	0.039	
Self-grooming	0.018	0.031	0.014	0.011	0.026	0.033	0.017	0.013	0.030	0.015	0.021	0.021	0.007	0.010	
Explore environment	0.011	0.040	0.020	0.027	0.058	0.052	0.048	0.034	0.090	0.087	0.045	0.048	0.032	0.024	
Locomotion	0.108	0.109	0.047	0.042	0.035	0.029	0.027	0.040	0.048	0.041	0.053	0.052	0.032	0.032	

Table I. Mean proportion of calves in view performing behaviours per observation week.

Each value in this table is the total count of each behaviour for the period and group indicated, divided by the total count for the heifers in view over the same period, resulting in the presented proportion value. The two right-most columns are summary statistics of the corresponding week-by-week data.

MEANp, mean proportion across all weeks per treatment. SEp, standard error of the mean proportion.

on average, between experimental treatment groups. The small standard errors indicate that proportions were consistent across weeks. Grazing, lying, locomotion and standing accounted for an average of 91% of the behaviours observed. Lying was the most frequently observed behaviour across the duration of the experiment, followed by grazing (Table 1, Fig. 2). Calf-calf social behaviours such as pushing, fighting and mounting were rarely observed.

Fig. 2 explores these behavioural patterns in more detail, illustrating by time quartile the behavioural distribution of the proportions of animals in view per treatment. Overall, this figure concurs with the averaged data presented in Table 1. By separating the quartiles, we can see that lying occurred most frequently in the middle of the day, across the second and third quartiles. Fig. 2 also shows that grazing behaviour tended to increase in the final quartile of the day, particularly for Replicate 1 +S calves. In exploring this further, two-way ANOVA regression shows that there was a replicate:treatment interaction on lying during Quartile 2 (P = 0.019) and Quartile 4 (P = 0.002; Supplementary table II). In Replicate 1, +S heifers spent less time lying and tended (P < 0.1) to spend more time standing, grazing and in locomotion in Quartiles 2 and 4 than did -S heifers, but the treatments did not differ in Replicate 2 (Fig. 2).

Behavioural differences between Week 1 and the following experimental weeks are apparent across both treatments and replicates (Fig. 2). During the first week, calves spent more time earlier in the day standing, grazing and moving around and less time lying than they did in subsequent weeks, resulting in a significantly higher proportion of calves spending the final quartile of this week lying (P = 0.003). Differences between Week 1 and the following experimental weeks were significant for lying across all quartiles, for standing and locomotion during Quartiles 1–3, and for grazing during Quartiles 2 and 4 (Table 1, Fig. 2;  $P \leq 0.031$ , see Supplementary tables III, IV, V and VI for individual *P*-values).

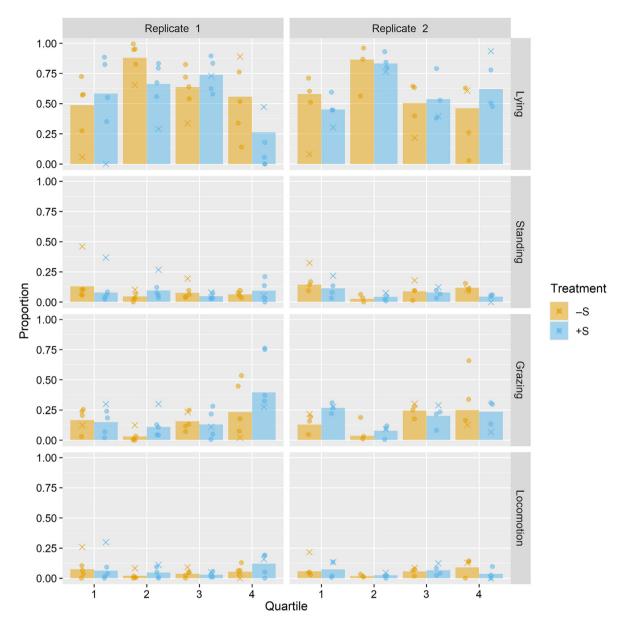
#### **Social interactions**

Differences in the relationships between cows and calves in the +S groups are apparent in the descriptive data (Table 2). Replicate 1 calves more frequently explored, attempted to suckle and received grooming behaviour from their cows than did Replicate 2 calves, and were also observed within 1 cow's length of the cows 2.5 times more often than were Replicate 2 calves. Within both replicates, most time spent in the proximity to cows and interactions with cows occurred during the third week of observations, or seventh experimental week.

Allogrooming was the most observed calf-calf social interaction, while kicking was not recorded at all (Table 3). Cross-sucking was observed in all groups except for the +S group in Replicate 1. Both -S groups were observed performing a higher count of calf-calf social interactions across the experimental period.

# Discussion

To our knowledge, this is the first study to document the effects of providing a socially enriched environment (in terms of rearing with older unrelated animals) on the behaviour of artificially reared, pre-weaned calves in a pasture-based dairy system. It provides a preliminary version of a management system that socially enrichens the developmental environment of calves, which may be more easily implemented on larger-scale pasture-based dairy farms than is transitioning to a cow-calf retainment model, and which could be adapted for indoor systems seeking to achieve similar aims. The present paper forms the first phase of a longitudinal study following the development of replacement heifer calves exposed to differing social and environmental conditions from 2 weeks of age to the first month of lactation after first calving at the age of 24 months. It is also one of the first to document the behavioural patterns of hand-reared calves at pasture.



**Fig. 2.** Proportion of calves in view performing lying, standing, grazing and locomotion behaviour across daily quartiles, by replicate. Bars denote data mean proportions of calves performing each respective behaviour over all observation weeks, while dots represent weekly proportion of calves performing each respective behaviour for each respective quartile. Crosses denote the mean proportion of animals engaging in each respective behaviour on Week I, which tended to be different from that on other weeks.

Contrary to expectations, there were few effects of housing calves with non-maternal adult cows on calf behaviour. Factors other than treatment, such as innate diurnal tendencies, may have a stronger effect on calf behaviour patterns during rearing than the presence of older conspecifics.

## Behavioural patterns of calves at pasture

Rearing dairy heifer calves from the age of 2 to 12 weeks in contact with dry non-maternal adult cows had few effects on the immediate behavioural patterns of the calves. The instinct to follow innate behavioural patterns may have influenced behaviour patterns more strongly than did the presence or absence of adult cows. Studies of hand-reared beef and dairy calves at pasture have generally been restricted to grazing or growth outcomes (e.g. Chambers 1959). The behavioural patterns observed across all groups of calves in this study are comparable to those in other studies of damreared beef calves at pasture (Reinhardt and Reinhardt 1981; Vitale *et al.* 1986; Veissier *et al.* 1989; reviewed by Whalin *et al.* 2021). In a study of such calves, followed from birth to 2 months of age, Vitale *et al.* (1986) noted

Week			Rep	licate l	Replicate 2						
	I	3	5	7	9	Total	I	3	5	7	Total
Calf explore cow	0	0	0	I	3	4	0	0	I	I	2
Calf receive agonistic	0	0	0	0	0	0	0	10 <sup>A</sup>	0	0	10
Calf suck cow	0	2	4	0	2	8	0	0	0	0	0
Calf groom cow	0	0	0	0	0	0	0	0	0	0	0
Calf receive groom	0	3	I	2	3	9	0	0	0	0	0
Behaviour total	0	5	5	3	8	21	0	10	I	I.	12
Proximity to Cow I <sup>B</sup>	2	18	I	I.	0	22	2	5	2	0	9
Proximity to Cow 2	3	9	I	5	4	22	5	7	0	0	12
Proximity to Cow 3	6	6	2	5	5	24	3	2	0	I.	6
Proximity total	П	33	4	11	9	68	10	14	2	I	27

#### Table 2. Group counts of interactions between +S calves and dry cows per observation week.

Each value represents the total number of times each interaction or behaviour was observed for the respective group and observation week, providing descriptive count data only. Data were collected only for animals in view.

<sup>A</sup>A single event in which one cow disturbed the group of calves.

<sup>B</sup>Proximity refers to the number of calves in +S groups observed within one adult cow's body length of a cow at each time point. Cows I-3 denote the three cows within each replicate paddock; as different cows were used in each replicate, a total of six cows are therefore represented in this table.

Table 3.	Group counts o	<sup>f</sup> calf–calf interactions	per observation week.
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Week		Replicate I							Treatment total			
	I.	3	5	7	9	Total	I.	3	5	7	Total	
+S												
Push	0	0	0	0	0	0	0	0	0	I.	I	I
Mount	0	0	0	0	I.	I.	0	0	0	0	0	I
Fight	4	2	4	0	2	12	0	0	0	2	2	14
Cross-suck	0	0	0	0	0	0	I	L	2	0	4	4
Allogroom	0	2	8	5	7	22	6	2	10	11	29	51
Week total	4	4	12	5	10	35	7	3	12	14	36	71
—S												
Push	0	I	0	0	I.	2	0	I	4	I.	6	8
Mount	0	I	I	I.	0	3	0	0	2	0	2	5
Fight	0	4	0	2	0	6	4	4	0	0	8	14
Cross-suck	2	0	I	I.	0	4	0	0	0	3	3	7
Allogroom	5	0	13	10	I	29	3	11	10	5	29	58
Week total	7	6	15	14	2	44	7	16	16	9	48	92

Each value represents the total number of times each interaction or behaviour was observed for the respective group and observation week, providing descriptive count data only. Data were collected only for animals in view. Given that +S groups housed three more possible social partners than did -S groups, in the form of non-maternal cows, comparison between treatments should be undertaken with caution. Count data for all calf–calf social behaviours listed in the experimental ethogram are listed aside from 'Kick,' which was not observed during data collection.

that the herd spent much of the middle of the day lying, while play and grazing activity peaked in early–mid morning and again in late afternoon.

The present study observed high proportions of calves across all treatments lying during Quartiles 2 and 3 (i.e. the middle of the day). These results align with those of Vitale *et al.* (1986) and Hutchison *et al.* (1962) and indicate that

when housed in small groups at pasture, dairy calves prefer to spend many of the hours between 0900 hours and 1600 hours lying down. Diurnal patterns characterised by peaks of lying behaviour in the middle of the day were not influenced by the presence or absence of adults in this study, suggesting that this behaviour may not be shaped by adult social modelling.

The present study did not record a morning grazing or activity peak; however, grazing and activity did increase during Quartile 4 of the present study, coinciding with mid-late afternoon and reflecting the findings of Vitale et al. (1986). Observed morning behavioural patterns may have been affected by behavioural disruption caused by artificial milk-feeding routines, or observation periods encompassing only daylight hours, which may not have been suitable for capturing the entire diurnal behavioural pattern. Play and grazing, for instance, may have been more concentrated during time periods outside of the observational period such as sunrise, sunset or after feeding (given the link between play and satiation of hunger), as reported by other studies (Reinhardt and Reinhardt 1981; reviewed by Whalin et al. 2021). Several limitations necessitated the restriction of observations to the daylight hours between morning and afternoon milk feeds, corresponding to an approximate time period from 0930 hours to 1530 hours. These included complications associated with pasture-based animal research (weather conditions, the need for daylight to capture video of a suitable quality, reliance on battery-operated recording equipment and remoteness of experimental fields) and with observing animal behaviour, particularly in extensive management systems (anticipatory behaviour associated with the presence of humans and time of day).

Few instances of play or social behaviour were recorded using 5-min scan sampling in this study, despite play and social behaviours being anecdotally observed in all groups. An alternative sampling method may have affected results pertaining to these more transitory behaviours. Count data for calf-calf social interactions support this contention. Transitory event behaviours such as kicking and pushing were rarely recorded; behaviours more likely to occur in bouts, such as cross-sucking and allogrooming, were observed up to 13 times in a day. Count data are higher for -S calf-calf social interactions than for +S calf-calf social interactions, potentially due to the presence of more social partners in +S groups (i.e. calves and cows). An alternative sampling technique such as targeted continuous sampling or observations of focal animals may provide a better documentation of calf social behaviours in pastoral settings.

Contrary to our hypothesis, there were also no significant effects of adult contact on grazing behaviour over the experimental period. Other studies have observed that social facilitation of grazing behaviour leads the calf to develop suitable grazing techniques within days of birth (Kerr and Wood-Gush 1987b; von Keyserlingk and Weary 2007; Arrazola *et al.* 2020). Foraging with experienced grazing partners up to 10 months older than experimental animals has also been shown to increase the consumption of novel feeds, increase grazing intensity, and reduce the latency to commence grazing in the hours to days after mixing (Velázquez-Martínez *et al.* 2010; De Paula Vieira *et al.* 2012; Shingu *et al.* 2017). Costa *et al.* (2016) and Hessle (2009) found that the effects of an experienced grazing companion were highest in the first 1–3 days postmixing. It is currently unclear how much influence is related to the age of the experienced conspecific, or to the relationship of the conspecific to the calf. However, current research suggests that the presence of an experienced companion may influence the development of grazing behaviour only in the short-term, an effect not captured in the present study.

Calves from both treatments spent more time standing, grazing and in locomotion, and less time lying, during the first week of the experiment (3 days post-mixing) than in subsequent weeks. All groups also laid down significantly more in the final quartile of Week 1 than in the subsequent weeks. This suggests ongoing adjustment from sheds to the paddock environment, which still held some novelty for the calves, and that calves tired by the end of the day.

The management system developed in this study provided calves with social enrichment in the form of adult contact, and environmental enrichment in the form of a pasturebased housing system rather than an indoor housing system. Thus, we are unable to separate the effects of social and environmental enrichment on the immediate development of replacement heifers. Both additions to the rearing environment provide complexity not found in common commercial indoor calf-rearing systems and may affect heifer development. Indeed, compared with more restrictive calf-rearing systems utilising individual housing, the group housing provided to both groups of calves may have provided adequate social enrichment, resulting in few treatment differences. Future phases of this longitudinal study will explore stress reactivity, social competence and production outcomes for the heifers described here; other future research should explore both environmental and social management choices that may be adopted to improve lifetime welfare. Further work on identifying early developmental periods in calves, and how best to provide suitable enrichment during these periods, should also be encouraged.

#### **Cow-calf relationships**

Interactions between cows and calves were rarely observed. The method of sampling at 5-min intervals may have failed to pick up these more transitory interactions. Calves may also have spent more time in the proximity to cows outside of the daylight hours, during which observations occurred. Regardless, the counts and nature of cow–calf interactions and proximity differed between the two +S groups, with more affiliative behaviours and shorter distances between cows and calves recorded descriptively, although not statistically, in Replicate 1 than Replicate 2. These results indicate that the Replicate 1 cows directed more affiliative behaviours (such as grooming) towards calves, suggesting that they were more suited to calf companionship. Individual variability among cows may have affected these relationships. All cows used in this experiment were selected for health, ease of handling and good temperament, but no testing was undertaken to assess individual-cow behavioural traits, meaning that any hypothesis concerning desirable traits for individual cows used as social models or companions for artificially reared calves remains untested.

When housed together, dairy dam-calf pairs have been observed allogrooming approximately 10% of the time, while allogrooming between non-paired animals in the same groups was observed approximately 0.4% of the time (Johnsen et al. 2015). This preference for dam contact may further account for the low frequency of cow-calf contact observed in the present study. However, in the absence of the dam, calves do use brushes to simulate maternal grooming, suggesting that the physical sensations created by such contact are pleasurable for the calf (reviewed by Whalin et al. 2021). Indeed, data from the present study also indicated that allogrooming may have been the most commonly performed calf-calf social interaction across the experimental period. No studies have compared grooming by the dam with grooming by non-maternal adults in the absence of the dam; however, the observed grooming interactions between Replicate 1 calves and cows indicated that these desirable interactions were occurring, with the potential for positive affective outcomes. For example, anecdotally, only Replicate 1 +S heifers attempted to suckle cows, and this was also the only replicate in which crosssucking was not observed.

A significantly higher proportion of Replicate 1 –S calves spent Quartiles 2 and 4 lying, while Replicate 1 +S calves tended to spend these periods grazing, standing or moving around their paddock. These differences were not apparent in Replicate 2. One explanation for these results is that the relationships between cows and calves in Replicate 1, where the cows directed more dam-like behaviour towards the calves, differed from those in Replicate 2, where few cow-calf interactions were observed. It is not clear how the calves in this system perceived the cows, namely, as a peer or as a dam. Potentially the closer relationships observed between Replicate 1 +S cows and calves could have improved social facilitation of behaviours such as grazing, or encouraged greater interaction with the rearing environment by promoting a positive affective state or feelings of safety. A less likely explanation for the differences between Replicates 1 and 2 is that the hedge separating the replicates caused the sun to set earlier for Replicate 1 than Replicate 2, possibly triggering the commencement of evening activity earlier for this replicate. Alternatively, these differences could simply be attributed to differences in group composition and behaviour.

Clearly, further research with increased replication is needed to fully elucidate the relationship between unrelated dry cows and calves in this system. Such research should also explore what traits make certain cows more suited to forming positive relationships with unrelated calves. Such traits could make cows more likely to take on the role of 'creche' supervisor and provide greater security to groups of unrelated calves, or be better suited to the role of social model. The short- and long-term effects of these individual cow differences (including higher rates of affiliative behaviour) on outcomes for the calves they are mixed with, including for calf affective state, should be explored.

## Limitations and future work

The nature of pasture-based dairy research limits the availability of animals for study, and suitable infrastructure and space for pasture-based calf-rearing in an experimental context. Although limited to two groups of 10 calves per treatment, our research design included detailed behavioural observations over periods of several hours, repeated across the weeks of the entire pre-weaning period, with two sets of animals (replicates) for each treatment (+S and -S). Each group of animals were housed in similar environmental conditions with comparable management. This is notable considering the logistical and financial constraints of studying animal behaviour in large, seasonal-calving, pasture-based dairy systems, including successfully managing rotational grazing of simultaneous small groups in experimental settings (Bransby 1989; Oksanen 2001; Verdon et al. 2018). The data generated in this study are of value despite this low replication, given the paucity of scientific literature from pasturebased dairy systems reporting on calf social behaviour and alternatives to cow-calf separation. This research provides a foundation on which future alternative calf-rearing systems can be built and offers insight into the behavioural patterns of calves at pasture, with or without the presence of adults.

The social dynamics of a dairy heifer's rearing environment can influence her behavioural adaptability, stress resilience, handling ease and social capabilities, with implications for the duration of her productive life, including her productivity and longevity in the herd (Van Reenen *et al.* 2013; Hedlund and Løvlie 2015). Depriving young dairy heifers of social opportunities in early life may be limiting their opportunities to reach their genetic potential (Cantor *et al.* 2019).

The present study assessed the effects of non-maternal adult influence on immediate behavioural patterns of pre-weaned dairy calves housed in groups of same-age conspecifics. Developing species-appropriate behaviours may have both immediate and long-term effects, not only on behaviours such as grazing, but also on the animal's ability to interact successfully with its peers or adjust calmly to novel environments such as the milking parlour. Future research will explore whether longitudinal effects are present in cattle reared with or without non-maternal adult contact, and, if so, whether these effects improve the lifelong behaviour and welfare of the animal. Differences in outcomes such as stress reactivity and social competency, linking previous research to early life experiences, will be areas of particular interest.

# **Supplementary material**

Supplementary material is available online.

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Data availability. Data may be made available upon reasonable request to the corresponding author.

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