Social Bonds in Dairy Cattle: The Effect of Dynamic Group Systems on Welfare and Productivity.

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Krista Marie McLennan

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SOCIAL BONDS IN DAIRY CATTLE: THE EFFECT OF DYNAMIC GROUP SYSTEMS ON WELFARE AND PRODUCTIVITY

Faculty of Applied Sciences, University of Northampton

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By

Krista Marie McLennan

BSc (Hons) Sparsholt College, University of Portsmouth

MSc University of Exeter

To my mother and father for their enduring encouragement to learn

To my husband for all his love and support, I thank you

Declaration

I declare that this thesis is my own work and that the research described in it is my own work, unless stated otherwise.

Krista M. McLennan
January 2013

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Publications

Publications

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McLennan, K. M., Littlemore, J., McCormick, W., (2012). Husbandry Practices in Large Dynamic Dairy Herds: The Effects of Social Support. In: *Proceedings of SET for BRITAIN, Biological and Biomedical Sciences Exhibition*, House of Commons.

Abstract

The recent increase in intensification of the UK dairy industry has led to the majority of cattle in the UK being housed in large, dynamic groups. Proposals for two large-scale dairies intending to house between 3,000 and 8,000 head of cattle have been met with considerable opposition by both producers and the public alike. Recent reports by both the Farm Animal Welfare Council and European Food Safety Authority have highlighted the continued welfare issues relating to dairy cattle, especially those housed in such large, dynamic groups. Conversely, with the current economic challenges being imposed on the UK dairy industry, there are many who see these systems as the future of dairying and discount the welfare concerns being highlighted. This project aimed to address one of the main welfare issues that receives scarce consideration when designing such systems; the social bonds of dairy cattle.

A herd of 400 Holstein-Friesian cattle, plus followers, were observed in cubicle housing under commercial conditions. Through the identification of preferential relationships using an association index, important social bonds between individuals were identified. The majority of relationships between dyads were however weak, short term associations appearing together no more than once throughout the observation period. These bonds were significantly stronger in younger cattle demonstrated through the closer proximity maintained and the higher association index scores between dyads. Between the ages of 7 & 11 months animals performed the most positive social behaviour and had the strongest dyad relationships.

In order to assess the strength of these positive relationships between dyads and to investigate the importance of these relationships to cattle, a short term (30 minutes) separation test from the remainder of the herd was carried out. Cattle's responses to the challenge were assessed both physiologically and behaviourally. A significantly lower heart rate ($p<0.01$) during the separation period was observed when cattle were separated with their preferred partner compared to the non-preferred partner, and significantly lower levels of behaviour suggestive of agitation ($p<0.05$) were observed when they were with their preferred partner compared to when they were with the non-preferred partner. These results suggest that cattle were receiving social support from their preferred partners allowing them to have a reduced stress response to the social isolation test.

As cattle aged and experienced regrouping, positive social bonds tended to disappear and cattle were more likely to have only weak associations. During long term separation (two weeks) from preferred partners, cattle showed significant behavioural, physiological and milk production changes. Upon subsequent reunion of preferred partners and consequential regrouping of individuals no further changes in behaviour, biology and milk production were observed, suggesting that separation rather than regrouping elicited a stress response. The bonds that had previously been evident between dyads were no longer present after the two weeks of separation. Subsequent relationships were also significantly weaker in focal cattle after separation of preferred partners and regrouping of animals.

These results highlight the importance of relationships to the welfare of cattle and in particular the psychological well-being of cattle in commercial dairies. There is a significant need to reduce regrouping where possible and promote a more stable grouping system that enhances social bonds and positive social behaviour such as allogrooming; a behaviour which is currently rare in commercial systems. This will improve the quality of life for dairy cattle and increase their ability to cope with environmental challenge such as at times of regrouping and separation.

In conclusion, social bonds do occur in domesticated dairy cattle and can be found when living in large dynamic group systems, but they are significantly affected by separation at the time of regrouping. These social bonds are important to the welfare and well-being of cattle; practices that promote stability and positive associations will be beneficial to the welfare of animals.

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List of abbreviations

ACTH	Adrenocorticotrophic Hormone
AI	Artificial Insemination
ANOVA	Analysis of Variance
BCS	Body Condition Score
BMSCC	Bulk-Milk Somatic Cell Count
bpm	Beats Per Minute
CAP	Common Agricultural Policy
CNS	Central Nervous System
CRH	Corticotrophin Releasing Hormone
d	Days
DEFRA	Department for Environment, Food, and Rural Affairs
DMI	Dry Matter Intake
EC	Electrical Conductivity
EFRAC	Environment, Food and Rural Affairs Committee
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAWC	Farm Animal Welfare Council
GLM	General Linear Model
HPA	Hypothalamus-Pituitary-Adrenal
hr	Hour
HR	Heart rate
kg	Kilogram
LSD	Least Significant Difference
m	Metres
MC	Moulton College
Mins	Minutes
ml	Millilitres
mm	Millimetres

mS	milliSiemens
ng	Nanogram
NMR	National Milk Records
NN	Nearest Neighbour
PP	Preferred Partner
PPL	Pence per litre
PR	Focal with familiar but non-preferred individual
R&R	Regrouping and Relocation
RP	Partner with familiar random individual
rpm	Revolutions Per Minute
SEM	Standard Error Mean
SCC	Somatic Cell Count
SD	Standard Deviation
secs	Seconds
TMR	Total Mixed Ration
UK	United Kingdom

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Chapter Two

Social Bonds in Dairy Cattle Housed in a Dynamic Group System.

2.1 Introduction

As outlined in the review (chapter 1), cattle (*Bos* species) are highly gregarious animals which live in matriarchal units and have been observed to form long-lasting social bonds with other individuals under feral conditions (Green *et al.*, 1989; Kimura & Ihobe, 1985; Lazo, 1994; Murphey, 1990; Reinhardt & Reinhardt, 1982; Reinhardt & Reinhardt, 1981). It is these positive social relationships that form the very basis of the structure and organisation of a herd, ensuring social cohesion and stability (Lazo, 1994; Mitani, 2009; VanDierendonck *et al.*, 2009; Wittemyer *et al.*, 2005). The social behaviours within these groups are primarily of a positive nature, with limited aggression and individuals maintaining close spatial proximity (Bouissou & Andrieu, 1978).

The existence of such social bonds in domestic dairy cattle housed in large dynamic groups under intensive commercial conditions has not yet been reported, despite them being noted in domestic cattle maintained in smaller, more stable groupings (Duve & Jensen, 2011; Val-Laillet *et al.*, 2009). Val-Laillet *et al.* (2009) observed individual cattle, within small groups of eight under commercial conditions, to choose a particular individual to feed next to independent of the position at the feed bunk. This suggested that the closeness in proximity between dyads was due to the existence of a social bond rather than a chance encounter. In addition, cattle reared together from an early age with full social contact appeared to form stronger associations compared to their conspecifics who had been grouped later on in life (Bouissou & Andrieu, 1978; Duve & Jensen, 2011; Raussi *et al.*, 2010). Even after mixing with unfamiliar individuals, a process very similar to that of regrouping in commercial dynamic herds, bonds formed between cattle reared together since birth were still present, whilst cattle that had been grouped later on did not remain associated with one another (Bouissou & Andrieu, 1978).

The EFSA (2009) report indicates that taking note of any social bonds that can form between cattle is important in ensuring their welfare. Social bonds can influence how an animal responds to its environment and so can impact on the individual's coping ability. Animal welfare considers both the physical and psychological health of an individual, particularly in relation to the animal's ability to cope with its environment (Broom, 1991). It is therefore not surprising that in the absence of positive relationships, health problems often occur in both humans (Birmingham *et al.*, 2009) and non-human animals (Waiblinger *et al.*, 2006). In order to maintain positive social relationships and bonds cattle must be able to recognise each other and form a memory of previous social encounters (Fraser & Broom, 1997). The changes in grouping under domestic commercial conditions (including the increase in group size) make this memory formation and thus potential bond formation more difficult to achieve. Consequently the potential for bonds within dairy cattle that live in intensive large dynamic groups requires investigation.

Spatial distribution and maintenance of proximity between pairs of individuals has been a widely used technique for assessing the social relationships between individuals (Aschwanden *et al.*, 2008; Bejder *et al.*, 1998; Durrell *et al.*, 2004; Huber *et al.*, 2008; Sibbald *et al.*, 2005). In cattle, the consistent choice of a particular individual to be in close proximity to, has been observed to be independent of the position at the feeder (Val-Laillet *et al.*, 2009). Furthermore, when at grass cattle tend to choose a familiar individual as their nearest neighbour rather than an unfamiliar herd member (Patison *et* *al.*, 2010; Takeda *et al.*, 2000). It can be considered from these results that the spatial proximity that cattle maintain between themselves is affected by their relationship and so proximity can be a reliable indicator of the existence of a social bond. As such, the information on nearest neighbour proximity and group membership has been used to develop association indices, giving an estimation of how much time two individuals may be seen together (Cairns & Schwager, 1987; White & Smith, 2007; Whitehead, 2008). This time spent together is then interpreted as an indication of the relationship strength between dyads (Lusseau *et al.*, 2008).

The aim of this stage of the study was to ascertain whether social bonds existed in dairy cattle when they were housed in a large dynamic group. The objective was to investigate cattle social preferences through nearest neighbour observations that could be practically and easily recorded on commercial farms by producers. Using these observations, association index values for each pairing observed could be used to determine whether the number of individuals showing a higher than average association index occurred more frequently than would be expected by chance. The influence of age (heifers versus cows) on social bonds and the general sociability of individuals were also investigated through proximity recordings and association indices.

2.2 Method

2.2.1 *Housing area, animals and management*

The main milking dairy herd at Stud Farm Moulton College, Northampton was observed to ascertain social preferences between July 2008 and February 2010. The herd consisted of 334 Holstein-Friesian cattle plus followers, with on average 229 multiparous and primiparous cattle being milked three times a day (approximately 0430-0800hr, 1300-1600hr, 2100-2330hr), reduced to twice a day in October 2008 (approximately 0500-0900hr and 1530-1700hr) (Mean parity = 2.09 ± 1.6 SD). All cattle were housed in a loose cubicle shed and managed on a cascade system whereby cattle moved down through groups according to their lactation curve. Cattle were managed by the producers in three distinct groups within the main milking herd: heifers, high yielders, and mid/low yielders, with a close observation and dry group outside of this (see table 2.1 for average group size and frequency of group changes calculated using the method developed by Jóhannesson, & Sørensen, (2000) for heifers, high yielding and mid/low yielding cattle). Pasture access was available during summer months and all cattle were fed on a total mixed ration (TMR) consisting of maize silage (54%), grass silage (10%), blend (20%), oats (9%), and lucerne (7%) for the high yielding cattle, and maize silage (51%), blend (18%) and grass silage (31%) for the mid to low yielding groups. Fresh food was delivered after each milking session and cattle had *ad libitum* access to water.

Table 2.1 Average group size per month within each distinct group during 2008-2010 and the frequency of group change during that period, calculated using the method developed by Jóhannesson & Sørensen, (2000).

Group	**Average group size per month between 2008-2010**	**Mean frequency (± SEM) of group change per month during 2008-2010**
Heifers	55	20.5 (± 1.5)
High yielders	112	46 (± 1.9)
Mids/Lows yielders	47	37.2 (± 2.3)

In order to determine and verify the existence of social bonds in cattle, four repetitions of nearest neighbour and proximity observations (based on methods carried out by Neisen *et al.* (2007; 2009a), Benham (1982), Durrell *et al.* (2004) and Whitehead (2008)), were carried out over two years. Details of cattle that were involved in each repetition are outlined below (see figure 2.1 for timeline of observations).

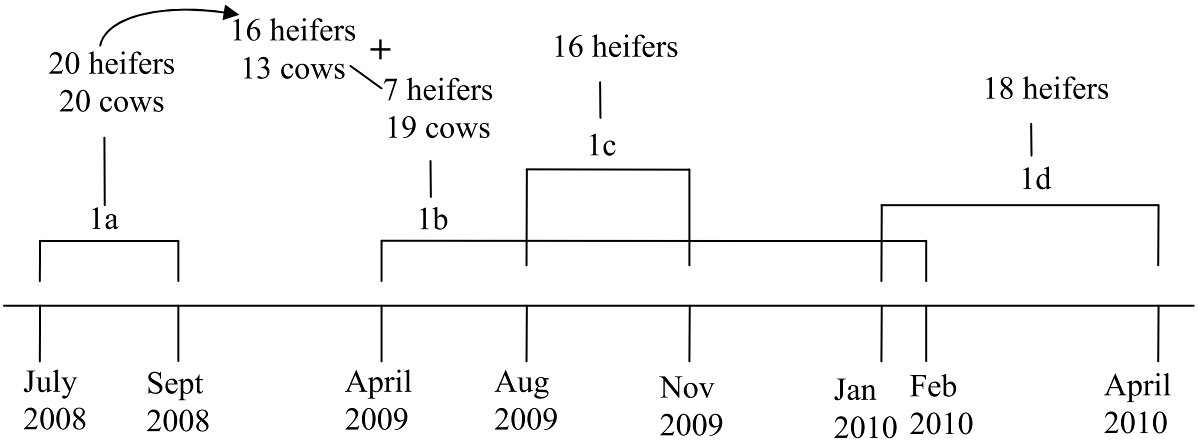


Figure 2.1. Timeline of nearest neighbour observations for all four repeats (1a, 1b, 1c, 1d) showing duration (months) and the number of animals involved in each observation.

2.2.1a. Observation 1a

Twenty multiparous and twenty primiparous cattle were chosen at random through a random number generation on Microsoft Excel 7.0 from the herd as a representative sample, and data collected between July 2008 and September 2008. All focal individuals remained within the herd and were not separated from their groups by the observer. Normal practices of regrouping did occur throughout the observations for four individuals whilst the others remained stable in their groups. A minimum of 10 nearest neighbour (NN) observations for all individuals (n=412) took place between the hours of 0900hr and 1500hr so as to avoid milking times.

2.2.1b. Observation 1b

Observation 1b took place between April 2009 and February 2010 and was an extension of 1a and included thirteen multiparous and sixteen primiparous cows from 1a and twenty-six new individuals (nineteen multiparous and seven primiparous) that had been identified as potential partners to focals in 1a. As in observation 1a all cattle remained within the herd and were not separated from the group unless it was part of the normal regrouping practice required by the farm. A minimum of fifteen nearest neighbour observations for each individual (n=781) took place between the hours of 0900hr and 1500hr avoiding milking times. The number of observations increased due to a change in methods (see section 2.2.2.). Five animals died during observations and therefore results for nearest neighbour observations for these individuals were removed from the analysis.

2.2.1c. Observation 1c

Observation 1c took place between August 2009 and November 2009 and included sixteen primiparous cattle; a new group brought in from rearing stock. A minimum of fifteen nearest neighbour observations for each individual took place between 0900hr and 1500hr avoiding milking times (n=243). All individuals remained within the herd unless required to be regrouped for management practices. Observation 1c took place in order to identify individuals that could be used in the short term separation test (chapter 3).

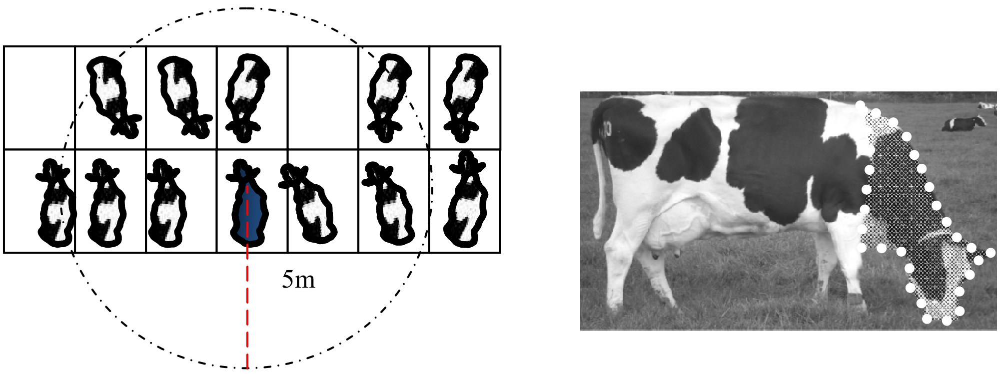
2.2.1d. Observation 1d

Observation 1d occurred before the short term separation test (chapter 3) could take place due to animal separation by the producer. Between January 2010 and April 2010 eighteen primiparous cattle (a new group brought in from rearing stock) were observed. Fifteen nearest neighbour observations for each individual took place between 0900hr and 1500hr avoiding milking times (n=256). All animals remained within the herd and remained stable throughout the observation period (no regrouping took place as part of normal management for these individuals).

2.2.2 *Data collection*

For each focal animal their first five nearest neighbours within five metres of their head (including the neck up to the withers) when indoors on the cubicle system (see figure 2.2a and 2.2b respectively), and the first five nearest neighbours within ten metres when at grass, were recorded. Neighbours had to have visual contact with the focal to be included. The proximity (in metres) of each neighbour from the focal individual was also estimated and used to asses which animals were the nearest to each focal. Distances were based upon findings from Benham, (1982), who observed cattle to spend 50% of their time in behavioural activities between 1.5 and 5m from the nearest neighbour, and Kondo *et al.* (1989), who observed the normal inter-animal distance under good grazing conditions to be approximately 10m. Neighbours were classed as associating with the focal individual if they were within the 5m (indoors) or 10m (outdoors) radius of the focal with no visual barrier between them. Proximity was estimated using cattle body length (approx. 2m) and cubicle measurements (1.2m wide) for practical reasons. The head and neck areas were chosen to take measurements from as they are the locations where most allogrooming have been noted to take place on, a potential sign of positive social relationships (Laister *et al.*, 2011; Sato *et al.*, 1991; Sato & Tarumizu, 1993). The activity of the focal animal was also recorded at the time of the observation as to whether they were resting, feeding, grazing, or performing other behaviours (e.g.

walking or drinking) along with their location (cubicles, feed face, alley ways, grazing) and current grouping (heifer, high, mid/lows, dry).



a) b)

Figure 2.2. Illustration of the nearest neighbour data collection method. (a) The first five nearest neighbours (patterned) from the focal animal (filled) were noted at five metres when indoors (N.B. not to scale), and at ten metres when outdoors. (b) Measurements were taken from the head, to include the neck and withers (inside the dotted line).

Focal animals were sampled two and a half days a week for five hours a day at a minimum of hourly intervals for observation 1a, and half hourly intervals for observations 1b, 1c, and 1d (based on intervals established by Neisen *et al.* (2009a)). It was likely that for each focal they would be sampled only once per day during the observation periods. To maintain independent group recordings, observations were carried out by area (see figure 2.3.) within the cubicle shed and sampled focals had to be at least 15m away from the previous focal so as to avoid recounting the previous focal's neighbours unless they had chosen to move. This was increased to 25m when at grass whereby focals were chosen systematically from right to left from the field gate. This was due to limited natural area separations. All individuals were able to be identified by their freeze brand number or by their ear tag number, both of which were matched for the majority of cattle. A number of focal individuals wore a coloured collar to help with identification from further afield. A total of 450 hours of nearest neighbour observations took place over the two years.

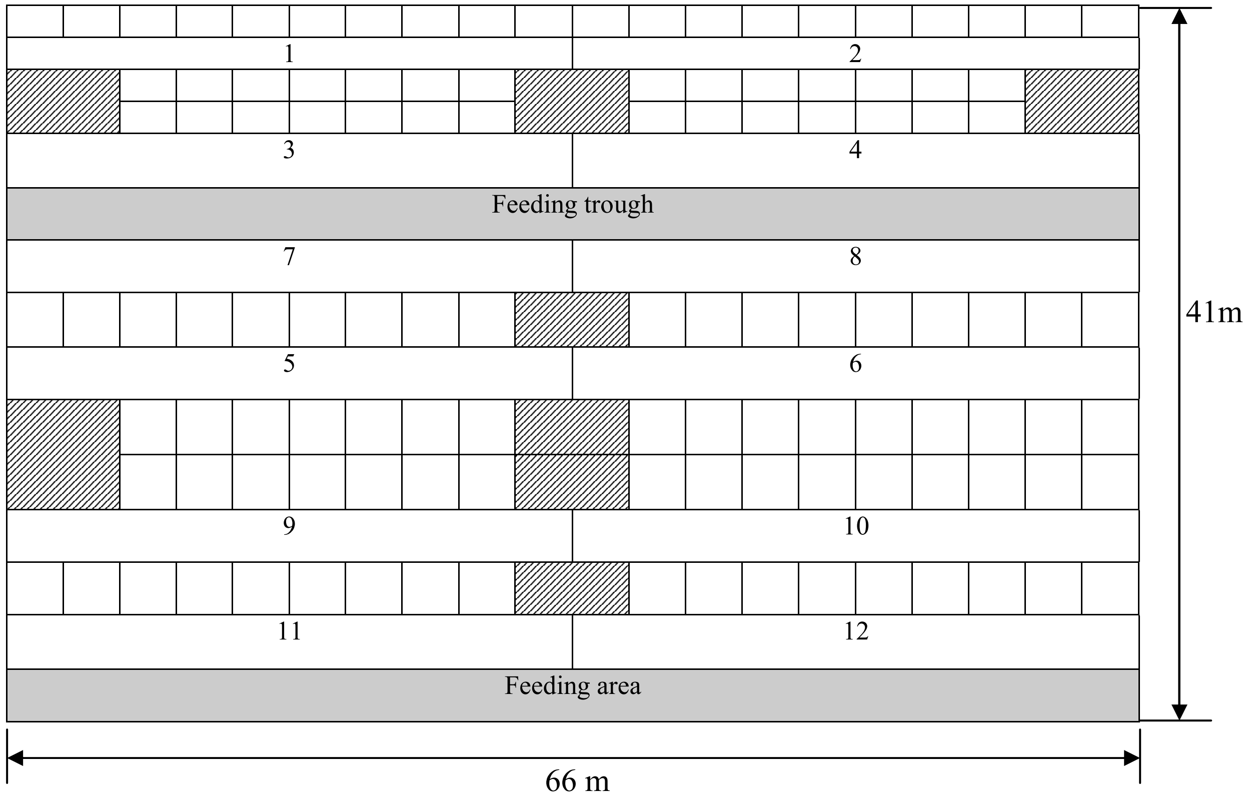


Figure 2.3. Diagram of cubicle housing showing order of the area's for data collection (not to scale).
Water troughs; Areas 1-4 High lactating cows; Areas 5-8 heifer high lactation group; Areas 9-12 Mid and Low lactation cows and heifers.

2.2.2a Individual social bond profiles

Due to the difficult nature of analysing social associations, an index of association adapted from Ginsberg & Young (1992) (see formula 2.1) was calculated for each pairing that focal individuals had to give a measure of inter-individual association. This index would give a value demonstrating the tendency to be found together and thus be used as an indicator of the potential strength of the relationship.

Formula 2.1

$$\textit{Index of association} = \frac{x}{NA}$$

where x is the number of times during which A and B are observed together and NA is the total number of observations of A.

This coefficient was favoured as it provides information regarding the tendency of the focal animal A to be found in proximity to each of its partners, and due to the large data set collected this was the most efficient way of calculating a preferred partner for each focal animal. The resulting index ranges from 0 to 1, with values of 1 indicating a pair to always be observed together, and values of zero to indicate a pair to never be observed together. Resulting indices were utilised for further analysis as described below.

2.2.3 *Statistical analysis*

All statistical analysis was carried out using Minitab version 13.20 and data presented as means ± SEM unless where otherwise stated. For all statistical analyses a significance level of $p<0.05$ was accepted. Any data which was not normally distributed was log transformed using $\log_{10}$ to normalise where required. Where this log transformation did not normalise data, non-parametric statistics were used. Details of particular methods and tests employed are outlined below.

2.2.3a. Association indices

The mean association index for each focal was calculated as outlined in section 2.2.2. As in Durrell *et al.* (2004), pairings that had a high association value (approximately two and half times or more the mean association index) were classed as preferred partners. This equated to three or more occasions where cattle were observed together out of 10 observations, and four or more occasions where cattle were observed together out of 15 observations. These parameters were then used to investigate on a herd basis the significance of these association values.

2.2.3b. Inter-individual social associations within the herd

The probability of observing a focal individual with another cow on three or four occasions, out of the 10 and 15 scans recorded respectively, was calculated using probability theorem as in Cooper *et al.* (2008). The chances of one cow associating with any other cow was 50/50 for the 10 observations, and 75/75 for the 15 observations (50 and 75 cows were used as this was the minimum number of different neighbours that one cow could be observed with during each observation period respectively). The chance of observing the same cow on the next observation with the focal animal was 1/50 or 1/75 respectively, or associating with a different cow 49/50 or 74/75 respectively. Thus, the probability value for observing one cow with another on two, three, four, or more occasions were calculated and are given in table 2.2. These probability values were used to determine the number of pairs that would be expected by chance to occur together on two, three, four, or more occasions, which were then compared to the number of pairs actually observed at that level. These were then analysed using Pearson's chi-square analysis to test whether as a herd there were associations occurring more than expected by chance alone.

Table 2.2. The probability of seeing a focal cow with another individual on two, three, four or five occasions, for ten and fifteen scans (rounded to three decimal places), calculated as per Cooper et al. (2008).

	Ten Scans (Obs 1a)	**Fifteen Scans (Obs 1b, 1c, 1d)**
Two occasions	≈ 0.419	≈ 0.384
Three occasion	≈ 0.027	≈ 0.027
Four occasions	≈ 0.001	≈ 0.001
Five occasions	≈ 0.000	≈ 0.000

2.2.3c. Individual social bond profiles

Statistical analysis of nearest neighbour data is problematic and so the association indices were utilised to investigate individual social bond profiles further. A Pearson's chi-squared test was carried out to examine the difference in the total number of focal heifers and the total number of focal cows that had at least one preferred partner, as this allowed for the assessment of the influence of age on social bond profiles. An independent two-sample *t*-test was used to analyse the difference between the total number of partners that heifers had compared to cows. To determine if associations were simply the result of sharing the same location within the cubicle shed a Pearson's chi-squared analysis was carried out on the number of pairings that were observed in the same area, compared to those occurring in different areas of the shed.

The spatial relations of associations were investigated by comparing the distance maintained to the first nearest neighbour when at grass compared to when in cubicles using a Mann-Whitney *U* test as data was not normally distributed and log transformation did not normalise the data. The difference between the average distance to the first nearest neighbour for heifers and cows was also analysed using a Mann-

Whitney *U* test due to unequal variances between the data sets, to investigate the differences in sociality due to age. A Spearman's correlation analysis was carried out to investigate the relationship between the focals mean association indices and the average distance that they maintained to their first nearest neighbour over the observation period.

2.2.4 *Ethical considerations*

The study was carried out on a commercial farm at Moulton College and involved the walking through cubicle sheds and in fields. Cattle were accustomed to the presence of humans as the farm was also a teaching farm and so students would often be walking through the sheds and fields as part of their training. Cattle were habituated to the presence of the researcher and the route taken for data collection within the sheds for one week before data collection started. Every caution was taken so as not to disturb cattle when walking through the sheds or fields and if any animal showed any signs of distress caused by the researcher's presence, the researcher would move away from the animal to a distance that allowed it to return to its activity and remove the signs of distress. Cattle that wore collars were checked twice daily by farm staff during milking and all collars were specially designed for use in cattle and fitted so that the collar was quick release if it were to get caught anywhere adhering to farm welfare standards.

2.3 Results

2.3.1 *Inter-individual associations within the herd*

With ten to fifteen observations per animal, a total of 1692 nearest neighbour observations were made with 5724 different pairings identified. The mean number of pairs for all cattle that each focal had were 44 different pairings with most pairings only appearing once. Seven cattle (5% of the observed individuals) were seen together with a specific partner five or more times (nine different pairings) with an association index score of 0.33 and 33 cattle (26%) with 47 different pairings were seen together with a specific individual at least four times (association score 0.27). Overall, there was a significant difference (χ^2 = 1472.24, df=4, $p<0.05$) in the number of pairs observed under each category compared to those expected by chance (see table 2.3 for individual observations).

Table 2.3. Data on the inter-individual associations within the her showing the probability of seeing a focal individual with another individual by chance. *The total number of pairings observed within each observation (x1 = seen together once only, x2 = seen together on two occasions, x3 = seen together on three occasions etc.), the average number of pairings focals had within each of the observations, and the total number of pairs seen only once, twice, three, four or five or more times together for all observations along with the expected number in brackets as calculated using the probability values from table 2.2. Pearson's chi-squared values along with the significance level are given for the difference between observed and expected number of dyad sightings.*

		Total pairings	**Average no. of pairings per individual**	**No. of neighbours x1 (*expected*)**	**No. of neighbours x 2 (*expected*)**	**No. of neighbours x 3 (*expected*)**	**No. of neighbours x 4 (*expected*)**	**No. of neighbours x 5+ (*expected*)**	**X^2**	***df***	***p*-value**
Obs 1a	Cows	578	28.9	487 (*320*)	78 (*242*)	11 (*15*)	2 (*1*)	0 (*0*)	202.89	*3*	$p<0.05$
	Heifers	689	34	513 (*380*)	135 (*289*)	25 (*18*)	11(*1*)	5 (*0*)	441.56	*4*	$p<0.05$
Obs 1b	Cows	686	53	548 (*404*)	113 (*263*)	19 (*18*)	6 (*1*)	0 (*0*)	167.13	*3*	$p<0.05$
	Heifers	800	50	674 (*471*)	104 (*307*)	16 (*21*)	5 (*1*)	1 (*0*)	265.60	*4*	$p<0.05$
	New focals	1241	48	991 (*729*)	200 (*477*)	41 (*33*)	7 (*2*)	2 (*0*)	311.87	*4*	$p<0.05$
Obs 1c	Heifers	734	49	562 (*431*)	135 (*282*)	28 (*20*)	11 (*1*)	1 (*0*)	202.80	*4*	$p<0.05$
Obs 1d	Heifers	940	52	765 (*326*)	144 (*361*)	25 (*25*)	5 (*1*)	0 (*0*)	2649.21	*3*	$p<0.05$
	Total	**5724**	**44**	**4582 (*3366*)**	**923 (*2198*)**	**165 (*154*)**	**47 (*7*)**	**9 (*0*)**	**1472.24**	*4*	$\mathbf{p<0.05}$

A large number of observations were made of neighbours appearing only once with focal animals (n=4582), and more of these type of relationships occurred than expected by chance (n=3366). Fewer observations were made of neighbours appearing twice (n=923) than was expected by chance (n=2198), and a higher number of observations were made of focal animals appearing with a specific neighbour on three (n=165), four (n=47) and five or more occasions (n=9) than was expected by chance (n=154, n=7, n=0 respectively) as shown in table 2.3 (see figure 2.4. for total distribution of number of occasions focal cattle seen with another individual by cattle type).

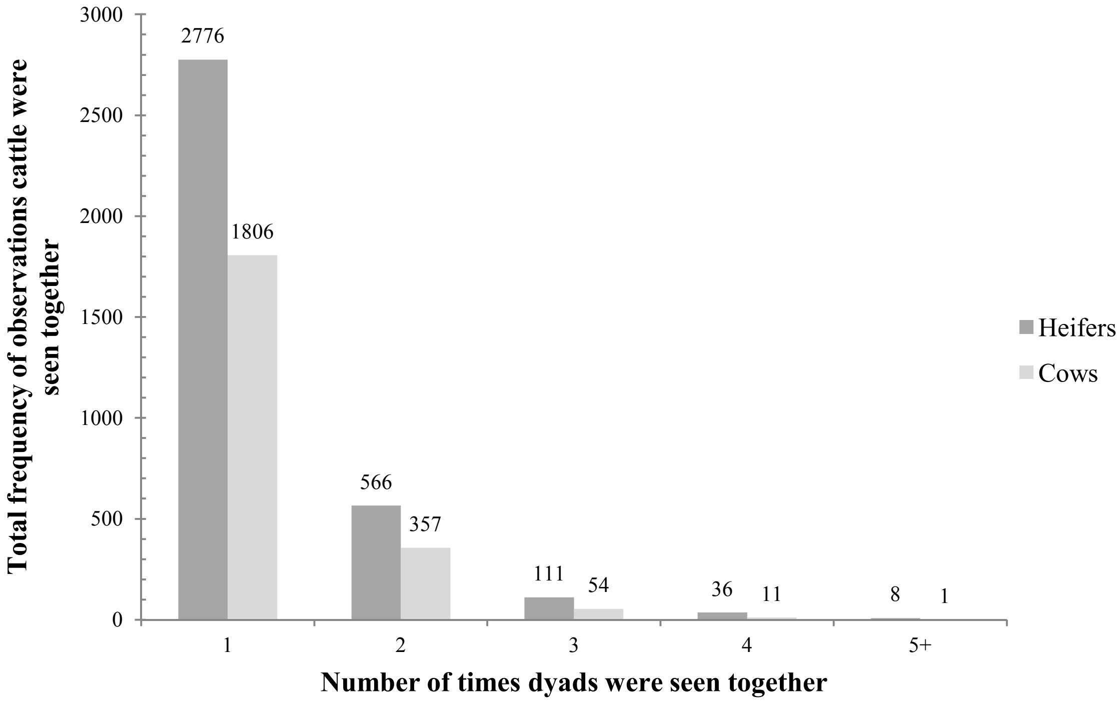


Figure 2.4 The distribution of the total number of occasions that focal heifers and cows were seen within a dyad between 2008 and 2010. *A large proportion of observations were made with pairings occurring only once together and a very small proportion of observations occurred where pairings were found together on 3 or more occasions.*

2.3.2 *Individual social bond profiles*

A total of 53 (41%) focal cattle were identified as having at least one preferred partner (mean of 1.43 ± 0.07 preferred partners, ranging from 1 to 3). There was no significant difference in the total number of preferred partnerships that heifers had compared to cows (T=0.88, df= 73, p=0.380; heifers 1.47 ± 0.09, cows 1.35 ± 0.10). There was a significant difference in the total number of focal cows compared to the total number of focal heifers that had at least one preferred partner (χ^2 = 6.88, df=1, p<0.05) (see figure 2.5.) with significantly more heifers having at least one preferred partner.

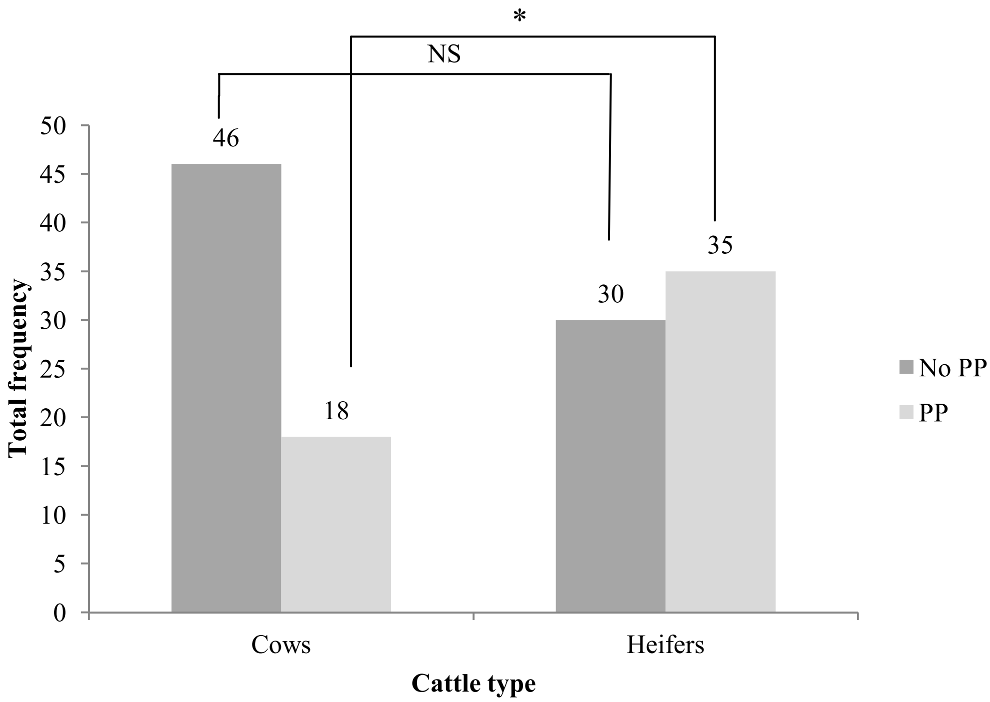


Figure 2.5. The total number of cows and heifers that had at least one preferred partner (PP) and the number of cows and heifers that had no preferred partners (No PP). *The total number of heifers that had at least one preferred partner was significantly higher than the total number of cows that had at least one preferred partner.*indicates significant difference at p<0.05*

A higher number of observations took place whilst animals were at rest (66%) compared to when feeding (28%), grazing (5%) or performing any other behaviour

(1%). Seventy one percent (n=29) of preferred partnerships were observed to appear in different locations between observations, compared to 29% (n=12) appearing in the same location, and this was significantly different (χ^2 = 7.05, df=1, p<0.05). Twenty five percent of preferred partnerships were observed to appear as the first neighbour position, 23% appeared as the second nearest neighbour and 18% appeared as the third and fourth nearest neighbour. Fifteen percent of preferred partnerships appeared as the fifth nearest neighbour.

As would be expected, distance increased as neighbour position increased. Mean distance to the first nearest neighbour was greater in cows (1.38 ± 0.26m) than in heifers (1.02 ± 0.1m) although this was not significantly different (p=0.8872) (see figure 2.6). Interestingly cattle were more spread out at grass as there was no significant difference (p=0.58) in the average distance of the first nearest neighbour when at grass (3.62 ± 0.19m) compared to the fifth nearest neighbour when indoors (3.15 ± 0.04m). Whether cattle were at grass (0.10 ± 0.002) or indoors (0.10 ± 0.004) however, did not impact upon the focal mean association index (p=0.38). There was a non-significant negative correlation between focal mean association index and the average distance to their first nearest neighbour for cattle indoors (r_s=-0.170, p=0.08); however for cattle at grass there was a significant positive correlation between mean association index and the average distance to their first nearest neighbour (r_s=0.803, p=0.000) (see figure 2.7).

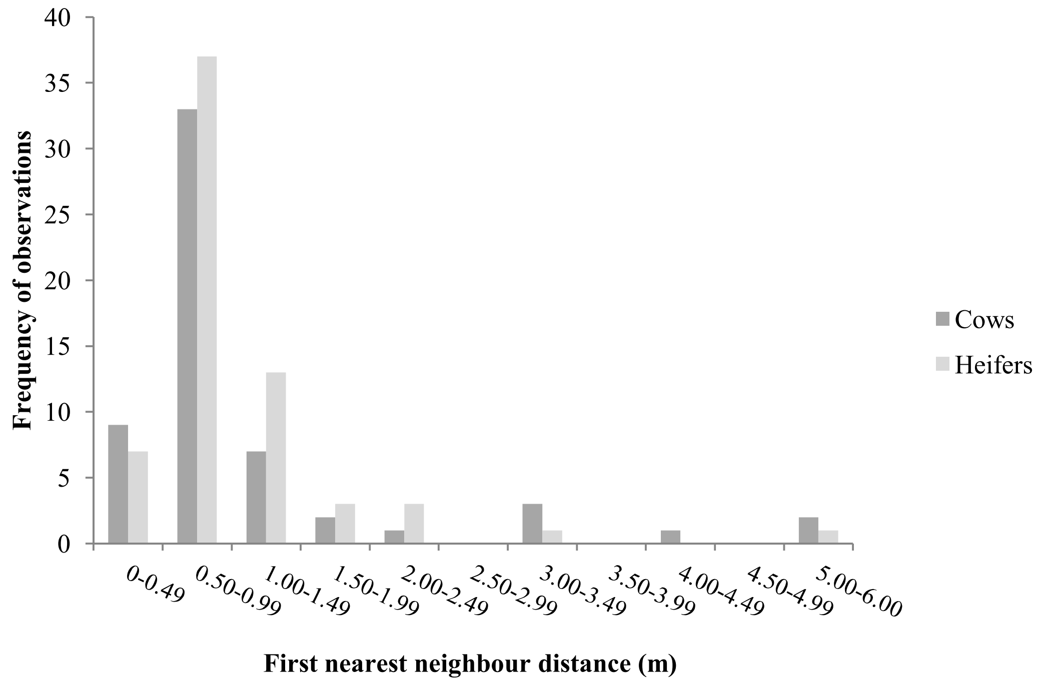


Figure 2.6. The distribution of the total frequency of observations of the mean distance (metres) to the first nearest neighbour for focal heifers and cows by cattle type.

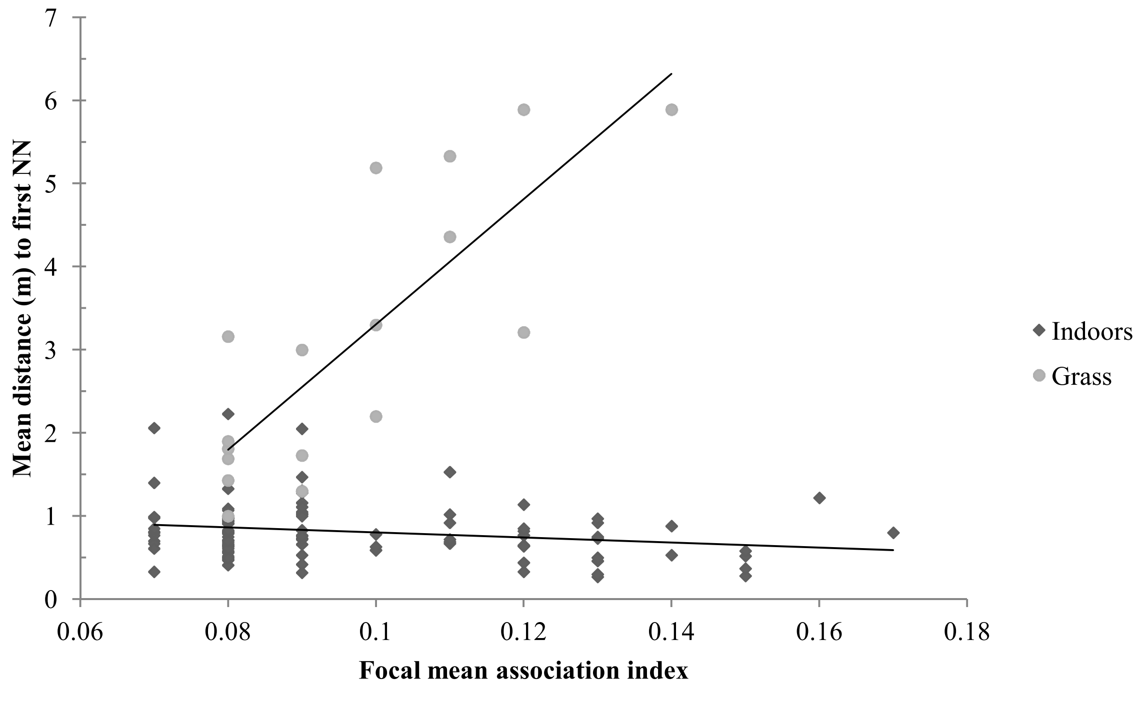


Figure 2.7. The correlation between focal mean association index (0 to 1) and the mean distance (metres) to their first nearest neighbour over multiple observations for heifers and cows when at grass or indoors.

2.4 Discussion

2.4.1 *The existence of social bonds in dairy cattle*

The main aim of this study was to ascertain whether dairy cattle housed in a large dynamic group system under intensive commercial conditions were able to form social bonds with their conspecifics as has been reported in both feral cattle and domestic cattle maintained in small stable groups (Bouissou & Andrieu, 1978; Duve & Jensen, 2011; Raussi *et al.*, 2010; Val-Laillet *et al.*, 2009). This was measured through the use of nearest neighbour and proximity recordings which were transformed into association indices. The results show that there were a number of dyads within the herd that had higher than average association index scores suggesting that a preferential relationship existed for some focal cattle with a particular social partner (chapter 4 and chapter 5 explore some of the reasons behind this). However very few cattle (5%) had particularly high association index scores (0.33 and above), and only 26% of individuals had a mean association index score of 0.27 with another individual, which was the cut off point for determining a preferred partner. Thus, a large proportion of the associations were very weak, with the majority of dyads seen together only once during the observation period. This would have had a significant impact on the average association index score lowering it to the point where it may have identified partners as a preferred partnership when they may have occurred by chance.

To control for this, the probability of seeing two cattle together on two, three, four and five occasions was calculated and the results for the total frequency of individuals that associated significantly more than that expected by chance verified that this level of association was unlikely to have occurred by chance alone. This confirmed that the arbitrary value of 0.27 was a good indicator of a preferential relationship between dyads. Furthermore, the value in this study is higher than values recorded by others in different species; pigs 0.10 (Durrell *et al.*, 2004); dolphins 0.09 (Ansmann *et al.*, 2012). Although this may not be the best method for assessing preferred or avoided companionships as it does not take into account the interdependent nature of social relationships (Asher *et al.*, 2009; Croft *et al.*, 2011), it is the most practical and simplest assessment of individual social relationships and so can easily be performed on farm.

Such a high number of weak associations in group living animals are not uncommon. This herd was very dynamic and animals changed groups on a regular basis with an average of 34.55 (± 1.69) changes occurring in group membership a month, and this may explain why there were a limited number of strong bonds observed. Furthermore, the collection of nearest neighbour data relied upon co-occurrence of a sample group. Due to the large number of animals in the herd, it did not take into account the entire group, thus giving only a snapshot into the relationships at that moment in time. This may have potentially under represented the full social structure of the herd (Asher *et al.*, 2009; Perreault, 2010). Benhajali *et al.* (2008) noted a high number of weak associations in horses with only ten out of 44 horses having a preferred partner. The stocking density was particularly high and the authors commented on the poor social organisation of the group which may have contributed to the lack of preferred partnerships noted. Poor stable groupings have also been noted in feral cattle populations, especially in herds that did not retain the primary bond between mother and calf (Kimura & Ihobe, 1985; Lott & Minta, 1983). Similar results have also been seen in an eland herd, where it was only mother-offspring relationships that appeared to have any strength, at least until the pair separated (Underwood, 1981). Due to the dynamic nature and high stocking density seen in intensive commercial dairy systems, this may explain why only a limited number of preferred associations were seen and a large proportion of the observations were weak associations between dyads.

Potentially, weak associations may demonstrate a very open social organisation and fluidity to the relationships that animals form (Bräger *et al.*, 1994). Plasticity in social relationships and grouping adaptability have been seen in other social systems, for example dolphins in two separate communities restructured their social system and joined together to form one cohesive group when their environmental conditions changed (Ansmann *et al.*, 2012). The plasticity in relationships seen in this study may also be due to the very different groupings that dairy cattle experience under commercial conditions compared to cattle in more natural environments; group sizes tend to be larger, close kin are rare and most likely unknown, and groups are often unstable under commercial conditions. Cattle have been seen to more readily form bonds with their close relations (Reinhardt & Reinhardt, 1982; Reinhardt & Reinhardt, 1981) and have been seen to form bonds when housed in small groups (Patison *et al.*, 2010; Takeda *et al.*, 2000). This is similar to the social nature of baboons, a highly sociable animal, whereby stronger bonds are more likely to exist and remain stable when groups are smaller and more close kin are available. When these conditions do not exist, relationships are less favourable, more dynamic and less consistent over time (Silk *et al.*, 2012), which may be the case with cattle in commercial systems.

2.4.2 *The characteristics of social bonds in dairy cattle*

2.4.2a Age

Preferential relationships appeared significantly more often in heifers than in cows and this is in line with the research by Bouissou & Andrieu (1978), Duve & Jensen (2011), and Raussi *et al.* (2010). They all noted bonds to be stronger in younger cattle, especially if they had been in contact with their peers since a very young age, and this is independent of kin relationship (Ewbank, 1967). In addition Val-Laillet *et al.* (2009) noted dyads which were often in close proximity were more likely to be composed of two primiparous cows than be of a mixed pair or composed of two multiparous cows.

This attraction to a partner of similar age may be due to the grouping of individuals and the level of familiarity that is present between pairs, as cattle are often raised in groups of similar age. Cattle often show a preference to be near to a familiar individual rather than to an unfamiliar individual. For example, when regrouping calves Færevik *et al.* (2007) noted during periods of lying they showed a preference to be in close contact with a familiar individual over unfamiliar individuals. In addition, Takeda *et al.* (2000) observed Japanese black cattle to choose familiar cattle over unfamiliar cattle for both their nearest neighbour and allogrooming partner.

Familiarity with an individual appears to be an important part of bond formation, more so than kin; Murphey (1990) observed young cattle to maintain close proximity to their mothers when visiting a shared resource, however they would then return to the group that they had been raised in rather than staying with their mother. This phenomenon has been documented in other species too, for example Mateo (2009) demonstrated the importance of odours in recognition and bond formation in Belding's ground squirrels.

When juveniles were exposed to odours of non-kin in the natal nest they were more likely to play with those individuals that were familiar by odour, compared to groups that were unfamiliar. In the current study, as heifers came from the same farm they were likely to have been in the same rearing group before entering the main milking herd which would have been made up of animals of a similar age. By having a separate group for the heifers away from older individuals when first entering the milking herd, heifers may have found it easier to recognise familiar individuals and be able to maintain bonds with certain partners from their original grouping. Heifers were also much more likely to be of a similar age compared to cows, which would have been extensively mixed with other cattle of varying ages and experience, making it more difficult to maintain bonds. This may suggest that the bonds recognised in this study may be active partnerships, in that they were associating strongly with familiar individuals that they had spent a significant amount of time with compared to individuals that they were unfamiliar with (Couzin, 2006). This may explain some of the variance between younger and older individuals, as heifers came from a more stable environment before entering the milking herd which was highly dynamic.

An increase in group size and the number of regroupings that cattle had previously experienced may also cause difficulty in bond formation and consequently fewer bonds between adults were observed (this is explored further in chapter 4). Cattle may have found it too difficult to remember each individual within the group. With regular changes occurring in group membership it would have become increasingly difficult for cattle to remember the specific relationships that they held with each other individual. Instead of using energy to constantly re-assess the relationships within the group, cattle may have changed their social strategy and adopted a more solitary social group system, a result reported in unstable feral cattle herds (Kimura & Ihobe, 1985; Lott & Minta, 1983). As group sizes become larger and more dynamic, the social environment becomes less stable and so an alternative social system must be adopted. Anderson *et al.* (2011) noted goats which experienced an increase in their group size adapted their social behaviour by reducing both their positive and negative social behaviours, resulting in a lower level of sociability in the largest group size. Although a reduction in negative behaviours at larger group sizes may be beneficial, the lack of positive social behaviours and bond formation may be more of a welfare concern as it may demonstrate poor psychological welfare.

These results, along with those of Gygax *et al*. (2009) and Neisen *et al.* (2009b), suggest that the consideration of bonds in younger animals, and in particular heifers that are joining the main herd may be of particular importance and should be considered to ensure welfare is maintained. By considering this pairing when integrating heifers into the main herd, mutual social support may have been reciprocally provided, thus easing their integration (Gygax *et al.*, 2009; Neisen *et al.*, 2009b).

2.4.2b. Proximity to nearest neighbour

Most of the nearest neighbour observations occurred whilst animals were at rest (66%). Under feral conditions relationships often consist of grazing partners (Reinhardt & Reinhardt, 1981). Due to the lack of access to grazing for domestic cattle they were unlikely to have been recorded performing this behaviour. Neisen *et al.* (2009b) recognise that resting areas appear to be where limited agonistic interactions take place and so positive associations are more likely to be noted in this area. Other locations such as the feed bunk or the walkways are much more dynamic as cattle are often displaced at the feeder and therefore are less likely to be in consistent contact with another individual (Endres *et al.*, 2005; Huzzey *et al.*, 2006). Reinhardt *et al*. (1978) did note that grazing partners would often lie in close proximity in the evening and associate as resting partners as well. This dual relationship was interpreted by the authors as showing cattle not making social contact on either a random or predictable basis but due to a voluntary bond between individuals, and that they had a particular individual which they preferred to spend their time with. This would suggest that relationships seen at resting may also likely to have occurred at grazing, given the opportunity. The positive relationship between average distance to the nearest neighbour and the mean association index for animals at grass demonstrates that this would have been likely. Furthermore, it helps to demonstrate the natural spacing of cattle compared to the negative, although not significant relationship when animals are indoors based on a cubicle system.

It may be that the associations noted whilst resting were more indicative of preferred resting location than preferred partners. For example, Durrell *et al.* (2004) found a large number of pigs showed an association in one particular pen but only a small number of those partnerships remained when the group was moved to a second pen suggesting that location was more important than the individual. However, in this study 71% of preferred partnerships occurred in different locations and so were not a result of a common preferred location. This is in agreement with the results by Val-Laillet *et al.* (2009) who observed cattle at the feed bunk and noted their locations and proximity to each other. They also noted cattle associated on a preferential basis and not as a result of shared resources and preferred locations.

When assessing an individual's sociability Sibbald *et al.* (2005) found sociability index scores calculated using the first, second and third nearest neighbours were highly correlated. Twenty five percent and 23% of preferred partnerships in this study appeared as the first and second nearest neighbour respectively. Ramseyer *et al.* (2009) also noted cattle and ewe-lambs that had a preferred partnership were spatially closer together and would frequently move in subgroups, suggesting further the importance of considering more than just the first nearest neighbour in order to get a better representation of an individual's social relationships.

Cattle also showed a trend towards having smaller distances between themselves and their first nearest neighbour the higher their mean association index score was. This would suggest that cattle which were more likely to form stronger bonds (have a higher mean association score) were also more likely to maintain closer proximity to their first nearest neighbour. This is in line with other research which has shown inter-individual distances to be shorter when animals are near to familiar individuals (Boissy & Dumont, 2002).

In addition, the mean distance to the first nearest neighbour was greater in cows compared to the distance that heifers maintained, although this was not significantly different. Aschwanden *et al.* (2008) commented that the quality of the bonds in goats significantly influenced the distances between individuals; dyads with more amicable bonds showed smaller distances. As fewer older cattle had a preferred partner and, although not significantly different, older cattle also had fewer preferred partners than heifers, it is possible that older cattle had a lower quality of social bond with others and therefore maintained greater distances. This reduced quality of bond could also explain why the strongest bonds were mainly observed in heifers when compared to cows. This reduction in bond quality could as mentioned previously, be the result of the continued regrouping and large groups experienced by the older cattle. Further, as heifers are likely to have come from the same group and therefore reared together, they are likely to be more familiar with each other and so have a better quality of social bond compared to cows.

Interestingly there was no significant difference in the distance to the first nearest neighbour when at grass and the fifth nearest neighbour when indoors. The increase in stocking density and the increase in group size as a result of increased intensification may make it difficult for cattle to maintain their preferred distance to their neighbours. Kondo *et al.* (1989) noted that the mean distance to the nearest neighbour in calf and adult cattle groups increased as the group size decreased and space allowance increased. This may explain the non-significant difference between the distances maintained to the first nearest neighbour when at grass and when in cubicles and it may also explain the reason why the preferred partner is not always the first nearest neighbour. The relatively similar number of observations of partners appearing as the first and second neighbour may be a result of the fact that cattle prefer to maintain a certain distance to their neighbours, and as the number of cattle increases and space allowance decreases, cattle without a preferred partner or preferential associations may simply fill in the space left by cattle wanting to maintain a certain distance when living in cubicles. The consequences of having non-preferred partners resting in closer proximity than would naturally be maintained when at grass may have negative consequences on cattle welfare as they may feel uneasy about having a random individual that close to them, however this needs further investigation in order to understand what consequences it could have for cattle's welfare.

2.4.3 *Practical implications*

These findings have practical implications on farms. When animals do not have the opportunity to form a social bond there could be negative consequences to their welfare. Social bonds increase cohesion and stability in the group which in turn reduces aggression and reduces social tension in cattle (Phillips, 2002). Improving the social environment and encouraging stability allows cattle to potential have the ability to cope with their environment better and so could relieve stress and ensure good welfare (Veissier & Boissy, 2007) through improved physical as well as psychological health. In addition, cattle's learning ability is better when a conspecific is present and cattle exhibit fewer signs of disturbance towards fear eliciting stimuli when a conspecific is present (Boissy & Le Neindre, 1997; Hagen & Broom, 2004). This would aid learning in younger individuals when introducing them to their new environment upon joining the herd. Training younger cattle to correctly use cubicles is important to health and welfare as incorrect use can lead to increased incidences of mastitis and lameness (Friend & Polan, 1974; Galindo & Broom, 2000; Galindo & Broom, 2002). Furthermore, when introducing cattle to the milking apparatus, a supporting conspecific could help producers to handle cattle and get them settled into the routine more quickly and safely, saving valuable time and increasing safety of stockpersons.

Encouragement for producers to offer an opportunity for cattle to form and maintain positive relationships in the commercial setting is now required. It will now be possible to take note of and adhere to EFSA's recommendation regarding the maintenance of social bonds as they have been demonstrated here to exist in dairy cattle housed under full commercial conditions. Although the majority of the relationships were weak, younger individuals were more likely to have associations and these were also stronger in younger cattle. The rarity and weakness of bonds in older cattle may be due to the lack of consideration given to them in the past and therefore their current environment, as like many other commercial systems, has not considered the social environment fully for dairy cattle to be able to maintain these important bonds.

2.5 Conclusion

In summary, the results of this study demonstrate that cattle under commercial conditions can and do form preferential partnerships with other cattle. This was indicated by the higher than average time that some dyads spent together. The observation that these relationships occurred at a higher frequency than was expected by chance alone indicated that dyads were associating due to a specific relationship between the pair. Younger cattle were more likely to be observed as having a preferred partnership and they were also likely to maintain closer proximity to others. Relationships in cattle appear to change as they age; this is further investigated in chapter 5.

Although spatial proximity may be a good indicator of a social bond, this does not necessarily confirm the importance of the relationship. Most association indices are based on a limited amount of time spent together and therefore the partnerships identified may only be short term associations (Lusseau *et al.*, 2008). Observations did not involve any specific interactions, just spatial proximity, therefore the nature of the bond cannot be determined (Lusseau *et al.*, 2008). In order to fully understand these relationships within cattle it is necessary to demonstrate the importance of this social bond in cattle's ability to cope with environmental challenges.

As the presence of an attachment figure can help to reduce the levels of stress experienced during environmental challenge (Boissy & Le Neindre, 1997; Færevik *et al.*, 2006; Hennessy *et al.*, 2002; Takeda *et al.*, 2003) an observation that tests these relationships in a stressful environment will help to further demonstrate the existence of these bonds in dairy cattle. Furthermore, it will demonstrate the importance of these relationships to cattle when attempting to cope with their environment when challenged, and so is explored in chapter 3.

Chapter Three

The Effects of Short Term Social Separation on the Behaviour, Production and Welfare of Dairy Cattle

3.1 Introduction

The welfare of an animal is closely linked to its ability to cope with challenge and how it perceives its environment (Veissier & Boissy, 2007). In humans, social support has been readily studied and been found to have positive impacts on an individual's coping ability during psychogenic stressors (Ditzen *et al.*, 2007; Ditzen *et al.*, 2008; Heinrichs *et al.*, 2003). Moreover, the mental health and well-being of people suffering from disease has also been seen to improve when being supported by others (Bloom *et al.*, 2001; Ironson & Hayward, 2008; Uchino, 2006). The elimination of potential stressors within the commercial dairy setting may be difficult to achieve but there is the potential ability to utilise the social support mechanism during periods of stress. This may help to alleviate any negative consequences that may be experienced by cattle when separated and promote positive physical and psychosocial welfare of dairy cattle leading to a better quality of life and subsequently higher welfare.

Short term separation of cows from the remainder of the group is a common practice in many dairies. Cattle are often held in a separate holding pen after milking when they are required for pregnancy scanning, artificial insemination or foot trimming. From an early age calves are highly motivated to gain full social contact with another calf compared to just partial contact demonstrating the need for social contact in cattle (Holm *et al.*, 2002). Subsequently, any form of separation or isolation can be a potent psychogenic stressor to cattle generating an acute stress response often resulting in behavioural (Boissy *et al.*, 2001; Piller *et al.*, 1999; von Keyserlingk *et al.*, 2008), endocrinological (Hennessy, 1997; Ruis *et al.*, 2002), immunological (Marsland *et al.*, 2002; Tuchscherer *et al.*, 2009) and other physiological changes (Færevik *et al.*, 2006; Phillips & Rind, 2001), which may be indicative of a reduced welfare status.

The strong emotional impact that social isolation and separation brings about has meant that it has been frequently implemented to validate and quantify the existence of a social bond between two individuals, as well as to explore the positive impacts of social support when confronted with such challenges (Færevik *et al.*, 2006; Price & Thos, 1980; Schweitzer *et al.*, 2010). For example, heifers restrained during social isolation exhibited signs of distress through increased vocalisations and struggling (Boissy & Le Neindre, 1997). The heart rates of heifers also increased during social isolation showing an autonomic stress response; on subsequent reunion of the isolated heifer with its peers, there was a marked decline on both the behavioural and autonomic response to the stressor. The rate of decline was, however, dependent on familiarity, with pen mates having a more pronounced effect than non-pen mates (Boissy & Le Neindre, 1997). Familiarity is important to bond formation, and appears to be important with regards to an individual's ability to reduce the stress response of another after a period of isolation; it does not however automatically confirm a bond between individuals. It could be argued that the distress caused by the separation of group mates may be indicative of the response to the isolation in itself and therefore does not fully address the concept of social support that may increase an individual's ability to cope with potential stressful conditions during that time.

Results by Færevik *et al.* (2006) however, do provide some evidence on the effects of social support during isolation. Six week old calves were exposed to two tests: 1) to observe the social preferences of calves using a Y-maze test; and 2) to observe the effects of separation stress when isolated on their own, with an unfamiliar calf, or with a familiar calf. During the Y-maze test, calves preferred to spend more time standing next to and exploring the areas near the familiar calf, representing a preferred companion. During the separation test, calves showed a reduced stress response when separated with a familiar calf compared to when either alone or with an unfamiliar calf (Færevik *et al.*, 2006). From this it can be deduced that from an early age cattle are able to form relationships with particular individuals and that these relationships are important to them during a time of social separation as they appear to lessen the stress response evoked, compared to another unfamiliar companion. Although familiarity did appear to offer some support during the social separation test, the level of familiarity and, therefore, the quantification of the bond between individuals was still not fully clarified.

In order to quantify the existence of a bond and the potential improvement in an individual's coping ability through support from its bonded partner, the effect of the presence of a familiar and previously identified preferred partner compared to the presence of a familiar but non-preferred partner during a period of social separation must be demonstrated. Having established that cattle under commercial conditions do form social bonds (see chapter two), the use of a short term separation test was utilised to help quantify that social bond further, as has been seen in previous studies mentioned above. The overall aim of this part of the study was to investigate the effects of short term social separation from those preferred group mates on the welfare, productivity and behaviour of dairy cattle. In order to fully understand the effects of social separation from their preferred partner and not just from the remainder of the herd, the use of the previously identified bonded individuals from study 1d (see section 2.2.1 for details) were used and cattle were exposed to social isolation whilst supported by their preferred partner and then by a non-preferred but familiar individual. This addressed some of the concerns with previous research as discussed above regarding the dissociation of the advantages of social support and the disadvantages of separation. As both animals were familiar with the focal, but one a preferred partner, this allowed for the potential effects of social support to be investigated, as well as to establish the importance of social bonds in cattle and how they might aid an individual's ability to cope with psychological stressors such as social isolation.

3.2 Materials and methods

3.2.1 *Housing area, animals and management*

Eleven lactating Holstein-Friesian cattle from the main milking herd at Stud Farm, Moulton College were used in the study: ten primiparous heifers, and one multiparous cow. These eleven individuals made up of six pairings (one animal was involved in two preferred partnerships) (see table 3.1), which had previously been identified as preferred partners from observation 1d. All cattle remained within the herd apart from when being socially separated for experimental observation and remained within the same lactation group throughout the observation period. All cattle were housed loose in a cubicle shed with *ad libitum* access to water and a total mixed ration (TMR) formulated to provide adequate nutrients for that particular lactation group. Fresh feed was delivered twice a day, after each milking period and cattle were allowed out to grass generally one hour after feeding until the next milking at 1530hr (approximately six hours at grass). See section 2.2.1 for more information.

Table 3.1. The makeup of the eleven preferred partner pairings that had been previously identified in study 1d (see section 2.2.1 for details). Numbers represent freeze brand numbers for ID.

Pair no.	Focal animal	Preferred partner
1	**1725**	1295
2	**1739**	1844
3	**1759**	1716
4 *	**1759**	1780
5	**1782**	1481
6	**1785**	1513

* *Denotes a reciprocal pairing*

3.2.2 *Experimental conditions and set-up*

The study took place between May and July 2010 and observations were conducted during normal milking hours, 0430-0900hr and 1600-1800hr with a total of 33 hours of observation. For five days a week over nine weeks, each morning and afternoon, one pairing was separated from the remainder of the group. The testing area used for separation of cattle was the shedding area and artificial insemination (AI) pens within the dairy (see figure 3.1 for layout). Cattle would exit the parlour after milking and walk along the corridor towards the shedding gates which would allow them into the test area. There was no specific order in which cattle entered the test area; this was based on their own natural progression from the milking parlour. This reduced any undue stress that may have been caused by forced movements, and reduced the impact on the producers. When all animals to be observed were in the test area, cattle were herded into the AI pens to allow for the attachment of the heart rate monitor and then released back into the test area. Due to the very nature of social separation, to minimise the stress experienced, cattle were observed for thirty minutes only while behaviour was recorded through the use of a video camera. This time limit also prevented cattle from experiencing excessive delays in the time they were away from resources and the amount of time that they spent standing after milking. Once behavioural observations were complete cattle were herded back into the AI pens so that the heart rate monitor could be removed and saliva and milk samples collected. Cattle were then released from the shedding area and allowed to travel back to the main milking herd under their own speed so that a runway test could be carried out. Cattle were never isolated on their own so as to reduce any stress experienced.

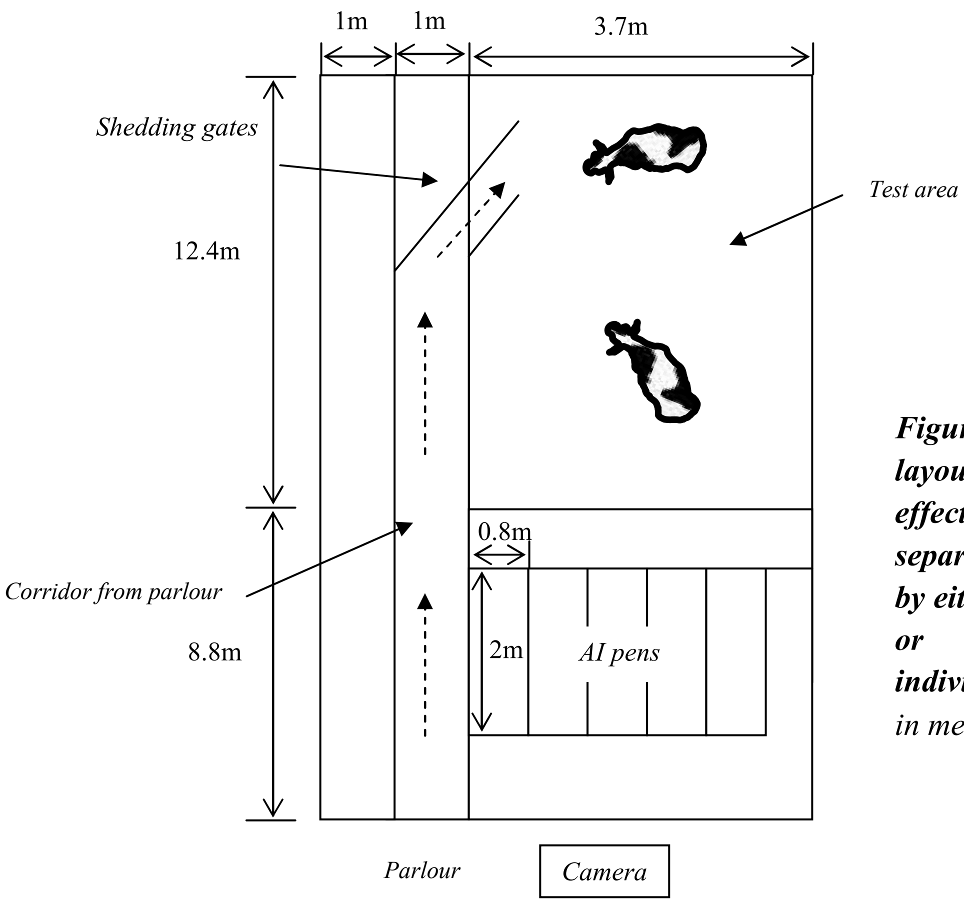


Figure 3.1 Experimental layout of pen to study the effect of short term social separation when supported by either a preferred partner or a random familiar individual *(dimensions given in metres, N.B. not to scale)*

There were three stages to the study, all of which were repeated three times. The first stage (PP) was carried out with the focal animal (A) and their preferred partner (B) being held within the shedding area. The second stage (PR) consisted of animal A and animal B, plus animal C, a familiar but non preferred individual. Animal A and animal C were held in the shedding area whilst animal B would be released from the AI pens back into the main herd. The third stage (RP) consisted of animals A, B, and C being held in the shedding area. Animal A would then be released leaving, animal B and animal C in the shedding area for observation.

3.2.3 *Measurements*

3.2.3a Behavioural observations

In order to assess the effects of short term separation on the welfare of cattle the behavioural response of heifers was monitored for signs of distress or changes in their response between treatments. From the video footage instantaneous behavioural sampling was carried out at 15 second intervals for each pair over the thirty minute separation period as defined in table 3.2 with the exception of vocalisation which was recorded on a continuous behavioural basis. This interval was chosen so that behaviour recordings coincided with the heart rate measurements and any unusual responses could be traced back using the video footage. In addition, the proximity between the two individuals was also recorded at 15 second intervals (<1 body length, 1-2 body lengths, >2 body lengths at the point of withers), as was the position of each individual within the pen (exit, middle, AI pens). This allowed comparisons between proximity measurements of preferred and non-preferred partners.

Table 3.2 Behavioural ethogram for short term social separation when supported by either a preferred partner or a random, familiar individual

Behaviour	Description
Walk/pace	Any forward movement that lasted for more than 4 steps at a variety of speeds including the trot or run.
Stand	The animal remains stationary in a passive manner and its head remains inside the barriers.
Social positive	Social licking/ grooming, nuzzling, head contact/resting on body.
Social negative	Head butting, pushing, nudging, causing displacement or submission within the other animal.
Investigate/ explore	Any part of the head (nostrils to ears) is put through the bars, under the bars, or held above the bars/walls and may be rested on the side. Usually the ears are forward and the animal is alert.
Agitation	Includes behaviours such as lifting both front feet off the floor and turning towards the left or right, stamping of the feet, one or two forward and backward steps or side to side movements, head toss where it is thrown up in the air and in a sideways motion, tail swish.
Vocalisation	Any audible sound that included the rumble, call or hoot. One vocalisation = 1 audible sound followed by an intake of breath.
Eliminate	Excretion of waste

3.2.3b Runway test

A runway test based on the method used by Gibbons *et al.* (2010), was carried out at the end of each observation period and assessed for both individuals. The runway (9m in length) consisted of the passageway situated between the shedding area where individuals were held and the home-pen area. This was the same passageway that cattle used to return to their home-pen after each milking period twice a day and so it was familiar to all animals.

On test days, at the end of the thirty minute separation procedure both individuals were released through the exit gate out of the holding area and into the runway. The test began for each animal once they had taken one step past the first post in the passageway and finished when they had taken one step with a front leg past the end of the runway area (last post in view of the camera). The latency to reach the end of the runway was taken from video recordings and used as a measure of social motivation to return to the remainder of the group. This latency to return was used to assess whether cattle held with their preferred partner were more or less inclined to access the remainder of the herd compared to when they were held with a non-preferred but still familiar individual (and their preferred partner was in the main herd).

3.2.3c Heart rates

The heart rates of cattle were measured to assess the autonomic stress response during short term separation. Differences in heart rate responses between treatments would provide numerical assessment of the importance of each partner, preferred or non-preferred, in providing support to ease the stress experienced. Heart rate was recorded at 15 second intervals in beats per minute (bpm) using a non-invasive heart rate monitor (Equine POLAR® RS800^{TM} heart rate monitor) that was modified for use in dairy cattle, similar to Hagen *et al.* (2005). The monitor was attached as per manufacturer's instructions to the left side with one electrode placed behind the shoulder blade near to the withers and the other in a ventral position by the heart. Electrodes were heavily moistened with warm water and ultrasound gel was applied under each electrode to enhance transmission. No animal was shaved or trimmed. Data was transmitted from the belt to the watch receiver where it was then downloaded to the Polar ProTrainer5^{TM} and then transferred to Microsoft Excel 7.0. Data was checked for abnormalities including zero values or values above 200 bpm, and then mean heart rate per minute were calculated, as in Stěhulová *et al.* (2008), ready for analysis. Collection of heart rates could only be collected for one individual in each stage to avoid cross over in readings. For stage 1 (PP) focal cattle (A) were monitored, for stage 2 (PR) focal cattle (A) were monitored, and for stage 3 (RP) partners (B) were monitored.

3.2.3d Cortisol

The physiological response to a potential stressor can be quantified through the measurement of glucocorticoid hormones, in particular cortisol. It is well established that levels of cortisol within the body are good indicators of the current welfare status of individuals as high levels of this hormone can indicate a high level of stress. Cortisol samples were taken using non-invasive techniques and were collected at the end of the separation period to assess the level of stress experienced due to the separation. Saliva samples were obtained using a similar technique to Cook *et al.* (1996), Negrão *et al.* (2004) and de Jong *et al.* (2000) whereby cattle were allowed to chew on a standard cotton ball held in the mouth with tongs until completely soaked, or for as long as the individual would allow. Swabs were then wrapped in cling film and placed on ice in a polystyrene box ready for transport to the laboratory. Foremilk samples (approximately 10ml) from all quarters were manually extracted into a 125ml airtight container, with a 50mm diameter opening, which were then placed on ice for transportation. Pooled samples from all four quarters were chosen so that the most representative physiological samples were collected.

The longest time between sample collection and sample separation in the laboratory was no longer than 2 hours. Samples were centrifuged using a SIGMA 2-16P Benchtop Centrifuge (Laborzentriugen GmbH, Germany) (milk at 4000 rpm for 30 mins and saliva at 1500 rpm for 20 mins) to remove fat and debris from the samples, as well as to extract as much saliva from the cotton bud as possible. Samples were stored in 1.5ml Eppendorf Safe Lock Tubes™ (Eppendorf, Germany) and subsequently frozen at -40°C in a Frigor -45°C chestfreezer (Snijders Sceintific B.V., The Netherlands) until required for assay: this was no longer than twelve months. All samples were allowed to thaw naturally for 30 minutes and mixed thoroughly before assay as per the manufacturers' instructions. A high-sensitivity human saliva commercial 96 well ELISA kit (DRG® Marburg, Germany) was used to determine cortisol levels. Its analytical sensitivity for detection was 0.012 ng/ml and samples were prepared as per the instructions and measured in duplicates. Plates were then analysed by a SPECTROStar^{Nano} Absorbance microplate reader (BMG LABTECH GmbH, Germany). Both saliva and milk samples were collected so that comparisons between the two could be made. It was hoped that the saliva samples could be used to investigate the potential use of milk samples for welfare assessment as it is readily available to both producers and researchers and thus can be collected without interfering too much with the animal and therefore influencing the results.

3.2.3e Production

In order to assess the potential impact on milk production that short term separation from the herd might have, and to investigate how any changes might be limited by the presence of a preferred partner, production data was collected. Individual milk yield (litres) was automatically collected by Crystal, Fusion Electronics BV (Fullwood Packo Group, The Netherlands) at each milking (am and pm). The Crystal software automatically records a number of management factors such as activity levels and milk volume produced (yield). The milk monitoring system employed is capable of measuring milk volume, conductivity (milliSiemens [mS]), and temperature (°C). This information was stored on a database within the farm's computer system and could be accessed to obtain the required information. Data was downloaded from farm records on a monthly basis and transferred to Microsoft Excel 7.0 so that it could be arranged into the required format for data analysis and where it could be checked for abnormalities. Data was collected to include the two milkings before separation and the two milkings after separation (24 hours either side) so that any changes between the time periods could be analysed and thus the effect of separation could be assessed. As milk production changes according to the time of day, it was important to compare both times (am and pm) rather than just adjacent days (Plaut & Casey, 2012).

3.2.4 *Statistical analyses*

All statistical analysis was carried out using Minitab version 13.20 and data presented as means ± SEM unless where otherwise stated. For all statistical analyses a significance level of $p<0.05$ was accepted. Any data which was not normally distributed was log transformed using the common logarithm $\log_{10}$ to normalise where required. Where log transformation did not normalise data, non-parametric statistics were used. Details of particular methods and tests employed are outlined below.

3.2.4a. Behavioural observations

Individual behaviours were compared for both the focal animal and its preferred partner, between stages. Normality was tested using the Anderson-Darling test. If normality was met a paired-samples *t*-test was used, and if normality assumptions were not met whereby the data was either not normally distributed or there was unequal variances, the non-parametric equivalent, the Wilcoxon's signed rank test was used. Individual behaviours for the non-preferred random animal between stages were compared using either the independent two-sample *t*-test when normality was met (as above) or the non-parametric equivalent Mann-Whitney *U* test when normality was not met, due to non-repeated measures.

Distance between dyads within stages was analysed using a one-way ANOVA for stage 1 (PP) and stage 2 (PR), and a Kruskal-Wallis test for stage three (RP) as data was not normally distributed after log transformation. Comparisons were made using Fisher's *a priori* least significant difference tests to further investigate where differences existed. Due to non-normally distributed data after log transformation, a general linear model was used to analyse the interaction effect of stage and the number of times cattle were within each of the proximity categories (<1 body length, 1-2 body lengths, >2 body lengths). To test for order effects that may have allowed habituation to the testing area, Spearman rank correlations between each repeat test session for all animals were carried out as data was not normally distributed.

3.2.4b Runway test

The latency to reach the herd upon release was analysed using non-parametric statistics as the data was not normally distributed after log transformation. The average time taken for cattle to return to the herd across the three stages was compared using a Kruskal-Wallis test, as was the difference in average time taken between cattle (focal animal A, preferred partner B, and random individual C). The effect of separation was analysed using a Wilcoxon's signed rank test for A and B due to paired-samples, and a Mann-Whitney *U* test for C due to independent samples. Spearman's rank correlations between each repeat test session for all animals were carried out to test for habituation across test sessions.

3.2.4c Heart rates

To avoid confounding effects of handling cattle on heart rate, the first ten minutes of data were removed from the analysis. Differences in mean heart rates per minute, peak heart rates, the latency to reach peak heart rate, and the latency to reach 80bpm (deemed to be resting heart rate), were compared between stage 1 (PP) and stage 2 (PR) using repeated measures analysis, and between stage 1 (PP) and stage 3 (RP), and stage 2 (PR) and stage 3 (RP) using independent analysis. Spearman's rank correlations between each test session for all animals were carried out to test for consistency across test sessions.

3.2.4d Cortisol

Cortisol samples for both milk and saliva were analysed to test for the differences in cortisol levels between stage 1 (PP) and stage 2 (PR). Milk cortisol samples were compared using a non-parametric Wilcoxon's signed rank test as data was not normally distributed, and saliva cortisol samples were compared by a paired-samples *t*-test due to repeated measures.

3.2.4e Production

To investigate general differences in total milk yield, milk conductivity, and duration of milking between focal animal A and the preferred partner B, and the condition experienced, a general linear model was used. The model included cattle identity (A/B), condition (PP, PR, or RP), and whether it was before or after separation. The effects of separation on each of the focal individuals were compared using a Wilcoxon's signed rank test to test for changes in yield and the duration of milking, and a paired-samples *t*-test was used to test for changes in conductivity before and after separation, between stages.

3.2.5 *Ethical note*

All ethical considerations were approved by MC ethics committee. All animals were observed for any signs of excessive distress or behavioural reactions that may result in injury. Any animal displaying behavioural signs of distress were removed from the study and released from the area back towards the herd as soon as was possible and safe to do so. Cattle were acclimatised to the use of the cotton wool swab before data collection started. Any animal that showed signs of excessive disturbance when collecting saliva or milk samples, collection was ceased and the animal released from the pen.

3.3 Results

3.3.1 *Behavioural observations*

Table 3.3 shows the mean frequency (± SEM) and significance level for differences between stages for all behaviours for focal animal A, the preferred partner B, and the random individual C. During the separation period, the behaviour with the highest frequency was standing for all animals and this did not differ significantly across stages. The frequency for 'investigate / explore' and 'walk / pace' was also relatively high for all cattle across the three conditions. In particular, for focal individuals (A), the mean frequency of 'walk / pace' behaviour, although not significantly different did show a tendency to increase when they were with a random individual compared to when with their preferred partner.

For both A and B there were significant differences in behaviours suggestive of agitation between stages, with higher frequencies observed when cattle were with the random individual. C did not show any differences. Both A and B had lower levels of agitation when in stage 1 (PP) than when with a random individual (stage 2 (PR) and stage 3 (RP) respectively). There was no significant difference in the frequency of both positive and negative social behaviours for all cattle across the three stages, although for B there was a trend towards significance with more negative behaviours being performed when with the random individual compared to when with the focal individual.

Table 3.3 Difference in mean frequency (± SEM) of behaviour during short term separation for focal (A), partner (B) and random cattle (C) between the three stages (PP – preferred partners, PR- focal animal with random individual, RP-partner with random individual).

	Stage (mean frequency ± SEM)			Test statistic	*p*-Value
Behaviours	**Stage 1 (PP)**	**Stage 2 (PR)**	**Stage 3 (RP)**		
Focals (A)					
Walk / Pace	14.45 ± 4.00	17.76 ± 6.00	-	T=-1.80	***0.09***
Stand	82.50 ± 2.73	82.33 ± 3.46	-	T=-0.10	0.93
Social Positive	1.77 ± 0.69	0.76 ± 0.28	-	W=74.50	0.18
Social Negative	0.91 ± 0.31	1.29 ± 0.37	-	T=-1.44	0.25
Investigate/explore	21.50 ± 2.21	18.29 ± 2.13	-	T=1.41	0.18
Agitation	4.05 ± 0.84	7.48 ±1.17	-	W=33.50	**0.01***
Vocalisation	14.50 ± 2.56	16.38 ± 2.70	-	W=78.50	0.33
Elimination	0.46 ± 0.14	0.76 ± 0.18	-	W=7.50	0.16
Partners (B)					
Walk / Pace	13.27 ±1.60	-	14.77 ±1.72	T=-1.11	0.29
Stand	82.60 ± 4.59	-	84.23 ± 4.11	T=-0.48	0.64
Social Positive	0.67 ± 0.27	-	0.62 ± 0.31	W=25.50	0.77
Social Negative	1.13 ± 0.40	-	1.77 ± 0.39	W=6.00	***0.06***
Investigate/explore	22.80 ± 3.26	-	19.00 ± 2.82	T=1.27	0.23
Agitation	2.33 ± 0.97	-	6.08 ± 1.35	W=5.50	**0.01***
Vocalisation	17.40 ± 4.93	-	7.62 ± 1.90	T=1.38	0.20
Elimination	0.47 ± 0.13	-	0.85 ± 0.27	W=7.50	0.31
Random (C)					
Walk / Pace	-	15.57 ± 1.91	11.54 ± 2.10	T=1.49	0.15
Stand	-	80.10 ± 4.26	84.00 ± 5.38	T=-0.61	0.55
Social Positive	-	0.52 ± 0.19	0.23 ± 0.17	W=392.00	0.27
Social Negative	-	2.05 ± 0.72	0.85 ± 0.25	W=389.50	0.42
Investigate/explore	-	22.57 ± 2.84	23.62 ± 3.86	T=-0.22	0.83
Agitation	-	6.38 ± 1.40	7.62 ± 2.09	T=-0.22	0.83
Vocalisation	-	22.33 ± 5.56	17.69 ± 5.27	T=0.64	0.53
Elimination	-	0.43 ± 0.16	0.69 ± 0.21	W=337.50	0.23

*Significant differences at $p<0.05$ are denoted by * in bold, and trends towards significance at $p\leq 0.09$ are in bold italics.*

There were no significant differences in vocalisations for focal animal A, preferred partner B, or random individual C ($p>0.05$), however; as can be seen in figure 3.2, C performed the most vocalisations overall with the highest frequency being when they were separated with animal A. B also performed the most vocalisations when separated with A and very few when separated with C. For animal A the number of vocalisations did not differ between when separated with either B or with C. Elimination behaviours did not differ between stages for A, B or C.

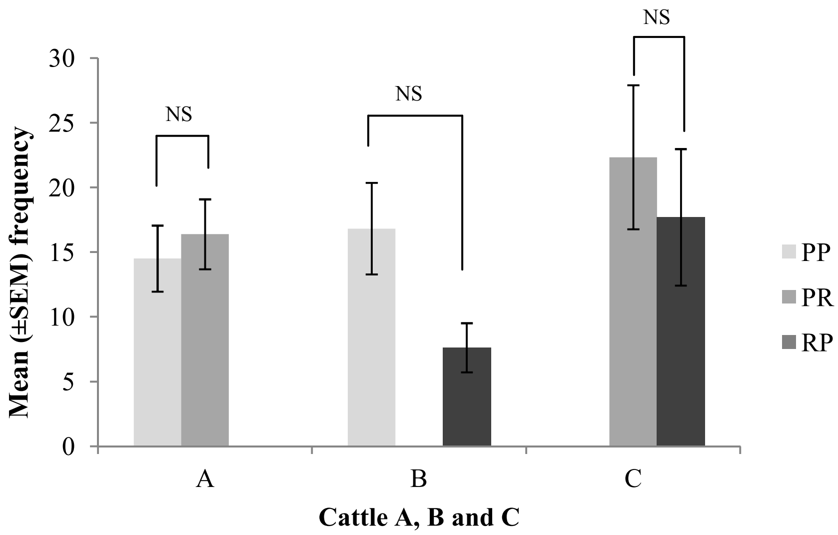


Figure 3.2. The mean (± SEM) frequency of vocalisations across the three stages (PP, PR, RP) for all cattle. *Analysis carried out on log transformed data due to non-normal distribution.*

A significant interaction effect between the stage and frequency of observations within proximity categories was evident (F=2.71, df=4, *p*=0.03). Dyads in stage 1 (PP) had significantly different frequencies between distances (F=39.53, df=2, *p*=0.00), as did dyads in stage 2 (PR) (F=21.76, df=2, *p*=0.00) and stage 3 (RP) (H=16.73, df=2, *p*=0.00). Figure 3.3 demonstrates where significant differences lie between proximity distances. For all cattle, the lowest frequency of observations was seen with cattle more than two body lengths apart, and dyads in stage 1 (PP) were observed to be in close proximity for the highest number of observations but this was not significantly different (*p*>0.05) to stage 2 (PR) or stage 3 (RP).

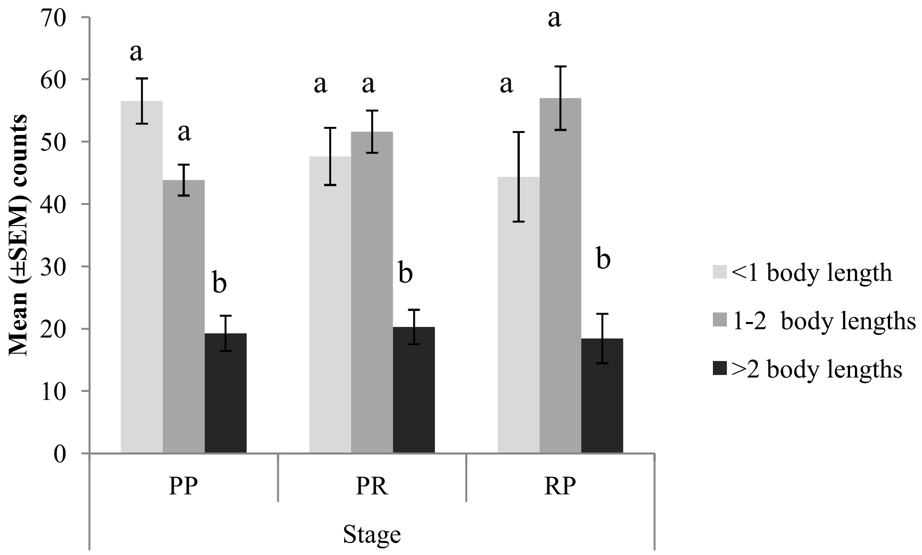


Figure 3.3. Mean (± SEM) number of observations where dyads were less than one body length, between 1 and 2 body lengths and more than 2 body lengths apart across stages. *Significant differences (p<0.05) within stages are shown by different letters.*

Overall, cattle were significantly more likely to be observed standing by the AI pens (F=114.87, df=2, p=0.000) compared either the middle and exit (see figure 3.4 for average frequency of observations seen in the different pen areas). The preferred position did not differ between stages (F=0.01, df=2, p=0.99) and did not differ between focal animal A, preferred partner B or random individual C (F=0.01, df=2, p=0.99).

There were no significant correlations between the repeat test runs of each session for cattle A, B, or C for all behaviours except for walking which had a significant positive correlation between run two and run three (r_s=0.367, p=0.04).

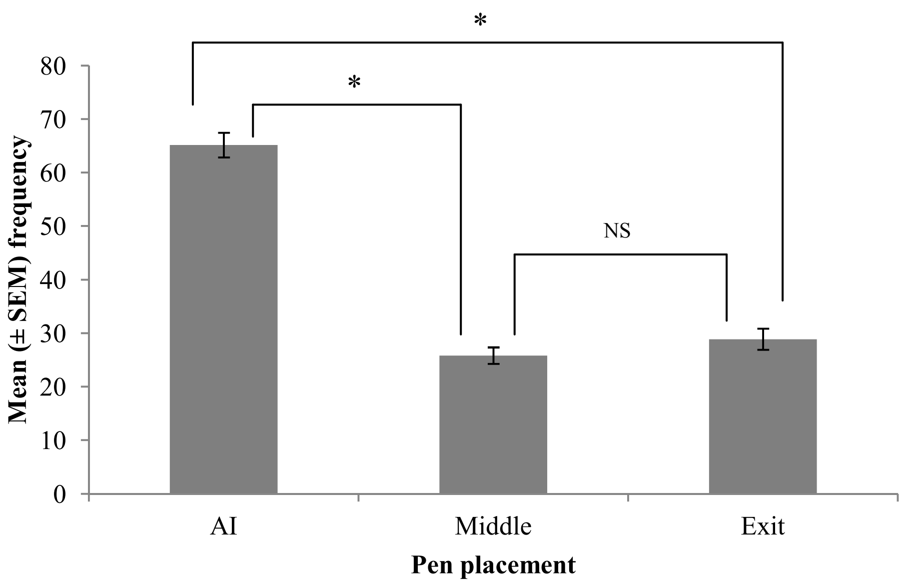


Figure 3.4 Mean (±SEM) frequencies for preferred placement in the observational pen when separated for all cattle observed. *Significant differences (p<0.05) denoted by *. NS = non-significant differences.*

3.3.2 *Runway test*

Between stages there was a significant difference (H=7.89, df=2, *p*=0.02) in the time taken to return to the herd. Cattle in the PP stage took longer (10.76 ± 0.58 secs) than when cattle were in the PR stage (9.93 ± 0.86 secs). Cattle in the RP stage took the longest time to return towards the herd (11.56 ± 0.92 secs). There was no significant difference between cattle A, B, and C, although there was a trend towards significance (H=4.76, df=2, *p*=0.09) with cattle A (11 ± 0.86 secs) and B (11.24 ± 0.78 secs) taking longer on average than random cattle C (9.53 ± 0.56 secs) to return to the herd.

Random cattle C took longer to return to the herd when with partners B than when with the focal individual A, although this was not significantly different (W=281.5, *p*=0.06). There was no significant difference in the latencies to return to the herd between stages for focal animals (A) and no significant difference in the latencies to return to the herd between stages for preferred partners (B). See figure 3.5 for mean ± SEM latency to return to the herd across the three stages for A, B, and C.

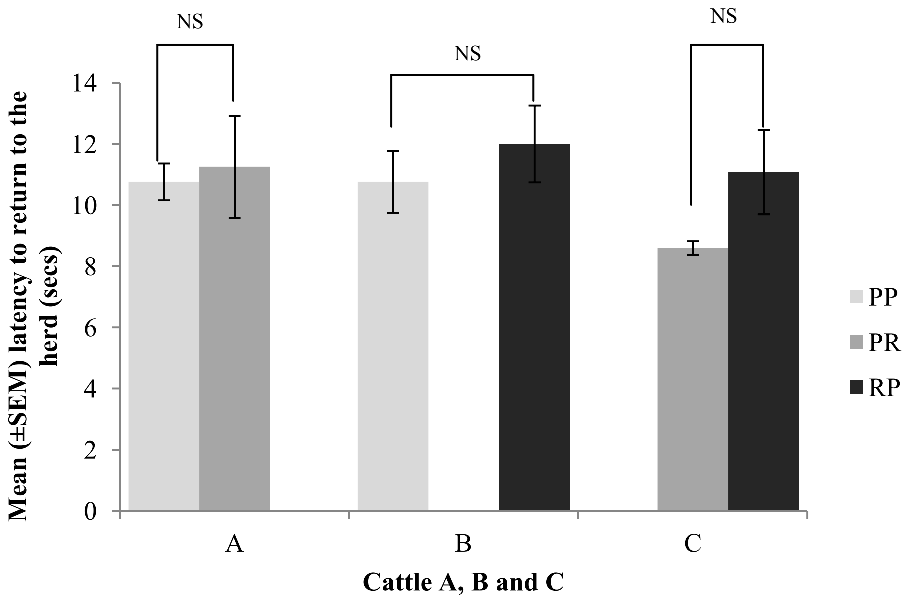


Figure 3.5. Mean (±SEM) latency (seconds) to return to the herd during the runway test across the three stages (PP, PR and RP) for focal animal A, preferred partner B, and random individual C.

There were no significant correlations between the repeat test runs of each session for cattle A, B, or C for the run-way tests.

3.3.3 *Heart rates*

Focal animals had significantly lower heart rates across the separation period when they were with their preferred partner (PP) compared to when with a random individual (PR) (see figure 3.6 and table 3.4). There was no significant difference in heart rates between focal animal A in stage 1 (PP) and its partners heart rate in stage 3 (RP) (see figure 3.7 and table 3.4), although there was a significant difference between the focal (A) cattle's heart rate in stage 2 (PR) and the partners (B) heart rate in stage 3 (RP) (see figure 3.8

and table 3.4). There was no significant difference between each of the three conditions for maximum heart rate, the latency to reach peak heart rate and the latency to reach 80 bpm (see table 3.4 for details and *p*-values).

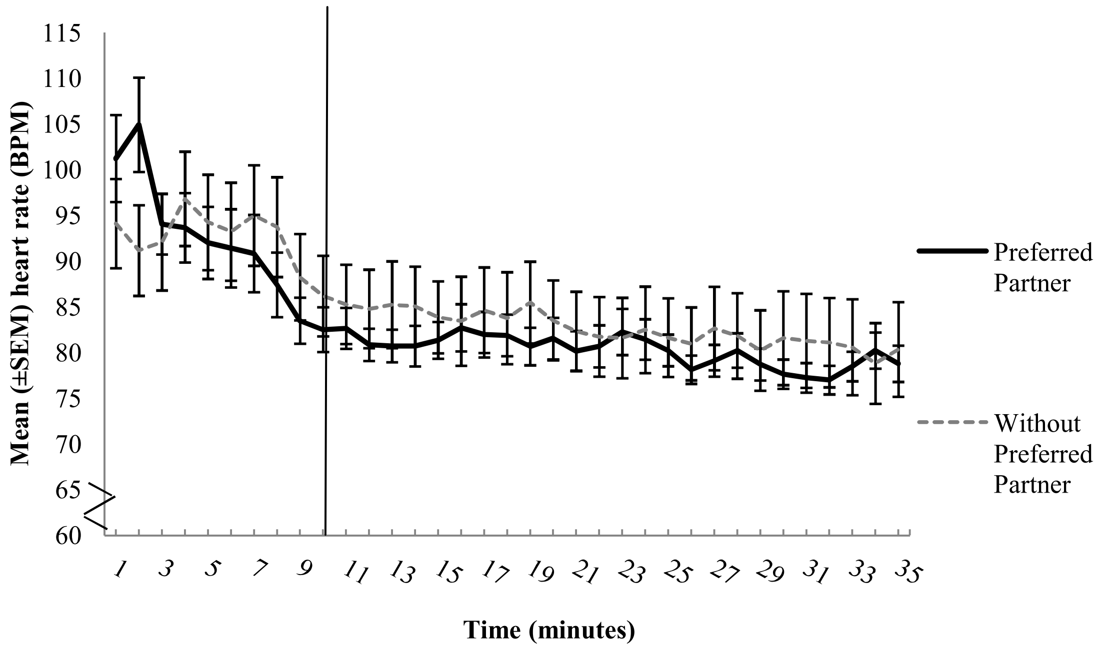


Figure 3.6 Mean heart rate ± SEM (bpm) for focal heifers (A) when socially isolated with their preferred partner (B) (PP) and without their preferred partner (PR) but with a random individual (C). *N.B. False origin. The vertical line represents the ten minute point where data before this time was not used in analysis.*

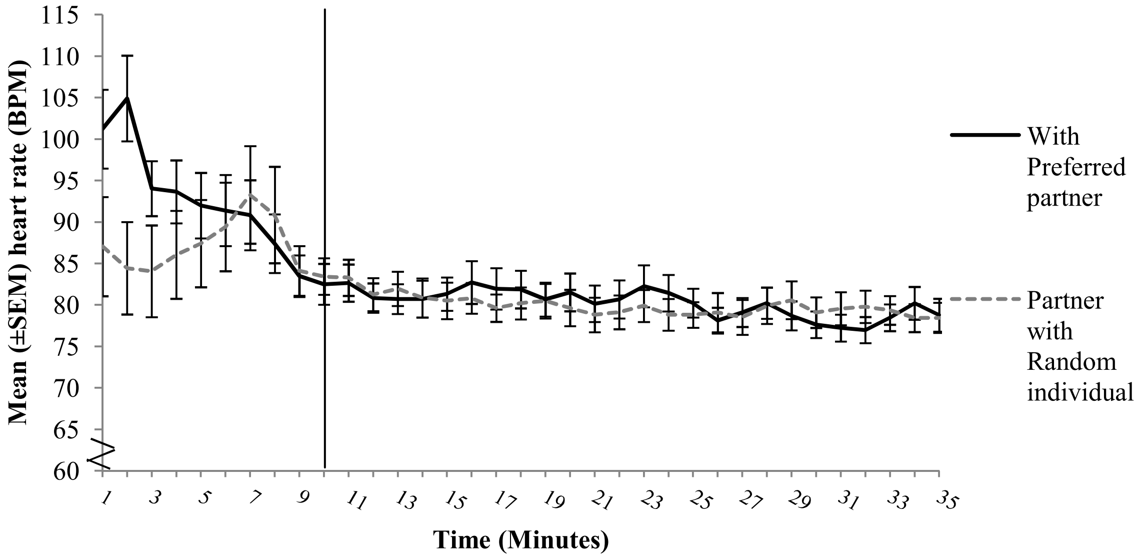


Figure 3.7. Mean heart rate ± SEM (bpm) of socially isolated focal heifers (A) when with their preferred partner (B) (PP) and their partners heart rate (B) when isolated with a random individual (C) (RP). *N.B. False origin. The vertical line represents the ten minute point where data before this time was not used in analysis.*

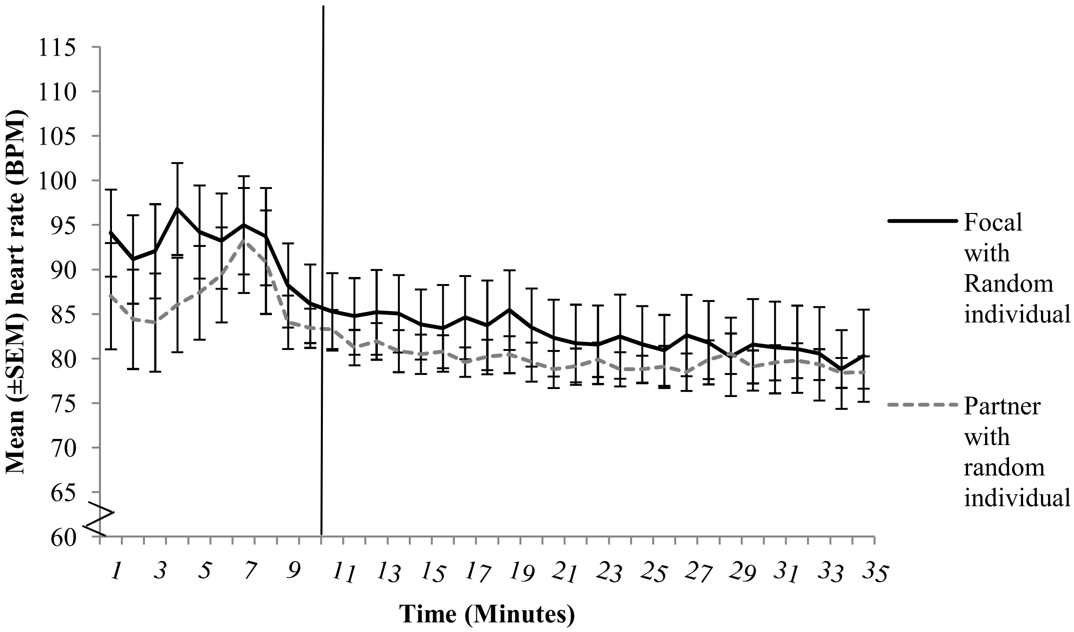


Figure 3.8 Mean heart rate ± SEM (bpm) of socially isolated focal heifers (A) with a random individual (C) (PR) and their partners heart rate (B) when with a random individual (C) (RP). *N.B. False origin. The vertical line represents the ten minute point where data before this time was not used in analysis.*

Overall, there was a significant positive correlation (r_s=0.264, *p*=0.000) between session PP and session PR. For animal A, during test session PP for the repeat testing's there was a significant positive correlation between run 1 and run 2 (r_s=0.318, *p*=0.00), a non-significant decline in heart rates between run 1 and run 3 (r_s=-0.105, *p*=0.256) and a significant decline in heart rates between run 2 and run 3 (r_s=-0.278, *p*=0.002). For animal A, for the repeat testing's for PR, there was a significant decline in heart rates between run 1 and run 2 (r_s=-0.447, *p*=0.00), a significant increase in heart rates between session 1 and 3 (r_s=0.634, *p*=0.000), and a significant increase between run 2 and run 3 (r_s=0.169, *p*=0.041). Animal B showed for the repeat test runs for session RP there was a significant negative correlation between run 1 and run 2 (r_s=-0.4004, *p*=0.00), a significant positive correlation between run 1 and run 3 (r_s=0.256, *p*=0.010), and a significant positive correlation between run 2 and run 3 (r_s=0.436, *p*=0.000).

Table 3.4. The mean (± SEM) heart rates (bpm), maximum heart rate (bpm), latency to reach peak heart rate (seconds) and latency to reach 80 bpm (seconds), across the three stages (PP = Focal animal (A) with preferred partner (B), PR = focal animal (A) with a non-preferred random individual (C), RP = the focals partner (B) with a familiar random individual (C)), during the thirty minute separation period.

Treatment	**Condition (Mean ± SEM)**			**Test statistic and *p*-value**		
	PP	**PR**	**RP**	**PP vs PR**	**PP vs RP**	**PR vs RP**
Mean heart rate (bpm)	80.2 ± 0.33	82.6 ± 0.37	79.9 ± 0.23	T=7.44, ***p*=0.00***	T=0.82, *p*=0.42	W=886.5, ***p*=0.00***
Maximum heart rate (bpm)	95.86 ± 3.44	99.62 ± 5.14	95.87 ± 2.91	W=100.5, *p*=0.88	T=0.00, *p*=1.0	W=391, *p*=0.95
Latency to reach peak heart rate (secs)	1137.1 ± 88.5	1147.1 ± 95.1	974 ± 155	T=0.08, *p*=0.93	W=444, *p=0.07*	W=445, *p=0.07*
Latency to reach 80 bpm (secs)	604 ± 182	400 ± 153	575 ± 257	W=114.5, *p*=0.22	W=443, *p=0.08*	W=423.5, *p*=0.26

* Denotes significant differences. Trends towards significant differences are in italics.

3.3.4 *Cortisol*

A number of samples were not able to be read effectively and therefore there were a limited number of replicates in each stage that could be used for analysis. Results for both animal A and animal B were thus pooled for each stage. From the data that could be analysed, the results showed that cortisol levels for milk between stage 1 (PP) (2.04 ± 5.39 ng/ml) and stage 2 (PR) (1.04 ± 1.60 ng/ml) were not significantly different (Z= 4, p>0.05). Cortisol levels for saliva between stage 1 (PP) (2.84 ± 4.8 ng/ml) and stage 2 (PR) (3.66 ± 5.05 ng/ml) were also not significantly different (T= -2.33, p>0.05).

3.3.5 *Production*

Total milk yield differed significantly across stages (F=7.28, df=2, p=0.001) with individuals in stage 3 (RP) having the highest average yield (16.04 ± 0.56 litres), and individuals in the stage 2 (PR) having the lowest average yield (13.17 ± 0.51 litres) across the four milking sessions. Yield also differed significantly (F=37.83, df=1, p=0.000) between focals and their partners with focal cattle having a lower average yield (12.57 ± 0.35 litres) than their partners (16.35 ± 0.50 litres) across the four milking sessions. There was no significant interaction affect between the three stages and between A and B (F=0.01, df=2, p=0.986).

When looking at the effects of separation on A and B, the yield for focal individuals significantly reduced (W=181.5, p=0.02) when separated with their preferred partner (-0.67 ± 0.55 litres) compared to when separated with a random individual (0.01 ± 0.37 litres). There was no significant changes in conductivity (W=87.5, p=0.95) and duration (T=0.61, p=0.55) between stages for A, and there was no significant change in yield

(W=28, p=0.132), conductivity (W=44, p=0.94) or duration (W=72, p=0.23) for individual B between stages.

Conductivity significantly differed (F=8.58, df=1, p=0.004) between focals and their partners with focals having lower conductivity levels (9.24 ± 0.04mS) than their partners, (9.46 ± 0.06mS), but did not differ between stages (F=1.75, df=2, p>0.05). Duration of milking was not significantly different between A and B (F=0.35, df=1, p>0.05) and was not significantly (F=2.68, df=2, p=0.07) affected by stages, although it did show a trend to be of longest duration for those in RP (335.9 ± 12.3 secs) and of the shortest duration in stage PR (300.9 ± 11.3 secs).

3.4 Discussion

3.4.1 *The effects of social separation on behaviour, physiology, and production*

The main aim of this research was to investigate the effects of short term social separation from preferred group mates on the behaviour, welfare and production of cattle. The general pattern of results confirms social separation to be a psychosocial stressor for cattle as has been seen in other studies (Boissy & Le Neindre, 1997; Hopster & Blockhuis, 1994; Müller & Schrader, 2005; Waiblinger *et al.*, 2006). The results also indicate that certain relationships between dyads appear to be important when providing social support during this type of stressor but there is also indication that differences exist between individuals in their ability to cope with social challenges, and this may be having an impact upon their welfare.

3.4.1a Behaviour

During the separation period cattle tended to be relatively inactive standing for the majority of their time near to the AI pens within the shedding area. This is similar to the results reported by Boissy & Bouissou (1995) who noted cattle to be relatively immobile during isolation; however, the inactivity they noted was also positively correlated with other fear-related reactions previously expressed. Such inactivity in the current study could suggest that when cattle were separated with a companion they were relatively unaffected by the stressor. However walking / pacing behaviours along with vocalisations were also displayed at relatively high frequencies, particularly in the initial stages of isolation. Together, the activity and passivity behaviours may suggest that cattle were both motivated to regain contact with their peers and so performed a number of reinstatement behaviours (Boissy & Bouissou, 1995), but when reunion attempts were unsuccessful they experienced prolonged bouts of passivity for the remainder of the separation. This two-stage reaction to such a stressor as social separation and isolation has previously been referred to as ‘protest’ and ‘despair’ (Hennessy, 1997; Siebert *et al.*, 2011) and has been seen in other species such as capuchin infants (Byrne & Suomi, 1999) and goats (Siebert *et al.*, 2011) over a two hour and 30 minute period respectively. Alternatively, these behaviours could be due to them ‘waiting’ to be released back into the main shed area with the remainder of the herd. As cattle were used to this area and had experienced a number of very similar separations when having vet checks, or when having their feet trimmed, they may have learnt that they will be released back into the main group at some point. However, correlations between test sessions demonstrated that cattle had not habituated to the testing area. In practical terms, it is important to be aware of particularly prolonged bouts of standing still which could be a general sign of depression and cause for concern regarding their welfare and therefore cattle should be checked on a regular basis while they may be waiting for vets to arrive.

Focal individuals performed higher frequencies of walking / pacing and vocalisation behaviours (although not significantly different between stages) and significantly higher frequencies of behaviours suggestive of agitation when they were separated with a random individual compared to when they were separated with their preferred partner. This could be suggestive of both the strength of the bond A has with B but also the potential support that A received from B whilst separated from the remainder of the herd. In support of this bond, cattle in the preferred partner stage tended to stand in closer proximity to each other (less than one body length apart) than cattle in the other two stages. This is similar to the results found by Waiblinger *et al.* (2006) and Færevik *et al.* (2006) who also noted that cattle tended to stay closer to bonded and familiar individuals, respectively.

3.4.1b Runway test

Latencies to return to the herd after separation have been demonstrated as a moderately repeatable indicator of the assessment of sociability in dairy cattle and thus could be suggestive of specific behavioural traits in cattle (Gibbons *et al.*, 2010). Gibbons *et al.* (2010) found that cows that had high latencies to reach the end of a runway area had fewer recordings with two nearest neighbours. In the current study cattle with the highest latencies to return to the herd were the preferred partner (B) and the random individual (C) when together (RP). This may be due to a lack of preferred relationship on both parts and would explain why each B and C had not been previously identified as having a preferred partner. Furthermore, it may suggest that although B may be A's preferred partner the relationship may not be reciprocal, in that A may not be B's preferred partner.

When the focal animal (A) was with its preferred partner (B), they had a medium latency to return towards the herd. This result may still be showing the level of sociability that these cattle had as they were with their preferred partner at the time of leaving the pen, which was not the case in the study carried out by Gibbons *et al.* (2010). In addition, cattle in stage 2 (focal animal A with a random animal C) were shorter in their latencies to return to the herd than when preferred partnerships (A and B) were together (stage 1) suggesting that cattle were trying to rejoin their preferred partner upon release. Interestingly, random cattle (C) who had not been necessarily identified as having a preferred partner before separation took place, had the shortest latencies to return to the herd compared to focal animals (A) and their partners (B). This is contradictory to previous results. There are two possible explanations for this: 1. random cattle had a preferred partner back in the herd that had not been identified in the earlier study phases, and 2. they were trying to remove themselves from the other two individuals. Random cattle were quickest to return to the herd when they were with A, and although not significantly different, this result was seen in the preferred partners (B) when they were also with A. This may suggest that A had an impact on the behaviour of the other two individuals and may have increased their desire to return to the main herd and remove themselves from A's presence. It is possible that individual A had a particular characteristic that meant that she was more likely to be seen with another individual because she was highly sociable, but along with this came a need to be with others. This may suggest that focal animals (A) were particularly needy individuals and may have experienced a higher level of stress than others when separated from the herd.

The influence of an individual's temperament on their social relationships needs investigating further in order to fully understand its influence on their behaviour. In addition, as animal A, B and C did not have significant correlations between repeat test sessions for each condition may suggest that cattle were not consistent in their responses and so results should be considered carefully.

3.4.1c Heart rates

Social separation also induced a physiological response by means of changes in the heart rate of cattle, as seen in other studies (Boissy & Le Neindre, 1997; Færevik *et al.*, 2006; Hopster & Blockhuis, 1994; Hopster *et al.*, 1995; Stěhulová *et al.*, 2008). In line with the behavioural results, focal individuals appeared to cope better with the social separation when they were with their preferred partner. Although the difference in heart rates between stage 1 (PP) and stage 2 (PR) were small, they were significantly different across time with focals (A) having a lower heart rate when they were with their preferred partner (B) compared to when with a random individual (C). Interestingly the partner's (B) heart rates were significantly lower than the focal's (A) heart rate when both were with a random individual (C), and although not significant the partners' (B) heart rates were lower when they were with a random individual (C) compared to the focal's (A) heart rate when they were separated with its preferred partner (B). These results suggest that some individuals respond to the same stressor at a higher level than others and that they may be more heavily reliant upon social contact and social support than their partners. It may be that partners perceived the stress of the separation differently than focals and therefore the emotional and physiological reaction differed between individuals. This would support the possibility of focal animals being particularly 'needy' individuals as was considered for the runway test. The use of heart rate variability in future observations may help to identify these differences.

Positive correlations between stages 1 (PP) and stage 2 (PR) demonstrate that cattle had not habituated to the testing area but that cattle were responding to the condition that they found themselves in. The correlations between repeat testing's within stages would also suggest that cattle had not habituated to the testing area as there were differences between repeats within stages. Furthermore these results suggest that the non-randomising of the order in which cattle experienced conditions did not have an effect on the results.

3.4.1d Cortisol

Within this study as in others (Verkerk *et al.*, 1998; Waiblinger *et al.*, 2006) cortisol levels were either not affected by the stress experienced or there was a failure to detect the level of change elicited by the ELISA kits. Although other studies have found milk to be a reliable indicator of cortisol concentrations in blood and that mammary uptake of cortisol from the blood is almost instantaneous (Termeulen *et al.*, 1981), the assay used for analysis was not specifically designed for cattle or for milk samples. It was however able to pick up levels in both milk and saliva suggesting its sensitivity range was adequate. Alternatively, it may be that the short term separation was not perceived to be a big enough stressor to threaten the metabolism of the body and so did not elicit a change in the HPA axis (Mormède *et al.*, 2007). In contrast, social isolation has been found to increase cortisol levels significantly in pigs, although the increase depended upon the different coping strategies employed by individuals with reactive pigs having a higher increase in saliva cortisol levels than proactive pigs (Ruis *et al.*, 2001). It should be noted that cattle in this study would have been familiar with the separation area and would have experienced similar separations from the group before. As such this may have allowed individuals to adapt to the situation and adjust their maintenance levels so that noticeable changes in stress responses may not have been detected due to their coping ability (Mormède *et al.*, 2007).

3.4.1e Production

Production differed significantly across stages with individuals in stage 3 (RP – B and C) having the highest average yield and individuals in stage 2 (PR - A and C) having the lowest average yield. This would suggest that when focal animals are with a random individual their yield along with the random individual's is reduced. This would tie in with previous results suggesting that animal C's response was impacted by animal A. However, in contrast to what might be expected for focal individuals, their yield significantly decreased when they were separated with their preferred partner compared to when they were with random individuals. For focal animals, there are two potential explanations for such results: 1) the separation that took place with their preferred partners was likely the first time separation of this nature had occurred and therefore this may have been the initial response to the separation; 2) focal individuals (A) had significantly lower milk yields than individuals B in general and therefore this could have been affecting the results between stages, although there was no significant interaction effects between stages and individuals A and B.

Electrical conductivity (EC) was significantly different between focals and their partners with focals having a lower EC level. EC did not differ significantly between stages. EC of milk is often used to monitor mastitis levels (Norberg, 2005). Mastitis infections are influenced by high levels of bacteria which are often observed in animals that are experiencing stress (Verbrugghe *et al.*, 2012). Marsland *et al.* (2002) note that stress can impact upon immune reactivity and can potentially cause susceptibility to infections. This is in contrast to focal animal A in that it would suggest that animal A was not particularly under high levels of stress. However, it would link in with the potential impact that focal animals (A) may have been having on their partners (B). As animal A was the focal animal observed, they may have been responsible for the relationship and if it was not mutual, the continual presence of A may have been affecting the health and welfare of B.

3.4.2 *Social support*

Focal cattle showed better coping abilities with the social separation stressor when they were supported by their preferred partner through a reduced stress response. However, the overall response to the separation did not appear to be particularly heightened in comparison to other studies where cattle have been completely isolated (Boissy & Le Neindre, 1997). It would appear that when cattle are separated with a companion they are able to receive social support from an alternative attachment figure other than their preferred partner. As both cattle were familiar to the focal individual the support provided by each of them attenuated the depressive response often seen when cattle have been completely isolated. Such alternative attachments have been seen in infant bonnet macaques when they were separated from their mother, a highly stressful experience; if they retained alternative attachment figures to their mother during the separation they showed fewer behavioural signs of depression (Boccia *et al.*, 1997).

The ability to provide and receive social support may be influenced by a number of factors including an individual's identity, whether they are familiar or not, the emotional state of both provider and receiver, and the ontogeny of the relationship at an individual level and at a dyad level (Rault, 2012). As an animal matures the relationships they form with others and their social behaviour may change and therefore their support mechanisms may also change (Hennessy *et al.*, 2006). For example Hopster *et al.* (1995) noted that multiparous cows were only mildly affected by the removal of their calves. As the majority of focal and partner cattle were heifers, this may indicate the potential dependence of younger individuals on their social environment for support at a time of stress. Furthermore, the mixing of multiparous and primiparous cattle has been shown to result in the disruption of grazing and social behaviour which subsequently leads to a reduced milk yield (Phillips & Rind, 2001). This may provide some explanation as to why random individuals which were more often than not multiparous did not provide as much support as the focals preferred partners which were at similar ages to themselves.

Provision of support or social buffering has been found to be costly. Langer *et al.* (2009) found that partners that were supporting and buffering human patients suffering with cancer reported adverse psychological outcomes. It is therefore possible that the provision of the support itself may result in adverse effects on the cattle that are providing the support to those that do not cope as well and this may explain some of the results observed in this study.

3.4.3 *Individual differences*

Cattle have been found to show consistent and repeatable responses both behaviourally and physiologically to a number of tests such as isolation, suggesting that they retain individual characteristics (Hopster & Blockhuis, 1994; Müller & Schrader, 2005; Schrader, 2002). An individual's genetics and environmental and early-rearing experiences can affect individual coping abilities and subsequently could affect their welfare when faced with stress. Broom & Leaver (1978) found differences in the social skills of isolation-reared and group-reared calves with isolation-reared calves spending more time alone than group-reared calves and isolation-reared individuals both initiating and receiving more negative social interactions when exposed to a social environment. Additionally, Purcell & Arave, (1991) found that group-reared calves took longer to complete a maze and showed higher stress levels when separated from conspecifics, thus isolation-reared calves may cope better with separation from the herd due to their lack of social skills. As there is a legal requirement (EU directive 97/2/EEC) within the UK to keep calves in contact with other calves from eight weeks of age, it would be expected that there would be a higher susceptibility to stress caused by isolation within the dairy industry.

Animals also tend to develop their own coping style which is often expressed through differences in behavioural and physiological stress responses, similar to those seen in the current study. This difference results from an individual's cognitive evaluation of the triggering stressor (Boissy *et al.*, 2001; Veissier & Boissy, 2007). Proactive individuals tend to try and remove themselves from stressors and have a tendency to develop routines and anticipate situations (Ruis *et al.*, 2001). These animals are predominantly influenced by the sympathetic nervous system (Koolhaas *et al.*, 1999) affecting physiological parameters such as heart rates, as was seen in focal individuals. Reactive animals appear to aim to reduce the emotional impact of the stressor and thus tend to adapt more easily to variable conditions (Ruis *et al.*, 2001) and are predominated by the parasympathetic nervous system (Koolhaas *et al.*, 1999). These different coping styles can impact upon the animal's emotional reactivity to their environment and certain stressors both in magnitude and duration (Ruis *et al.*, 2001) and may explain some of the differences in response of cattle to the separation carried out in the current study. More importantly the long term consequences of stress such as reduced immunity as resources are used elsewhere (Moberg & Mench, 1999), may become more of an issue for those individuals that cannot adapt as well as others and thus these particular individuals may be at more of a risk for poor welfare. Further research is required to look at the longer term effects of such different coping styles and how cattle respond to everyday husbandry practices and to consider the potential breeding of fewer proactive individuals.

3.4.4 *Practical implications*

There are a number of practical implications to this study. When separating individuals for a short period of time, providing them with a companion is advised. It appears that both a preferred partner and a non-preferred companion can provide some form of support. It is important however to observe all individuals within the holding pen on a regular basis for any adverse effects of the separation. In particular, animals that may be showing signs of excessive inactivity may be of some concern as they may be experiencing a high level of fear and so should be handled with care.

There is also the possibility that some highly sociable cattle may have adverse effects on the well-being of others around them. Care needs to be taken when housing and grouping individuals together. The temperament of individuals should be considered where possible. Stable and consistent groupings will ensure that animals pair with those individuals that are able to cope with their relationship. As a conspecific can also help another increase their learning ability, pairing individuals together will ensure that cattle are habituated quickly to their routine and any changes within their environment will be more easily dealt with.

These results were however observed in younger cattle due to their higher level of association with others. It may be that younger cattle need extra time and higher consideration when settling in the herd and the provision of support may be more important to them at this time due to the high stress levels they are likely to be experiencing when first entering the main milking herd.

3.5. Conclusions

In summary, there appears to be a complex relationship involving a number of factors that influence the kind of effects that short term separation can have on individual cattle. Short term separation in itself did produce a stress response within cattle, although the results have highlighted some individual differences within responses. Results also indicate that previously identified bonds between dyads were important with regards to providing support as responses for both behaviour and physiology were lessened by the presence of a preferred partner when compared to a familiar individual. Furthermore, these bonds can be confirmed and quantified as significant due to the element of support demonstrated. However, there appears to be a potentially negative effect on the individuals that are providing support to other cattle and this needs further investigation.

Chapter Four

The Effects of Long Term Separation from Preferred Groups Mates, and Subsequent Reunion, on Behaviour, Production and Health Parameters in Dairy Cattle

4.1 Introduction

In chapter two it was demonstrated that dairy cattle housed in commercial conditions were able to form preferential partnerships. In particular, heifers spent significantly more time with at least one other group member than was expected by chance. Chapter three further demonstrated that these preferential partnerships appeared to be important to cattle when experiencing short term separation, by providing a form of social support during this stressor. Both behavioural and physiological responses to the short term separation stressor were reduced by the presence of a preferred partner. This suggested that partners offered the opportunity to enhance an individual's ability to cope with their environment compared to another familiar non-preferred conspecific. In order to fully understand and appreciate the value of these bonds to cattle it was necessary to investigate the longevity of the relationship. Furthermore, it was necessary to investigate how these preferential relationships may be affected by long term separation. A higher number of dyads than would be expected by chance were only seen together once suggesting that for some cattle they did not have a preferential relationship with another member of the group. Therefore factors such as long term separation may be influencing this and thus needed investigating.

4.1.1 Long term separation and stress

Under commercial conditions, the most likely cause of long term separation is during regrouping, which can be a period of extreme stress and cause significant effects on health, behaviour, productivity and welfare in dairy cattle (de Groot *et al.*, 2001; Dobson & Smith, 2000; Hagnestam-Nielsen *et al.*, 2009; von Keyserlingk *et al.*, 2008). Previous investigations on regrouping have often focused upon the introduction and mixing of cattle, observing how both the introduced and resident cattle of the group respond to the changes in their social environment. Animals entering the herd have been observed to receive the majority of aggressive acts from resident animals (Alexander & Irvine, 1998; Beilharz & Zeeb, 1982; Mench *et al.*, 1990). This aggression is likely to continue until a stable hierarchy has been founded, which may take anywhere from 15 days to 13 weeks (Hasegawa *et al.*, 1997; Mench *et al.*, 1990). Often, there is a decrease in time spent feeding as displacements at the feed barrier increase, and time spent resting is reduced (Hasegawa *et al.*, 1997; von Keyserlingk *et al.*, 2008). Consequently, milk production is affected (Hasegawa *et al.*, 1997; Phillips & Rind, 2001) as energy required for milk production is transferred elsewhere to enable the individual to cope with the challenges in their environment (Moberg & Mench, 2000). In addition, higher levels of cortisol are often seen in individuals entering the herd suggesting a higher level of stress in these animals and thus a reduced welfare status (Hasegawa *et al.*, 1997; Mench *et al.*, 1990; von Keyserlingk *et al.*, 2008). The potential for a companion to provide support during regrouping has already been demonstrated with integration into the herd being eased by the presence of a conspecific at that time (Gygax *et al.*, 2009; Neisen *et al.*, 2009b).

During regrouping not only are cattle introduced into a new group with unfamiliar individuals, they also are potentially separated from their bonded partner. This aspect has not been fully considered to date. The breaking of a social bond through separation evokes an emotional response that often manifests itself through an increase in reinstatement behaviours such as escape attempts and vocalisations as was seen in the chapter three, in agreement with Boissy & Le Neindre (1997). This strong emotional response at the beginning of the separation period generates an acute stress response resulting in physiological as well as behavioural changes, as was documented in chapter three. Prolonged bouts of separation from bonded partners can lead to signs of poor psychological well-being and a depressed immunity (Boccia *et al.*, 1997; Gordon *et al.*, 1992) leading to a reduced welfare status. On the other hand, animals have been seen to adapt to long term separation from bonded partners, with the stress response subsiding after time and routine activities being resumed (Newberry & Swanson, 2001). The ability to adapt to the separation and resume routine activities is an important consideration for animal welfare, although the context that this can occur in needs to be investigated.

4.1.2 The effects of reunion after separation

Due to the nature of regrouping (the mixing of individuals into a new group with or without their preferred partner), there may also be times when cattle previously separated from their preferred partner are reunited (after a period of separation preferred partners are joined back together). It is unclear however whether a bond previously formed is remembered after a long period of separation, and if so, whether reunion with a preferred partner within the group at the time of regrouping is enough to provide support at this time.

The separation of a calf from its mother results in a behavioural response suggestive of stress in both parties; however upon reunion of the bonded pair this response is reduced in both mother and calf (Sandem & Braastad, 2005; Solano *et al.*, 2007). This would suggest that the bond had not been broken and cattle recognised and remembered their young. This was after 4 days of being together (Sandem & Braastad, 2005) and with fence line contact after 90 days of being together (Solano *et al.,* 2007). Conversely, in cows and also goats, if mothers are separated from their young for 24 hours after five minutes of contact immediately postpartum, upon reunion with their offspring mothers reject their young which is suggestive that the mother-young bond had not been established in that initial short period (Hudson & Mullord, 1977; Ramírez *et al.*, 1996).

The separation periods outlined in the studies above were fairly short compared to how long cattle are likely to be apart when regrouped before reunion occurs. To the best of the author's knowledge, evidence for the effects of long term separation (two weeks or more without repeated regrouping) has not been reported in cattle. When considering other species, juvenile rhesus monkeys that experienced 18 weeks of separation from their natal group before being reunited had a lower health status with a reduced immunity and reduced weight gain compared to controls which had remained in the group (Gordon *et al.*, 1992). Upon reunion with their natal group, cortisol levels rose in both the reintroduced and the resident animals, with reintroduced individuals having a higher level than resident individuals (Gordon *et al.*, 1992). This demonstrates that both introduced and resident animals are affected by changes in their social groupings and also that the bonds that may have been previously formed within the group were broken after such a long period of separation. Furthermore, limited support was offered by the group upon reinstatement of previous group members.

In order to fully understand and appreciate the value of social bonds in cattle, a better understanding of the effects of long term separation without an element of regrouping or mixing at that moment in time, is required. Furthermore, the ability for a previously identified preferred partner to provide support to an individual at a time of regrouping after separation has occurred is also required. As demonstrated previously in chapter three, preferred partners have a particular ability to reduce the stress response in focal cattle during short term isolation from the herd, more so than a familiar but non-preferred partner. What is not clear is whether a period of long term separation will have a negative impact upon the preferential relationship and reduce its ability to provide a form of social support at the time of regrouping. Therefore, the main aim of this part of the study was to investigate the effects of a long period (two weeks) of separation from an identified preferred partner on the behaviour, production and health parameters of cattle, whilst still maintaining a stable group membership, for example no new individuals introduced. In addition, the influence of a previously identified preferred partner upon an individual's ability to cope with an environmental stressor was investigated by examining the behaviour, production and health of cattle at the time of regrouping when reunion occurred.

4.2 Materials and methods

4.2.1 Housing area, animals and management

Farm A

As the previous studies had highlighted younger individuals as having a stronger bond, 67 Holstein-Friesian replacement heifers and calves on farm A were observed between Jan 2011 and November 2011. Cattle ranged in age from 4-30 months and all animals were housed in loose straw yards at Arthingworth Farm, Leicestershire, UK. Ten focal animals were randomly (through a random number generation on Microsoft Excel 7.0) selected where possible from each of the existing groups so that a range of ages and grouping experiences were represented. Due to commercial constraints, focal animals had to be chosen from existing groups so could not be matched for age or size between groups (see table 4.1 for group details). Fresh feed was delivered twice daily at approximately 0900hr and 1600hr and consisted of a compound feed and *ad libitum* access to straw and water. Cleaning out and the delivery of fresh straw and bedding was provided every other day during which time cattle were held in a holding area where feed was then delivered. During summer months all cattle had access to pasture.

Table 4.1 Group details for focal animals on farm A. *The total number of animals within each group and the mean age of each of the groups (months) are also given. Control groups did not experience any separation or regrouping whilst treatment groups were separated for a two week period.*

Group	Condition	Number of focal animals	Total number of animals in group	Mean age
1	Control	4	6	4 months
2	Control	6	6	6 months
3	Treatment	7	7	7 months
4	Treatment	10	37	13.9 months
5	Treatment	10	20	9.6 months
6	Control	10	10	17.7 months
7	Control	10	24	22.1 months
8	Control	10	28	25.2 months

Farm B

One-hundred-and-twenty-three lactating Holstein-Friesian primiparous and multiparous dairy cattle were observed from July 2008 to November 2011 on farm B and consisted of the continued observation of animals that had been previously observed in studies one and two (See section 2.1.1 for more details). Cattle were housed in a cubicle shed at Stud Farm, Moulton College, Northamptonshire, UK. As this was a commercial unit there was no possibility of controlling when animals were separated from preferred partners and regrouped; however movements between groups were recorded and noted from farm records.

4.2.2 Experimental design and treatment

Farm A

Between January 2011 and March 2011 nearest neighbour observations took place on cattle from farm A using the same methods as outlined in section 2.2.2. Preferred partnerships were identified after 135 hours of observation using the same parameters as outlined in section 2.2.3 in that they had to have been observed together for a minimum of four out of fifteen observations. Cattle were allocated to be either a control group or a treatment group (see table 4.1.). Five groups acted as controls in that they remained stable during observations and no separation was carried out by the observer. Three groups were allocated as treatment groups whereby preferred partnerships were separated for two weeks before being reunited. Two weeks of separation was chosen as the long term period as production parameters have been seen to be affected for this length of time when regrouping occurs (Hasegawa *et al.*, 1997) suggesting that this is a sensitive period after a stressful event such as separation. Cattle with more than one preferred partner were separated as far as was practically possible from the partner(s) that they had the strongest association with. As far as was possible half of the focal animals were removed from the group and relocated to a separate pen, whilst the other half remained within the group but separated from their preferred partners. No other animals were mixed in with the groups and so only the effects of separation would be observed (see figure 4.1 for example of how groups were separated). Separation took place using the crush and with the help of the stockperson so as to keep stress levels to a minimum. This separation procedure was no different to what cattle already experienced as part of normal farm management.

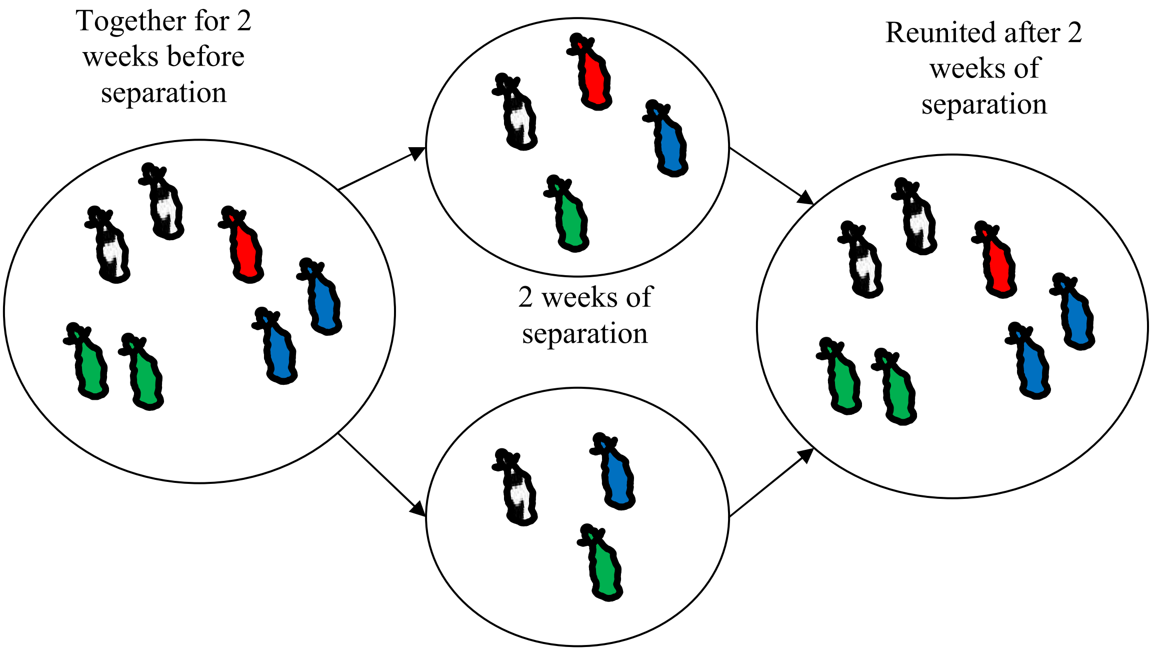


Figure 4.1. Diagrammatic example of how groups were separated on farm A so that preferred partners were separated from each other but remained with familiar group mates. All animals were then reunited to form the original group with no new animals added.

Farm B

All focal animals still available for observation from study one (see chapter 2) continued to be followed from November 2010 to November 2011 and nearest neighbour observations made. In total 123 cattle were followed between May 2008 and November 2011 at farm B and changes in preferred partnerships noted between observations. Records on movements during this time period were taken from farm records and long term (minimum two weeks) separation from previously identified preferred partners noted. These individuals were used to investigate the effects of long term separation on production (yield and quality), fertility and somatic cell count parameters as these could not be obtained from the cattle on farm A who were replacement stock only. To qualify for long term separation analysis, previously identified dyads had to have been separated for a minimum of two weeks without being reunited with each other at any point within these two weeks. In order to qualify for reunion analysis previously identified partnerships had to have been separated for a minimum of two weeks before being reunited. Only the first reunion of a pair was considered as new partners may have been identified after this time (see figure 4.2 for example).

Focal 13 and 57 would qualify for separation analysis here as they were together with their preferred partner for two weeks before separation took place which lasted for two weeks before being reunited with them.

Focal 13 and 57 would qualify for reunion analysis here as they were separated from their preferred partners for two weeks before being reunited with them which also lasted two weeks.

Focal	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
13	Together	Together	Together	Separated	Separated	Together	Together	Separated	Separated	Separated
57	Together	Together	Separated	Separated	Separated	Separated	Together	Together	Separated	Separated
125	Together	Together	Together	Separated	Separated	Separated				
125	Together	Together	Together	Together	Together	Separated	Separated	Separated	Separated	Separated
125	Together	Together	Together	Together	Together	Together	Together	Together	Separated	Separated
125	Together	Separated	Separated	Together	Together	Together	Separated	Together	Together	Separated
160	Together	Together	Separated	Together	Together	Together	Separated	Separated	Separated	Separated
274	Together	Together	Together	Separated	Together	Separated	Separated	Separated	Together	Together
522	Together	Together	Separated	Separated	Separated	Together	Separated			
525	Together	Together	Together	Separated	Together	Together	Together	Separated	Together	Together
525	Together	Separated	Separated	Separated	Separated	Separated	Together	Separated	Separated	Separated
621	Together	Together	Separated	Separated	Together	Separated	Together	Together	Separated	Together
629	Together	Together	Together	Separated	Together	Separated	Separated	Separated	Together	Together
662	Together	Together	Together	Separated	Separated					

Focal 629 would not qualify for separation analysis here as they were not separated for the minimum of two weeks before being reunited with one another.

Figure 4.2. Example of how animals were chosen for inclusion in separation and reunion analysis.

4.2.3 Measurements

4.2.3a Behaviour before, during and after separation

In order to assess the impact of long term separation from preferred group mates on the behaviour and welfare of cattle, a total of 88 hours of behavioural observation took place on farm A. Treatment groups were observed on a rotational basis and observed throughout the day (0800 – 1800hr), for two and a half days a week for six weeks. This allowed all treatment focal animals to be observed five to seven times throughout the observation period in ten minute sampling blocks. Observations were carried out two weeks before animals were separated, for two weeks during separation, and for two weeks after separation, between March 2011 and April 2011. This allowed for any changes resulting from both separation and reunion to be monitored. Observations were only recorded for those three groups which underwent separation using the behaviours outlined in table 4.2, focals acted as their own controls between time periods to account for individual variation in behaviour. Instantaneous focal animal sampling at 2 minute intervals for a total of ten minutes per group was employed. The two minute interval was chosen as this was the average time it would take to note down the behaviour and position of all focals in the group. Event behaviours (see table 4.2) were recorded on an *ad libitum* basis. The observer remained outside of the group in a position that would not disturb the cattle from carrying out their normal daily behaviours; however cattle may have been disturbed by normal daily routines carried out by the stockperson and this could not be controlled for.

Allogrooming behaviour has been considered an important aspect in the maintenance of social relationships and has been used as an indicator of preferential bonds in cattle (Sato *et al.*, 1993; Val-Laillet *et al.*, 2009). It generally occurs at a relatively low rate in cattle under commercial conditions (Takeda *et al.*, 2000; Val-Laillet *et al.*, 2009) and so was not used to assess relationships in study one. Allogrooming is a socio-positive behaviour and known to have a calming effect on those receiving the grooming (Laister *et al.*, 2011) suggesting that it provides a positive affective state and so should be considered when observing positive behaviour in cattle, hence its inclusion in this part of the study. By recording this behaviour it was also possible to investigate the longevity of the bond, and how long term separation would affect this positive behaviour.

Table 4.2 Behavioural ethogram for instantaneous focal sampling during long term separation from, and reunion of, preferred group mates.

Behaviours	
Alert	Head up, ears forward facing to direction of interest.
Drink	Taking in water through the lips or with the tongue into the mouth.
Eat	Feeding on either straw in round bale or concentrate feed at feeder.
Investigate	Explore surroundings, through sniffing and touching with nose
Lying	Lying in sternal or lateral recumbency
Negative social	Push, head butt, chase, or displace another individual
Positive social	Licking another cow, chin resting on another individual
Standing	Fully upright position with a relaxed posture; may or may not be chewing cud.
Vocalise	One long or several short calls
Walking	Four beat motion in a forward direction
Events	
Negative social	As above
Positive social	As above

Allogrooming was recorded for all animals on both farms continuously on an *ad libitum* basis throughout observations from March 2010 to November 2011. Allogrooming behaviour was defined as licking movements by one cow, the actor, carried out on the body of another, the receiver. Licking movements were characterised by repetitive movements of the tongue and head in and up-an-back motion whilst in direct contact with the skin of the receiver. Recording of each bout started as soon as the tongue made contact with the skin, and finished if there was a gap of more than ten seconds between one tongue movement and the next. Both frequency and duration of allogrooming events were recorded along with the identification of the performer and receiver. Grooming was categorised to occur on the head, neck, chest area, back or legs (see figure 4.3).

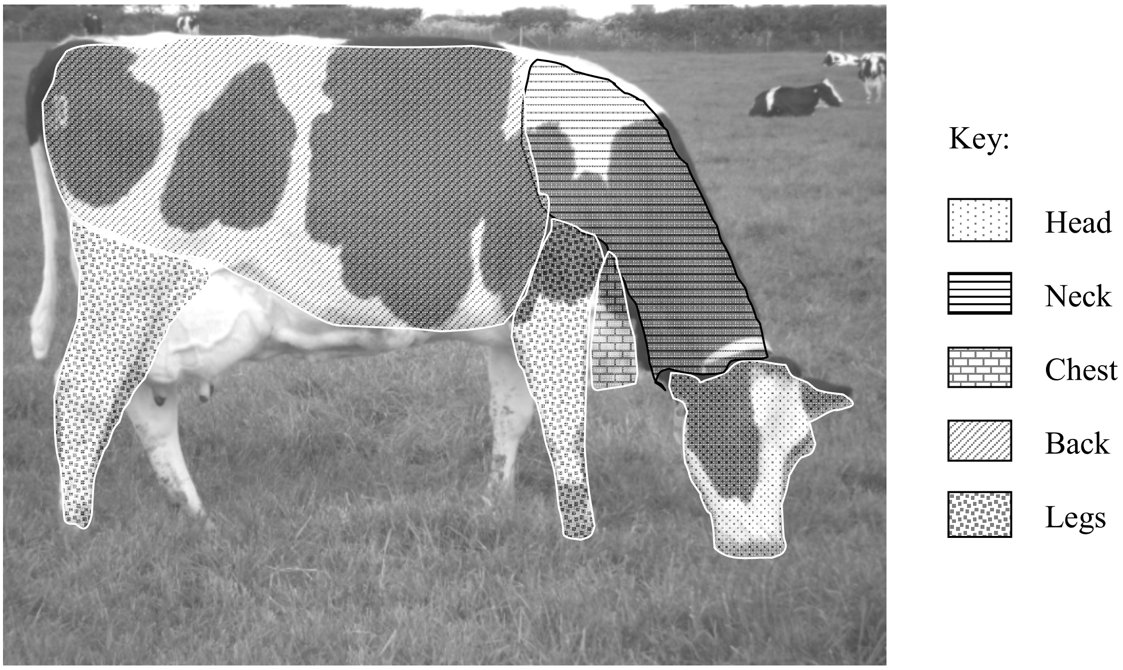


Figure 4.3. The different areas of the body that were observed when collecting data on allogrooming behaviour. *See key for body area definitions.*

The location of where the allogrooming behaviour took place was also recorded: at the feeder, in the walk ways (alley), the lying cubicles, when in straw yards (straw), or in the field. A small number of bouts were also recorded at the drinking trough and in the holding pen but due to lack of data in these areas they were removed from analysis.

4.2.3b Production

In order to investigate the effects of long term separation from preferred group mates on production, both yield and quality parameters were assessed. Furthermore, the impact of reunion and regrouping was also assessed by investigating the changes that occurred in production. Production is often affected by stress as energy is diverted to biological functions that are required more than milk production (Moberg & Mench, 2000) and so this can be used as part of a welfare assessment of cattle.

Yield

Individual milk yield (litres) was automatically collected by Crystal, Fusion Electronics BV (Fullwood Packo Group, The Netherlands) at each milking (am and pm) for cattle on farm B. The Crystal software automatically records a number of management factors such as activity levels and milk yield at each milking. The milk monitoring system employed is capable of measuring milk yields, conductivity, and temperature. This information was stored on a database within the farm's network and could be accessed to obtain the required information. Data was downloaded from farm records on a monthly basis and transferred to Microsoft Excel 7.0 so that it could be arranged into the required format for data analysis and where it could be checked for abnormalities. Four weeks of milk yield data before the date of separation or reunion and up to four weeks after the date of separation and reunion was used for analysis where possible. This was to assess the extent of the change as previous observations have reported effects lasting between two (Hasegawa *et al.*, 1997) and six weeks (Phillips & Rind, 2001). Furthermore this would relate to the monthly milk quality reports.

Quality

Milk quality parameters were collected and analysed on a monthly basis by the National Milk Records (NMR). NMR supply milk recording services to a large proportion of the UK's dairies and is responsible for recording individual cow performance and information on production that can be used by producers to improve their management of cattle (National Milk Records, 2012). These records came in a paper format and were copied into Microsoft Excel 7.0 so that they could be used for analysis. The monthly records for focal individuals before they were separated from or reunited with their preferred partner, were collected and compared to the monthly records from after separation, or on reunion.

4.2.3c Health parameters

There is a clear relationship between health and welfare in animals (Hughes & Curtis, 1997). It must however be remembered that health is not just the absence of disease; an individual that is able to use its biological resources to remain healthy is unlikely to be under any form of distress (Moberg & Mench, 2000). By assessing the impact that long term separation and reunion may have on the health of individuals will augment the ability to evaluate the impact that it may be having on the cattle's welfare.

Body condition score and weight

Body condition score (BCS) was recorded on a monthly basis for 86 animals in total using the chart by Edmonson *et al.* (1989) for body condition scoring Holstein's. Body condition is used by producers to assess the health status of their animals on a regular basis, and so this was used for mature animals to represent this management technique. Cattle on farm A were weighed (kg) as part of regular management approximately every month and so weights were recorded from farm records between the periods Jan-April 2011 and Sept-Nov 2011 for all focal animals. The weight recordings were however more accurate, especially for rearing stock that may be differing in their conformation as they grow and so body condition scoring was not appropriate to use for these individuals.

Somatic cell count and fertility

Somatic cell count and fertility measures were collected on a monthly basis by the NMR. As for milk quality parameters, these records were transferred to Microsoft Excel 7.0 so that they could be formatted for use in analysis. The monthly records for focal individuals before they were separated from or reunited with their preferred partner, were collected and compared to the monthly records from after separation or reunion. These two parameters were chosen due to their link with health and welfare (Cutullic *et al.*, 2012; Dobson *et al.*, 2001; Verbrugghe *et al.*, 2012).

4.2.4 Statistical analysis

All statistical analysis was carried out using Minitab version 13.20 and data presented as means ± SEM unless where otherwise stated. For all statistical analyses a significance level of $p<0.05$ was accepted. Any data which was not normally distributed was log transformed using the common logarithm $\log_{10}$ to normalise where required. Where log transformation did not normalise data, non-parametric statistics were used. Details of particular methods and tests employed are outlined below.

4.2.4a Behaviour before, during and after separation

The proportion of time spent performing each of the behaviours was calculated for each ten minute session for each treatment individual. Individual behaviours were compared using a repeated measures general linear model with the proportion of time spent performing each of the behaviours as the response and the GLM model including condition (before, during and after separation) and focal identification, with the focal identification as the random factor to count for repeated analysis. A GLM was favoured due to data not being normally distributed. Pearson's chi-squared analysis was used to investigate the difference in total frequency of both positive and negative social behaviours between the three conditions (before, during and upon reunion).

Allogrooming behaviour was analysed for both duration (seconds) and frequency across conditions. Duration data was log transformed to normalise its distribution before all analyses. The differences in both duration and frequency of grooming behaviour between farms were compared with an independent two-sample *t*-test and a Pearson's chi-squared analysis respectively. Allogrooming behaviour from both farms was analysed for the different body parts identified using a one-way ANOVA for duration, followed by Fishers *a priori* least significant difference (LSD) test for pair-wise comparisons between the different body areas. The frequency of grooming bouts on body areas was analysed by Pearson's chi-squared analysis. Due to a lack of data for the 'legs' this body area was removed from both the duration and frequency analysis. The influence of location on the duration and frequency of allogrooming bouts was also investigated across the two farms. A one-way ANOVA followed by Fishers *a priori* LSD test for pair-wise comparisons between the different locations within the housing area was used to analyse duration, and Pearson's chi-squared for frequency analysis. The effects of whether cattle were together with their partner, separated from their preferred partner or experiencing multiple regrouping on allogrooming behaviour was analysed for differences in both duration and frequency using a one-way ANOVA and a Pearson's chi-squared, respectively.

4.2.4b Production

A number of production parameters were analysed as part of the overall assessment of the effects of separation and reunion. These parameters included milk yield and milk quality. See below for individual parameters.

Yield

Forty-five focal individuals qualified for analysis before and after separation and 19 individuals qualified for analysis regarding the effect of reunion on milk yield. Due to non-normally distributed data after log transformation, a Wilcoxon's signed rank test was carried out to analyse the individual difference in milk yield (per milking) before and after separation over a period of eight weeks (four weeks before separation and four weeks after separation). In order to investigate differences within the four week period before separation and the four weeks after separation, and due to non-normally distributed data after log transformation, a Kruskal-Wallis test was carried out followed by a multiple comparisons post hoc test for Kruskal-Wallis (Siegel & Castellan, 1988). To investigate the effect of reunion on milk yield over an eight week period (four weeks before reunion and four weeks after reunion) a paired-samples *t*-test was carried out due to repeated measures. A Kruskal-Wallis test was also carried out to investigate the differences in yield within weeks due to non-normally distributed data after log transformation.

To investigate the impact of the social environment being experienced by cattle on the monthly average milk yield, data from 2008 to 2011 was investigated using a one-way ANOVA with multiple comparisons carried out using Fishers *a priori* LSD test. Cattle were grouped as to whether they were together with their preferred partner, experiencing long term separation from their preferred partner, experiencing multiple separation and reunion from their preferred partner, or were a control individual in that they had no preferred partner identified during this period.

Quality

Sixty-four individuals qualified for separation analysis and 36 individuals qualified for reunion analysis. To investigate the effect of long term separation on milk quality, the difference in quality parameters from the month before separation compared to the month after separation were analysed using a Wilcoxon's signed rank test on each of the quality parameters milk yield (kg), fat percentage (%) and pence per litre (PPL). A paired-samples *t*-test was used to analyse the difference in protein percentage (%) from the month before separation to the month after separation, as data was normally distributed. In order to investigate the effects of reunion with a preferred partner on milk quality, a paired-samples *t*-test was carried out on all quality parameters to compare the values from the month before reunion with the month after reunion due to repeated measures and data being normally distributed.

4.2.4c Health parameters

A number of health parameters were included in the overall assessment and included body condition score and weight, somatic cell count, and fertility. See below for individual parameters.

Body condition score and weight

Data investigating the effect of long term separation on BCS were analysed by farm. For farm A, the effects of long term separation on BCS was investigated by analysing the effects of separation compared to control animals using a Mann-Whitney *U* test as data was not normally distributed after log transformation. For farm B the effects of different social environments (together with preferred partner, separated from preferred partner and experiencing multiple regroupings) was analysed using a Kruskal-Wallis test as data was not normally distributed after log transformation. The difference in BCS from the months before separation compared to the month after separation was compared using a paired-samples *t*-test due to repeated measures and data being normally distributed.

To investigate the effects of long term separation on body weight, data from groups that had experienced separation were compared to control groups using a Mann-Whitney *U* test due to non-normally distributed data after log transformation. The changes in weight over the six week period was analysed using a one-way ANOVA with multiple comparisons carried out using Fishers *a priori* LSD test.

Somatic cell count (SCC)

Fifty-one individuals qualified for separation, and 27 for reunion. The effects of long term separation on somatic cell count ('000 cells/ml) were investigated using a Wilcoxon's signed rank tests, comparing the SCC before separation with the SCC after separation as the data was paired and not normally distributed after log transformation. Reunion effects on SCC were analysed using a paired-samples *t*-test to compare the previous month's SCC with the month after reunion SCC as data was paired and normally distributed.

Fertility

The fertility of 31 individuals was compared to assess the effect of long term separation. Both the number of inseminations and the interval between inseminations from the month before separation was compared to those from the month after separation using a Wilcoxon's signed rank test due to data not being normally distributed after log transformation. Only eight individuals qualified to assess the effect of reunion on fertility measures. A paired-samples *t*-test was carried out to investigate the difference in the number of inseminations and for the interval between inseminations, with the month before reunion compared to the month after reunion as data was paired and normally distributed.

4.2.5 Ethical note

All ethical considerations were approved by MC ethics committee. All animals were observed for any signs of excessive distress or behavioural reactions that may have resulted in injury or distress. Any animal displaying such behaviours or signs of distress were monitored closely and removed from the study. All cattle were monitored throughout the study by stock persons and the researcher for signs of ill health or injury and intervention was made where required. All cattle were handled under the supervision of the stock person and no excessive force was used to separate animals. The procedures that cattle were exposed to were no different to what normally takes place on farm for general husbandry practices.

4.3 Results

4.3.1 The effects of long term separation, and subsequent reunion, from preferred group mates on behaviour

The results of the behaviour analysis are given in table 4.3. The behaviours alert, eat, lying, negative social, positive social, other, stand, vocalise, and walk were not significantly different between conditions. Both positive social and standing behaviour showed a trend ($p<0.07$) towards significant difference. Positive social behaviour was performed for the least amount of time when cattle were reunited with their preferred partners. A very similar and higher amount of time was spent performing positive behaviours before they were separated from their preferred partner and when experiencing separation. Cattle stood for a longer proportion of their time when they were experiencing long term separation and the shortest proportion of time standing when they were reunited with their preferred partner.

Drinking was significantly reduced during separation and after reunion when compared to before separation. Cattle spent a higher proportion of time investigating their surroundings before they were separated and spent the lowest amount of time investigating their surroundings when they were separated from preferred partners.

Table 4.3. Mean (± SEM) proportion of time spent performing each of the behaviours, compared between the three time conditions using a repeated measures general linear model using focals from farm A as the random factor.

Behaviour	Mean (± SEM) before separation	Mean (± SEM) during separation	Mean (± SEM) upon reunion	GLM *p*-value
Alert	0.17 (0.00)	0.22 (0.06)	0.17 (0.00)	0.121
Drink	0.27 (0.17)	0.22 (0.01)	0.23 (0.02)	0.009*
Eat	0.60 (0.02)	0.59 (0.01)	0.60 (0.01)	0.782
Investigate	0.25 (0.01)	0.21 (0.01)	0.21 (0.01)	0.000*
Lying	0.84 (0.02)	0.81 (0.02)	0.77 (0.02)	0.108
Negative social	0.19 (0.01)	0.20 (0.01)	0.21 (0.01)	0.817
Positive social	0.23 (0.01)	0.24 (0.02)	0.19 (0.01)	0.064
Standing	0.37 (0.01)	0.40 (0.01)	0.36 (0.02)	0.062
Vocalise	0.24 (0.03)	0.17 (0.00)	0.19 (0.02)	0.376
Walking	0.20 (0.08)	0.19 (0.01)	0.19 (0.01)	0.397

** Denotes a significant difference (p<0.05).*

The frequency of positive behaviours was not performed at significantly different frequencies between conditions (X^2=3.72, df=2, p>0.05), however the number of negative social behaviours observed increased through each time period with the highest number of negative social behaviours being performed when animals were reunited with their preferred partners (X^2=21.21, df=2, p<0.05) (see figure 4.4).

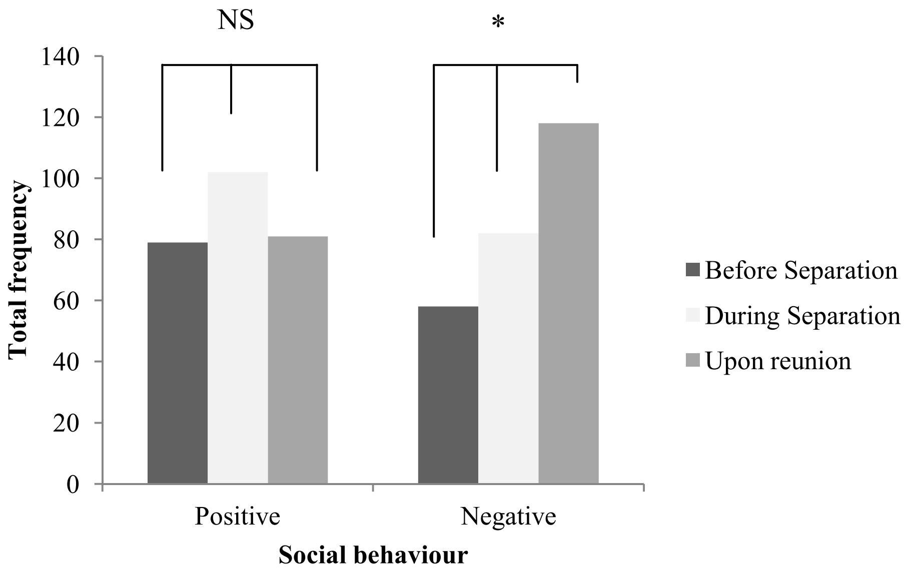


Figure 4.4. Total frequency of positive and negative social behaviour in the three conditions. ** denotes a statistically significant difference (p<0.05) between conditions.*

The duration of allogrooming bouts varied from a minimum of 2secs to a maximum of 262secs. Mean duration did not significantly differ between farm (T=0.16, p=0.874), however cattle on farm A (N=167) performed significantly more (X^2=5.73, df=1 p<0.05) bouts of allogrooming compared to farm B (N=126). The majority of allogrooming bouts were performed on the neck and head (see figure 4.5), with a significant difference in both duration (F=4.63, df=3 p=0.004) and frequency (X^2=166.7, df=1 p<0.05) between body areas (see table 4.4.). The duration (F=3.52, df=4 p=0.008) and frequency (X^2=165, df=6 p<0.05) of allogrooming data was also significantly different between locations, with the majority of bouts taking place at the feeder (see table 4.4.).

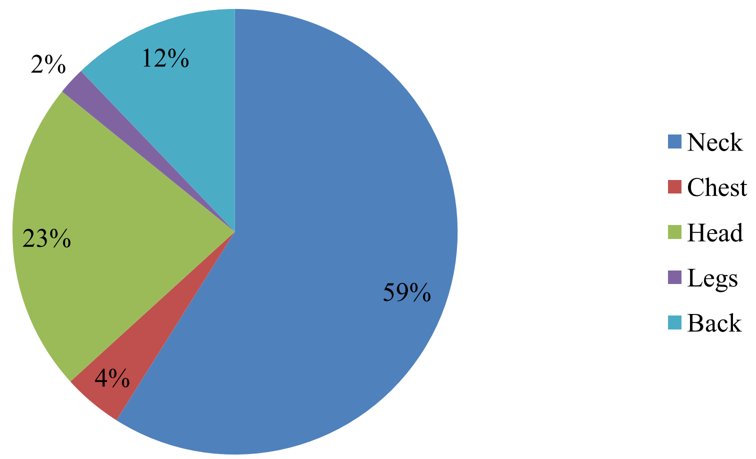


Figure 4.5. The percentage of allogrooming bouts performed for each of the different areas of the body (see figure 4.1 for area definitions). *Data is collated from both farms between March 2010 to November 2011.*

Table 4.4. The mean (±SEM) duration (seconds) and total frequency of, allogrooming events in different locations, and for different parts of the body. Data represents results from both farms, across all ages of cattle.

Location	Number of allogrooming events	Duration (seconds) of allogrooming events (Mean ± SEM)
At the feeder	93	29.95 ± 3.76^{ab}
In the cubicles	44	32.8 ± 4.96
In the alley	26	44.73 ± 7.67
On straw	77	53.14 ± 6.62^{a}
In the field	44	52.48 ± 8.03^{b}
Part of body		
Neck	150	47.89 ± 4.32^{c}
Chest	16	32.94 ± 7.61
Head	91	30.24 ± 3.45^{c}
Back	30	49.47 ± 9.7
Legs	6	40.3 ± 10.6

Multiple comparisons were carried out using Fishers a priori LSD test. Where superscript letters are the same, a significant difference ($p<0.05$) was found between different locations (a, b) and parts of the body (c), for the mean duration of allogrooming.

Whether cattle were together with their preferred partner, apart from their preferred partner for a long period of time or going through multiple regroupings, had a significant effect on the frequency of allogrooming bouts both performed (X^2=64.4, df=2 p<0.05) and received (X^2=114.3, df=2 p<0.05) (see figure 4.6), but no significant effect on duration of grooming bouts performed (F=0.45, df=2 p=0.637) or received (F=1.51, df=2 p=0.225). The highest number of bouts were both performed and received when cattle were together with their preferred partner, and the least amount of grooming was performed or received when experiencing multiple regroupings.

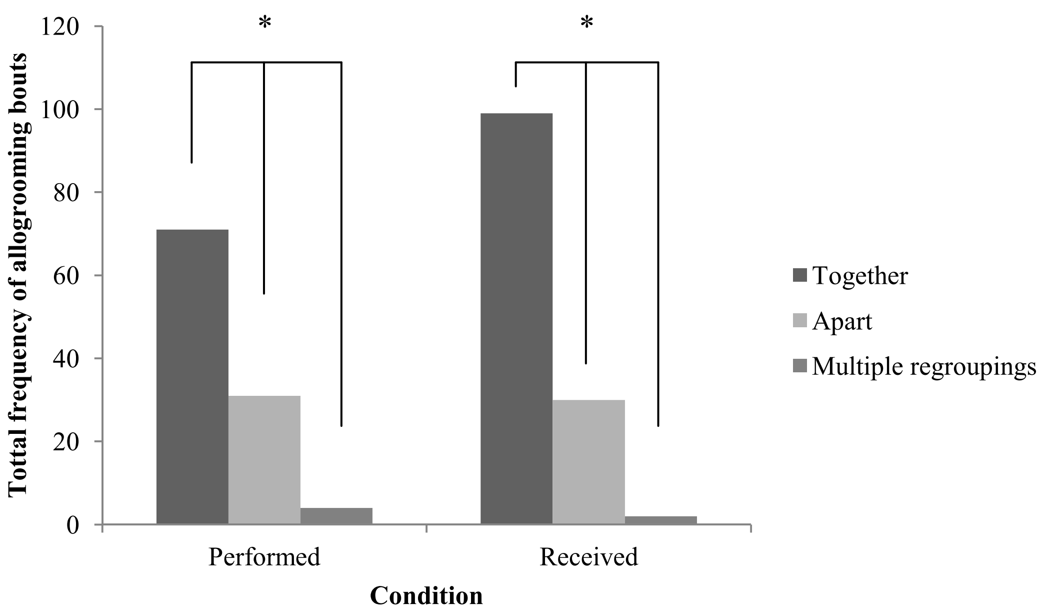


Figure 4.6. Total frequency of allogrooming bouts performed and received when focal cattle are with their preferred partner (together), when they are apart from their preferred partner long term (apart), or when they have been experiencing multiple regroupings (multiple regroupings). *Data is from both farms between March 2010 to November 2011. *denotes a significant differences (p<0.05) between the three conditions.*

4.3.2 Effects of long term separation, and subsequent reunion, on production parameters in the main milking herd

A number of production parameters were identified and assessed in order to fully understand the impacts of long term separation on production in the main milking herd. This included the milk yield and the milk quality. See below for results.

4.3.2a Yield

Long term separation had a significant effect on milk yield (F=4.84, df=7, p=0.000) (see figure 4.7). Cattle had a higher yield during the first four weeks when they were with their preferred partners (13.13 ± 0.09 litres) compared to last four weeks when they were separated from their preferred partner (12.53 ± 0.10 litres), a 4.6% decrease in yield over the time period. Yield differed significantly between weeks (H=35.56, df=7, p=0.000) with yield in weeks -1 (p<0.05), -2 (p<0.05), -3 (p<0.05) and -4 (p<0.05) after separation being significantly lower from the retrospective weeks before separation (see figure 4.8 for differences in mean milk yield before and after separation between weeks). When cattle were reunited with their preferred partners, milk yield was significantly (p=0.001) lower (12.90 ± 0.16 litres) compared to before reunion (13.10 ± 0.15 litres). However there was no significant difference (H=7.06, df=7, p=0.423) between the respective weeks before and after reunion on milk yield.

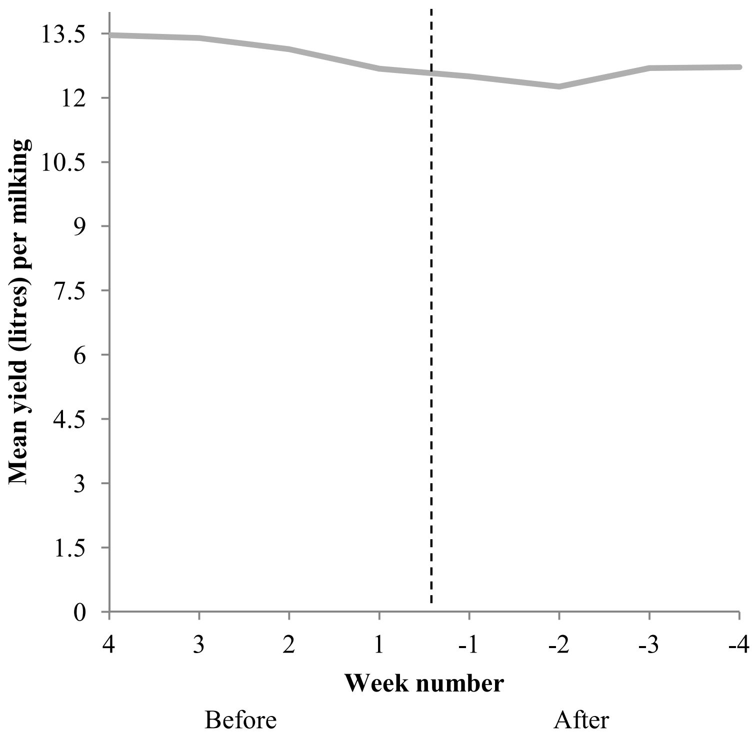


Figure 4.7. Mean milk yield (per milking) before and after long term separation. *Mean milk yield (per milking) was significantly higher before separation (weeks 4 to 1) compared to after separation (weeks -1 to -4) (p<0.001, df=7). Dashed line indicates when separation took place.*

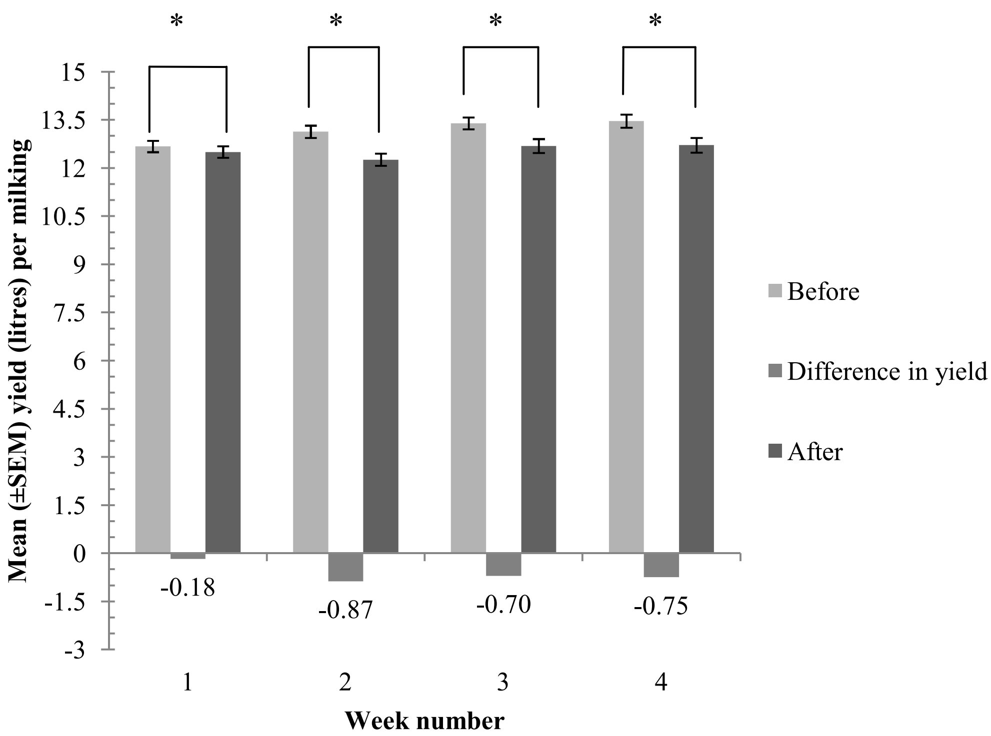


Figure 4.8 The effect of long term separation on mean (±SEM) milk yield (per milking) over an eight week period (four weeks before separation, and four weeks after separation). *Long term separation from preferred groups had a significant effect on mean milk yield (per milking) ($p<0.000$, df=7) between weeks. Differences between retrospective weeks are given below the horizontal axis to show where the effects of separation occurred. *denotes a significant difference ($p<0.05$) in yield before and after separation for particular weeks.*

The average monthly yield of cattle between 2008 and 2011 was significantly different between social environment conditions (F=2.96, df=3, p=0.033). Cattle that were together with their preferred partner produced on average a higher milk yield (14.76 ± 0.781 litres) compared to when cattle were separated from their partner (11.55 ± 0.82 litres), when they were experiencing multiple regroupings (11.59 ± 0.88 litres), and compared to those cattle that did not have a preferred partner identified (12.41 ± 0.54 litres) (see figure 4.9).

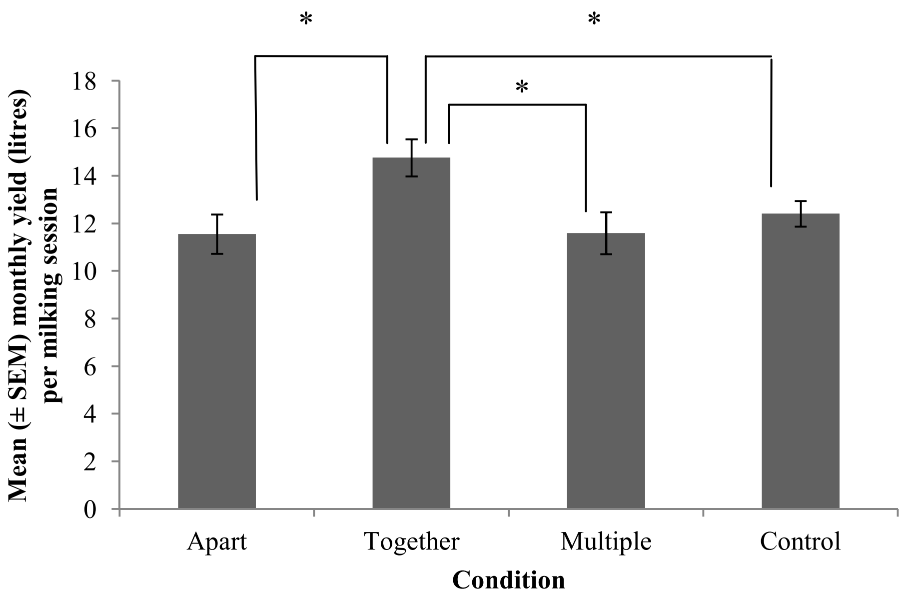


Figure 4.9. Monthly mean (±SEM) milk yield per milking session for cattle when they are apart from their preferred partner, together with their preferred partner, going through multiple regoupings, compared to cattle that did not have any preferred partners (control). *Where there are significant differences between mean monthly yield per milking session, these are denoted as * at p<0.05.*

4.3.2b Quality

Long term separation had a significant effect on all quality parameters (see table 4.5). Significantly less milk (kg) was produced after separation had occurred compared to the month before separation (W=322, *p*=0.001). All other parameters increased significantly after separation had occurred (W=905, *p*=0.05 %Fat; T=4.04, *p*=0.00 %Prot; W=1265, *p*=0.001 PPL). When cattle were reunited with their preferred partners there was no significant difference between all of the quality parameters (T=0.51, *p*=0.615 kg; T=0.48, *p*=0.633 %Fat; T=0.72, *p*=0.480 %Prot; T=0.68, *p*=0.499 PPL).

Table 4.5. The effects of long term separation and reunion on mean (± SEM) milk quality parameters.

	Mean ± SEM Before Separation / reunion	Mean ± SEM After separation / reunion	*p* - value
Separation			
kg	27.61 ± 1.30	25.94 ± 1.30	0.001*
%Fat	3.85 ± 0.09	4.13 ± 0.12	0.05*
%Prot	3.23 ± 0.06	3.46 ± 0.05	0.000*
Pence Per Litre (PPL)	23.89 ± 0.24	24.11 ± 0.24	0.001*
Reunion			
kg	28.46 ±2.2	29.46 ± 2.28	0.615
%Fat	3.88 ± 0.14	3.82 ± 0.12	0.633
%Prot	3.29 ± 0.08	3.24 ± 0.08	0.480
Pence per litre (PPL)	23.76 ± 0.36	23.53 ± 0.37	0.499

**Denotes a significant difference ($p<0.05$) in milk quality parameters from before to after separation and reunion.*

4.3.3 The effects of long term separation, and subsequent reunion, on health parameters in the younger cattle and on the main milking herd

Body condition score and weight, somatic cell count, and fertility were the parameters investigated as a sign of health in both younger cattle and the main milking herd. See below for the results of the effects of long term separation from preferred group mates on the health of cattle.

4.3.3a Body condition score and weight

For animals on farm A, weight gain was significantly (W=301.0, p=0.0056) lower in animals experiencing long term separation (182.2 ± 72.1 kg) compared to control animals (247.09 ± 25.7 kg). There was a significant difference (F=41.80, df=2, p=0.000) in weights over time for those individuals experiencing separation from preferred group mates (see figure 4.10) with weight gain increasing once cattle had been reunited. This difference in weight did not correspond with the results for BCS in that there was no significant (W=624.5, *p*=0.77) effect on BCS for those animals experiencing separation (3.65 ± 0.2) compared to control groups (3.63 ± 0.4).

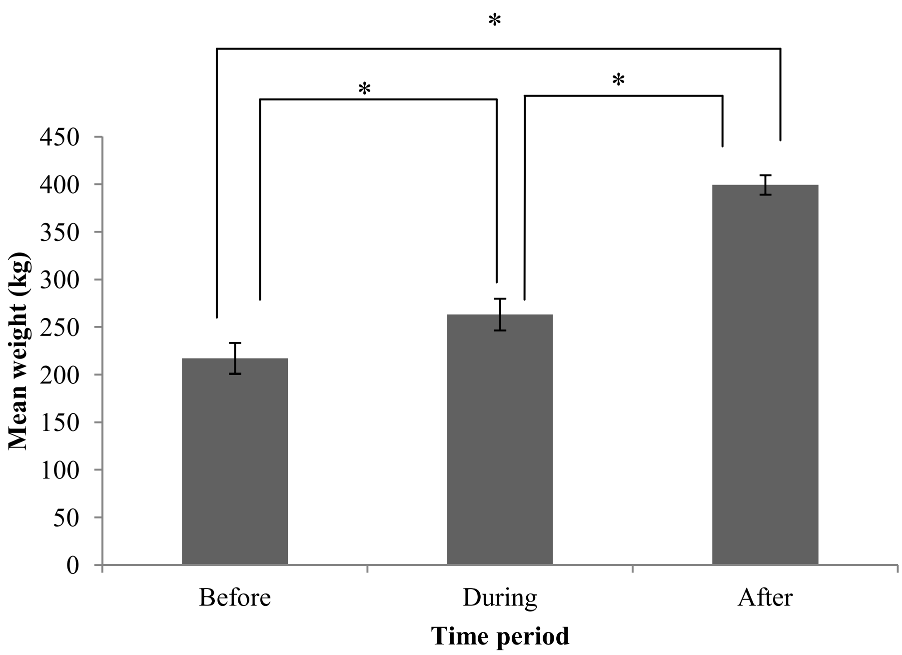


Figure 4.10. Mean (±SEM) weight (kg) of individuals on farm A, before separation, when experiencing two weeks of separation and after separation upon reunion to partners. *Significant differences at p<0.05 are denoted by *.*

For farm B, whether cattle were together with their partner (3.04 ± 0.44), when they were separated long term (3.06 ± 0.49), or whether they were experiencing multiple regrouping (2.97 ± 0.44), did not have a significant effect (H=1.42, df=2, *p*=0.4941) on BCS. Furthermore when comparing the pre-separation month's BCS (3 ± 0.53) with the post-separation month's BCS (3 ± 0.44), there was no significant difference (T=1.14, *p*=0.267).

4.3.3b Somatic cell count

Long term separation had a significant effect on SCC (W=875, *p*=0.022) with SCC being significantly higher after separation (661 ± 240 '000 cells/ml) compared to before separation (362.3 ± 94.7 '000 cells/ml). Reunion with preferred partners however did not have a significant effect on SCC (T=-1.85, *p*=0.08) from before reunion (138.1 ± 46.7 '000 cells/ml) compared to after reunion (263.6 ± 82.4 '000 cells/ml). There was however a trend towards an increase in SCC after reunion.

4.3.3c Fertility

No significant difference occurred in the number of inseminations (W=169, *p*=0.354) and the interval between inseminations (W=51.5, *p*=0.701) as a result of long term separation. The number of inseminations carried out did however increase from before separation (1.94 ± 0.34) to after separation (2.13 ± 0.43) and the interval between inseminations also increased from before separation (16.10 ± 3.64 days) to after separation (21.29 ± 6.13 days). The reunion of cattle to their preferred partner also did not have a significant effect on the number of inseminations required (T=0.16, *p*=0.879) or the interval between inseminations (T=-0.83, *p*=0.433).

4.4 Discussion

4.4.1 The effects of long term separation, and subsequent reunion, from preferred group mates and reunion on behaviour

Most of the behaviours displayed by cattle were not affected by the long term separation from their preferred partners. This may suggest that cattle were able to continue with the majority of their activities without requiring their preferred partner as has been suggested by Newberry & Swanson (2001). This apparent ability to continue with regular activities is supported by the previous findings in chapter three regarding short term separation where it appeared that any familiar conspecific was able to provide some form of social support during that particular environmental challenge.

Similar to short term separation, long term separation from preferred group mates also brought about a high frequency of standing. Although not significantly different between conditions, standing behaviour showed a trend towards being higher during separation compared to both before and after. This increase in standing behaviour is often seen in regrouped animals (Hasegawa *et al.*, 1997; von Keyserlingk *et al.*, 2008) and has been related to fear (Boissy & Bouissou, 1995) and so the high level of standing observed during separation may be an indication that cattle were uneasy about the change in their environment. Furthermore, an increase in time spent standing can increase the chance of foot problems and lameness (O'Connell *et al.*, 2008) causing welfare issues, and so must be monitored closely.

The higher frequency of standing in the separation period may have influenced the results for investigative behaviour which decreased during separation. This behaviour was also lower after cattle had been reunited compared to before separation took place. Cattle appear to feel safer exploring their surroundings when peers are present. As such a low level of locomotive activity may indicate both a high level of fear and reduced social motivation (Müller & Schrader, 2005) which may be due to the absence of preferred relationships. Cattle may have reduced their investigative behaviour and increased their time spent standing as they felt that they could not explore their surrounding as much when a preferred partner was not present.

When being reunited there was an increase in the amount of negative behaviours performed, as is often seen at regrouping. This may have influenced the lack of investigative behaviours due to having to be aware of others around them who may have been defending their resources from introduced cattle. This could also explain the observed differences in drinking behaviour, which was lower in animals during separation and upon reunion. It is likely that this difference in drinking behaviour was more due to climatic conditions rather than the separation. The result of increased negative behaviours would suggest the possibility that cattle did not maintain their social bonds after two weeks of separation and so being returned to the group may have meant that new relationships would have had to of been formed. It may have also increased the stress levels of individuals, both resident and re-introduced, which would have meant that support from previous preferred partners may have been lacking. The behaviours displayed are similar to what would be expected at regrouping time and so long periods of separation should be avoided where possible in order to ensure the welfare of cattle.

Under controlled two week separation on farm A positive social behaviours such as grooming or resting in contact with another, although not significantly different between conditions, did show a trend towards being performed at a higher level before and during separation when compared to after separation when partners were reunited. The grooming behaviour seen before separation could be due to the bonds that existed between cattle. Grooming is often seen as a method of reaffirming a bond between individuals (Schneider & Krueger, 2012; Val-Laillet *et al.*, 2009) but can also offer a relaxation and comfort giving property (Pellis & Pellis, 2010). This may explain the high proportion of time spent grooming others even though focal animals were separated from their preferred partners during separation. Grooming has been seen to slow the heart rates of those receiving the grooming (Laister *et al.*, 2011) and cattle that are lame receive more grooming than non-lame cattle (Galindo & Broom, 2002). The fact that grooming behaviour decreased upon reunion may be due to the increase in negative behaviour that was seen and may represent a regrouping reaction in cattle. Although cattle were reunited with their peers after two weeks of separation, they needed to re-establish their place in the group. Negative behaviours were at their lowest in both before and during separation suggesting that groups were cohesive and stable until reunion occurred and thus a change in social environment occurred, particularly for resident individuals.

When evaluating the allogrooming behaviour overall from both farms, allogrooming behaviour was performed and received at the highest frequency when cattle were together with their preferred partner, compared to when separated from their partner or when experiencing multiple regroupings, similar to the results on other positive behaviours. This change in positive grooming behaviour over time may be due to the instable nature of dynamic groups. This is also suggested from the results on allogrooming between farms. Farm A had the highest frequency of allogrooming compared to farm B, although there was no difference in the bout duration between farms. As farm A housed the replacement heifers and had much more stable groupings it would be expected that grooming would be performed more often in this setting compared to within the main milking herd. Furthermore groups on farm A were smaller and so it is likely that memory formation was better and so bonds were stronger, helping cohesion.

Menke *et al.* (1999) also noted a reduction in social licking frequency when cattle had been separated for a long period of time suggesting that separation had interfered with the bonding mechanisms that had been in place previously. Reduction in such a socio-positive behaviour that demonstrates cattle's ability to experience affective states is of great concern to the welfare of cattle. Promoting allogrooming and thus bond formation could be an important aspect of measuring welfare in dairy cattle, and could be used as an indicator of stable groupings if it were performed on a regular basis. In addition, Hinch *et al.* (1982) noted that there were particular individuals that appeared to dominate the grooming activity in a herd of Hereford cattle, with two individuals grooming 56% of the total herd. It was suggested that these particular individuals may play an important role in group cohesion (Hinch *et al.*, 1982). As such it is important to consider group dynamics carefully, as removal of a key groomer could be problematic for stability and cohesion, and consequently potentially increase social tension (Phillips, 2002). This could have negative effects on the welfare of cattle as the group's social structure and organisation may change dramatically with the removal of key individuals.

The majority of grooming bouts for both farm A and farm B together took place at the feeder. This is in line with other research by Val-Laillet *et al.* (2009). In addition, and potentially as a consequence of this positioning, most bouts of grooming were performed on the head and neck region. Grooming in this area by others may also hold a hygiene function due to cattle's inability to reach this area of themselves.

4.4.2 The effects of long term separation, and subsequent reunion, from preferred group mates and reunion on production

Long term separation significantly reduced the volume of milk produced (litres) and this reduction in yield lasted for at least four weeks. The yield for the four weeks after separation was significantly lower than the retrospective previous weeks, apart from the first week either side. This drop in milk production suggests that separation from preferred partners caused a challenge that required energy that would normally be required to produce milk to be transferred elsewhere. When animals experience stress, they need to transfer energy to resources that are more vital to their survival (Moberg & Mench, 2000). The reduction in milk would suggest that this transfer took place when animals were separated. As cattle were included simply on the fact that they had been separated from their preferred partner, there was no control as to whether it was their partner that was regrouped or whether it was the focal that was regrouped, therefore some of the changes in milk production could be due to regrouping more than separation and so must be considered with caution. Furthermore, individual lactation curves may have an effect on the outcome of results and could not be controlled for.

In addition to the above result, the monthly average yield (litres) for cattle was at its highest when cattle were with their preferred partner compared to when they were apart or experiencing multiple regroupings. Interestingly, monthly yield is higher when cattle identified as having a preferred partner are with their preferred partner compared to the monthly average yield of cattle that had no preferred partner identified. This may suggest that the existence of a relationship and the presence of that preferred partner allow cattle to produce higher volumes of milk. This link between a positive relationship and the production of higher volumes of milk in cattle may be due to the positive effects that these relationships appear to have on cattle. Val-Laillet *et al.* (2009) noted that social relationships affected the spatial proximity between individuals at the feed bunk in that individuals maintained a closer distance to each other at the feed area. The increase in milk yield when cattle are with their preferred partner may be due to the ability to access the feed area more and therefore gain more energy which can be converted to milk. Even though Val-Laillet *et al.* (2009) observed cattle to displace each other more the closer their proximity, it was also noted that cattle would groom each other more the closer their proximity.

Cattle will trade feed quality for a place to feed that is away from a non-preferred partner (usually a more dominant individual) (Rioja-Lang *et al.*, 2009) and so it is likely that cattle will choose minor displacements along with social grooming from preferred partners over a displacement from a more dominant individual. Cattle this way are able to gain better quality food and are probably likely to be able to eat a good amount of food despite the number of displacements. Furthermore, the results regarding allogrooming to be performed at the feed bunk are in agreement with Val-Laillet *et al.* (2009) who noted that the preferred partners often seen at the feed bunk also allogroomed each other the most. As allogrooming can relieve social tension (Phillips, 2002) the performance of a high frequency of allogrooming between a pair that often displace each other at the feed area may help to affirm their relationship and so each of the dyad are able to feed freely.

Upon reunion, the milk yield of cattle again dropped over the four weeks. As it was not possible to control how long cattle had been separated from each other, it is likely that they had been separated for longer than the minimum of two weeks and so had likely been unable to maintain the relationship with their preferred partner. Reunion is also likely to have happened due to regrouping which can be extremely stressful for cattle and so as discussed earlier, energy will have been transferred to other resources than being used for milk production. There was no significant difference between weeks and so the reunion or potential regrouping did not cause as much impact on yield as the initial separation had caused.

In line with the above results, the amount of milk recorded (kg) by NMR was significantly reduced after separation. The amount of milk that was lost due to separation might cost the producer a significant amount of profit if a number of animals were regrouped or separated from the partners in one go. However the price per litre that was paid each month, along with both the fat percentage and protein percentage increased after separation. The increase in quality parameters may be associated with the reduction in yield that was observed (Løvendahl & Chagunda, 2011). There was however no significant effect on any of the quality parameters when cattle were reunited with each other. This is in contrast to the results for separation.

4.4.3 The effects of long term separation, and subsequent reunion, from preferred group mates and reunion on health

For cattle under the controlled two week separation on farm A, weight gain was significantly affected by separation. Cattle experiencing the separation from their preferred partners gained less weight than control cattle during the same period. Cattle did gain weight upon reunion of their preferred partner but this did not match the weight gain of control individuals. The potential stress experienced by cattle when separated from their preferred partner appeared to affect weight gain. Replacement heifers are a valuable source for dairy producers. This loss of weight or slow weight gain, in replacement animals as a potential effect of separation from preferred partners could have negative impacts on the health and welfare of the cattle, but also on rate of herd replacement. If young heifers are served by their weight, losing weight could cause setbacks and difficulty in insemination and thus fertility levels.

On the contrary, long term separation did not affect body condition score both on farm A and farm B. This result has a number of potential concerns. If weight is significantly affected by separation but body condition score is not, the practical implications for this are problematic. Cattle could be losing weight but not enough to affect their body condition score and so may go unnoticed by producers. For older animals this may not be too much of an issue, but for younger animals, particularly replacement heifers, this could cause difficulties for producers. Secondly, the difference in the body condition score result and that for weight may be due to the calves and youngsters being affected more by the separation than older cattle. As the younger cattle were too small to be effectively condition scored on a regular basis, they were not observed for condition but only observed for weight; therefore the younger individuals may be affected more by the separation procedure than older individuals who may have already experienced some regrouping. The assessment of body condition score is subjective compared to the direct measurement of weight and so the measurement of weight and its reduction due to separation should be considered as the main assessment to take note of.

Somatic cell counts are often a sign of an individual's health, in particular regarding udder health and potential infection by mastitis pathogens. Somatic cell counts were significantly higher after long term separation compared to before separation. They also increased, although not significantly, after reunion. This would suggest that the separation and likely the regrouping of cattle had a negative effect on cattle's udder health. As stress levels increase, so do corticosteroids and this can impair the cattle's immune system and therefore they are less able to fight infections they exposed to (Boccia *et al.*, 1997; Gordon *et al.*, 1992). It could however be that the high SCC caused regrouping and subsequent reunion of individuals as cattle were separated into a close observation group if they were undergoing treatment for mastitis.

Although the fertility of focal individuals was not significantly affected by long term separation from their preferred partners, there was an increase in the number of inseminations and the interval between inseminations required. This may suggest that cattle took longer and more inseminations to be confirmed positive in pregnancy when they had been separated from their preferred partners. Individuals under stress are likely to divert energy requirements away from non-essential functions such as reproduction (Moberg & Mench, 2000). However, the data available for this analysis was very limited and no clear conclusions can be drawn from these results on fertility. As for separation, reunion appears to have had no significant influence on fertility measures.

4.4.4 Practical implications

There are a number of practical implications from this part of the study. In particular it would appear, as for the previous studies, that younger cattle may be more sensitive to the separation and regrouping process. Extra care surrounding these individuals needs to be considered. Further investigation of this formed the next and final stage of the study, looking at the influence of age on the parameters described above.

At critical periods in cattle rearing such as when reaching the weight for insemination, limited stress and limited changes in their social environment may be beneficial. Although fertility was not significantly affected by separation, the occurrence of having to do more inseminations later on after separation occurred would suggest that limiting regrouping around this period may be beneficial for the health and well-being of the cow so that her energy can concentrate on the foetus.

In order to avoid loss in production, both through the reduction in yield and the increase in somatic cell count which is often used as a quality control parameter for processors, the avoidance of separating preferred partners when regrouping occurs would be beneficial. As has been seen by Gyax *et al.* (2009) and Neisen *et al.* (2009b), pairing heifers at the introduction of regrouping eased integration, and so if pairs consisted of preferred partners, the potential for social support is increased as it was in study two (chapter three). Being able to regroup animals at the same time will require good management of groups so that multiple animals are ready for regrouping at the same time. If animals are carefully managed so that they can be regrouped together this may help in the management of those individuals within a group and will ensure that the avoidance of separating preferred partners is feasible.

Keeping individuals together allows for bonds to be continued and therefore stable relationships can be formed and maintained. These relationships encourage positive behaviours between individuals and allow animals to investigate and explore their surroundings in comfort. Smaller, more stable groups make this easier and so negative impacts on health and welfare are minimised as positive relationships help animals to cope with their changing environment. This might be why there were very limited excessive behavioural responses to separation observed as they were still with familiar individuals.

4.5 Conclusion

In summary, long term separation from preferred group mates has negative consequences on behaviour, health, production and welfare. The minimisation of separation and regrouping of animals will improve the welfare of animals and in turn prevent negative consequences on health, behaviour and production. The fact that upon reunion there were no major significant improvements on the negative effects of separation would suggest that separation of two weeks could cause bond formation to break and so if cattle need to be removed from a group for reasons such as sickness, they should not be held for any longer than needed, and where possible, less than two weeks. This will help to ensure that these important bonds are not broken and their positive impact on an animal's coping ability is maintained. The impact of long term separation was more significant than reunion suggesting that it is the separation from their preferred partner that causes the most stress. It is possible that these effects seen from long term separation would suggest that these preferential relationships are bonds that exist over time but after long term separation become difficult to maintain.

Chapter Five

How Age Affects the Formation of Social Bonds, Behaviour, Production and Health Parameters at Regrouping

5.1 Introduction

Results from chapter two, three and four suggested that there were differences in the social behaviour of cattle that were influenced by their age. In chapter two, younger cattle (heifers) were more likely to form a preferential association with at least one other member of the group. These bonds tended to be stronger amongst dyads that appeared together more often and the association indices were higher in heifers than they were in cows. This validated why heifers were utilised in the short term separation observation, chapter three. During short term separation it appeared that a number of the familiar but non-preferred partners did not show the same heightened stress response as some of the focal individuals. This may be due to the differences in ages, and likely, experience between these two cattle as focal individuals tended to be heifers, whereas familiar non-preferred partners were cows. Furthermore, in the long term separation observation (chapter four) the level of allogrooming was observed more often in cattle on farm A (rearing herd) compared to cattle on farm B (main milking herd). This suggests that younger cattle performed more grooming behaviour, a socio-positive behaviour indicative of a preferential relationship (Val-Laillet *et al.*, 2009; Waiblinger *et al.*, 2006) and this may be some of the reasoning behind the strong relationships observed in younger cattle from chapter 4.

Under feral conditions, the matriarchal structure and organisation of cattle groups mean that animals of different ages will be together; young calves are reared alongside their mothers, peers and older siblings. The group would consist of very young, sub-adult, adult and much older individuals. Under commercial conditions the groupings of individuals are based on age, lactation stage, reproduction status or weight in order to create homogenous groups for ease of management (St-Pierre & Thraen, 1999). This begins at eight weeks of age at the most as the EU directive (97/2/EEC) states calves must be housed together as a group. As cattle mature the relationships in an animal's life change and it isn't until they join the main milking herd that they will encounter individuals of considerably different ages when regrouping occurs.

This very dynamic social system can impact on the development of a relationship, and the influence of age on changes in social environment has previously been recognised in cattle. For example, multiparous cattle are only mildly affected by the removal of their calves compared to primiparous cattle (Hopster *et al.*, 1995); however, the mixing of multiparous and primiparous cattle causes considerable disruption to grazing and social behaviour (Phillips & Rind, 2001). The ability to cope with repeated changes in social environment through regrouping and relocation has also been demonstrated to be influenced by age; Veissier *et al.* (2001) found calves aged between 3 and 4 weeks old to show an increased sensitivity to ACTH and a modified daily rhythm of activity when regrouped. The authors did, however, report that there was no clear evidence that calves were significantly stressed by the regrouping and relocation and so concluded that they habituated to the process of regrouping (Veissier *et al.*, 2001). Conversely, Raussi *et al.* (2005) found heifers aged 11 months at first regrouping to not habituate to the relocation and regrouping process. They observed aggression to be consistently induced by the regrouping process and the behaviour of heifers was significantly affected, as heifers would change their activity more often than controls. This would suggest that cattle of different ages dealt with the process of regrouping and relocation differently.

Further investigation into these differences in ability to cope with dynamic social systems that appear to be influenced by age is required in order to have a better understanding of how cattle deal with such social challenges. Thus, the aim for this final part of the study was to investigate the affect that age has on bond formation in cattle, and how age might influence the impact on health, production and behaviour at regrouping. This was carried out by returning to some of the previous data from chapter two and chapter four and investigating how age may have influenced the results. A better understanding of how age may influence the ability of individual cows and calves to cope with social challenges will enable practical recommendations to be implemented so that high standards of welfare can be maintained.

5.2 Materials and methods

5.2.1 Housing area, animals and management

Animals included in this part of the study were also involved in chapter two and chapter four (see section 2.2.1, and section 4.2.1 respectively). A total of 198 cattle were available for observation throughout the study period of 2008-2011 from farm A (rearing stock to include calves, Arthingworth, Leicester) and farm B (main milking herd, Pitsford, Northampton). Table 5.1 details the age categories used for analysis.

Table 5.1. The age category for animals used within data analysis across farm A and farm B. *The actual age range, along with the farm that cattle were based at are also given.*

Age category	Actual age range	Farm
Calf	<4-6 months of age	A (rearing stock)
Youngster	7-11 months of age	A (rearing stock)
Replacement Heifer	12-23 months of age	A (rearing stock)
Heifer	24 months and within first lactation	B (main milking herd)
Cow	In her second or later lactation(s)	B (main milking herd)

5.2.2 *Measurements*

The data for this section of the study included the analysis of data previously collected in chapters two and four as it was not possible to have a control group that had not experienced regrouping due to commercial constraints. Overall herd parameters for production and health were used along with information from animals that had not been identified to have a preferred partner to help ascertain potential relationships. Therefore, the social bonds between cattle were determined as for chapter two (see section 2.2.2. for more details) and included data from July 2008 through to November 2011. The behaviour of cattle on farm A experiencing separation was collected as in chapter four (see section 4.2.3 for details) and allogrooming behaviour for cattle on both farms A and B were collected as in chapter four (see section 4.2.3 for details). Production parameters were also investigated including daily milk yield and monthly milk quality. The data used was collected as described in section 4.2.3. For yield, the average monthly yield for 110 focal cattle between 2008 and 2011 were calculated for the month before regrouping, the month of regrouping and the month after regrouping. For milk quality, the data from the month of regrouping, the month after regrouping, and the month before regrouping for 103 focal cattle between 2008 and 2011, were used for analysis. Health parameters were included in the overall assessment and included body condition score, somatic cell count, and fertility. The data was collected as described in section 4.2.3 for all parameters. For somatic cell count and fertility data from the month of regrouping, the month after regrouping, and the month before regrouping for 103 focal cattle between 2008 and 2011, was used for analysis. Only the number of inseminations that cattle had was used for analysis in this part of the study due to lack of data available on the number of days between inseminations.

5.2.3 Statistical analysis

All statistical analysis was carried out using Minitab version 13.20 and 15.0, and data presented as means ± SEM unless where otherwise stated. For all statistical analyses a significance level of $p<0.05$ was accepted. Any data which was not normally distributed was log transformed using the common logarithm $\log_{10}$ to normalise where required. Where log transformation did not normalise data, non-parametric statistics were used. Details of particular methods and tests employed are outlined below.

5.2.3a Bond formation

The effect of age on bond formation was investigated by exploring the association between the number of preferred partners an individual had and the average association index score of each focal with their age (years) using a Spearman's rank correlation. In order to investigate how regrouping may affect bond formation and preferential relationships, an individual's average association index score from the first observation was compared to their average association index score on the second observation, a time period which allowed for regrouping to have occurred. This was carried out using a Wilcoxon's signed rank test to test before and after for the same individual as data was not normally distributed. The effect of age on this change in average association strength was investigated further by analysing the difference between age categories through the use of a Kruskal-Wallis test followed by a multiple comparisons *post hoc* test for Kruskal-Wallis (Siegel & Castellan, 1988). In order to have a better understanding of the dynamics of social relationships and the influence of age, the impact that long term separation had on bond formation was investigated by comparing the average association strength before and after long term separation (outlined in section 4.2) by age. The changes in average association strength across time were analysed using a general linear model with the model including time (before or after) and age category (replacement heifer, 12-23 months; youngster, 7-11 months). Categories of age were used to reflect grouping under normal management.

5.2.3b Behaviour

The influence of age on behaviour at regrouping was investigated by comparing the difference in behaviour when cattle were reunited between the two different age categories of replacement heifer and youngsters. Only these two groups were observed for behavioural analysis due to movement in control groups before the end of the separation period for management reasons. The proportion of time cattle spent drinking, eating, lying down, standing, walking, and being alert was compared using a Mann-Whitney *U* test as it was comparing two different samples that were not normally distributed. The difference between age categories and the frequency of both negative and positive behaviours performed was analysed using a Pearson's chi-squared analysis.

The influence of age on allogrooming behaviour was analysed for both duration (seconds) and frequency. Duration was log transformed and a one-way ANOVA performed to investigate differences between the four age categories (cow, heifer, youngsters, calf), followed by Fisher's *a priori* LSD test for pair-wise comparisons. Pearson's chi-squared analysis was used to analyse differences in frequency of allogrooming between the four age categories.

5.2.3c Production

A number of production parameters were investigated. These included the milk yield and milk quality. Details of analysis are outlined below for each parameter.

Yield

In order to investigate how regrouping affected monthly average yield across age (years), the difference between time periods (the month before regrouping compared to the month of regrouping, the month of regrouping compared to the month after regrouping, and the month before regrouping compared to the month after regrouping) were calculated and used for analysis. Each time period was analysed separately to fully investigate how regrouping affected milk yield across different ages using Kruskal-Wallis tests.

Quality

As for yield, in order to investigate how regrouping at different ages (years) affected the quality parameters of milk yield (kg), %protein, %fat, and price paid for the milk (pence per litre, PPL), the difference between time periods for each of the quality parameters were calculated and used for analysis. Each time period was analysed separately by age using a Kruskal-Wallis test, apart from %fat for the month of regrouping and after regrouping as data was normally distributed and so a one-way ANOVA was utilised.

5.2.3d Health Parameters

A number of health parameters were investigated. This included somatic cell count for cattle on farm B only, and the fertility of cattle on both farm A and farm B. See below for details on analysis carried out for each health parameter.

Somatic cell count

In order to investigate whether there was a significant difference between age groups on somatic cell count (SCC) level when experiencing regrouping the difference between each of the time periods was calculated and used for analysis, as for yield and quality parameters. As data was not normally distributed a Kruskal-Wallis test was employed to test for changes in somatic cell counts due to regrouping between age groups (year categories).

Fertility

To investigate the influence of age (years) on fertility levels at regrouping, the changes in the number of inseminations that cattle had from the month before regrouping, to the month of regrouping, and to the month after regrouping were analysed using a Kruskal-Wallis test as data was not normally distributed.

5.3 Results

5.3.1 The influence of age on bond formation

Cattle on farm A had significantly higher mean association index values (0.23 ± 0.02) compared to cattle on farm B (0.09 ± 0.001). The correlation between age (years) and average association strength (AI) was negative and significant on both farms (farm A r_s= -0.506, p=0.000; farm B r_s= -0.165, *p*=0.027); as cattle got older they had a lower average association strength and so fewer bonds (figure 5.1). Age also had a negative correlation between the number of preferred partners that cattle had (Farm A r_s= -0.338, *p*=0.000; farm B r_s= -0.208, *p*=0.005) with younger animals having a higher number of preferred partners (figure 5.2).

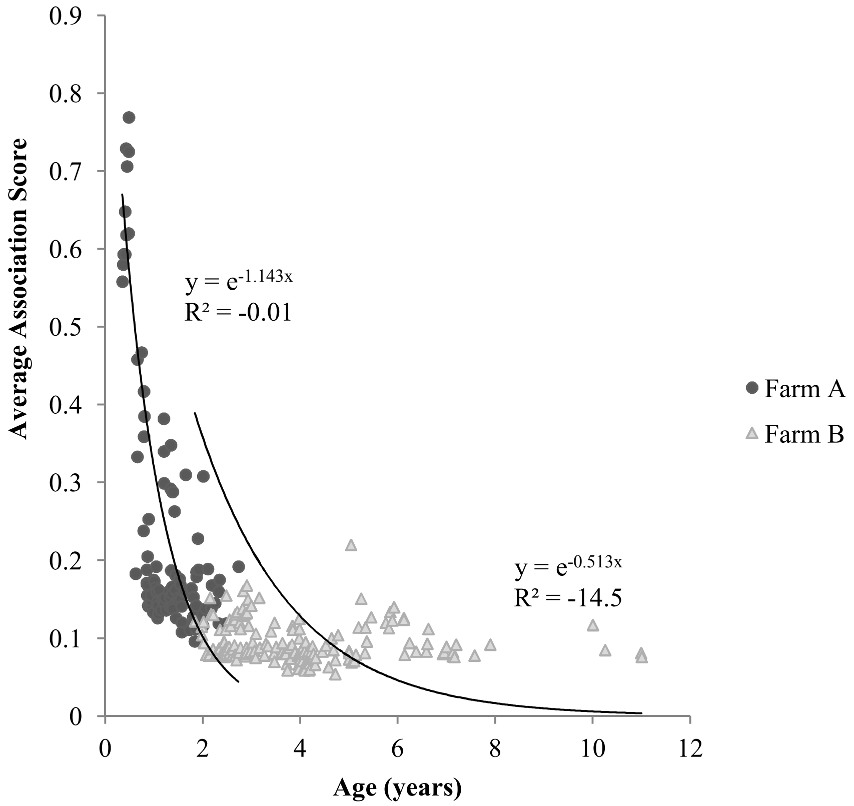


Figure 5.1. The negative relationship between age (years) and the average association indices score (mean association indices score 0.15 ± 0.01; mean age 2.98 ± 0.11 year) for farm A (rearing stock) and farm B (main milking herd). *Exponential trend line is shown, plus equation and R value.*

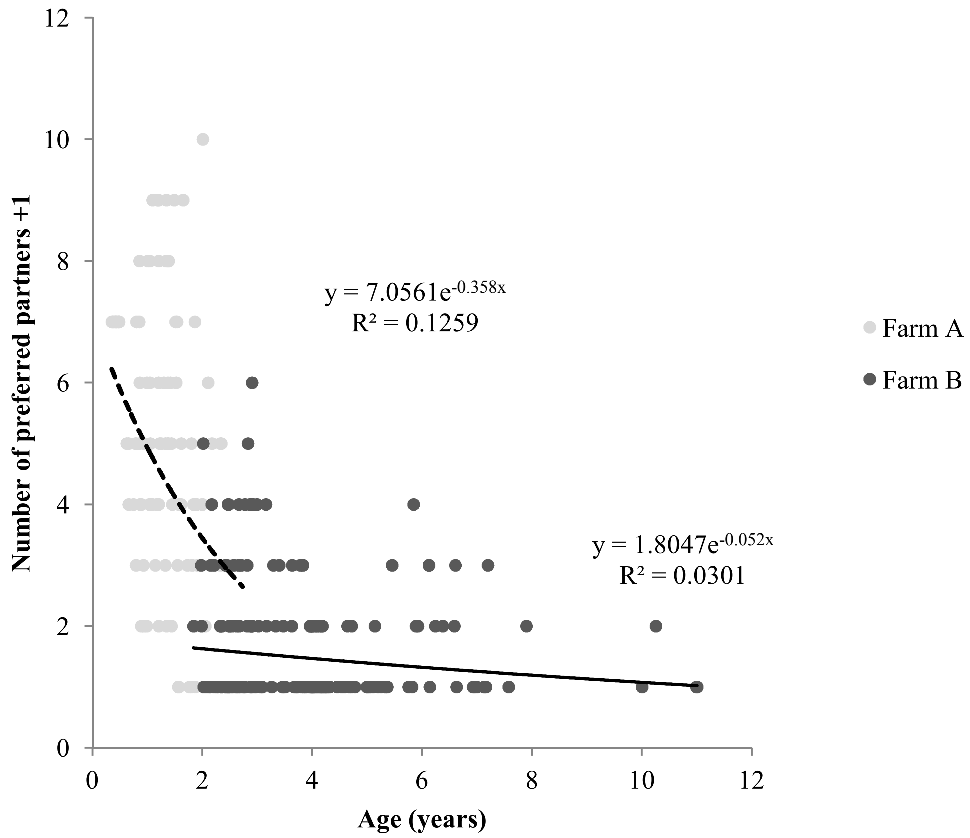


Figure 5.2. The negative relationship between age and the number of preferred partner's (plus 1 so as to provide curve) cattle had (mean number of preferred partners A 3.99 ± 0.21; B 0.69 ± 0.08 and mean age A 1.31 ± 0.05; B 3.97 ± 0.13 years). *Exponential trend line is shown, plus equation and R value.*

Over time, as animals would have experienced regrouping and aged, their average association index fell significantly (W=70, *p*=0.000). The mean association index for cattle on their first observation was 0.22 ± 0.02 compared to mean association score of 0.10 ± 0.00 on their second observation. The influence of age on this change in association score showed a significant difference between age categories (H=50.14, df=4, *p*=0.000) and following multiple comparisons it was possible to see that the mean association index of younger cattle fell significantly more than older individuals between the two observations (see figure 5.3).

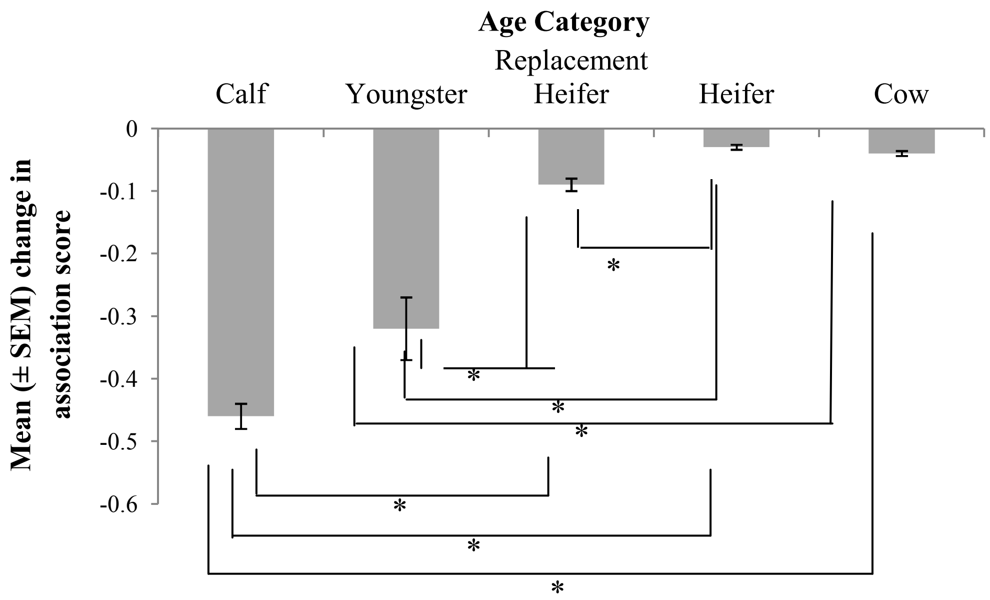


Figure 5.3. Multiple comparisons between age category and the mean (±SEM) change in association score from the first observation to the second observation. *Significant differences are denoted by * at p<0.05. Non-significant comparisons are not shown.*

A very similar result was found when investigating the effects of long term separation and regrouping on average association index score. The average association score of younger animals fell significantly (F=25.76, df=1, *p*=0.000) from before separation to after separation and regrouping. In general younger cattle had higher association index scores compared to older cattle (F=26.59, df=1, *p*=0.000), however there was a significant interaction affect of age and time, with younger individuals decreasing in association score significantly more so than older cattle (F=18.71, df=1, *p*=0.000) (see figure 5.4).

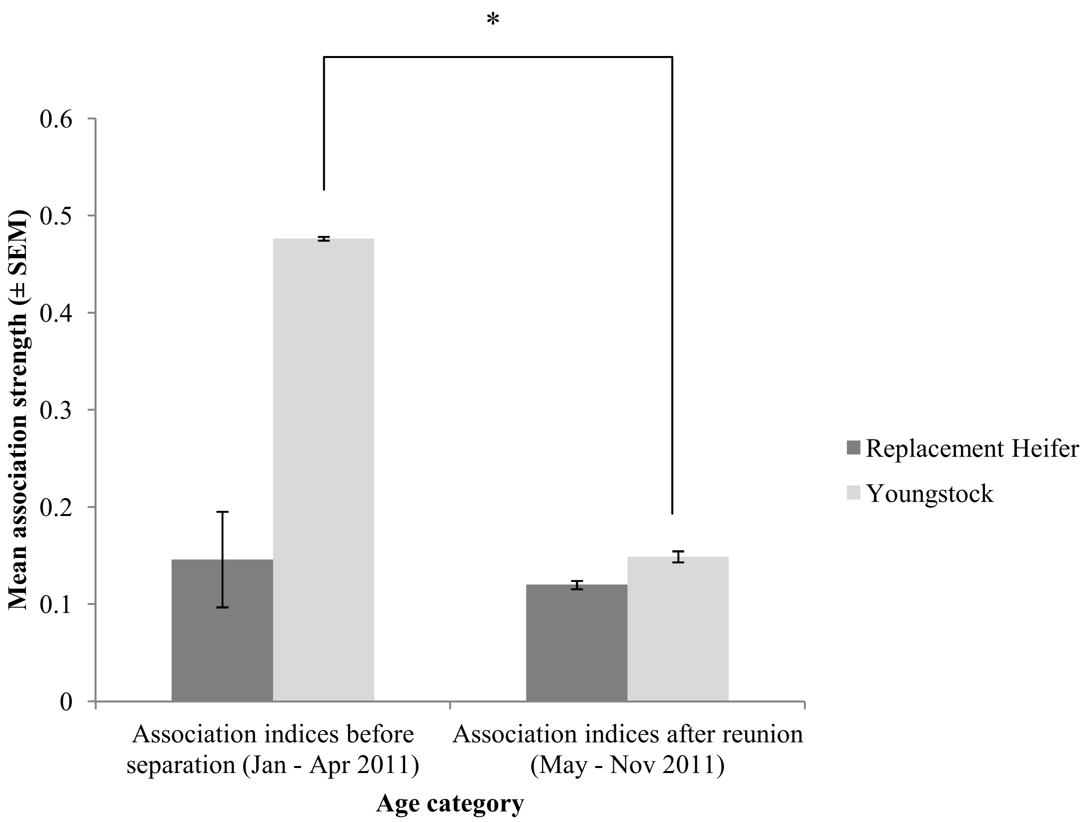


Figure 5.4. Relationship between age category (Youngster, 7-11 months; replacement heifer, 12-23 months) and mean (± SEM) association indices score, before and after long term separation.

5.3.2 The influence of age on behaviour at regrouping.

Age had a significant influence on the amount of time spent eating, standing and being alert at regrouping. Younger animals spent more time being alert and spent more time standing than older cattle. Younger cattle spent less time eating than older cattle. All other behaviours were not significantly affected by age at regrouping (see table 5.2 for *p*-values). There was no significant difference ($p<0.05$) in the frequency of positive or negative behaviours as a result of regrouping between age categories (see figure 5.5).

Table 5.2. The influence of age (youngster, 7-11 months: replacement heifers 12-23 months) on mean (± SEM) proportion of time spent performing behaviours upon regrouping of individuals and the significant difference between age groups.

Behaviour	Mean (± SEM) proportion of time		*p*-value
	Youngster	Replacement heifers	
Alert	0.25 ± 0.02	0.17 ± 0.00	**0.019***
Drinking	0.25 ± 0.02	0.21 ± 0.02	0.380
Eating	0.56 ± 0.02	0.66 ± 0.02	**0.002***
Lying	0.75 ± 0.03	0.83 ± 0.04	0.109
Standing	0.38 ± 0.02	0.29 ± 0.01	**0.005***
Walking	0.18 ± 0.01	0.21 ± 0.01	0.109
Investigate	0.22 ± 0.02	0.17 ± 0.00	0.267

* denotes significant difference at $p<0.05$

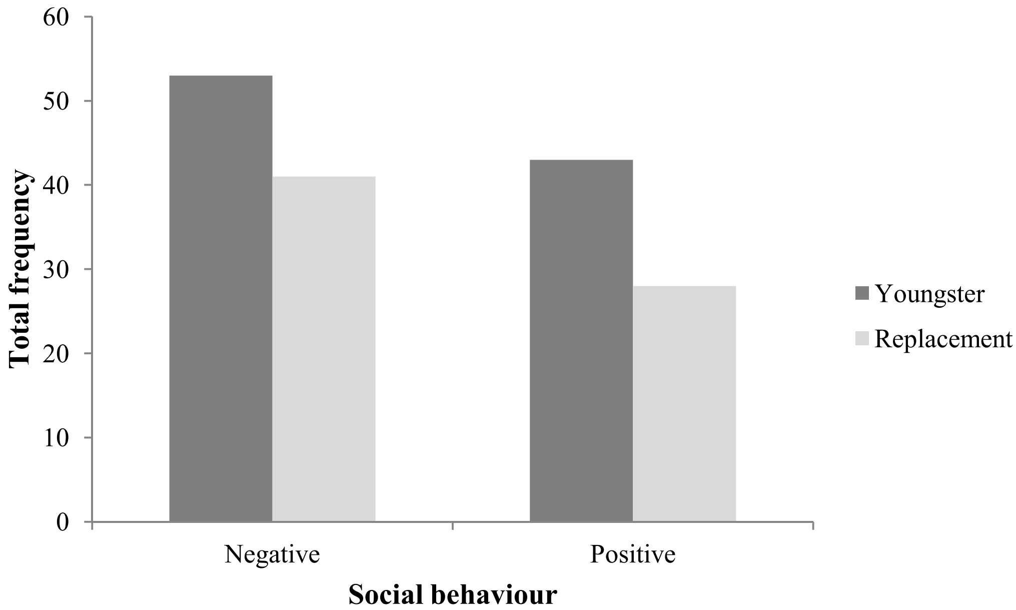


Figure 5.5. Total number of negative and positive social behaviours performed when cattle were regrouped and thus reunited with preferred partners, by age category (Youngster, 7-11 months: replacement heifer, 12-23 months).

Ninety-three percent of allogrooming bouts occurred between the same age group of animals, for example cows allogroomed cows, and heifers allogroomed heifers, with only 7% occurring between different age groups. There was no significant difference in allogrooming duration for both performer age (F=1.06, df=3, p=0.368) and receiver age

(F=1.13, df= 3, *p*=0.335), however animals on farm A classed as youngsters performed and received significantly (X^2=118.0, *p*<0.05; X^2=115.1, *p*<0.05 respectively) more allogrooming bouts than the other three age groups (cow, heifer, and calf) (see figure 5.6). The number of preferred partners that the performer had, significantly affected both the frequency (X^2=42.9, *p*<0.05) and the duration (F=6.86, *p*=0.001) of allogrooming bouts. The higher the number of preferred partners a focal had the lower the frequency of grooming bouts. The highest duration of grooming was observed for animals that had between four and eight preferred partners with the lowest duration between zero and three preferred partners. However the number of preferred partners that the receiver had did not have a significant influence on grooming bout duration (F=2.12, df=2, *p*=0.124), although it did have a significant effect on the frequency of allogrooming bouts (X^2=55.6, *p*<0.05) received. The smallest total number of bouts was observed in animals that had more than eight preferred partners (See table 5.3 for more details).

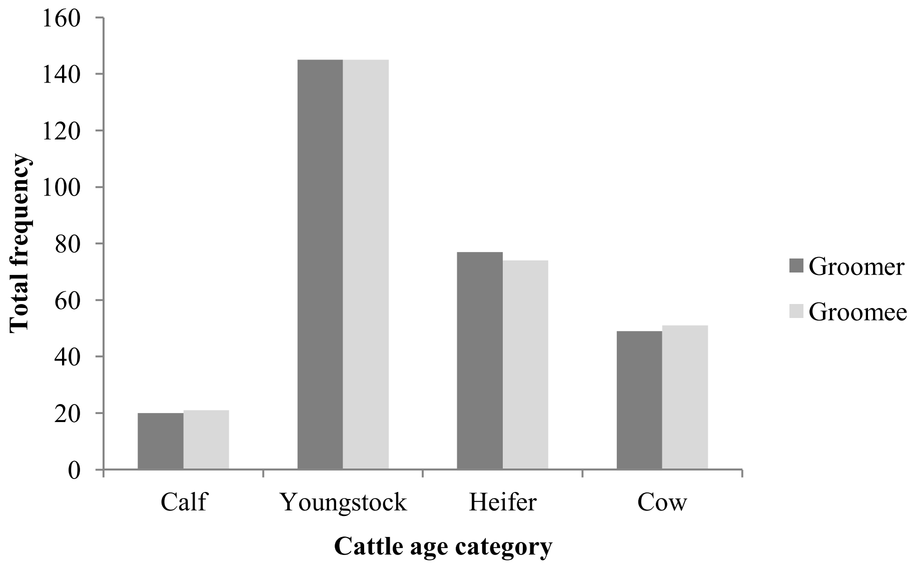


Figure 5.6. Total number of allogrooming bouts performed and received by age of cattle (calf, 4-6 months; youngster, 7-11 months; heifer, 12-23 months and within first lactation; cow, within second or later lactation(s)).

Table 5.3 The number of preferred partners and the frequency of allogrooming performed and received, along with the mean (±SEM) duration of allogrooming bouts performed and received. *Data is from both farms across all ages. The least number of bouts were performed in dyads that had more than eight preferred partners which may be an indication of extreme networking.*

Number of preferred partners	**0-3**	**4-8**	**>8**
Allogrooming frequency			
Groomer	66	63	10
Groomee	84	60	10
Allogrooming duration (Mean ± SEM)			
Groomer	32.23 ± 5.05	55.49 ± 6.60	33.3 ± 11.00
Groomee	33.88 ± 3.91	44.60 ± 6.39	69.50 ± 20.9

5.3.3 The influence of age on production parameters at regrouping

5.3.3a Yield

Age had a significant effect on the changes in milk yield that occurred due to regrouping. There was a significant difference in the changes in milk yield (3.64% decrease in yield) between the month before regrouping occurred and the month in which regrouping occurred (H=28.64, df=6, *p*=0.000) with younger cattle generally having the smallest amount of change in yield. The changes that occurred from the month of regrouping to the month after regrouping was also significantly different (H=30.72, df=6, *p*=0.000) (5.162% increase in yield) as was the difference between before regrouping and after regrouping (H=37.53, df=6, *p*=0.000) (1.34% increase in yield). As can be seen from figure 5.7, as cattle aged they became more sensitive to regrouping and had larger negative changes in their milk yield until they reached above the age of approximately eight years, and the variation from the mean within the age group also increased as animals aged.

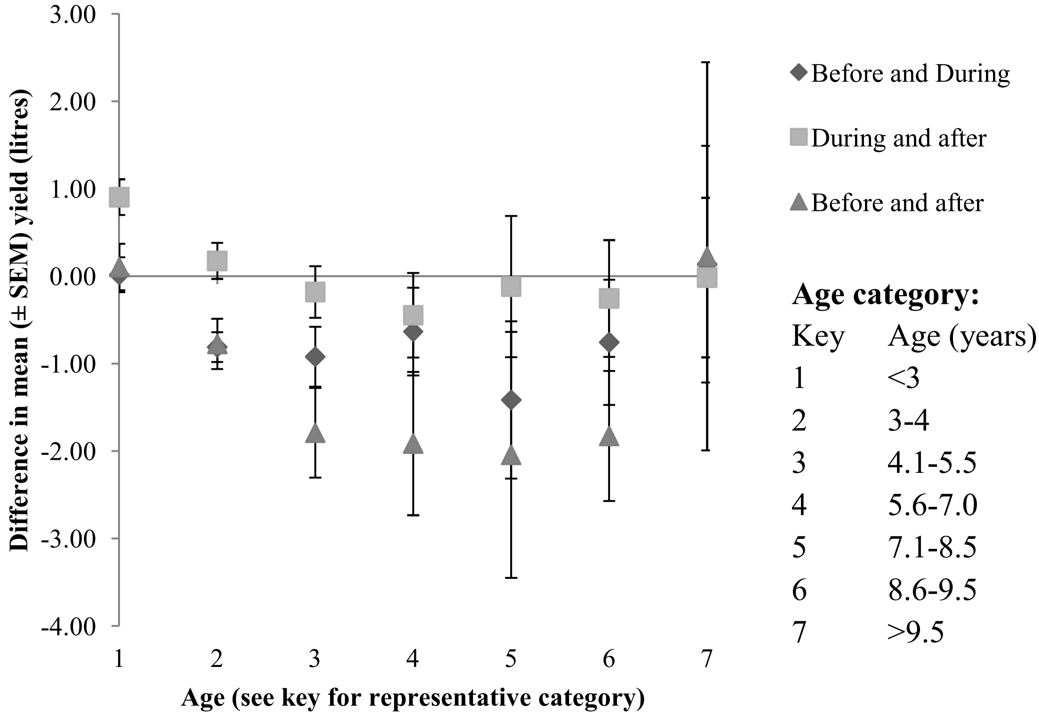


Figure 5.7. The relationship between age (years) and the mean (± SEM) change in milk yield (litres) due to regrouping of cattle. *The large error bars are likely due to the smaller number of cattle within the older age ranges. The average lactation number for cattle on these two farms was 3.5 and so there would have been a very small number of individuals over the age of seven.*

5.3.3b Quality and milk recorder data

Milk production (kg) dropped as a result of regrouping (see figure 5.8) and age had a significant influence on the amount that it dropped by from the month of regrouping to the month after regrouping (H=22.93, df=4, p=0.000), and between the month before regrouping and the month after regrouping (H=22.53, df=4, p=0.000). Older individuals decreased their production more than younger individuals. There was however no significant difference in age groups when comparing the change in milk production from the month before regrouping to the month of regrouping, although it did show a trend towards significance (H=8.26, df=4, p=0.083).

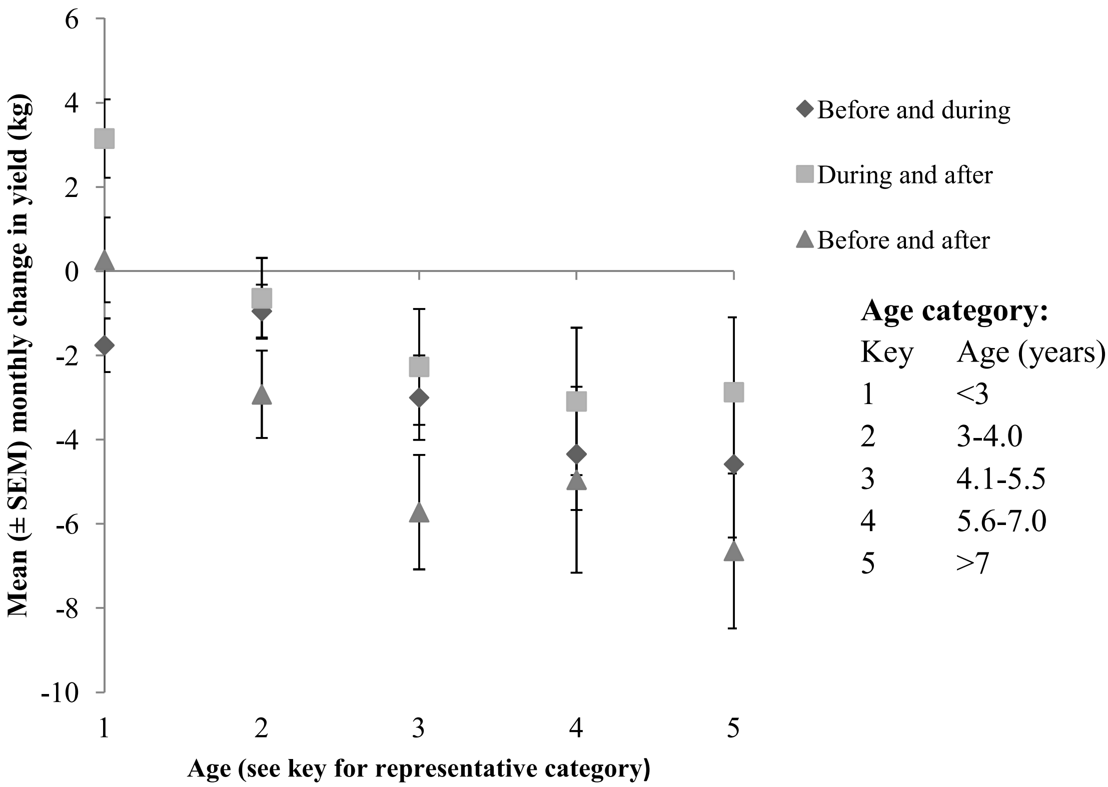


Figure 5.8. The relationship between age and mean (± SEM) milk production (kg) change when experiencing regrouping. *As for the change in yield (litres) there were fewer individuals available for analysis in the older categories and so may explain the large error bars seen graphically.*

Age effected the change in %protein from the monthly average before regrouping to the monthly average when regrouping took place (H=10.20, df=4, *p*=0.037) with younger and middle aged individuals (3-5 years) dropping in %protein over the time when regrouping took place whilst older individuals on average increased in the amount of %protein. However there was no significant difference in age groups when comparing the change in %protein from the month of regrouping to the month after regrouping (H=2.32, df=4, *p*=0.677), and there was no significant difference between age groups when comparing the change in %protein from the month before regrouping to the month after regrouping (H=3.92, df=4, *p*=0.416) (see table 5.4 for details).

Table 5.4 Average (± SEM) %protein change across the stages of regrouping** (the month before & the month of regrouping; the month of regrouping and the month after regrouping; and the month before regrouping compared to the month after regrouping)* ***between different ages of cattle.

Age (years)	Mean (± SEM) %protein before and when regrouping	Mean (± SEM) %protein when and after regrouping	Mean (± SEM) %protein before and after regrouping
<3	-0.01 ± 0.03	-0.08 ± 0.05	-0.02 ± 0.05
3-4	-0.02 ± 0.05	-0.02 ± 0.04	0.04 ± 0.04
4.1-5	-0.42 ± 0.39	-0.02 ± 0.05	0.00 ± 0.04
5.1-7	0.03 ± 0.03	0.03 ± 0.05	0.00 ± 0.08
>7	0.15 ± 0.04	-0.03 ± 0.05	0.14 ± 0.04

%Fat change was also not significantly affected by age when comparing the difference between the month before regrouping and the month of regrouping (H=2.75, df=4, p=0.601) and when comparing the difference between values for the month of regrouping and after regrouping (F=1.01, df=4, p=0.405). Although the difference between age groups across the time before and after regrouping was not significantly different (H=8.27, df=4, p=0.082) there was a trend towards significance with the youngest and oldest individuals dropping in %fat whilst those between the ages of three and seven years increased in their %fat (see table 5.5 for details).

Table 5.5 Average (± SEM) %fat change across the stages of regrouping** (the month before & the month of regrouping; the month of regrouping and the month after regrouping; and the month before regrouping compared to the month after regrouping)* ***between different ages of cattle.

Age (years)	Mean (± SEM) %fat before and month of regrouping	Mean (± SEM) %fat month of and after regrouping	Mean (± SEM) %fat before and after regrouping
<3	-0.07 ± 0.15	-0.06 ± 0.13	-0.17 ± 0.14
3-4	-0.11 ± 0.10	0.07 ± 0.08	0.17 ± 0.09
4.1-5	-0.04 ± 0.10	0.04 ± 0.12	0.08 ± 0.10
5.1-7	0.13 ± 0.20	-0.15 ± 0.17	0.07 ± 0.20
>7	0.13 ± 0.27	-0.37 ± 0.26	-0.22 ± 0.18

Age had a significant influence on the change in price paid (pence per litre, PPL) across time. There was a significant difference in price paid for milk between before and the month of regrouping time across the age categories (H=13.76, df=4, p=0.008), from the month of regrouping to the month after regrouping (H=25.14, df=4, p=0.000), and from the month before regrouping and the month after regrouping (H=22.29, df=4, p=0.000). Figure 5.9 demonstrates that cattle below 3 years of age had a decrease in the price that was paid for the milk compared to older cattle that appear to be more consistent in their average price paid per litre.

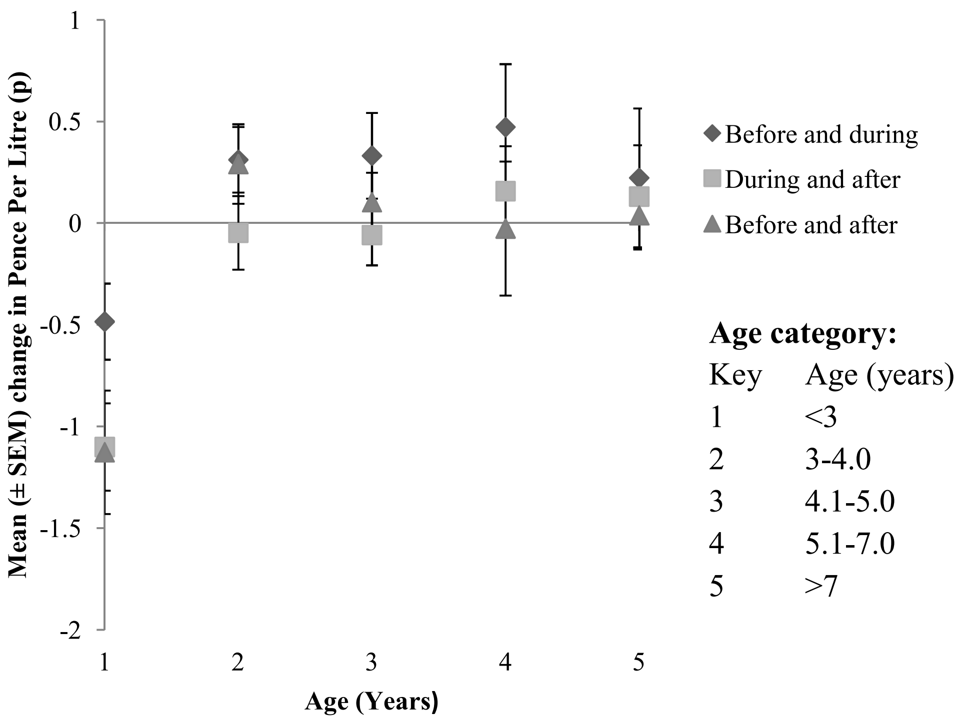


Figure 5.9. The relationship between age (years) and the average (± SEM) change in price paid per litre (Pence Per Litre; PPL) between the stages of regrouping *(the month before regrouping compared to the month of regrouping; the month of regrouping compared to the month after regrouping; and the month before regrouping compared to the month after regrouping).*

5.3.4 The influence of age on health parameters at regrouping

5.3.4a Somatic cell count

The degree of change on somatic cell count (SCC) between the months before regrouping to the months after regrouping was significantly different between age groups (H=17.51, df=4, p=0.002) with younger cattle (aged between 2 and 5 years) showing a decrease in SCC whilst older cattle (5 years or more) showed an increase in SCC. The change in SCC between the month before regrouping and the month of regrouping was not significantly different between age groups (H=1.49, df=4, p=0.829), and it was not significantly different between age groups from the month of regrouping to the month after regrouping (H=8.74, df=4, p=0.068), although there was a potential trend towards a significant difference with younger cattle dropping in SCC compared to older cattle.

5.3.4b Fertility

There was no significant difference in the number of inseminations given from the month before regrouping to the month of regrouping across the different age groups (H=6.81, df=4, p=0.146). There was no significant difference across the age groups in the number of inseminations given from the month of regrouping to the month after regrouping (H=1.09, df=4, p=0.895), and there was no significant difference on the number of inseminations given before the month of regrouping to the month after regrouping across the different age categories (H=1.11, df=4, p=0.892). In general older cattle required more inseminations than younger cattle but the difference in age groups was not significant (see figure 5.10).

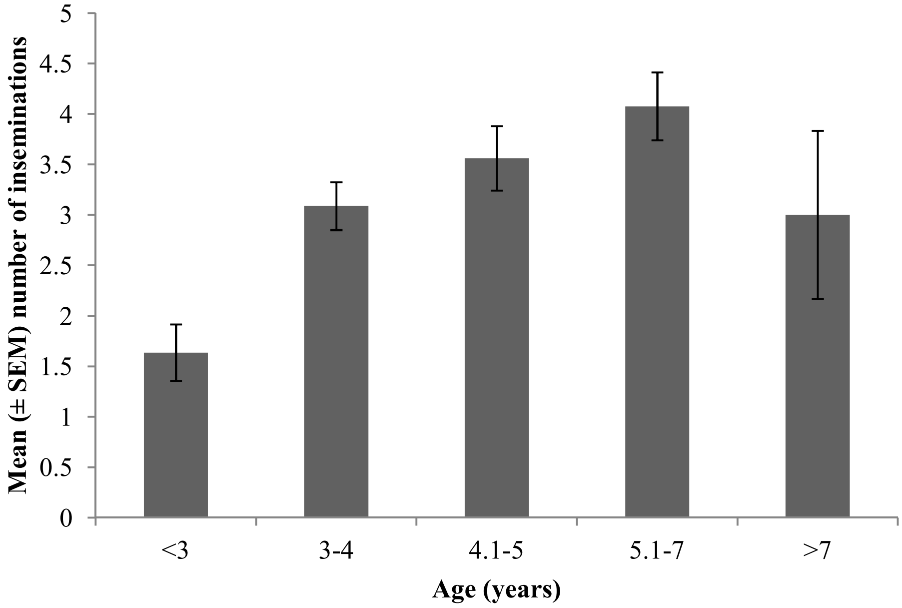


Figure 5.10. The mean (±SEM) number of inseminations cattle received across the different ages (years) of cattle when experiencing regrouping.

5.4 Discussion

5.4.1 The effects of age on bond formation at regrouping

Within this study age had a significant effect on bond formation. As cattle got older their mean association index score reduced and the number of preferred partners that cattle had also reduced. This relationship was also recognised in chapter two where heifers were more likely to have at least one or more preferred partners compared to cows. However, there was no difference in the number of partners that heifers and cows had on farm B which is likely due to the very weak bonds seen on the commercial unit (farm B) compared to on the rearing unit (farm A). These results are similar to Raussi *et al.* (2010) who observed younger cattle, in particular calves that have been together from an early age, to have stronger bonds than cattle grouped later on in life. Calves tended to stay closer together and perform less aggression towards one another compared to cattle that were grouped later in life, even after regrouping (Raussi *et al.*, 2010).

As calves reared in commercial conditions are separated very early on from their mothers and then grouped with animals of very similar age, they are likely to replace the mother attachment figure with an alternative, their peers (Veissier & Le Neindre, 1989). This is a process that naturally occurs at the time of weaning where cows will start to prevent their calves from suckling more often and thus calves' attraction to other calves increases, making peers an important part of a young calf's social group (Veissier *et al.*, 1990a). However, after repeated regrouping and relocation calves show a lack of interest in unfamiliar calves and reduce the amount of sniffing of unfamiliar calves as they are regrouped (Veissier *et al.*, 2001). This has been suggested as a sign that calves give up forming relationships with others due to the repeated regrouping and relocation (Færevik *et al.*, 2006). This may also be the case with cattle on commercial dairy units. As animals age they experience more regrouping and relocation as they move through the different groups and so give up forming strong associations and so a number of weak associations are evident as was seen in chapter two.

As cattle aged and experienced changes in their social environments, their mean association index score fell. This fall in association indices appeared to be more severe in younger cattle compared to older cattle. This result appeared after cattle had experienced the long term separation from preferred group mates that occurred in chapter four. This change in association indices would suggest that previous social relationships were not maintained after the long term separation. Younger cattle started with higher mean association index values and so this may be one of the reasons why such a dramatic fall in mean association index values was seen. Furthermore, younger cattle started off in much smaller groups and as they aged they were mixed in to a much larger group; in observation one younger cattle were in much smaller groups than they were in observation two. Smaller groups tend to be more stable and due to a lower number of potential partners, bonds may be stronger in smaller groups (Schweitzer *et al.*, 2011). However, this is in contrast to the results observed by Færevik *et al.* (2007); where calves in larger groups (16) compared to smaller groups sizes (8) were found to be in closer proximity with others and appeared to have a preference for a familiar individual when in a larger group. This may however be due to cattle requiring more support in larger groups in order to gain access to resources and the two group sizes compared by Færevik *et al.* (2007) were still both small compared to the group sizes on farm A and farm B.

The fall in mean association index value as cattle aged would suggest that even from an early age, as cattle experience change in their social environment they will change their sociability. A fall in mean association scores from one observation to the next would suggest that cattle rarely held onto the same bonds as from their previous observations and were associating more randomly than they had done before in smaller groups. This may be a part of the regrouping process and in order to adjust to such a dynamic social environment cattle have to amend the associations that they make. This process of change in social associations may get easier as cattle age as cattle give up forming preferential partnerships and so adapt to a more solitary lifestyle under commercial conditions.

5.4.2 *The effects of age on behaviour at regrouping*

After cattle had experienced long term separation from preferred group mates they were reunited with their home group which would have involved some elements of regrouping as over this time it was evident that social relationships had changed as was seen in the fall in mean association indices. For the period where regrouping had taken place there were differences in eating behaviour, standing, and being alert between the different age groups. Younger cattle spent less time eating and more time standing compared to their older counterparts and younger animals were more alert than older individuals when in separate groups and so was not an artefact of dominance. This change in behaviour between different aged animals may suggest that younger individuals may be more sensitive to changes in their social environment and so may benefit more from stable groupings and where this is not possible, the presence of a preferred partner during times of stress may help them to cope with the change. In contrast older individuals appear to no longer respond to this regrouping process. This may be due to habituation to such practices; however due to older individuals unlikely to have a preferred partnership, the effects of separation and regrouping are reduced.

There was no significant difference between animals of different ages and the number of negative behaviours seen. This is in contrast to results often reported for regrouping adult cattle where there is an increase in the amount of aggression shown at regrouping (Brakel & Leis, 1976; Hasegawa *et al.*, 1997; Mench *et al.*, 1990). This may suggest that cattle were familiar enough with those that they were reunited with that it reduced the negative effects of regrouping. This would support the need for relatively stable groups and to avoid repeated regrouping of animals so that they are able to become familiar with the remainder of the group.

Ninety-three percent of all allogrooming took place between animals of the same age group with the remaining 7% carried out between animals in different age groups. This would be expected due to the groups consisting of cattle of similar ages, although individuals could access others of different ages through barriers on both farms. Youngsters performed and received the most allogrooming. As grooming is thought to be an indicator of positive associations, the large amount of grooming to take place between the same age groups and within the youngster age category in particular, strengthens the results observed regarding their social bond formation. The strongest associations were seen in younger cattle and so younger individuals may form these bonds through positive behaviour like allogrooming. Observing both strong bonds and a high level of positive social behaviour in young animals would suggest that at this age, the social environment that they are in becomes highly important to them and they will work to maintain those relationships through behaviours such as allogrooming. Further investigation into the social relationships of cattle at this age may help identify certain time points where sociability levels change and so understanding of how this may affect their behaviour and welfare can be found. It appeared that cattle aged between 7-11 months of age had the most changes in their social behaviour and welfare due to regrouping.

In addition to the influence of age, the social associations and in particular the number of preferred partners that cattle had significantly affected both the duration and frequency of allogrooming bouts. The more partners that individuals had the fewer bouts of grooming occurred. The highest mean duration of allogrooming was seen in individuals that had between four and eight preferred partners whilst the lowest was seen for individuals that had between zero and three preferred partners. In a short observation carried out on a small stable herd of beef cattle by the BBC (2010), it was noted that when the dominant individuals were removed from the group, one particular individual, that was lower in age to the cattle removed, would frequently walk around the group grooming multiple partners in what was thought to be a bid to change her position within the group. For the young cattle in the current study, the high level of grooming observed and the high number of preferred partners may suggest that social relationships may start to change as they mature and in particular between 7 and 11 months of age there appears to be a significant shift in the grooming and association behaviour. Establishing a position within the group and learning which relationships are the most important may begin during this period and therefore an increase in grooming behaviour may be a part of the formation of different relationships, both for the groomer and for those receiving the grooming. The function of grooming at this time needs further investigation as it could have different meanings for each individual depending upon their current social status. In addition, this is around the same time when feral cattle may start to wean their young ready for the new offspring to be born (Bouissou *et al.*, 2001). It could be suggested that at this age the social group and structure of that group is of high import to cattle and so measures to ensure that groups remain relatively stable should be implemented at this time so that affiliative bonds can be formed.

5.4.3 The effects of age on production at regrouping

The age of cattle significantly affected the changes that occurred in average milk volume (yield) per milking and the overall amount of milk produced (kg) at regrouping. Younger cattle decreased production levels, both yield and kg, less than older individuals. As cattle aged, the loss in yield that occurred due to regrouping increased.

As milk production increases with age (Khan & Shook, 1996; Løvendahl & Chagunda, 2011; Zanella *et al.*, 1998) older individuals will be producing more milk and so will have higher demands on their body (Brun-Lafleur *et al.*, 2010). Changes in diet have a higher degree of effect on milk yield for high producing cattle compared to lower producing cattle (Brun-Lafleur *et al.*, 2010). Thus older cattle, as higher producing individuals are more likely to have excessive strain on their body at the time of regrouping and therefore larger changes in yield may occur, compared to younger, lower producing cattle.

Conversely, other quality parameters decreased in younger cattle more significantly compared to older individuals. %Protein decreased as a result of regrouping in younger cattle whilst it increased in older cattle. Brun-Lafleur *et al.* (2010) noted that primiparous individuals decreased their protein yield and protein content more than multiparous cattle in response to changes in their diet. They suggested that this might be due to primiparous cattle having higher energy demands for growth and maintenance compared to multiparous cattle. As regrouping often results in a decrease in time spent at the feeder and a decrease in time spent resting (Hasegawa *et al.*, 1997; O'Driscoll *et al.*, 2006; von Keyserlingk *et al.*, 2008), energy intake is likely to be lower and therefore production is likely to suffer. The change that occurred at regrouping may be because younger cattle needed more protein for maintenance compared to older cattle and so the influence of regrouping stress may have impacted further on them than on older individuals. The change in %protein only occurred when comparing %protein from the month before regrouping and the month of regrouping. There was no significant difference in age groups and the change in %protein when comparing the month of regrouping to the month after regrouping, and when comparing the overall change from the month before regrouping to the month after regrouping. This would suggest that %protein changed when regrouping occurred but the effects did not last long and levels returned to those from before regrouping by the month after regrouping. This is in agreement with chapter three which highlighted separation to be the main influence on animal welfare. In addition, a stable hierarchy, indicated by a reduced amount of aggression, is often achieved between 15 (Hasegawa *et al.*, 1997) and 45 (Sato *et al.*, 1990) days after regrouping has occurred. This may have allowed younger cattle to return to protein levels seen before the regrouping took place relatively soon after regrouping as they were able to find a position to feed and therefore restore their energy requirements.

The effect of regrouping on %fat was not significantly different between age groups. There was however a trend for middle aged cattle to drop slightly in their %fat in the month after regrouping when compared to %fat of the month before regrouping. These results would suggest that quality parameters may not be significantly affected by the regrouping process and so the effect on yield should be of most concern. In addition, fat percentage is the least repeatable trait and variance in animals increases as lactation progresses (Løvendahl & Chagunda, 2011) which was also seen in this study for most of the production parameters. Variability in composition of milk is influenced by biological factors, pathological and physiological changes (Forsbäck *et al.*, 2010) and so changes that may come about due to the regrouping process may be highly variable between individuals and so this may explain why no significant changes were observed in fat percentage change.

The change in price paid per litre of milk when regrouping took place was significantly different between age groups with younger cattle (less than three years of age) having a decrease in the price that was paid for their milk between all stages of regrouping compared to older cattle. The price (pence per litre) paid for the milk that younger cattle produced before regrouping took place was higher than the price they were paid for the milk they produced after regrouping. This may be due to the changes in the quality parameters that were observed previously; however, as they did not decrease the amount of milk that they produced as much compared to older cattle, this change in price paid per litre may not have a significant overall economic effect on the producer. Further research would be required to fully understand the impact of these results on the economic outcome for the producer.

5.4.4 The effects of age on health at regrouping

Younger cattle showed a decrease in somatic cell count due to regrouping, whilst older cattle showed an increase in somatic cell count. Younger cattle may have been regrouped because of a high somatic cell count as the decrease was observed between the months before regrouping took place and the month after regrouping. This would suggest that regrouping took place due to a high somatic cell count level and therefore after receiving treatment a drop in somatic cell counts would have been observed. Whereas for older cattle, an increase in somatic cell count appeared between the months before regrouping and the month after regrouping took place. This would suggest that the somatic cell count was higher the month after regrouping compared to before and so regrouping did not take place due to a high number of somatic cells in older cattle. This would suggest that the high somatic cell count level observed was likely due to the regrouping process. Previous research by Haskell *et al.* (2009) also found that somatic cell counts increased with increasing lactation number. These results together would suggest that as cattle aged they naturally increased in somatic cell count numbers as part of the increasing number of lactations.

Although there was no significant difference in the number of inseminations required across the age groups as a result of regrouping, in general older cattle required more inseminations than younger cattle. This would be expected, as cattle age their fertility lowers and thus is one of the most common causes of early culling in cattle (Brickell & Wathes, 2011).

5.4.5 Practical implications

Regrouping is a regular occurrence on larger dairy farms as producers try to manage a group rather than an individual to aid efficiency (St-Pierre & Thraen, 1999). However, there are a number of consequences to this management system; keeping the number of regroupings as low as possible will reduce the negative consequences observed in this study and the previous studies for this project. Where regroupings need to take place, providing familiar individuals to others during this process may reduce some of these negative consequences. Furthermore, if preferred partnerships have been identified, keeping these individuals together at such a stressful time will also reduce these negative consequences and provide some support, thus improving the animal's welfare.

Keeping similar aged cattle together allows for stronger bond formation and for the performance of positive behaviours such as allogrooming. The results would suggest that cattle of similar ages should be housed together and for their groups to remain stable, but also that age of cattle impacts upon their response to changes in their social environment and so this must be considered. In particular younger cattle require extra care when regrouping or making any changes to their social groups. When maturing from a calf to a sub-adult, young cattle appear to have a significant change in their social structure and extra care needs to be taken at this time not to disturb groups as much as possible. Although older cattle appear to not be affected by regrouping as much as some of the younger individuals, they have far fewer positive associations and are more sensitive to changes in terms of production compared to younger individuals. Care therefore needs to be taken when moving older cattle between groups on a regular basis so as to avoid significant losses in production and to ensure positive interactions and associations that will improve psychological well-being.

5.5 Conclusion

In summary, this study has shown age to have significant effects on how animals appear to cope with changes in their social environment. When regrouping occurs, changes are exhibited in all animals but younger individuals appear to be more sensitive to the effects and therefore extra precautions should be taken for these individuals. However this does not appear to be the case in terms of production in that younger cattle appeared to be less affected by regrouping compared to older cattle in this case. Although the results are from two different groups, they do give a better understanding of the factors that might affect the changes that occur at regrouping. Having a better understanding of these factors allows for enhanced solutions to be put into place so that the welfare of the cattle can be improved. Furthermore, the results highlight the complexity of the affects that changes in social environment have in cattle at a range of ages, and that thought needs to be taken when regrouping animals at all stages of their life.

Chapter Six

General Discussion

6.1 Introduction

The UK dairy industry has faced a number of challenges in recent years including changes in policies, abolition of milk quotas, lowering milk prices, and an increase in competition from abroad. There has, however, also been an increase in the global demand for milk products. Coupled together, these factors have prompted a change in the way dairy products are produced which has lead to a further increase in intensification. In order for the UK dairy industry to thrive in such a dynamic climate, large-scale dairies may be the way forward (EFRAC, 2011). With the majority of dairy cattle in the UK currently housed in already large and dynamic group systems, there has been a need to further the understanding of such management practices. To ensure that the welfare of dairy cattle is not compromised under such intensive systems a better understanding of the impacts they may have on the behaviour and welfare of dairy was required. In particular, the social behaviour of cattle, which has lacked serious consideration when designing intensive housing for cattle, has been highlighted by the European Food Safety Authority (EFSA, 2012) as a high welfare concern. This is concurrent with the report made by the FAWC (2009) that also raised concerns regarding the continuous regrouping of dairy cattle. A project investigating the potential effects of current large dynamic group management systems on the social behaviour and positive social relationships in dairy cattle, would assist in ensuring high standards of welfare are maintained and that positive welfare indicators can be utilised on farm.

The overall aim of this thesis was to assess the impacts of large dynamic group systems on the social bonds of dairy cattle and to evaluate how the formation and breaking of these bonds under such dynamic management systems affects the behaviour, welfare and productivity of the dairy cow. There were four main areas of the study; 1) to ascertain whether dairy cattle living in large dynamic group systems were able to form social bonds; 2) to analyse the effects of short term social isolation from preferred group mates on behaviour, welfare and productivity; 3) to analyse the effects of long term social separation from preferred group mates followed by the reunion with those preferred group mates after long term separation, on behaviour, welfare and productivity; and 4) to evaluate how age affects the formation of social bonds, behaviour, welfare and productivity, at regrouping. From these four objectives practical recommendations that could be instigated into commercial practice were proposed throughout each of the respective chapters.

This chapter aims to provide an overall summary of the results presented in previous chapters demonstrating their significance and relationship to current scientific knowledge. Furthermore, it will highlight areas that have raised further questions over the social behaviour of dairy cattle and recommend further investigation where needed. By using the overall results, practical recommendations are put forward that can be easily implemented on farm.

6.2 Social bonds in dairy cattle

The first aim of the project was to ascertain whether dairy cattle were able to form social bonds while housed in large dynamic group systems. Social bonds in adult cattle have been previously noted in feral populations (Lazo, 1994; 1995; Reinhardt & Reinhardt, 1982; Reinhardt & Reinhardt, 1981) but had not been reported for cattle in commercial dairy units. The results from chapter two established that these positive social relationships did exist in commercial dairy cattle that were being housed in larger than average, dynamic groups. However, the relationships observed were mainly weak and strong associations were rare. In addition, relationships were more likely to be observed in younger cattle. Chapter five also verified the existence of social bonds in dairy cattle and further highlighted that the age of cattle affected the strength of the bonds that they had. The older cattle became the fewer bonds they had and the weaker those bonds were. Nevertheless, it was also demonstrated within chapter five that the separation of bonded individuals, and subsequent regrouping, changed this important social relationship with a sharp decrease in association value after regrouping had taken place.

Where allogrooming has been considered a potential sign of a significant social bond, the performance of this behaviour has been reported at very low frequencies in the commercial setting (Takeda *et al.*, 2000; Val-Laillet *et al.*, 2009). The results from chapter four and five concur that allogrooming frequency is fairly low under commercial settings and this may explain the lack of strong bonds in commercial dairy cattle that were noted. The results from chapter four show that allogrooming took place more frequently when cattle were in stable groups compared to when either separated from their preferred partner or when they experienced multiple regroupings. This supports the hypothesis that allogrooming is an important aspect of the social behaviour of dairy cattle and in particular for the formation and maintenance of social bonds. Furthermore, in chapter five animals aged between 7 and 11 months old performed a significantly higher amount of allogrooming than others. At this age (7-11 months of age) cattle also had a high number of preferred partners. The results may suggest that during this time cattle are forming social bonds that are important to them. Any regrouping taking place after this age has been demonstrated to have negative consequences on cattle in that they do not habituate to repeated regrouping and relocation (Raussi *et al.*, 2005). To ensure the performance of positive interactions and the continuation of these important social bonds, every effort must be made to maintain cattle in stable groups after 7-11 months of age.

6.3 Social separation and reunion

The process of separating cattle from their preferred group mates led to changes in their behaviour, health, welfare and productivity. For short term separation in chapter three, cattle showed a stress response similar to that seen in other separation and isolation observations (Boissy & Le Neindre, 1997; Piller *et al.*, 1999; von Keyserlingk *et al.*, 2008). Boissy and Le Neindre (1997) had previously noted that the presence of a familiar animal reduced the stress response observed in cattle isolated from their peers. The results from chapter three further this knowledge to show that the presence of a preferred partner can provide more support during a stressful time than that provided by just a familiar individual. The results also highlighted individual differences in the coping ability of cattle with those individuals identified as having a preferred partner having a higher stress response to the separation than cattle that had not been identified as having a preferred partner. These cattle with a lower stress response were either a partner of the focal individual or were a random member of the herd. This raises questions regarding the temperament of cattle and whether there may be individuals that require more social support during environmental challenge than others. Le Neindre and Sourd, (1984) had previously noted differences in the behavioural response of Salers and Friesians during social isolation suggesting that Friesians did not appear to be as affected by the isolation from the social group compared to Salers. In addition, the impacts on the supporting individuals of cattle that require more support than others needs to be investigated as the results from chapter three suggested negative consequences to the individual that was undertaking the supporting position.

The results from chapter four demonstrate that long term separation also had negative consequences on cattle's behaviour, health, welfare and productivity. Social bonds that had been previously identified appeared to have been broken after long term separation had occurred. Additionally, upon reunion cattle did not appear to regain their social relationships, reflected in their reduced social association index value. This suggested that cattle were not as sociable after the separation period compared to before separation had occurred. Further analysis of these results in relation to the age of cattle in chapter five showed that there appeared to be different coping abilities among cattle that were influenced by their age. However, the results were somewhat complex showing different responses for different age groups. Younger cattle had significant changes occur in their behaviour during the separation and regrouping process and their health with regards to weight was more significantly affected than older cattle. However with regards to production parameters, the older cattle showed a higher response with a more pronounced loss of production due to the separation and regrouping process.

The awareness of the negative effects that common management practices such as regrouping have on dairy cattle raises concerns regarding the separation of bonded individuals. The results from both chapter four and five argue that it is the separation of individuals that is the main source of stress and not the regrouping process as has been so readily focused upon in past research. When cattle were reunited with their preferred group mates where similar elements to regrouping took place, there was a limited response compared to when cattle were only separated from their bonded partner.

Furthermore, chapter four demonstrates that production parameters were affected more by the separation procedure compared to when they were reunited with their partner. As both separation and reunion contained elements of regrouping, the evidence for whether it is the separation of individuals causing the negative effects or the regrouping phase is not clear. After long term separation positive social bonds are no longer present and so preferred partners can offer no support during this time, a result that has been seen in this study as well as in the studies by Gygax *et al.* (2009) and Neisen *et al.* (2009b). Therefore, preferred partners are no longer able to help cattle cope with changes in their social environment.

6.4 Welfare implications

The ability to express natural behaviour is imperative to maintaining high standards of welfare (Spinka, 2006; Wechsler & Lea, 2007). When animals are able to perform highly motivated behaviours, for example rooting in pigs and dust bathing in hens, it stimulates positive emotional circuits within their brains (Edwards, 2010). Being able to experience positive emotional states can enhance animal welfare and promote the welfare of sentient beings (Spinka, 2006). The housing systems employed by the majority of dairy producers in the UK have, unfortunately, seriously lacked consideration towards the social behaviour of cattle and this has been one of the most affected behaviours by domestication and intensification (Phillips, 2002). Where it may be that large-scale dairies can provide adequate health programs that ensure the physical health of their animals involved, there is no current evidence on the psychological well-being of cattle. How these housing systems and the lack of thought given to the social organisation and behaviour of cattle may have impacted upon their psychological well-being has now only just been investigated through this study.

Allowing and promoting positive social bonds and allowing for positive social behaviours such as allogrooming will allow animals to experience positive affective states and so enable a better coping ability and therefore a better quality of life, and this has been demonstrated throughout the previous chapters. Through providing adequate space and stable social groupings, the size of a group may not be significant to the social behaviour and welfare of cattle and will still allow for highly motivated social behaviours to be performed. It is the predictability and a sense of controllability about the social environment that will be important, and as a result cattle will have higher positive expectations (Veissier & Boissy, 2007). Evidence from chapter five suggests that cattle appear to 'give-up' forming social bonds and so may have a current sense of negative expectation about their social environment. As it appears to be the separation of these important bonds that brings about a higher response suggestive of stress, this negative emotional state that cattle experience may prohibit further bond formation and thus along with it, the positive affective state of the animal. Therefore dairy cattle experiencing repeated regrouping and separation may have negative emotional welfare which exhibits itself through a change in behaviour, health and productivity. Stable groupings will provide support and reduce negative affective states whilst promoting positive experiences such as allogrooming and thus aiding coping ability and promoting a better quality of life.

6.5 Practical solutions

In order to improve the welfare of dairy cattle and to prevent negative social experiences that may be associated with large-scale dairies, the social behaviour and structure of a herd must be considered. Producers need to assess current facilities and resources in order to find a solution that will work for their herd as the management of a farm with little regrouping will require careful planning. However, there are crucial aspects of the results that must be considered when assessing housing requirements of individual herds. The main aspect of this report, along with both the FAWC and EFSA reports, is the reduction in the number of separations due to regrouping. This will in turn aid the formation of a stable group and thus provide a constant and secure social environment. Where regroupings cannot be avoided, providing a supportive partner will reduce the negative consequences of regrouping and if that partner is a preferred partner, it will aid the transition further and reduce the affects of separation improving positive emotional welfare. Furthermore, if separations do occur for reasons such as ill health, where separation may be longer than two weeks provision again of a supporting partner will reduce separation stress and may aid recovery. More than two weeks apart appears to be a time point when bonds are broken, although this needs further investigation to confirm this time frame.

There is a need to assess individual characteristics and coping abilities. Temperament testing of cattle may enable identification of individuals that may be a source of social disruption and those that appear to be more 'needy' in their coping abilities (see Gibbons *et al.* (2010) for methods of temperament testing that can be easily carried out on farm). The breeding of cattle that do not require emotional social stability may be beneficial for herds that are likely to carry out regular and repetitive regrouping. Alternatively, identification of individuals that can provide support within groups may be a better method of enhancing positive welfare.

As age had significant affects on cattle's ability to cope with social challenges in differing ways, care needs to be taken throughout the animals' lives. Cattle between 7 and 11 months of age may be able to cope better with challenges such as regrouping as they appear to be in a period where bonds are formed. After this age social challenges become more problematic, and as cattle age and the presence of supportive positive bonds disappear, coping ability is reduced. Replacement heifers are central to the continuation of the dairy herd. If the social environment is carefully managed from this time, their ability to cope with challenges they may face later on such as herd integration, may be enhanced.

The occurrence of frequent allogrooming and the presence of strong, long lasting social bonds are an indicator of group stability and thus a positive indicator of welfare. The occurrence of such positive behaviours would indicate that cattle are not deprived of important sources of pleasure and that their other needs are being met (Boissy *et al.,* 2007). This would allow for a positive assessment of welfare to be given.

6.6 Future Research

Research into positive emotional states and positive social environments of cattle and other farm animals is limited and has long been overshadowed by research into the negative aspects of social behaviour such as aggression. This project has offered important positive social considerations of cattle housed in current large dynamic group systems. It does, however, raise further questions regarding the positive social behaviour in cattle and how current and future housing systems may affect their social behaviour and welfare. Knowledge and understanding of the affective states experienced by cattle during times of separation and regrouping along with those experienced during bond formation, is required.

Cattle do appear to be socially adaptable through the changes that occur in their social structure and individual relationships. Vasopressin receptor distribution patterns in the brain have been demonstrated to partly determine the social system of voles and may explain the plasticity of social bonds and social systems seen in this species (Nair & Young, 2006). Oxytocin, a neurosecretary hormone involved in affiliation behaviour has also been observed to increase prosocial choices and attention towards affiliates in rhesus macaques (Chang *et al.*, 2012). The link between oxytocin and milk let down (Belo & Bruckmaier, 2010) may have a significant part to play in bond formation and so scientific investigation into exactly how the bonds observed in this current project are formed, will allow potentially for easier identification of bonds between individuals. The pairing of individuals may be able to be manipulated so that positive social experiences can be felt during times when they may have previously formed stress.

Further investigation into the social networks of cattle in larger systems will also be beneficial, as will comparisons between systems with a more stable type of grouping such as automatic milking systems with a more dynamic group system where individuals need to be maintained in different social groups. Large-scale dairies may provide an opportunity to have more stable social groups with fewer separations as groups will be large enough that a number of animals are likely to be in sync with one another. In addition, the full influence of age on cattle's ability to cope with challenge and the changes that occur throughout the life of dairy cattle with regards their social behaviour will also be beneficial. Further study of particularly large groups such as those seen in the US, may give understanding to the full impacts of group size. The need for a preferred partner may no longer be required when cattle are in very large groups as there is evidence in other species that suggests that it is the number of animals around that provide support (Kikusui *et al.*, 2006).

6.7 Conclusions

In conclusion, this project has aided the understanding of dairy cattle social behaviour and the impact that large dynamic groups may have on the social behaviour and welfare of cattle. The main findings of the project are that dairy cattle are able to form positive associations under commercial practices, but that commonly utilised management techniques can have a serious consequence on these positive relationships. In particular there is a change in the strength and longevity of social bonds when separation and regrouping occurs. These relationships are more likely to form in younger cattle and are likely to be stronger in younger cattle. Serious consideration on the effects of management on the social behaviour of rearing stock, especially replacement heifers needs to be performed. These social relationships are able to provide support during social challenges such as short term separation. Long term separation from a preferred group partner, however, causes significant changes in the social behaviour and welfare of cattle. The separation and splitting of partners at regrouping has more serious negative consequences than the actual regrouping. It is thus extremely important that as suggested by the EFSA and FAWC that social bonds in cattle are given consideration when grouping animals and any form of separation and regrouping has to be very careful considered regarding the negative impacts that it has on the welfare of cattle. It may not be visible, except for where severe aggression may take place that the welfare of cattle is compromised by this process, as it is likely that the negative impacts are more psychological rather than physical.

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