Strategies for maximising health, welfare and performance in first lactation heifers

Final report for AgriSearch (Project D-40-08)

December 2011

Research team: Alastair Boyle, Niamh O'Connell and Conrad Ferris

Report prepared by Alastair Boyle and Niamh O'Connell

Agri-Food and Biosciences Institute, Agriculture Branch, Hillsborough, County Down, Northern Ireland BT26 6DR

LIST OF CONTENTS

EXECUTIVE SUMMARY

The overall objective of this research was to develop optimum management strategies for dairy herd replacements during the post-calving period, with a view to improving welfare and productivity. This involved investigating different regrouping strategies for freshly calved heifers, such as altering the time of day animals are mixed into the dairy herd, examining the effect of previous social experience with older animals, and determining if an extended 'recovery' period after calving improves welfare and productivity. In all experiments the animals' behaviour was recorded directly after mixing into the post-calving group and also after fresh feed was provided, and other welfare and performance parameters were also measured.

In Study 1, the influence of time of day on the welfare and production performance of primiparous cows was assessed. After calving, twenty-eight primiparous Holstein Friesian dairy cows were either introduced to an established group of resident cows between 06:00-08:00 hours ("AM") or between 16:00-18:00 hours ("PM"). The size of the resident group remained constant at 18 animals (12 multiparous cows and 6 primiparous cows).

Heifers in the AM treatment spent more time in receipt of aggressive behaviours such as threats ($P<0.05$), butts ($P<0.01$) and chases ($P<0.05$) immediately after mixing compared to those in the PM treatment. During the feeding periods, heifers in the AM treatment were observed feeding for longer ($P<0.05$), showed less pen exploration ($P<0.05$) and also received more butts ($P<0.05$). No significant treatment effects were shown on overall feed intake levels, milk yield, milk cortisol levels or on body weight or condition score loss. No significant treatment differences were shown in overall time spent lying, with heifers in both treatments lying for less than 4 hours during the first 24 hours in the group. Taking into consideration the reduction in received aggression and the lack of adverse effects on performance, these results suggest that primiparous cows should be introduced into the main dairy herd after evening milking.

The effect of previous social experience on the welfare and production performance of primiparous cows was assessed in Study 2. Twenty nulliparous Holstein Friesian dairy cattle were assigned to one of two treatments (‘Mixed’ and ‘Unmixed’). The ‘Mixed’ treatment involved housing experimental animals with non-lactating multiparous cows for three weeks prior to their expected calving date. In the ‘Unmixed’ treatment, experimental animals were housed with other nulliparous animals during the 3-week period prior to their expected calving date. During this pre-calving period animals in both treatments were housed in groups of 10 animals, with the ‘Mixed’ group comprising of seven multiparous non-lactating cows and three experimental nulliparous cows. The experimental animals were added to a resident group that contained 15 lactating animals (10 multiparous cows and 5 primiparous cows) within 24 h of calving.

‘Mixed’ animals received fewer butts ($P<0.05$) after mixing, and these animals also showed increased locomotion during this period ($P<0.05$). After feeding, ‘Mixed’ animals performed more ‘shouldering’ of other animals and increased locomotion ($P<0.05$), and received less butts ($P<0.001$). Also, during this period animals in the ‘Unmixed’ treatment were located in the cubicles to a greater extent ($P<0.01$). Animals in the ‘Mixed’ treatment were located to a greater extent in the front passage after feeding ($P<0.001$).

Overall, it appeared that giving primiparous cows experience with dry multiparous cows prior to calving improved their welfare when mixed into a group containing older animals. After calving it was suggested that increased levels of aggression and locomotion performed by ‘Mixed’ animals, along with increased time spent near the front passage, reflected increased ‘confidence’ levels.

In Study 3, the effect of being housed in a primiparous cow group after calving on the welfare and performance of animals when subsequently introduced into the main herd was examined. After calving, primiparous Holstein Friesian cows were either retained in a separate primiparous group for 2 weeks before being integrated with a 'resident' group containing mature cows ('Separate Group'), or were integrated with a resident group within one day of calving ('Direct Introduction'). The size of resident groups remained constant at 16 animals (10 multiparous cows and 6 primiparous cows).

Animals in the Separate Group treatment exhibited increased levels of exploratory behaviour, increased lying and were located more in the cubicles and less in the front passage of the pen upon introduction to the Resident group compared to animals in the Direct Introduction treatment ($P<0.01$). Animals in the Direct Introduction treatment received more butts ($P<0.05$) and showed increased durations of avoidance behaviour during this period ($P<0.05$). After feeding (where comparisons were standardised to weeks 3-6 of lactation) animals in the Direct Introduction treatment received significantly more butts ($P<0.05$) compared to Separate Group animals. Treatment had no significant effect on overall lying behaviour, with heifers in both treatments lying for less than 6 hours during the first 24 hours after introduction to the post-calving group. No significant treatment effects were shown on overall milk yield, milk cortisol levels or on weight or condition score loss.

The findings showed that retaining primiparous cows in a separate group for 2 weeks after calving led to them receiving significantly less aggression when mixed into the resident group. This reduction in received aggression appeared to continue after feeding, suggesting positive effects on welfare.

The effect of an extended time period in a straw pen after calving on the welfare and performance of animals when introduced into the main herd was assessed in Study 4. Primiparous cows were housed in a straw pen for a period of either 12-24 hours (Short Duration) or 36-48 hours (Long Duration) after calving. These experimental animals were then introduced to established 'resident' groups that contained 16 animals (10 multiparous cows and 6 primiparous cows).

After mixing into the resident group, animals in the Long Duration treatment spent a greater amount of time lying compared to those in the Short Duration treatment ($P<0.05$). Primiparous cows in the Long Duration treatment exhibited more butting ($P<0.05$) and exploratory behaviour ($P<0.05$) after mixing than those in the Short Duration treatment. No significant treatment effects were shown for milk yield, body weight or condition score loss.

Retaining primiparous cows in a straw pen for a 36-48 hour period after calving appeared to improve welfare through promoting lying behaviour in the post-calving period and when introduced into the resident group. Furthermore, these animals appeared to be more 'confident' and this was expressed through increased butting behaviour towards resident animals.

In summary, the research suggests that it is beneficial from a welfare perspective to introduce calved dairy heifers into a lactating group containing older animals after evening milking, and also to mix them with older animals prior to calving. Furthermore, retaining heifers in a straw-bedded calving pen for slightly longer than normal, and in a 'primiparous cow group' during the initial post-calving period also appears to be beneficial for welfare.

GENERAL INTRODUCTION

There has been increased research into devising optimum programmes for rearing dairy herd replacements in recent years, and this is probably due to the realisation that these animals represent the future of the dairy herd (Greter *et al.,* 2008). Producing high quality replacement dairy heifers efficiently is of integral importance to dairy farmers as replacement costs can represent 15-20% of total milk production costs (Heinrichs, 1993; Annexstad, 1986). Typically in the UK, farmers will have replacement rates of up to 30%, which, with a herd size of 100 cows, means 30 heifers are required annually (DairyCo, 2008). In Northern Ireland it costs £1220 to rear a dairy heifer to the point of calving (CAFRE, 2008). The average dairy cow herd size in Northern Ireland is 74 cows (DARD, 2008/09) and replacement rates are 28% (Mayne *et al.,* 2002), meaning that dairy farmers invest over £25,000 annually in replacement dairy heifers. This level of investment means that it is critical that the heifers are managed correctly during the pre- and post-calving periods to ensure optimal milk yield, continued growth and successful breeding.

The transition period in dairy cow production can be defined as the period from 3 weeks before to 3 weeks after parturition. This period is particularly important in terms of potentially dictating the future productivity, health and profitability of the animal (Drackley, 1999). During this period, newly-calved heifers have to deal with various stressors. These include the pain associated with calving and the subsequent removal of the calf (Hydbring *et al.,* 1999; Daniels *et al.,* 2007). Dairy animals often also experience metabolic stress after calving associated with moving from fibrous/forage diets to high energy lactation diets (Goff and Horst, 1997). Environmental changes are also important during this period. For example, entering the milking parlour for the first time is a stressful event for heifers, primarily due to the novelty of the situation and the close contact with stockpersons (Van Reenen *et al.,* 2002). These stresses are also often compounded by stress associated with entering a new housing environment and joining a new social group in the form of the main milking herd (Kondo and Hurnik, 1990; Knierim, 1999).

Evidence suggests that social stress associated with joining the main milking herd has a significant adverse effect on welfare and productivity (Bøe and Faerevik, 2003). For example, heifers mixed with cows after calving are often subject to high levels of aggression and show increased stress hormone levels and reduced milk yield (Krohn and Konggaard, 1982; Phelps, 1992; Phillips and Rind, 2001; Neisen *et al.,* 2009). The problems faced by heifers are often associated with the fact that they are smaller, more timid and of lower social rank than older animals (Sambraus, 1970; Lamb, 1976; Wierenga, 1990). In this situation, first parturition cows also have to compete with unfamiliar and more experienced cows for resources such as feeding places (Fraser and Broom, 1990; Gonzalez *et al.*, 2003).

One option to reduce problems faced by heifers when integrated with the main dairy herd is to house them separately during their first lactation, however this is not a viable option for many famers. There has been limited research investigating ways in which the welfare and productivity of heifers could be improved through altering the way in which they are introduced to the herd. For example, research by O'Connell *et al.* (2008) and Neisen *et al.* (2009) found welfare benefits when heifers were introduced to the dairy herd in pairs rather than as individuals.

However there has been a general lack of further, properly replicated, research to identify the effects of different methods of integrating heifers with the main herd on welfare and productivity.

The overall objective of this research was to develop optimum management strategies for dairy herd replacements, with a view to improving welfare and productivity. This involved conducting studies to identify the benefits of different regrouping strategies when integrating freshly calved primiparous cows into a group containing older animals.

The specific aim of the first study was to determine the effect of time of day when introducing primiparous cows into a group containing older cows ('resident group') on welfare and productivity. This was achieved through introducing these animals into the resident group either after morning or evening milking. The specific aim of the second study was to determine if prior experience of being housed with non-lactating cows improves welfare and productivity in primiparous cows when integrated into a group containing older cows after calving. This was attained by mixing nulliparous cows with non-lactating cows three weeks prior to calving. The specific aim of Studies 3 and 4 was to assess the effect of different 'recovery' periods after calving on welfare and productivity of primiparous animals. In Study 3 this was assessed through forming 'primiparous cow groups' for a 2-week period after calving before integrating animals with a group containing older animals. In Study 4 primiparous cows remained in their calving box (straw bedded) for either a 12-24 hour period or 36-48 hour period after calving before being integrated with a group containing older animals.

Study 1

Are there benefits in introducing dairy heifers to the main dairy herd in the evening rather than the morning?

Abstract

Twenty-eight primiparous Holstein Friesian dairy cows were assigned to one of two treatments after calving. These experimental heifers were introduced to an established group of resident cows either between 06:00-08:00 hours (i.e. after morning milking, "AM") or between 16:00-18:00 hours (i.e. after evening milking, "PM"). The size of the resident group remained constant at 18 animals (12 multiparous cows and 6 primiparous cows). There were five resident groups in total, and 2-3 non-experimental primiparous cows in each group were replaced by AM and by PM heifers as they calved. Fresh TMR was provided daily between 10:00 and 10:30 hours, and concentrate feed was offered in the milking parlour. The behaviour of the experimental heifers was assessed over a 2-hour period immediately after mixing into the resident group, and also after feed provision one day each week during the first month in the group. In addition, time spent lying was assessed each week for one month using data loggers attached over 24-hour periods. Lying and location of the group was assessed by direct observations during the 2-hour period prior to evening milking on 2 consecutive days each week for one month. The time spent feeding was recorded automatically using computerised feeding gates. Milk production, milk cortisol levels, and changes in body condition and live weight were assessed over the first month after calving. Heifers in the AM treatment spent more time in receipt of aggressive behaviours such as threats ($P<0.05$), butts ($P<0.01$) and chases ($P<0.05$) immediately after mixing compared to those in the PM treatment. During the feeding periods, heifers in the AM treatment were observed feeding for longer ($P<0.05$), showed less pen exploration ($P<0.05$) and also received more butts ($P<0.05$). No significant treatment effects were shown on overall feed intake levels, milk yield, milk cortisol levels or on body weight or condition score loss. However feed intakes were higher in the AM treatment during the second week after mixing ($P<0.05$), and automated recordings showed that AM animals spent longer average periods of time feeding ($P<0.01$). No significant treatment differences were shown in overall time spent lying, with heifers in both treatments lying for less than 4 hours during the first 24 hours in the group. The PM heifers showed reduced lying behaviour compared to resident animals or AM heifers during evening observations ($P<0.01$). In conclusion, the reduction in received aggression and the lack of adverse effects on performance (milk production, weight/condition loss) suggest that heifers should be introduced to the main dairy herd after evening rather than morning milking. Further research to determine the relative importance of time of day and time interval since feeding on behaviour immediately after mixing would be beneficial.

Introduction

The regrouping of dairy cows can cause an increase in aggressive behaviour, with negative effects on welfare and productivity. For example, Brakel and Leis (1976) and Kondo and Hurnik (1990) observed an increase in agonistic contests in dairy cows during the 1-2 hour period after regrouping, with agonistic interactions almost doubling in the regrouped cows to 9.6 per cow/hour compared to 4.9 per cow/hour prior to regrouping. Decreases in milk yields have also been observed due to regrouping, with daily yields declining from 43.4 kg to 39.7 kg (von Keyserlingk *et al.,* 2008). The negative effects of regrouping can be exacerbated for dairy heifers when they are being integrated into the milking herd for the first time, due to timidity and lower social ranking than the older cows (Lamb, 1976; Wierenga, 1990; Gonzalez *et al.,* 2003). Thus, in addition to suffering high levels of aggression, these heifers might also have difficulty competing for feeding and lying places (Schein and Fohrman, 1955; Gonzalez *et al.,* 2003).

Welfare and productivity can be improved by altering the way in which heifers are introduced to the milking herd. For example, introducing heifers as pairs rather than as individuals has been shown to reduce aggression (Neisen *et al.,* 2009). However, this option may not be practical for commercial producers, and other aspects of regrouping strategy should be investigated. Lamb (1976) suggested that introducing animals to the main milking herd in the evening may reduce levels of aggression. This agrees with data from sows showing reduced levels of aggression when regrouped in the evening rather than morning (Csermely and Wood-Gush, 1987; Barnett *et al.,* 1996).

The aim of this study was to assess the effects of introducing recently-calved heifers into the milking herd (12 multiparous cows and 6 primiparous cows) either after morning or evening milking on measures of welfare and performance.

Material and methods

Animals, Management and Housing

Twenty-eight Holstein Friesian (**HF**) heifers were used as experimental animals in this study. These heifers had been reared in groups with cubicles from 7 weeks of age, with the exception of the period from April-September (inclusive) when they were at pasture. Experimental heifers were moved to the main dairy unit approximately one month prior to calving. They were moved in small batches according to expected calving date and added to a dynamic group kept in cubicle housing. The size of this group ranged from 15 to 20 heifers and animals in this group had access to 20 cubicles and 7 Calan gate feed boxes. Heifers were moved to individual straw-bedded calving pens (5.9 × 3.3 m) prior to calving (based on predicted calving dates and daily assessments by experienced stockpersons). Discretionary calving assistance was given. After birth, the calves remained with their dams for 6-12 hours. All heifers were housed individually with their calves during calving and the subsequent suckling period. The heifers calved between September 2008 and January 2009 (at an average age of 25 months).

Experimental pens had 19 cubicles in 3 rows and solid concrete floors (Figure 1). The cubicles (2.17 m long and 1.21 m wide) had rubber mats and were bedded with sawdust. Concrete passageway floors were cleaned a minimum of 4 times daily using an automated scraper. Feed was accessed via one of six Calan gates and animals were able to access feed from any of the gates.

During the treatment period heifers were offered TMR *ad libitum* that comprised 60% concentrate and 40% forage. This was offered between 10.00 and 10.30 hours. The forage component of the diet consisted of 60% grass silage and 40% maize silage on a DM basis. Heifers were offered 1.0 kg of concentrate in the milking parlour (0.5 kg in the morning and evening). Heifers were milked in a 50 point rotary parlour twice daily (at approximately 06:00 and 16:00 hours).

Treatments and Experimental Groups

The present experiment included two treatments: AM: Heifers (n = 14) added to the resident group after morning milking (between 06:00-08:00 hours); and PM: Heifers (n = 14) added to the resident group after evening milking (between 16:00-18:00 hours).

Treatments were balanced for genetic merit (Predicted Transmitting Ability (PTA) for kg fat + protein, kg fat and kg protein), sire, pre-calving body weight and body condition score. A total of five replicate or 'resident' groups were used, with each group being established 5 days before the first experimental heifer was introduced (please refer to Figure 1). At establishment, each resident group contained twelve mature HF cows (average 149 DIM; range 2-9 lactations) and six primiparous non-experimental HF heifers. The group size and cow:heifer ratio within the resident group was maintained throughout the study by removing a non-experimental heifer from the group prior to each experimental heifer being added. The process of replacing all 6 non-experimental animals with experimental animals was completed over an average period of 20 days across all replicates. In replicates 1 to 4 three non-experimental heifers were replaced by animals in the AM treatment, and three by animals in the PM treatment. In replicate 5, two non-experimental animals were replaced by animals in the AM treatment and two by animals in the PM treatment.

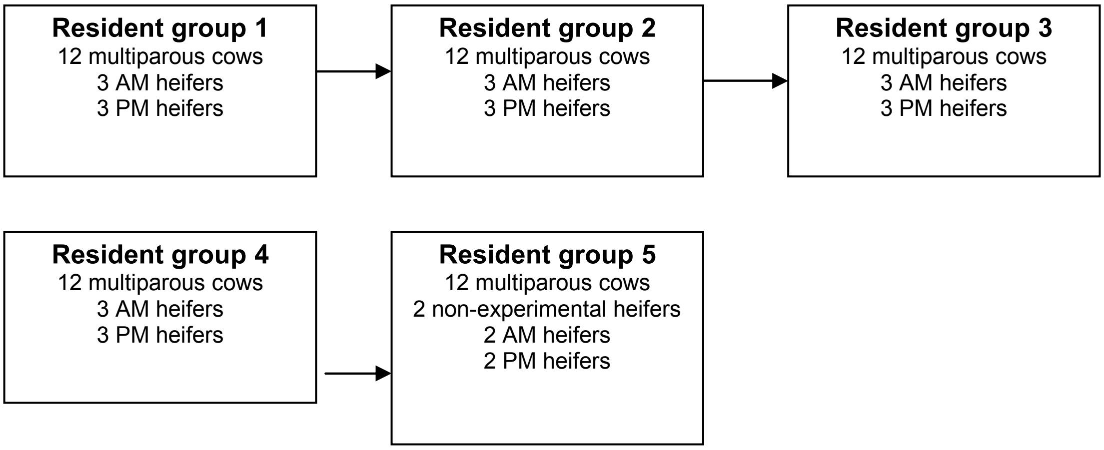

Figure 1 Structure of resident groups (Study 1)

In both treatments, heifers were introduced to the group approximately 30 minutes after all animals in the resident group had returned from milking. Non-experimental heifers were removed from the resident group immediately prior to either the AM or PM milking (e.g. non-experimental heifers were removed from the group prior to AM milking when AM heifers were being added to the group). In each resident group no more than one experimental heifer was introduced on a particular day. The interval being successive heifers being introduced to the group ranged from 1 to 7 days, therefore there was continual disruption to the social group throughout the experimental period. During this period, the order in which experimental animals were introduced into the resident groups was balanced across treatments.

Measurements

Behaviour after mixing and feeding

Each experimental heifer was observed for a continuous 2-hour period immediately after joining the resident group. In addition, each heifer was observed during four 5-minute periods (at 30 minute intervals) during the 2-hour period after feeding on one day each week for one month after introduction to the group. All animals were fed between the hours of 10:00-10:30.

Behaviour was recorded by direct observation using a handheld data recorder (Psion Organiser II, Model LZ64, Noldus Information Technology, The Netherlands) using the ethogram shown in Table 1. The ethogram contained four mutually-exclusive categories of behaviour: the state of the animal (in terms of lying or standing), the location of the animal in the pen, and behaviours performed by the animal ("activity") and received by the animal. The duration and frequency were recorded for all aggressive behaviours performed (i.e. head to head contact, butting, threatening, chasing, shouldering, avoiding) and for all received behaviours. Only the duration for the state, location and all other "activity" behaviours was recorded.

Lying behaviour

Direct observations of lying and standing behaviour, and of location of experimental heifers in the pen, were performed. These observations were used to assess lying locations of heifers. The group was scanned at 10-minute intervals for 2 hours (13:00-15:00 hours) on two consecutive days each week of the trial. In each scan, the state (i.e. lying or standing) and location of each animal in the group was recorded. Locations recorded included feed boxes, front cubicles, middle cubicles, rear cubicles, front passageway, side passageway and rear passageway (see Figure 2). These observations finished approximately one hour before PM milking (Wierenga and Hopster, 1990; Dippel *et al.,* 2004).

Data loggers (DL) (Tinytag Plus, Re-Ed volt, Gemini data loggers (UK) Ltd., Chichester, UK) were used to record the time spent lying by experimental heifers. Loggers were fitted below the hock of the right hind leg of each experimental animal while in the milking parlour, and were secured using Vetwrap™ bandage (Andover, Healthcare, Massachusetts, USA). Lying behaviour was monitored for 24 hours after heifers were introduced to the group and also for one 24-hour period per week in the following month. The data loggers were programmed to record whether the animal was lying or standing at 30-second intervals and validated. They were validated on four occasions during each 24-hour measurement period, with two lying/standing bouts being recorded by direct observations over a period of 2 hours. Data indicating lying bouts of less than two minutes were regarded as anomalies and converted into standing periods (as suggested by Blackie *et al.,* 2006).

Feeding behaviour

Feeding behaviour of each heifer was recorded continuously from introduction to the group until day 28. Feeding behaviour was recorded using the electronic Calan gate system (American Calan; NH, USA) with each gate allowing access to a feed box (length 120 cm, depth 104 cm, width at top 118 cm, width at base 63 cm) mounted on a weigh scale and linked to an automated cow identification system (Griffith Elder; Bury St Edmunds, UK). Opening of the Calan gates was controlled by a transponder fitted to the neck collar of each animal. Experimental heifers were habituated to this system approximately one month prior to calving. Data from the Calan gates were used to calculate individual dry matter intake (DMI), number of meals/day, average duration of each meal, time spent eating and intake rates. Any interval of <6 min between the end of a previous intake period and the beginning of a subsequent intake period was ignored and the data were treated as one continuous meal (Patterson *et al.,* 1998).

Milk cortisol

Milk samples for cortisol analysis were taken from each animal on a weekly basis for a period of one month after introduction to the resident group. Samples were collected during the AM and PM milking of each sampling day, and these samples were assayed separately. Milk samples were refrigerated at 4°C after collection and de-fatted. Milk samples were de-fatted by centrifugation (2500 rpm, 20 mins @ 4°C) within 4 hours of collection, and the skim milk fraction stored at -20°C until analysis (Verkerk *et al.,* 1996; Fukasawa *et al.,* 2008).

Milk cortisol concentrations were measured using a commercial ELISA kit for cortisol (Cortisol EA65, Oxford Biomedical Research, Inc., Oxford, MI, USA). Samples were prepared as follows: 100 µl of skimmed milk was mixed with 900 µl of ethyl ether. After the vortex stage, the organic phase was transferred into a test tube and evaporated with a nitrogen stream. The residue was dissolved in 200 μl of extraction buffer supplied with the kit, and 50 μl of sample was assayed in duplicate into each ELISA well (Fukasawa *et al.,* 2008).

Milk production

Milk yield (kg) of each experimental animal was recorded at each morning and evening milking between day 6 and day 35 after calving. Milk composition (fat, protein, lactose) was measured weekly during the experiment, with samples taken during two successive milkings (AM and PM) and a weighted composition for the 24 hour sample period subsequently calculated. Milk composition was estimated by UKAS accredited tests using a microscan model 605 (Foss Electric, Hillerød, Denmark). The energy value of milk (MJ/kg) was calculated using the following equation ((0.0384 × fat) + (0.0223 × protein) + (0.0199 × lactose) – 0.108) (Tyrrell and Reid, 1965). The value obtained from this equation was then multiplied by the daily milk yield to obtain the total milk energy output (MJ/day).

Body weight and condition score

The live weight and body condition score (1-5, using increments of 0.25; Edmonson *et al.,* 1989) of each heifer was recorded on a weekly basis for one month after calving. Live weight and body condition score loss were calculated by subtracting values recorded at one month after calving from those recorded at day 1 after calving.

Statistical Analysis

Data were analysed using Genstat 11.1 (Payne *et al.*, 2008). The influence of treatment on behaviour during the first 2 hours after introduction to the group was analysed by ANOVA, with the random effects being replicate and group within replicate, with group being a mean of animal behaviours within a treatment in each replicate. The influence of treatment on behaviour after feeding was assessed by REML Variance Components Analysis (fixed effects were week, treatment, and treatment within week, and random effects were replicate, group within replicate, and week within group within replicate). This model was also used to assess treatment effects on the total time spent lying within 24-hour periods (recorded by data loggers), and on the average proportion of AM, PM and resident animals lying, and on the proportion of these animals lying in different locations (observed through direct observation).

The number of lying bouts per 24-hour period and mean hours lying per bout (measured using data loggers) were analysed by REML Variance Components Analysis using treatment as a fixed effect, and replicate and group within replicate as random effects. Treatment effects on average milk cortisol levels were analysed by REML Variance Components Analysis using a model which took milk yield into account (fixed effects were milk yield, week, treatment and treatment within week, and the random effects were replicate, group within replicate, and week within group within replicate). The effect of treatment on the feeding behaviour of heifers was analysed by ANOVA, with random effects being replicate and animals within replicate. Analysis was conducted on average values for all 4 treatment weeks and for each week separately.

The influence of treatment on production performance and live weight was analysed by REML Variance Components Analysis (fixed effects were time period (day of lactation or week), treatment and treatment within day of lactation/week, and random effects were replicate, animal within replicate, and time period (day of lactation or week) within animal within replicate). ANOVA (random effects, replicate and animal within replicate) was used to determine treatment effects on live weight and body condition score loss. For all REML analysis where week/time time period has been used as a factor, main treatment effects and also interactions between treatment and time period will be reported, but main effects of time will not be reported. For all statistical models, residual values were plotted and visually assessed for normality. Some behaviours were performed too infrequently for statistical analysis and therefore results will not be presented. These included ‘threatening’, ‘chasing’ and ‘receiving avoid’ after both mixing and feeding, ‘shouldering’ after mixing, and ‘head to head contact’, ‘avoiding’, ‘allogrooming’, ‘receiving threat’ and ‘receiving chase’ after feeding.

Table 1 Ethogram of post mixing and post feeding behaviours recorded

Behaviour	Description
Activity	
Head to head contact	Contact between two individuals using the front of their heads
Butting	When the cow uses the front of her head to make vigorous contact with another cow
Threatening	When a cow turns towards or approaches another individual with her head down and then lunges without making contact
Chasing	When a cow actively moves towards another individual causing this individual to retreat
Shouldering	Displacement of an individual using the shoulder
Avoiding	When a cow actively moves away from another individual irrespective of whether an interaction has occurred between the two individuals
Allogrooming	Mutual grooming between two individuals
Motionless	No legs moving and head not in contact with any substrate
Feeding	Head in Calan gate and feeding
Explore-feeder	Nosing any part of the Calan gate box with head
Explore-general	Nosing any substrate in pen including floors, walls and railings, but not the feeder or another cow
Social-cohesive	Licking another animal or rubbing heads
Social-investigative	Nosing another animal without displaying agonistic or cohesive behaviour
Ruminating	Regurgitating and chewing boluses of food
Drinking	Drinking at water trough
Grooming	Focal animal grooming itself
Sniffing cow	Sniffing any part of another animal (with no physical contact)
Location	
Cubicle	Focal animal located in cubicle
Front-passage	Focal animal located in front passageway
Rear/side passage	Focal animal located in rear or side passageway
State	
Lying	Lying down
Standing	Standing up
Received Behaviours	
Receive butt	When the focal animal receives butting behaviour
Receive avoid	When a non-focal animals moves away from the focal animal
Receive threat	When the focal animal receives threatening behaviour
Receive chase	When the focal animal receives chasing behaviour
Receive shoulder	When the focal animal receives shouldering behaviour
Receive nose	When the focal animals receives nosing behaviour
Receive sniff	When the focal animal receives sniffing behaviour

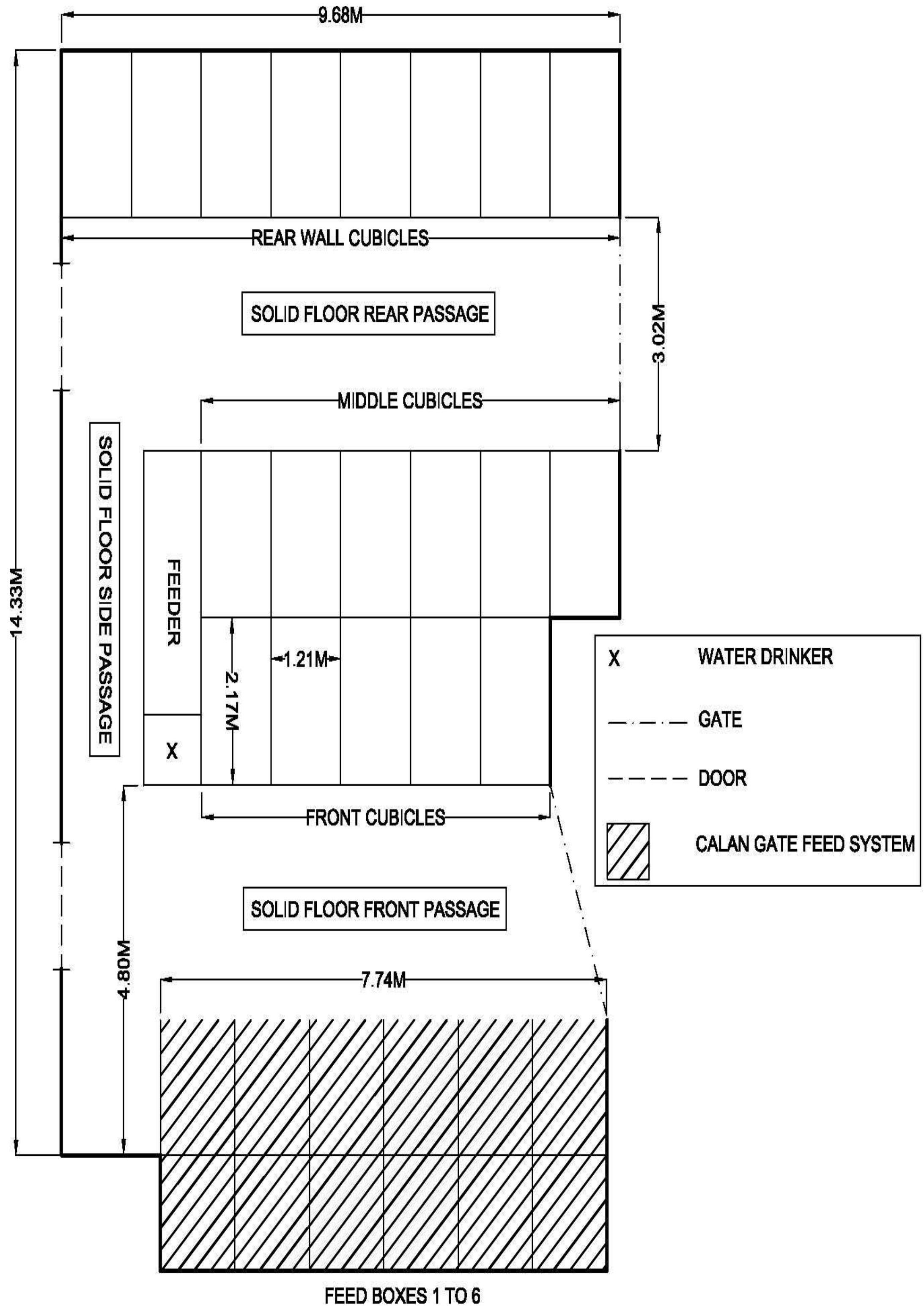

Figure 2 Layout of cubicle accommodation where the 18 animals within each replicate were housed.

Results

Behaviour

Direct observations after mixing

The influence of treatment on the duration spent performing or receiving different behaviours or in different locations is presented in Table 2. Heifers in the AM treatment spent a greater duration in receipt of threats (P=0.048), butts (P=0.007) and chases (P=0.033) after mixing than those in the PM treatment. When analysed on a frequency/minute basis heifers in the AM treatment received more butts compared to PM heifers (butt: AM 0.16, PM 0.05, SED 0.013/minute, $F_{1,4}$ 75.01, P=0.001).

Direct observations after feeding

The behaviour of heifers and behaviours directed towards heifers after feeding are presented in Table 3. Heifers in the AM treatment were observed feeding for a greater duration of time (P=0.013), and also spent more time in receipt of butts than those in the PM treatment (P=0.023).

There were significant interactions between treatment and week in the duration of exploring (Figure 3), grooming (Figure 4) and drinking (Figure 5) (exploring: (AM) week 1, 2.19; week 2, 2.96; week 3, 3.06; week 4, 2.61; (PM) week 1, 7.98; week 2, 5.35; week 3, 3.46; week 4, 12.78; SED 2.279, $F_{3,16.1}$ 3.55, P=0.038; grooming: (AM) week 1, 0.34; week 2, 1.93; week 3, 3.02; week 4, 0.86; (PM) week 1, 2.22; week 2, 1.07; week 3, 1.21; week 4, 1.45; SED 0.848, $F_{3,16.4}$ 3.80, P=0.031; drinking: (AM) week 1, 0.44; week 2, 0.52; week 3, 4.19; week 4, 0.06; (PM): week 1, 1.52; week 2, 0.36; week 3, 0.85; week 4, 1.96; SED 1.165, $F_{3,16.6}$ 3.52, P=0.038).

Table 2 Influence of treatment on the time spent performing and receiving different behaviours and in different locations and states after mixing (% observation time)

Behaviour	Treatment		SED	$F_{(1,4)}$	P
	AM	PM			
Activity					
Social-investigative	0.77	1.27	0.439	1.29	0.319
Social-cohesive	0.17	1.06	0.330	7.31	0.054
Allogrooming	0.04	0.24	0.155	1.74	0.258
Explore-general	13.90	14.10	1.140	0.03	0.863
Explore-feeder	1.66	1.57	0.418	0.04	0.850
Feeding	13.80	21.00	4.840	2.19	0.213
Ruminating	30.50	26.20	6.090	0.49	0.522
Motionless	28.10	21.00	5.850	1.49	0.289
Grooming	0.89	2.82	0.707	7.41	0.053
Drinking	0.94	1.05	0.240	0.22	0.663
Sniffing cow	0.40	0.35	0.119	0.21	0.671
Avoiding	0.11	0.04	0.060	1.53	0.284
Butting	0.14	0.20	0.114	0.28	0.623
Head to head contact	0.40	0.02	0.146	6.71	0.061
Location					
Cubicle	25.30	33.40	9.510	0.74	0.439
Front-passage	29.10	37.90	6.680	1.74	0.257
Rear/side passage	38.50	21.50	8.350	4.13	0.112
State					
Lying	6.90	5.70	7.790	0.02	0.890
Standing	93.10	94.30	7.790	0.02	0.890
Receive					
Nose	0.12	0.16	0.051	0.39	0.566
Sniff	0.12	0.08	0.021	3.22	0.147
Threat	0.02	0.00	0.008	7.94	0.048
Shoulder	0.06	0.02	0.024	2.84	0.167
Butt	0.38	0.17	0.042	25.23	0.007
Chase	0.06	0.01	0.017	10.22	0.033

Table 3 Influence of treatment on the time spent performing and receiving different behaviours and in different locations and states after feeding (% observation time)

Behaviour	Treatment		SED	F	P
	AM	PM			
Activity					
Social-investigative	0.12	0.16	0.050	$F_{(1,3.7)}$ 0.87	0.406
Social-cohesive	0.07	0.24	0.063	$F_{(1,4)}$ 7.20	0.055
Explore-feeder	4.43	4.77	2.650	$F_{(1,3.7)}$ 0.01	0.929
Feeding	29.24	21.78	1.887	$F_{(1,4)}$ 17.91	0.013
Ruminating	31.83	32.80	3.266	$F_{(1,3.8)}$ 0.08	0.788
Motionless	23.86	24.75	2.684	$F_{(1,4)}$ 0.04	0.854
Sniffing cow	0.03	0.08	0.042	$F_{(1,3.9)}$ 1.47	0.293
Shouldering	0.05	0.08	0.044	$F_{(1,3.9)}$ 0.39	0.566
Butting	0.61	0.65	0.186	$F_{(1,4)}$ 0.23	0.659
Location					
Cubicle	51.50	56.53	7.606	$F_{(1,4)}$ 0.44	0.542
Front-passage	44.15	36.67	5.648	$F_{(1,3.9)}$ 1.26	0.325
Rear/side passage	3.91	6.27	3.232	$F_{(1,4)}$ 0.61	0.477
State					
Lying	44.29	37.54	6.143	$F_{(1,4)}$ 1.20	0.334
Standing	55.71	62.46	6.143	$F_{(1,4)}$ 1.20	0.334
Receive					
Nose	0.02	0.02	0.010	$F_{(1,4)}$ 0.00	0.952
Sniff	0.01	0.00	0.005	$F_{(1,4)}$ 1.48	0.290
Shoulder	0.06	0.07	0.021	$F_{(1,4)}$ 0.33	0.597
Butt	0.29	0.17	0.032	$F_{(1,3.8)}$ 13.72	0.023

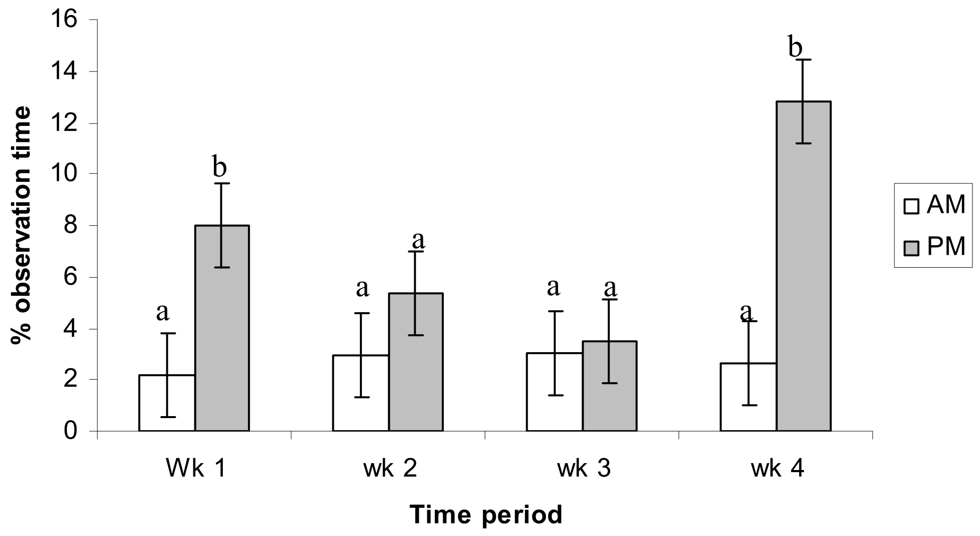

Figure 3 Interaction between treatment and week for exploring (% observation time)

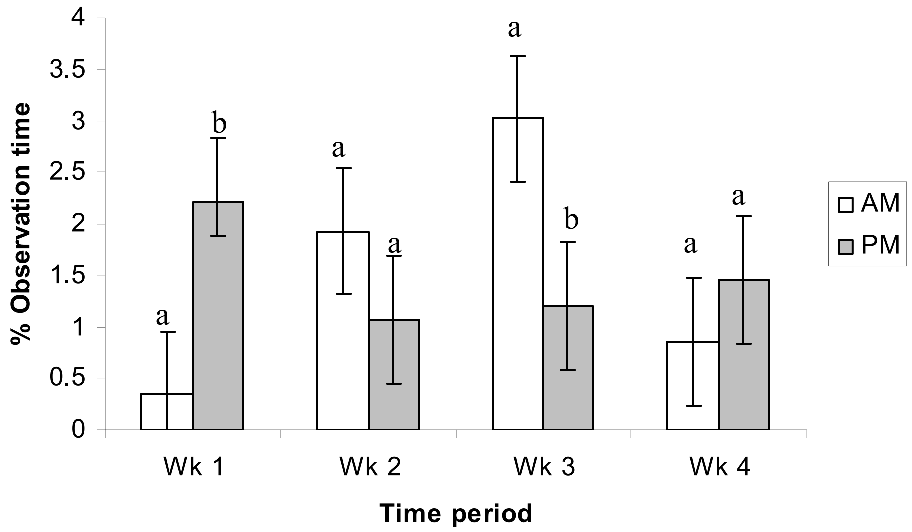

Figure 4 Interaction between treatment and week for grooming (% observation time)

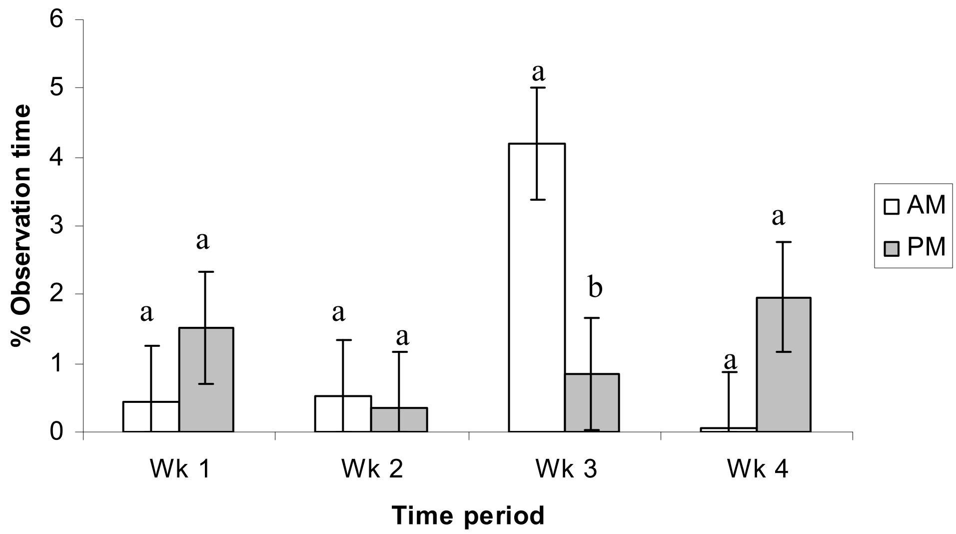

Figure 5 Interaction between treatment and week for drinking (% observation time)

Lying behaviour

There was no significant treatment effect on time spent lying as recorded by data loggers (P=0.917) (Table 4). The average mean lying time was 7.5 hours per 24 hour-period, and this was reduced to <4 hours per 24-hour period after the initial mixing period.

In terms of lying behaviour during evening observations, PM heifers spent a lower proportion of time lying than AM heifers or resident cows (P=0.002). There was no significant difference in the proportion of time spent lying between AM heifers and resident cows during this period. A significant interaction was found between treatment and week in the proportion of animals located in the front passage (Figure 6) (AM: week 1, 0.09; week 2, 0.06; week 3, 0.10; week 4, 0.09; PM: week 1, 0.10; week 2, 0.06; week 3, 0.06; week 4, 0.16; Resident cows: week 1, 0.09; week 2, 0.08; week 3, 0.07; week 4, 0.09; SED 0.025, $F_{6,32.3}$ 2.52, P=0.041).

Table 4 Lying behaviour parameters measured through automated data loggers or by direct observation

	Treatment heifers		Resident cows	SED	F	P
	AM	PM				
Data loggers						
Total hours lying (hours)	7.57	7.51		0.524	$F_{(1,4)}$ 0.01	0.917
No. of Lying Bouts/day	14.46	12.73		0.954	$F_{(1,3.8)}$ 3.27	0.148
Mean hours lying per bout	0.59	0.65		0.070	$F_{(1,4)}$ 0.70	0.450
Direct observation (proportion of animals)						
Lying	0.45[b]	0.34[a]	0.42[b]	0.022	$F_{(2,8.6)}$ 14.88	0.002
Rear cubicles (facing wall)	0.30	0.25	0.21	0.044	$F_{(2,7.4)}$ 2.46	0.152
Rear Passage	0.02	0.04	0.02	0.009	$F_{(2,8.3)}$ 1.82	0.222
Middle cubicles	0.22	0.18	0.19	0.030	$F_{(2,8.1)}$ 0.84	0.467
Side passage	0.02	0.02	0.02	0.007	$F_{(2,8.1)}$ 0.58	0.583
Front cubicles	0.14	0.19	0.22	0.044	$F_{(2,8.1)}$ 2.17	0.176
Feed boxes	0.21	0.22	0.26	0.023	$F_{(2,8)}$ 3.04	0.104

Data logger measurements were taken over five 24-hour periods for treatment heifers only (post mixing and one day each week for the first 4 weeks). Direct observations were recorded between 13:00 and 15:00 hours.

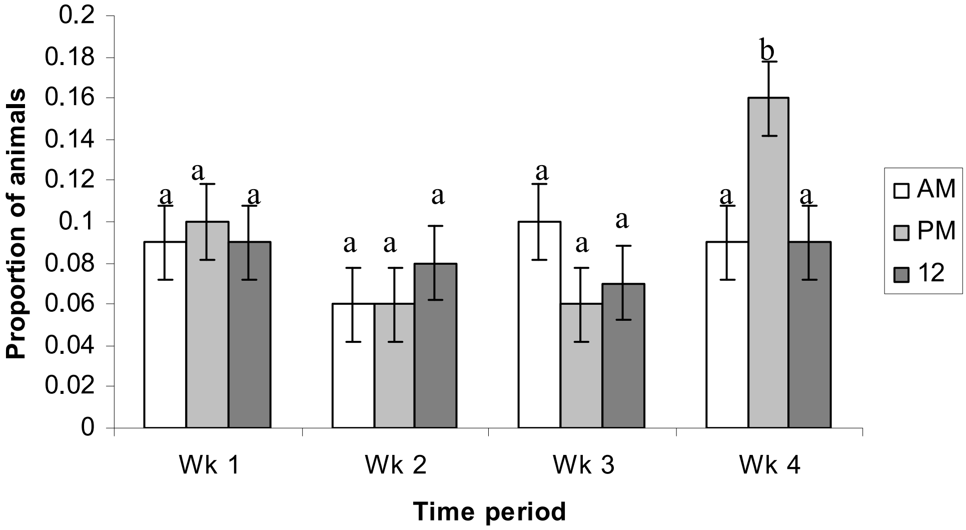

Figure 6 Interaction between treatment and week for the proportion of animals (AM, PM, resident animals) located in front passage

Feeding behaviour

Treatment had no significant effect on average daily food intake levels during the first month after calving (AM 14.3, PM 13.6, SED 0.54 kg DM, $F_{1,21}$ 1.51, P=0.233). However, during week 2 heifers in the AM treatment had a significantly higher intake and a greater number of meals per day than those in the PM treatment (feed intake: AM 14.4, PM 13.0, SED 0.51 kg DM, $F_{1,22}$ 7.94, P=0.010: meals/day: AM 12.0, PM 10.5, SED 0.72, $F_{1,22}$ 4.38, P=0.048). There was also a tendency for the number of meals per day to be greater with heifers in the AM treatment when averaged over the month compared with the PM treatment (AM 12.2, PM 11.2, SED 0.52, $F_{1,21}$ 4.26, P=0.052). There was also a significant treatment effect on the total time spent eating each day, with AM heifers eating for significantly longer during the first month after calving compared to PM heifers (AM 171, PM 151, SED 6.561 min/day, $F_{1,21}$ 9.26, P=0.006). This appeared to be due to significant treatment effects observed during week 1 (P=0.007) and week 2 (P=0.011). Treatment had no significant effect on the average duration of each meal (AM 14.37, PM 14.69, SED 1.100 min, $F_{1,21}$ 0.09, P=0.771) or eating rate (AM 88.7, PM 94.8, SED 5.64 g DM/minute, $F_{1,21}$ 1.17, P=0.291) during the first month after calving.

Milk Cortisol

There was no significant difference between treatments in levels of milk cortisol recorded, with AM and PM heifers both exhibiting concentrations of 0.64 (SED 0.144 ng/ml, $F_{1,4.1}$ 0.00, P=0.991).

Production Performance

Treatment had no significant effect on milk production performance (Table 5). Average daily milk yield for both treatments during the first 4 weeks after calving was 25.4 kg.

Live Weight and Body Condition Score Losses

No significant difference was found between treatments in total live weight loss (AM -16.9, PM -19.4, SED 7.130 kg, $F_{1,22}$ 0.12, P=0.729) or total condition score loss (AM -0.20, PM -0.14, SED 0.073, $F_{1,22}$ 0.54, P=0.472). There was also no significant difference between treatments in average live weight (AM 516, PM 528, SED 13.29 kg, $F_{1,21.9}$ 0.76, P=0.392) or average body condition score (AM 2.72, PM 2.75, SED 0.063, $F_{1,22}$ 0.15, P=0.702) during the first month after calving.

Table 5 Feeding behaviour, production performance, body tissue and cortisol levels of the experimental heifers during the first month after introduction to the group

Parameter	Treatment		SED	F	P
	AM	PM			
Intake and feeding behaviour					
Daily food intake (kg)	14.3	13.6	0.54	$F_{(1,21)}$ 1.51	0.233
Meals/day	12.2	11.2	0.52	$F_{(1,21)}$ 4.26	0.052
Daily eating time (min)	171	151	6.561	$F_{(1,21)}$ 9.26	0.006
Milk production					
Milk yield (kg/day)	25.0	25.7	1.277	$F_{(1,22)}$ 0.33	0.570
Fat (g/kg)	44.8	44.1	2.340	$F_{(1,23.8)}$ 0.03	0.862
Protein (g/kg)	36.8	35.5	0.667	$F_{(1,21.6)}$ 3.75	0.066
Lactose (g/kg)	45.9	46.2	0.581	$F_{(1,22.3)}$ 0.41	0.530
Fat yield (kg/day)	1.12	1.13	0.075	$F_{(1,19.6)}$ 0.07	0.788
Protein yield (kg/day)	0.90	0.92	0.044	$F_{(1,20.7)}$ 0.23	0.640
Fat + protein yield (kg/day)	2.02	2.06	0.114	$F_{(1,20.1)}$ 0.22	0.646
Milk energy content (MJ/kg)	3.34	3.35	0.095	$F_{(1,21.6)}$ 0.06	0.812
Milk energy output (MJ/day)	83.09	85.94	4.719	$F_{(1,20.1)}$ 0.46	0.506
Body tissue					
LWT (kg)	516	528	13.29	$F_{(1,21.9)}$ 0.76	0.392
Condition Score	2.72	2.75	0.063	$F_{(1,22)}$ 0.15	0.702
LWT loss (kg)	16.9	19.4	7.130	$F_{(1,22)}$ 0.12	0.729
Condition Score loss	0.20	0.14	0.073	$F_{(1,22)}$ 0.54	0.472
Stress hormone					
Milk cortisol (ng/ml)	0.64	0.64	0.144	$F_{(1,4.1)}$ 0.00	0.991

Discussion

Behaviour After Mixing

In the current study, PM heifers had fewer aggressive behaviours directed towards them from resident cows than AM heifers (3 versus 9.6 butts/hour, respectively). The levels observed in the AM treatment are similar to the 7-10 agonistic encounters per hour previously reported (Brakel and Leis, 1976; Knierim, 1999; Neisen *et al.,* 2009). Heifers which encounter lower levels of aggression are likely to have improved welfare (Neisen *et al.,* 2009). The findings of the current study agree with previous research with pigs which showed that regrouping animals in the morning led to significantly more aggression than regrouping in the evening (Csermely and Wood-Gush, 1987; Barnett *et al.*, 1996). Research is limited as to how time of day of regrouping affects the behaviour of cattle. Nakanishi *et al.* (1991; 1993) found that the introduction of a strange cow into a group after dark still led to increases in aggression, although levels of aggression on consecutive days were not as high as when animals were introduced during the daytime. Lamb (1976) suggested that evening introduction of cattle to groups may reduce levels of fighting due to lower levels of general social behaviour. This agrees with previous observations that cattle are less socially active in the evening, and spend most of their time lying and ruminating (Nakanishi *et al.,* 1993; Johansson *et al.,* 1999).

It should be noted that time of day was confounded with time interval since feeding in the current study. Hunger has been shown to stimulate aggression in cattle (Vieira *et al.,* 2008), and it is possible that this contributed to increased aggression after mixing in the AM treatment. This design, where TMR was provided once per day, was chosen for the current study as it reflects the practice in many commercial enterprises. Further research would be useful, however, to determine the extent to which time interval since delivery of fresh feed affects aggression directed towards newly-introduced animals. In addition, animals were continually replaced within resident groups in the current study, making the groups socially unstable. This factor may have increased the overall level of agonistic behaviours expressed by the resident animals (Kondo and Hurnik, 1990). However, freshly-calved dairy animals are often added to socially unstable groups in commercial operations, and the treatment design was chosen to reflect this.

Behaviour After Feeding

The level of agonistic behaviour declined between the mixing period and subsequent observations. This concurs with Kondo and Hurnik (1990) who found that agonistic interactions primarily occurred during the 2-hour period after regrouping, with aggression levels declining to pre-mixing levels by 3-15 days after mixing (Hasegawa *et al.,* 1997; Fernandez *et al.,* 2007). However, during the post-feeding period heifers in the AM treatment did receive an increased number of butts. It is unclear why this treatment effect occurred, however it could be related to the fact that these animals spent more time at the feed boxes, and, as a result, may have been involved in more competitive interactions. Increased levels of feed-related aggression can occur during the 30-45 minute period after the delivery of fresh feed or during the 2-hour period on return from milking (Friend and Polan, 1974; Oloffson, 1999).

In weeks 1 and 4, heifers in the PM treatment exhibited increased levels of pen exploration during the feeding observation periods. Research with other species suggests that exploratory behaviour may reflect a foraging motivation (Day *et al.,* 1995; Stern and Andresen, 2003). It is possible that PM heifers were motivated to feed during this period, but were not as confident as AM animals in competing for feed, and thus showed increased exploratory behaviour.

Lying Behaviour

Previous research has shown that integration of dairy heifers into a herd causes a significant reduction in lying time of heifers after mixing, with animals lying for 1.5 hours per 12 hours, compared with pre-mixing lying times of over 7 hours per day (Krohn and Konggaard, 1979; Knierim, 1999). It is pertinent to note that in the current study the heifers in both treatments lay down for less than 4 hours during their first 24 hours in the group, which is similar to Knierim's (1999) findings. Reduced lying times in heifers can have a detrimental effect on hoof health, which in turn has negative animal welfare implications (Singh *et al.,* 1993; Galindo *et al.,* 2000). There was no significant treatment effect on overall time spent lying, with both AM and PM heifers lying for an average of 7.5 hours per 24 hour period. This is broadly in agreement with Singh *et al.* (1993) who recorded lying times in heifers of 8.39 hours per day six weeks after being mixed with adult cows.

Direct observations of lying behaviour indicated reduced lying by PM animals between 13:00 and 15:00 hours. The reason for this occurring in the PM animals is unclear, but it is possible that these animals used this opportunity to feed when other animals were lying. Although only observed during week 4, this may also explain why these animals were located in the front passageway to a greater extent than other groups.

Feeding Behaviour

Dry matter intake can have a significant effect on milk production and on condition score change (Grant and Albright, 1997). While there was no significant overall difference in DMI between the two treatment groups in the current study, AM animals had higher intakes during week 2. Heifers in the AM treatment also spent significantly more time feeding each day over the one-month observation period compared to heifers in the PM treatment. This was also observed in direct observations after feeding, where AM heifers were observed feeding for significantly longer than PM animals. In fact, AM heifers consumed one meal per day more than PM heifers, which equated to an extra 14 min of feeding time. As previously stated, these effects may reflect an increased willingness to compete for food among AM animals. It is also possible that introducing animals to a new group in a new environment during the PM period confuses feeding patterns, although further research is needed to determine if this is the case.

Production Performance

Lawson (1999) found no difference in milk yield for dairy heifers introduced to a group after evening rather than morning milking. This is similar to the current study where no difference was found in milk yield between the two treatment groups.

Milk Cortisol

Treatment had no significant effect on the concentration of milk cortisol. Fukasawa *et al.* (2008) observed that cows in the early stage of lactation (days 7-90) had a baseline milk cortisol level of 0.4 ng/ml. That study also found that cortisol levels then declined as lactation progressed, indicating that early lactation may act as a stressor for cows. These results are lower than those observed in the current study (AM 0.65, PM 0.64 ng/ml), which may suggest that the onset of lactation coupled with the added stress of being integrated into the herd increased cortisol levels.

It should be noted that milk cortisol concentrations may only be a useful indicator of stress when samples are obtained during the period of increased plasma cortisol concentrations (Verkerk *et al.,* 1996). In the current study milk cortisol levels were not measured directly after mixing, and therefore we may have missed periods of peak stress. Furthermore, increased standing and locomotion levels can affect cortisol levels (Gonzalez *et al.,* 2003), and this, in conjunction with the heifers being introduced into a new group, may have over-ridden any treatment effects.

Conclusions

Introducing heifers to the resident group after PM milking appeared to improve welfare during the initial mixing period by reducing levels of aggression to which they were exposed. However, this treatment did not promote an increase in overall lying behaviour. Heifers in the AM treatment had higher feed intakes during week 2 after mixing, and spent longer periods of time feeding, and it is suggested that this reflects an increased willingness to compete for food. However, milk yield did not differ between treatments. Overall, the reduction in received aggression, coupled with a lack of adverse effects on production performance, suggests that it is beneficial to introduce heifers into the main dairy herd after PM milking. Further research is needed, however, to determine the extent to which treatment differences in aggression received immediately after mixing reflect differences in time of day or time post feeding.

Study 2

Does housing nulliparous cows with multiparous animals prior to calving influence welfare and performance after calving?

Abstract

Twenty nulliparous Holstein Friesian dairy cattle were assigned to one of two treatments ('Mixed and Unmixed') pre-calving. The 'Mixed' treatment involved housing experimental animals with non-lactating multiparous cows for three weeks prior to their expected calving date. In the 'Unmixed' treatment, experimental animals were housed with other nulliparous animals during the 3-week period prior to their expected calving date. During this pre-calving period animals in both treatments were housed in groups of 10 animals, with the 'Mixed' group comprising of seven multiparous non-lactating cows and three experimental primiparous cows. The experimental animals were added to a resident group that contained 15 animals (10 multiparous cows and 5 primiparous cows) within 24 hours of calving. The behaviour of the experimental animals was assessed immediately after the mixing period and also after feeding during the first month after introduction to the resident group (days 2, 4, 10 and 1 day in weeks 3 and 4). In addition, data loggers were attached to the animals for a 24-hour period on five occasions during the first month post calving to assess lying behaviour. Lying and location of the group was assessed by direct observation on two consecutive days each week during the 2-hour period prior to evening milking. Milk production, serum cortisol levels and changes in body condition and live weight were assessed during the first month after calving. 'Mixed' animals received fewer butts ($P<0.05$) after mixing, and these animals also showed increased locomotion during this period ($P<0.05$). After feeding, 'Mixed' animals performed more 'shouldering' of other animals and increased locomotion ($P<0.05$), and received fewer butts ($P<0.001$). Also during this period animals in the 'Unmixed' treatment were located in the cubicles to a greater extent ($P<0.01$). Animals in the 'Mixed' treatment were located to a greater extent in the front passage after feeding

(P<0.001). During observations prior to evening milking, both treatment groups were located in the rear passage significantly more than resident cows (P<0.05). No significant treatment effects were shown for milk yield, serum cortisol levels or body weight or condition score loss. Overall, giving primiparous cows experience with dry multiparous cows prior to calving appeared to improve their welfare when mixed into a group containing older animals after calving through leading to lower levels of received aggression. It is suggested that the increased levels of aggression and locomotion performed by these animals, and the increased time spent near the front feeding passage after feeding, reflected increased 'confidence' in 'Mixed' animals.

Introduction

Primiparous dairy cows (heifers) are frequently subject to aggression following integration into the main dairy herd, and this can have a negative impact on their welfare and production performance (Krohn and Konggaard, 1982, Neisen *et al.*, 2009). This is primarily due to newly-introduced heifers being more timid and having a lower social rank than older cows (Sambraus, 1970; Lamb, 1976). This may also mean that newly-introduced heifers find it more difficult to access key resources such as feeding and lying places (Wierenga, 1990). In addition, other stressors such as entering the milking parlour and abrupt changes in diet may further compound the stress associated with integration to the herd (Van Reenen *et al.*, 2002; Goff and Horst, 1997).

Abeni and Bertoni (2009) proposed that if heifers cannot be kept in a separate group after calving, they should be mixed with dry cows for a period of time prior to calving. It has been suggested that this strategy could reduce aggression after calving by providing heifers with a period of pre-exposure to opponents (Jensen and Yngvesson, 1998; Hartmann *et al.*, 2009). However in commercial situations it may not always be possible to house freshly-calved heifers with the same cows that they were housed with prior to calving. Nevertheless, in this situation mixing heifers with mature non-lactating cows prior to calving may allow them to gain experience of social regrouping, and in interacting with older animals, and this could also improve welfare and performance after calving. For example, piglets which had been mixed three to four times prior to being regrouped as gilts at five months of age had improved social skills and this led to significant reductions in aggression and time spent fighting (van Putten and Buré, 1997). In addition, research with dairy cows suggests that previous experience of regrouping reduces the adverse effects of this practice on milk yield (Sowerby and Polan, 1978). However, there is limited research on the effects of mixing nulliparous cows with multiparous animals during the pre-calving period, on their behaviour after calving. Nevertheless, some authors have examined the effect of mixing heifers multiple times. Bouissou (1975) found that during their first encounter with unfamiliar animals the level of agonistic behaviour was 25% higher than when the same animals experienced a further four encounters with unfamiliar animals. In contrast, Raussi *et al.,* (2005) found no reduction in agonistic interactions as a result of repeated regrouping in heifers.

The aim of this study was to examine the effect of housing nulliparous dairy cattle with multiparous dairy cows during the pre-calving period on welfare and performance after calving when heifers were integrated into a group of lactating animals containing unfamiliar individuals.

Materials and Methods

Animals, Treatments, Management and Housing

This experiment involved 20 experimental Holstein Friesian (HF) dairy cattle. Following weaning at approximately seven weeks of age, these animals were housed in cubicle accommodation until March/April (a period of approximately two months), then they were turned out to grass until October, with the experimental animals managed as part of a much larger group of nulliparous dairy cattle. Following their second season at grass, animals were housed again in October, until calving between January and March (at an average age of 25 months). Experimental animals (together with other non-experimental animals) were transferred in small groups (of 3–6 animals, according to calving date) from a heifer rearing facility to a dairy cow house approximately one month prior to their expected calving date, where they were housed as part of a larger dynamic group. The size of this group ranged from 15 to 20 animals, with animals having access to 30 cubicles. At three weeks prior to their expected calving date, the experimental animals were removed from the larger dynamic group and allocated to one of two treatments.

The two treatments (n=10 animals per treatment) were designed to examine the effect on animal welfare and performance during the post-calving period arising from mixing nulliparous dairy cattle with non-lactating multiparous dairy cows prior to calving. Treatments examined involved housing nulliparous dairy cattle with either non-lactating multiparous cows for three weeks before calving (treatment 'Mixed'), or with other nulliparous cattle for three weeks prior to calving (treatment 'Unmixed'). Each treatment was replicated four times. Treatments were balanced for genetic merit (Predicted Transmitting Ability (PTA) for kg fat plus protein, kg fat and kg protein), sire, body weight and condition score.

During this 3-week pre-calving period, animals in both treatments were housed in groups of 10, with each group having access to 10 cubicles. With the Mixed treatment, the group comprised seven multiparous non-lactating cows and three experimental nulliparous animals. Each of the Mixed and Unmixed treatment groups were 'dynamic' as experimental and non-experimental animals were added or removed from the groups according to different calving dates. Pens used in this period were similar to those used after calving, as described later.

The experimental animals were moved to individual straw bedded calving pens (5.9 × 3.3 m) 24–48 hours prior to calving (based on predicted calving dates and daily assessments by experienced stockpersons) and discretionary calving assistance given. Calves remained with their dams for 6-12 hours.

After calving, experimental animals were introduced to a resident group containing 10 multiparous (mean lactation number, 3.1) HF dairy cows (average 122 DIM) and 5 non-experimental primiparous HF cows. These resident group animals were all lactating and were unfamiliar to the experimental heifers. A total of four resident (or replicate) groups were used, with each resident group being established five days before the first experimental heifer was introduced. The group size and cow:heifer ratio within each resident group was maintained throughout the study by removing a non-experimental primiparous cow from the group immediately before an experimental primiparous cow was added. The process of replacing all 5 non-experimental animals with experimental animals was completed over a period of 18 days (on average) across all replicates. On days when an experimental animal was due to be introduced into the group, a non-experimental heifer was removed immediately prior to morning milking, with the experimental animals introduced after the remainder of the group returned to the pen from milking (between approximately 06:00 and 06:30 hours).

In replicates (resident groups) 1 and 3, two non-experimental primiparous cows were replaced by animals from the Mixed treatment, and three by animals from the Unmixed treatment. In replicates 2 and 4, three non-experimental primiparous cows were replaced by animals from the Mixed treatment and two by animals from the Unmixed treatment (Figure 7). In each resident group no more than one experimental heifer was introduced on a particular day. Groups were continuously disturbed until the final non-experimental heifer was replaced. The interval between replacing animals within individual groups ranged from 1 to 15 days.

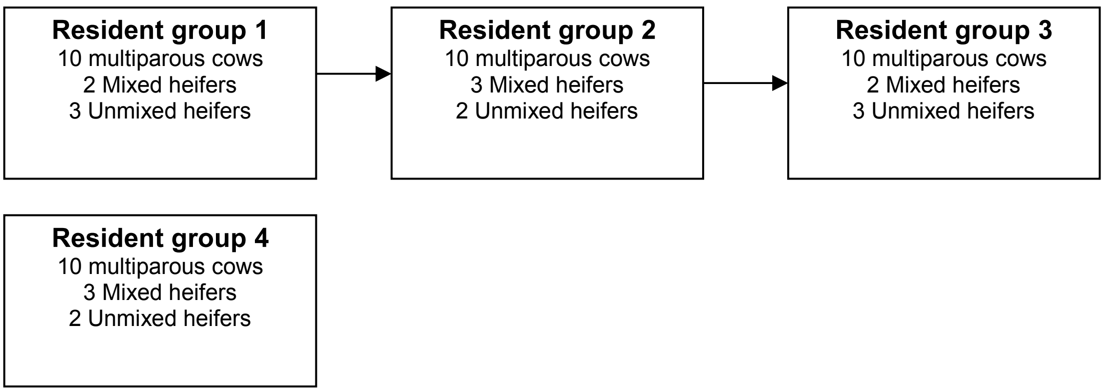

Figure 7 Structure of resident groups (Study 2)

The resident groups were housed in pens with 16 cubicles, arranged in 3 rows, and with solid concrete floors (Figure 8). Out-of-parlour feeders were present in all pens but were not used. Concrete passageways were cleaned a minimum of four times daily using an automatic scraper, and feed was accessed via an open feed barrier. During the 3-week pre-calving period the experimental groups were offered a total mixed ration (TMR) *ad libitum*, comprising 80% forage and 20% concentrate on a DM basis, together with 100 g/cow/day of dry cow mineral. After calving, animals within each resident group were offered a TMR comprising 50% concentrate and 50% forage (DM basis) on an *ad libitum* basis. The forage component of the diet consisted of 65% grass silage and 35% maize silage on a DM basis. Experimental animals were also offered 1.0 kg of concentrate in the milking parlour (0.5 kg in the morning and evening). Heifers were milked in a 50-point rotary parlour twice per day (at approximately 06:00 and 16:00 hours).

Research was conducted under the Animals (Scientific Procedures) Act 1986, and following agreement from the Agri-Food and Biosciences Institute Ethical Review Committee.

Measurements

All measures were undertaken during the first month (approximately) after the experimental animals had been introduced to the resident group.

Behaviour after mixing and feeding

Each experimental animal was observed continuously for a 2-hour period immediately after being introduced to the resident group. In addition, experimental animals were also observed during four 5-minute periods at 30-minute intervals during the 2-hour period after fresh food was offered on days 2, 4 and 10 and on 1 day during weeks 3 and 4 after introduction to the resident group.

Behaviour was recorded by direct observation using a handheld data recorder (Psion Organiser II, Model LZ64, Noldus Information Technology, The Netherlands) using the ethogram presented in Table 6. The ethogram contained three mutually-exclusive categories of behaviour: the location of the animal in the pen (measured as duration of time), behaviours performed by the animal ("activity") and behaviours received by the animal. The duration and frequency were recorded for all aggressive behaviours performed (i.e. butting and shouldering) and for all received behaviours. Only the duration of all other "activity" behaviours was recorded.

Table 6 Ethogram of post mixing and post feeding behaviours recorded

Behaviour	**Description**
Activity	
Butting	When the cow uses the front of her head to make vigorous contact with another cow
Shouldering	Displacement of an individual using the shoulder
Locomotion	All four legs moving and head not in contact with any substrate
Feeding	Head extended through the feed barrier and feeding
Exploring-feeder	Nosing any part of the feed barrier
Ruminating	Regurgitating and chewing boluses of food
Drinking	Drinking at water trough
Grooming	Focal animal grooming itself
Location	
Cubicle	Focal animal located in cubicle
Front-passage	Focal animal located in front passageway
Rear/side Passage	Focal animal located in rear or side passageway
Received Behaviours	
Receiving butt	When the focal animal receives butting behaviour
Receiving shoulder	When the focal animal receives shouldering behaviour

Lying behaviour and location

The methods used to assess these parameters are the same as used in Study 1.

Serum cortisol

Blood samples for serum cortisol analysis were taken from the tail vein at 25 and 49 hours after introduction to the group. Samples were taken after morning milking, between the hours of 06:00 and 06:30, and were then refrigerated at 4°C. Blood samples were centrifuged within 2 hours of collection (12 min, 3000 rpm @ 4°C) and the serum was stored at -20°C until analysed.

Cortisol concentrations were measured using a commercial ELISA kit (Cortisol EA65, Oxford Biomedical Research, Inc., Oxford, MI, USA). Samples were prepared as follows: 100 μl of serum was mixed with 1000 μl of ethyl ether. After the vortex stage, the organic phase was transferred into a test tube and evaporated with a nitrogen stream. The residue was dissolved in 100 μl of extraction buffer supplied with the kit. The sample was diluted further by adding 990 μl of diluted extraction buffer to 10 μl of the previously dissolved solution. After the vortex stage the samples were assayed in duplicate into each ELISA well.

Milk production

Please refer to Study 1 for experimental protocol.

Body weight and condition score

Please refer to Study 1 for experimental protocol.

Statistical Analysis

Data were analysed using Genstat 11.1 (Payne *et al.*, 2008). Unless otherwise stated, all analysis was conducted using REML Variance Components Analysis. Behaviour during the first two hours after introduction to the group was analysed using treatment as a fixed effect, and replicate and group within replicate as random effects. The influence of treatment on behaviour during the 2-hour period after feeding was analysed using time period (measurements were taken over 5 time periods), treatment and treatment within time period as fixed effects, and replicate, group within replicate and time period within group within replicate as random effects. In these analyses the term ‘group’ refers to the mean of animal behaviours within a treatment in each replicate.

Lying parameters recorded by data loggers (total hours lying, number of lying bouts per day and mean hours lying per bout) were analysed using treatment as a fixed effect and replicate and group within replicate as random effects. Lying parameters recorded by direct observations (location and synchronicity) were analysed using week, treatment and treatment within week as fixed effects, and replicate, group within replicate and week within group within replicate as random effects. Mann-Whitney U tests were used to compare the level of stress hormones (cortisol) between the two treatments at 25 and 49 hours after mixing.

The influence of treatment on production performance, live weight and condition score was analysed using time (day of lactation or week), treatment, and treatment within time, as fixed effects, and replicate, animal within replicate, and time (day of lactation or week) within animal within replicate as random effects. The effect of treatment on live weight and body condition score loss was analysed using treatment as a fixed effect and replicate and animal within replicate as random effects. For all REML analysis where week/time period was used as a factor, main treatment effects and also interactions between treatment and time period will be reported, but main effects of time will not be reported. For all parametric statistical models, residual values were plotted and visually assessed for normality. Some behaviours were performed too infrequently for statistical analysis and results are not presented. These included ‘shouldering’, ‘drinking’ and the location ‘cubicle’ after mixing, and ‘exploring feeder’, ‘ruminating’, the location ‘rear/side passage’ and ‘receiving shoulder’ after feeding.

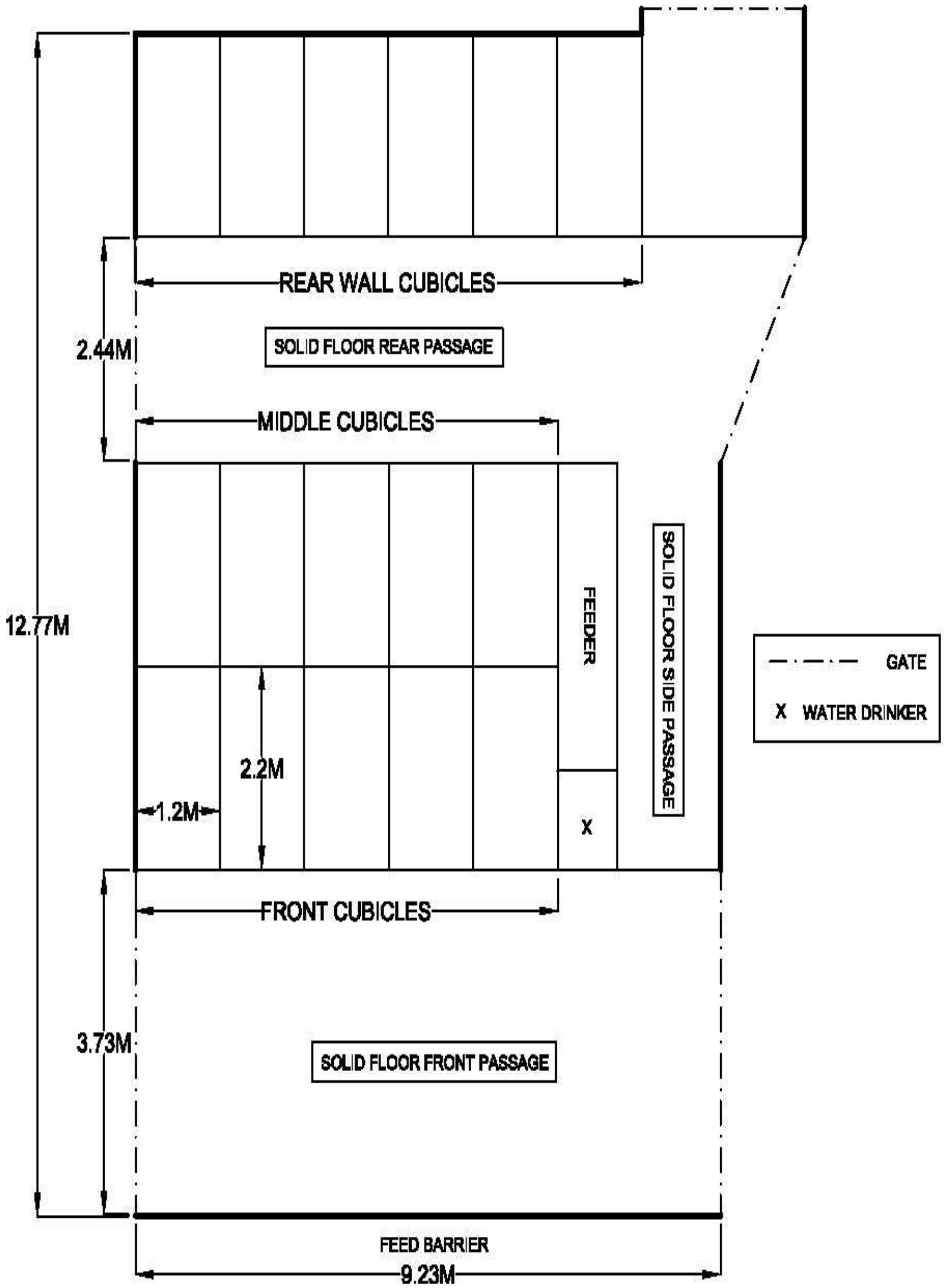

Figure 8 Layout of cubicle accommodation where the 15 animals within each replicate were housed

Results

Behaviour

Direct observations during the two hour period after mixing

The influence of treatment on the duration of time spent performing or receiving different behaviours, and the time spent in different locations within the pen is presented in Table 7. Primiparous cows in the Mixed treatment exhibited increased levels of locomotion compared to animals in the Unmixed treatment (P=0.042). There was a tendency for Unmixed animals to spend more time in receipt of butts (P=0.07) compared to those in the Mixed treatment. When analysed on a frequency/min basis, Unmixed animals received significantly more butts compared to Mixed animals (Mixed 0.03, Unmixed 0.15, SED 0.032/min, $F_{1,3}$ 12.96, P= 0.037).

Table 7 Influence of treatment on the time spent performing and receiving different behaviours and on time spent in different locations during the two hour period after mixing (% observation time)

Behaviour	Treatment		S.E.D.	F	P
	Mixed	Unmixed			
Activity					
Butting	0.34	0.06	0.129	$F_{(1,3)}$ 4.44	0.126
Locomotion	7.72	5.27	0.361	$F_{(1,1.5)}$ 46.31	0.042
Feeding	23.19	22.16	9.490	$F_{(1,3)}$ 0.01	0.920
Explore- feeder	3.85	0.98	1.528	$F_{(1,2.6)}$ 3.53	0.169
Ruminating	29.82	29.77	10.410	$F_{(1,3)}$ 0.00	0.997
Grooming	1.73	0.88	0.521	$F_{(1,3)}$ 2.67	0.202
Location					
Front-passage	60.21	35.42	13.210	$F_{(1,2.8)}$ 3.52	0.163
Rear/side passage	8.82	12.95	2.128	$F_{(1,2.8)}$ 3.78	0.154
Receive					
Shoulder	0.01	0.07	0.027	$F_{(1,3)}$ 4.60	0.121
Butt	0.08	0.35	0.097	$F_{(1,3)}$ 7.69	0.07

Direct observations after feeding

Table 8 shows behaviours performed and received by primiparous cows after fresh food was offered. Mixed animals showed increased durations of locomotion (P=0.017) and shouldering (P=0.039) behaviour compared with Unmixed animals. When analysed on a frequency/min basis the performance of both shouldering and butting differed significantly between treatments (shouldering: Mixed 0.03, Unmixed 0.01, SED 0.008/min, $F_{1,3}$ 14.05, P=0.033; butting: Mixed 0.08, Unmixed 0.01, SED 0.027/min, $F_{1,2.5}$ 24.83, P=0.024). Primiparous cows in the Mixed treatment spent less time in the cubicles (P=0.003) and more time in the front passage (P<0.001) than animals in the Unmixed treatment.

Table 8 Influence of treatment on the time spent performing and receiving different behaviours and on time spent in different locations during the two hour period after fresh food was offered (% observation time)

Behaviour	Treatment		S.E.D.	F	P
	Mixed	Unmixed			
Activity					
Butting	0.48	0.05	0.232	$F_{(1,3)}$ 3.90	0.143
Shouldering	0.16	0.07	0.041	$F_{(1,2.7)}$ 14.37	0.039
Locomotion	4.01	3.24	0.236	$F_{(1,6.1)}$ 10.54	0.017
Feeding	44.66	36.13	4.542	$F_{(1,1.8)}$8.51	0.115
Drinking	2.73	1.85	0.863	$F_{(1,3.8)}$ 2.71	0.179
Grooming	1.18	1.12	0.618	$F_{(1,3.5)}$ 4.70	0.105
Location					
Cubicle	36.90	50.05	5.704	$F_{(1,3.4)}$ 55.22	0.003
Front passage	55.54	44.70	4.998	$F_{(1,3.1)}$ 197.10	<0.001
Receive					
Butt	0.05	0.53	0.077	$F_{(1,5.5)}$ 73.41	<0.001

Unmixed animals spent more time in receipt of butts (P<0.001) compared with Mixed animals. When analysed on a frequency/min basis Unmixed primiparous cows received significantly more butts compared to Mixed animals (Mixed 0.03, Unmixed 0.26, SED 0.054/min, $F_{1,3}$ 28.76, P=0.013).

Lying behaviour (assessed using data loggers)

Treatment had no significant effect on lying behaviour (total hours lying: Mixed 8.15, Unmixed 8.60, SED 0.244, $F_{1,2.4}$ 3.37, P=0.187; number of lying bouts: Mixed 12.74, Unmixed 11.33, SED 0.583, $F_{1,3}$ 5.87, P=0.094; mean hours lying per bout: Mixed 0.70, Unmixed 0.81, SED 0.043, $F_{1,3}$ 6.11, P=0.089). It is worthy to note that during the first 24-hour period following integration with the group the average lying time across both treatments was <5 hours.

Afternoon lying and location observations

Primiparous cows in both treatment groups spent a greater proportion of time in the rear passageway compared to resident cows (P=0.027) (Table 9). A significant interaction was found between treatment and week for the time spent at the feed barrier. During week 1 animals in both treatment groups were observed at the feed barrier for less time than 'resident' animals, while in week 2 both resident and Mixed animals were observed at the feed barrier for longer than Unmixed animals (Figure 9) (Mixed: week 1, 0.15; week 2, 0.29; week 3, 0.29; week 4, 0.31; Unmixed: week 1, 0.16; week 2. 0.13; week 3, 0.23; week 4, 0.27; Resident cows: week 1, 0.37; week 2, 0.37; week 3. 0.31; week 4, 0.31; SED 0.053, $F_{6,23.1}$ 2.81, P=0.033).

Table 9 Influence of treatment on afternoon lying and locations measured by direct observation during the first month after introduction to the group

	Experimental heifers		Resident cows	SED	F	P
	Mixed	Unmixed				
Direct observation (proportion of time animals observed lying or in locations)						
Lying	0.51	0.58	0.51	0.027	$F_{(2,5.7)}$ 4.37	0.07
Rear cubicles (facing wall)	0.29	0.22	0.14	0.062	$F_{(2,7.2)}$ 3.42	0.090
Rear Passage	0.04[a]	0.04[a]	0.01[b]	0.011	$F_{(2,9.2)}$ 5.51	0.027
Middle cubicles	0.18	0.27	0.18	0.055	$F_{(2,5.9)}$ 2.01	0.216
Side passage	0.01	0.02	0.01	0.007	$F_{(2,10.1)}$ 0.87	0.448
Front cubicles	0.17	0.19	0.26	0.068	$F_{(2,7.3)}$ 1.10	0.382
Front passage	0.05	0.04	0.06	0.015	$F_{(2,5.5)}$ 0.62	0.573

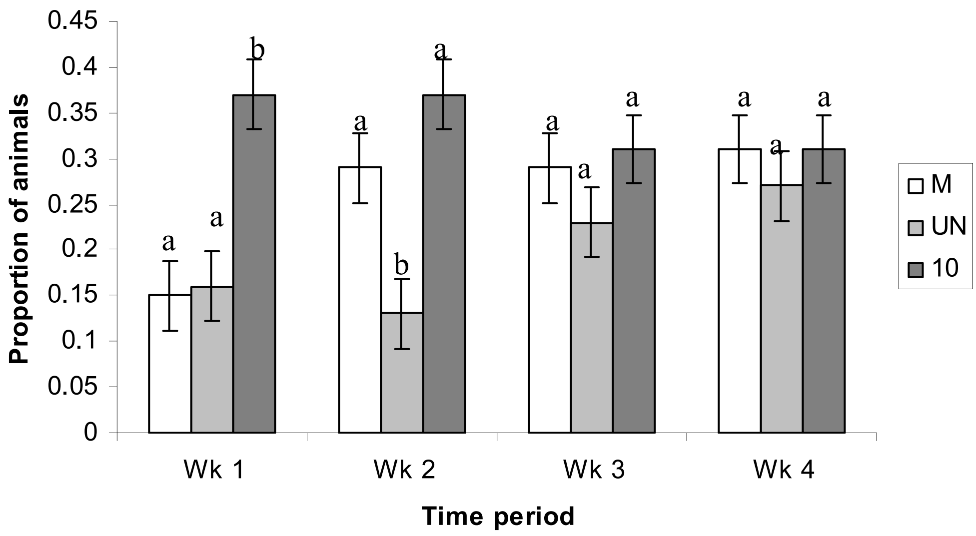

Figure 9 Interaction between treatment and week for the proportion of animals (Mixed, Unmixed, resident animals) at feed barrier

Cortisol

There were no significant treatment effects on levels of serum cortisol during the first 25 or 49 hours after mixing (Mann Whitney U test, 25 h: U=46.5, P=0.810, Median (Q1,Q3) Mixed 9.2 ng/ml (0,11.8), Unmixed 7.7 (0,11.2); 49 h: U=35.0, P=0.267, Median (Q1,Q3) Mixed 7.4 ng/ml (4.1, 13.3), Unmixed 5.7 (0.11.2)).

Production Performance

Treatment had no significant effect on any of the milk production parameters as shown in Table 10.

Table 10 Production performance and body tissue levels of the experimental heifers during the first month after introduction to the resident group

Parameter	Treatment		S.E.D.	F	P
	Mixed	Unmixed			
Milk production					
Milk yield (kg/day)	29.3	28.2	1.563	$F_{(1,15)}$ 0.44	0.516
Fat (g/kg)	46.5	43.2	2.001	$F_{(1,12.7)}$ 3.09	0.103
Protein (g/kg)	34.8	34.5	0.064	$F_{(1,13.5)}$ 0.29	0.598
Lactose (g/kg)	47.0	46.6	0.661	$F_{(1,14.7)}$ 0.42	0.528
Fat yield (kg/day)	1.35	1.24	0.082	$F_{(1,16.5)}$ 2.01	0.175
Protein yield (kg/day)	1.01	0.99	0.048	$F_{(1,14.4)}$ 0.29	0.598
Fat + protein yield (kg/day	2.36	2.22	0.121	$F_{(1,13.4)}$ 1.23	0.287
Milk energy content (MJ/kg)	3.39	3.24	0.085	$F_{(1,16.8)}$ 2.97	0.103
Milk energy output (MJ/day)	98.8	91.9	5.32	$F_{(1,14.4)}$ 2.86	0.112
Body tissue					
LWT (kg)	526	529	15.9	$F_{(1,13)}$ 0.04	0.853
Condition Score	2.72	2.71	0.078	$F_{(1,16.1)}$ 0.02	0.889
LWT loss (kg)	28.3	33.1	4.229	$F_{(1,15.7)}$ 1.28	0.275
Condition Score loss	0.33	0.25	0.092	$F_{(1,18)}$ 0.77	0.391

Live Weight and Body Condition Score Losses

No significant difference was found between treatments in total liveweight loss (Mixed: -28.3, Unmixed: -33.1, SED 4.229 kg, $F_{1,15.7}$ 1.28, P=0.275) or condition score loss (Mixed: -0.33, Unmixed: -0.25, SED 0.092, $F_{1,18}$ 0.77, P=0.391) during the first month after calving. There was also no significant difference between treatments in average live weight (Mixed: 526, Unmixed: 529, SED 15.9 kg, $F_{1,13}$ 0.04, P=0.853) or average body condition score (Mixed: 2.72, Unmixed 2.71, SED 0.078, $F_{1,16.1}$ 0.02, P=0.889), during the first month after calving.

Discussion

Behaviour During the Two Hour Period After Mixing

Animals in the Mixed treatment received fewer aggressive behaviours than Unmixed animals during the 2-hour period after mixing (1.8 versus 9 butts/hour respectively), with the levels observed in the Unmixed treatment similar to the 7-10 agonistic encounters previously reported by other authors (Brakel and Leis, 1976; Knierim, 1999; Neisen *et al.*, 2009). It is unclear why Mixed animals received less aggression from resident animals, although it may have been related to the increased level of locomotion they displayed. Individuals with prior experience of regrouping appear to become involved in fewer acts of aggression when subsequently regrouped, and it is suggested that this is achieved through learning to avoid aggression by active withdrawal, avoidance and increased locomotion (Bouissou, 1975; van Putten and Buré, 1997). Thus it is possible that the previous social experience of nulliparous Mixed animals with non lactating multiparous cows prior to calving allowed them to actively adapt their behaviour to the new situation of being regrouped into the milking herd (Wechsler and Lea, 2007). Although these results suggest increased social learning in the Mixed treatment, it is worth noting that unreported observations suggested no differences in levels of aggression to which heifers were exposed in pre-calving treatment groups.

Behaviour After Feeding

Primiparous cows in the Mixed treatment appeared more willing to leave the cubicles, move around the pen, and particularly move close to where feed was available, namely the front passage. This may have reflected increased ‘confidence’ of these animals as a result of greater experience of socialising with older animals. Alternatively, Unmixed animals experienced increased levels of aggression, and may have used the cubicles as a safe area to escape this aggression. This concurs with Metz and Mekking (1984) and Potter and Broom (1987) who suggested that low ranking animals use cubicles as a refuge from aggression.

Previous research has shown that after social order has been formed within a group of cattle, agonistic behaviour primarily occurs after feeding, specifically during the 30-45 minute period after the delivery of fresh feed or during the 2-hour period on return from milking (McPhee *et al.*, 1962; Friend and Polan, 1974; Grant and Albright, 1997; Oloffson, 1999; Langford *et al.*, 2011). Within the current experiment there was evidence that the Unmixed animals were at a disadvantage compared to other animals in the group during the post feeding period, with this being reflected in them receiving higher levels of aggression and showing reduced locomotory behaviour compared to Mixed animals. It is pertinent to note that although primiparous cows in the Mixed treatment spent more time in the feed passage than the Unmixed animals, they actually received lower levels of aggression and indeed were more overtly aggressive towards other animals. These animals may have been engaged in more aggression because they were located in an area where aggression was more likely to be exhibited, namely at the feed barrier, or because they had gained experience of how to compete in the feeding area, particularly when housed with non lactating cows pre-calving.

Location and Lying Behaviour

Afternoon lying and location observations indicated that Mixed animals and resident cows spent a greater proportion of time at the feed barrier during week 2 compared to Unmixed animals. This suggests that the Unmixed primiparous cows remained less prepared to feed at the barrier, even after peak feeding time had passed (Friend and Polan, 1974). This may have been due to the adverse experience of receiving increased aggression after fresh feed had been presented in the morning, although it is not clear why this was observed in only one out of the four observation weeks. Similarly, it is unclear why experimental animals from both treatments spent more time in the rear passage compared to the resident animals, however Potter and Broom (1987) found a tendency for animals that were not using a cubicle or feeding, to stand more in the cubicle passage as opposed to the feeding area.

Introducing primiparous cows into a herd can cause a significant reduction in their lying time, particularly during the period after mixing when animals have been observed to lie for as little as 87 minutes per 12-hour period and 5 hours per 24-hour period (Krohn and Konggaard, 1979; Knierim, 1999). In the current study animals in both treatments were observed to lie for less than five hours during the first 24 hours after introduction to the group, supporting the research mentioned above. It is also known that decreased lying times in primiparous cows can lead to reductions in hoof health (e.g. increased sole lesions), which can, in turn, have negative implications for the welfare of the animal (Singh *et al.*, 1993; Galindo *et al.*, 2000). However, treatment had no effect on the total time spent lying, with both Mixed and Unmixed primiparous cows lying for an average of 8.4 hours per 24-hour period, similar to the 8.39 hours per day observed by Singh *et al.* (1993) 6 weeks after heifers were mixed with adult cows.

Stress Hormones

While cortisol levels in dairy cows are normally between 3.0-3.5 ng/ml during the first month post calving (Uchida *et al.*, 1993), cortisol levels in the experimental animals in the present study were higher than these, although they were unaffected by treatment. This may suggest that animals in both treatments found the situation of being grouped with mature cows stressful. Indeed Krohn and Konggaard (1982) found primiparous cows grouped with older cows to have a cortisol concentration of 5 ng/ml during the first week, while those kept in a separate group had a concentration of 3.7 ng/ml. Cortisol levels in both treatments in the current study, particularly in the 49-hour period, were higher when compared with the levels observed by Krohn and Konggaard (1982) at day 2 after regrouping (4 ng/ml) for primiparous cows grouped with multiparous cows. This may suggest that the current experimental set-up was particularly stressful in the post-calving period.

Production Performance

There appears to be little published research on the effects of mixing primiparous cows with older animals prior to calving on subsequent production performance. Although animals in the Unmixed treatment were subject to increased levels of aggression, especially after fresh food was offered, this appeared to have no effect on production performance parameters or body tissue losses between the two treatments.

Conclusions

Mixing nulliparous cows with non-lactating multiparous cows prior to calving appeared to improve welfare after mixing by reducing levels of aggression to which they were exposed. This treatment effect was also evident after feeding during the first month in the group. During this time period animals in the Mixed treatment not only received less aggression, but were more overtly aggressive towards other animals. These animals also appeared more ‘confident’ in their behaviour by spending less time in cubicles and more time in feeding areas, however there was no significant treatment effect on milk production, stress hormones or overall lying behaviour. It is suggested that this increased ‘confidence’ reflects increased social experience gained in the pre-calving period. Overall, these results suggest that there are beneficial effects on welfare during the post-calving of mixing heifers with dry cows prior to calving.

Study 3

Should primiparous cows be housed separately from multiparous animals during the two-week period after calving?

Abstract

There is increasing interest in identifying optimum management strategies by which to introduce primiparous dairy cows to the milking herd after calving. In this study, thirty primiparous Holstein Friesian cows were assigned to one of two treatments during the post-calving period. The animals were either retained in a separate primiparous group for 2 weeks before being integrated with a 'resident' group containing mature cows ('Separate Group'), or were integrated with a resident group within one day of calving ('Direct Introduction'). The size of resident groups remained constant at 16 animals (10 multiparous cows and 6 primiparous cows). All measures were taken from primiparous cows within the first 6 weeks of calving. The behaviour of these animals was assessed after mixing into the separate group (as appropriate) and the resident group, and also after feeding in the resident group. Lying and location of the group was assessed by direct observation during the 2-hour period prior to evening milking during weeks 3 to 6 after calving. In addition, data loggers were attached to experimental animals over 24-hour periods to assess time spent lying (weeks 1-6). Milk production and milk cortisol levels, in addition to changes in live weight and body condition, were also assessed (weeks 1-6). Animals in the Separate Group treatment exhibited increased levels of exploratory behaviour, increased lying and were located more in the cubicles and less in the front passage of the pen upon introduction to the Resident group compared to animals in the Direct Introduction treatment ($P<0.01$). Animals in the Direct Introduction treatment received more butts ($P<0.05$) and showed increased durations of avoidance behaviour during this period ($P<0.05$). After feeding (where comparisons were standardised to weeks 3-6 of lactation) animals in the Direct Introduction treatment received significantly more butts ($P<0.05$) compared to Separate Group animals. Treatment had no

significant effect on overall lying behaviour, with heifers in both treatments lying for less than 6 hours during the first 24 hours after introduction to the post-calving group. During the afternoon lying and location observations it was observed that primiparous cows in both treatments spent a higher proportion of time in the rear cubicles facing the wall, compared to resident cows ($P<0.01$), and that Direct Introduction animals spent a lower proportion of time in the middle cubicles compared to both the Separate Group animals and resident cows ($P<0.01$). No significant treatment effects were shown on overall milk yield, milk cortisol levels or on weight or condition score loss. In conclusion, retaining primiparous cows in a separate group for 2 weeks after calving led to them receiving significantly less aggression when integrated with a group containing mature cows than those introduced directly after calving. These animals continued to receive less aggression after feeding suggesting prolonged beneficial effects on welfare.

Introduction

Primiparous dairy cows are exposed to a number of stressors during the post-calving period. For example, integration into the main dairy herd for the first time can have an adverse effect on their welfare, and indeed regrouping has been shown to negatively affect production performance (Krohn and Konggaard, 1982; von Keyserlingk *et al.,* 2008; Neisen *et al.,* 2009). To address this issue it has been suggested that it may be desirable to group primiparous cows separately from the main herd during the post-calving period, as they are often smaller and have a lower social status than mature cows (Lamb, 1976; Dawson and Carson, 2004). Indeed, studies have highlighted a number of production and welfare benefits associated with housing heifers in a separate group during the post-calving period (Krohn and Konggaard, 1979; Phelps, 1992; Payne and Aikman, 2007). However, housing constraints on many farms mean that it is not always feasible to keep primiparous cows separate from the rest of the herd for the whole of their first lactation. Limited research has been carried out to examine the effects of keeping primiparous cows in a separate group for a period of time after calving, and before integration with the main herd. O'Connell *et al.* (2008) examined the effect of retaining heifers individually in a straw pen for a period of seven days prior to being introduced to a group containing mature cows, but found no significant effect on milk yield. Østergaard *et al.* (2010) observed that when primiparous and multiparous cows (housed together) were kept in a separate group from the main herd for one month after calving, there were positive effects for milk production and health for primiparous cows only, when introduced into the main milking group. However, that study did not examine aggressive or lying behaviour, or stress hormones levels.

The aim of the present study was to assess if housing primiparous cows separately for two weeks after calving leads to welfare and performance benefits when integrated with a group containing mature cows compared to animals introduced directly to this group after calving.

Materials and Methods

Animals, Management and Housing

Thirty primiparous Holstein Friesian (HF) cows were used as experimental animals in this study. These animals were reared in groups, and had access to cubicles from seven weeks of age, with the exception of the periods from April-September (inclusive) when they were at pasture. Experimental nulliparous cows were moved in small batches from a heifer rearing facility to the main dairy production unit approximately one month prior to calving and placed in a dynamic group in a pen with free access to cubicles. During this pre-calving period cows had *ad libitum* access to a forage diet comprising grass silage, maize/silage and chopped straw (60, 20 and 20% respectively, on a dry matter (DM) basis), together with an additional 120 g/day of dry cow mineral.

Experimental cows were moved to individual straw-bedded calving pens (5.9 × 3.3 m) 24-48 hours prior to calving (based on predicted calving dates and daily assessments by experienced stockpersons). Discretionary calving assistance was given. After birth, calves remained with their dams for 6-12 hours, with the dam continuing to be penned individually with their calves during this time. These experimental cows calved between September and December 2009.

Animals in both treatment groups were introduced to their resident group (see later) between 06:00 and 06:30 hours (after milking). The resident groups were housed in pens with 16 cubicles in 3 rows and solid concrete floors (see Figure 8). Out of parlour feeders were present in all pens but were not used. Concrete passageways were cleaned a minimum of four times daily using an automatic scraper, and cows accessed food via an open feed barrier. After calving, cows were offered a total mixed ration (TMR) *ad libitum* comprising 40% concentrate and 60% grass/silage (DM basis), together with 4 kg of concentrate in the milking parlour (2.0 kg in the morning and evening). Primiparous cows were milked in a 50-point rotary parlour twice daily (at approximately 06:00 and 16:00 hours).

Treatments and Experimental Groups

The effect on welfare and performance of retaining primiparous cows in a separate group for a 2-week period after calving, prior to being introduced to a group containing multiparous cows, was assessed in a two treatment (n=15 experimental animals per treatment) experiment involving five replicates (Figure 10). The experimental period lasted for 6 weeks after calving.

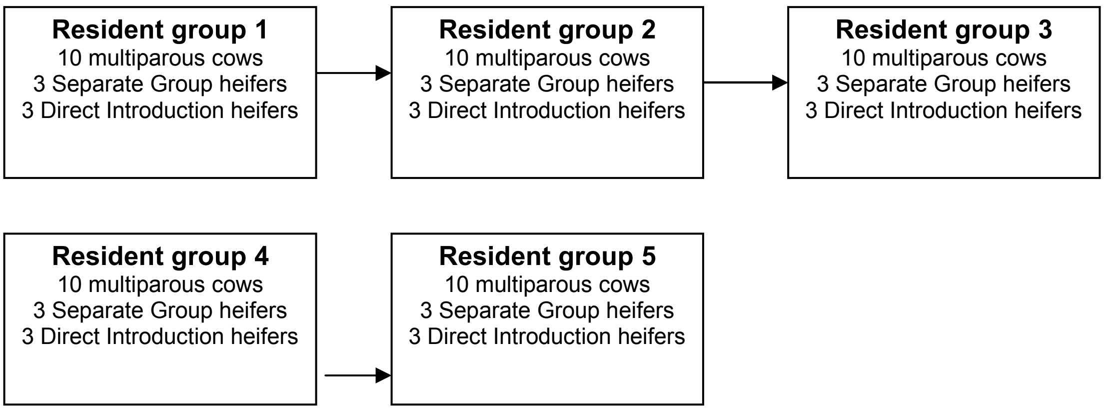

Figure 10 Structure of resident groups (Study 3)

Treatments are described as follows:

Separate Group (SG) (n=15) – Primiparous cows retained in a separate 'primiparous group' for two weeks after calving prior to being introduced to the resident group.

Direct Introduction (DI) (n=15) – Primiparous cows added to the resident group within 1 day of calving.

Treatments were balanced for genetic merit (Predicted Transmitting Ability (PTA) for kg fat + protein, kg fat and kg protein), sire, body weight and condition score pre calving. Group size in the Separate Group treatment was kept constant at 6 primiparous cows, with this being a dynamic group. These animals were housed in a solid floor pen similar to the resident pen, but with access to only 6 cubicles, 3 of which were in the front and 3 in the middle (Figure 8). A total of five replicate or 'resident' groups were used, with each group being established five days before the first experimental heifer was introduced. Each resident group contained ten multiparous HF cows (average 3.7 lactations) and six non-experimental (HF) primiparous cows. The group size and cow:heifer ratio within the resident group was maintained throughout the study by removing non-experimental primiparous cows from the group as new experimental primiparous cows were added. The process of replacing all six non-experimental animals within a replicate group, with experimental animals, was completed over an average period of 20 days. In each of the five replicates, three non-experimental primiparous cows were replaced by primiparous cows from the Separate Group treatment, and three by primiparous cows from the Direct Introduction treatment. During this period, the order in which experimental animals were introduced into the resident groups was balanced across treatments. The interval between the introduction of successive experimental animals into the resident group ranged from 1 to 14 days, resulting in continual disruption to the social group throughout the experimental period.

Measurements

Behaviour after mixing and feeding

Each experimental primiparous cow was observed for a continuous two-hour period immediately after being introduced into their post-calving group, while animals in the Separate Group treatment were also observed again during the two-hour period after joining the resident group. In addition, experimental animals were also observed during four 5-minute periods at 30-minute intervals during the 2-hour period after fresh food was offered. These latter observations were taken on one occasion each week during weeks 3, 4, 5 and 6 post-calving.

Behaviour was recorded by direct observation using a handheld data recorder (Psion Organiser II, Model LZ64, Noldus Information Technology, The Netherlands) using the ethogram shown in Table 11. The ethogram contained four mutually-exclusive categories of behaviour: the state of the animal (in terms of duration of time spent lying or standing), the location of the animal in the pen (also measured as duration of time), and behaviours performed by the animal ("activity") and received by the animal. The duration and frequency was recorded for all aggressive behaviours performed (i.e. butting, shouldering, avoiding) and for all received behaviours. Only the duration of all other "activity" behaviours was recorded.

Lying behaviour

Direct observations of lying and standing behaviour, and the location of the experimental animals in the pen were also performed over the duration of the study (weeks 3-6) (Refer to Figure 8). Lying behaviour using data loggers (DL) was monitored for the first 24 hours after experimental animals were introduced to their post-calving group and also for a single 24-hour period each week thereafter for a period of six weeks. Please refer to Study 1 for experimental protocol used to record these parameters.

Table 11 Ethogram of post mixing and post feeding behaviours recorded

Behaviour	Description
Activity	
Butting	When the cow uses the front of her head to make vigorous contact with another cow
Shouldering	Displacement of an individual using the shoulder
Avoiding	When a cow actively moves away from another individual irrespective of whether an interaction has occurred between the two individuals
Locomotion	All four legs moving and head not in contact with any substrate
Motionless	No legs moving and head not in contact with any substrate
Feeding	Head in the feed barrier and feeding
Explore-feeder	Nosing any part of the feed barrier
Explore-general	Nosing any substrate in pen including floors, walls and railings, but not the feed barrier or another cow
Social-investigative	Nosing another animal without displaying agonistic or cohesive behaviour
Ruminating	Regurgitating and chewing boluses of food
Drinking	Drinking at water trough
Grooming	Focal animal grooming itself
Location	
Cubicle	Focal animal located in cubicle
Front-passage	Focal animal located in front passageway
Rear/side Passage	Focal animal located in rear or side passageway
State	
Lying	Lying down
Standing	Standing up
Received Behaviours	
Receive butt	When the focal animal receives butting behaviour
Receive nose	When the focal animals receives nosing behaviour

Milk cortisol

Milk samples for cortisol analysis were taken from each animal within 24 hours of being introduced to their post-calving group and during weeks 2, 3 and 6. Please refer to Study 1 for experimental protocol.

Milk production

Milk yields (kg) of individual animals were recorded at each morning and evening milking between days 6 and day 42 after calving. Please refer to Study 1 for experimental protocol.

Body weight and condition score

The live weight and body condition score (1-5, using increments of 0.25; Edmondson *et al.,* 1989) of each experimental animal was recorded on a weekly basis for the first six weeks post calving. Live weight and body condition score loss were calculated by subtracting values recorded at week 6 after calving from those recorded at day 1 after calving.

Statistical Analysis

Data were analysed using Genstat 11.1 (Payne *et al.*, 2008). The influence of treatment on behaviour during the first two hours after introduction to the group was analysed by ANOVA, with the random effects being replicate and group within replicate (a comparison was made between Separate Group and Direct Introduction animals after mixing in their respective post-calving groups, and also between treatments when introduced to the main resident group). In this analysis the term 'group' refers to the mean of animals behaviours' within a treatment and replicate. This model was also used to access time spent lying in the first 24 hours after introduction into their post-calving group (recorded by data loggers). The influence of treatment on behaviour during the post-feeding period for the 3-6 week post-calving period (i.e. standardised for stage of lactation) was assessed by REML Variance Components Analysis (fixed effects were time period (week), treatment and treatment within time period, and the random effects were replicate, group within replicate and time period within group within replicate). This model was also used to assess treatment effects on total time spent lying within 24-hour periods in weeks 3-6 and 1-6 (recorded by data loggers), and on the proportions of these animals lying in different locations in weeks 3-6 e.g. front passage, side passage and rear passage (observed through direct observation).

The number of lying bouts per 24-hour period and mean hours lying per bout (recorded using data loggers) were analysed by REML Variance Components Analysis using treatment as a fixed effect, and replicate and group within replicate as random effects. This model was also used to assess treatment effects on the average proportion of Separate Group, Direct Introduction and resident animals lying and on the proportions of these animals lying in different locations in weeks 3-6 e.g. feeding at the barrier, front cubicles, middle cubicles and rear cubicles facing wall (observed through direct observation). Treatment effects on average milk cortisol levels were analysed by REML Variance Components Analysis using a model which took milk yield into account (fixed effects were milk yield and treatment, and random effects were replicate and group within replicate).

The influence of treatment on production performance, live weight and condition score was analysed by REML Variance Components Analysis (fixed effects were time period (day of lactation or week), treatment and treatment within day of lactation/week, and random effects were replicate, animal within replicate and time period (day of lactation or week) within animal within replicate. REML Variance Components Analysis (fixed effect was treatment and random effects were replicate and animal within replicate) was used to determine treatment effects on live weight and body condition score loss.

For all REML analysis where time period/week has been used as a factor, main treatment effects and also interactions between treatment and time period will be reported, but main effects on time period will not be reported. For all statistical models, residual values were plotted and visually assessed for normality. Some behaviours were performed too infrequently for statistical analysis and included butting, shouldering, avoiding and receiving nosing after mixing in the separate heifer group, and avoiding, social investigative, the location rear/side passage and receiving nosing after feeding (weeks 3-6).

Results

Behaviour

Comparison of behaviour after mixing into separate group and resident group immediately after calving

Experimental cows in the Separate Group treatment performed more exploratory behaviour ($P<0.05$), and spent more time in the front passage ($P<0.05$) compared to those in the Direct Introduction treatment (Table 12). However experimental cows in the Direct Introduction treatment expressed increased locomotory behaviour ($P<0.05$), spent more time motionless ($P<0.05$) and spent more time in receipt of butts, compared to cows in the Separate Group treatment ($P<0.01$). There were no significant effects of treatment on any of the other behaviours or locations of cows within the pen.

Comparison of behaviour of Separate Group and Direct Introduction animals after mixing into the resident group

Primiparous cows in the Separate Group treatment spent more time exploring during the first two hours after introduction to the resident group compared to cows in the Direct Introduction treatment ($P<0.01$), while the latter spent more time avoiding other animals ($P<0.05$) (Table 13). Animals in the Separate Group treatment spent significantly more time in the cubicles ($P<0.01$) and less time in the front passage ($P<0.01$), and spent more time lying ($P<0.01$) and less time standing ($P<0.01$), than those in the Direct Introduction treatment. However, animals in the Direct Introduction treatment spent more time in receipt of butts ($P<0.05$) than those in the Separate Group treatment. When analysed on a frequency/minute basis, Direct Introduction animals also received more butts (butt: Separate Group, 0.03; Direct Introduction, 0.12; SED 0.020 min^{-1}, $F_{1,4}$ 23.09, $P=0.009$).

Table 12 Effect of treatment on the time spent performing and receiving different behaviours and in different locations and states during the two-hour period after experimental cows were introduced to the post calving primiparous cow group (SG) and cows in the Direct Introduction (DI) were introduced to the resident cow group (% observation time)

Behaviour	Treatment		SED	$F_{(1,4)}$	P
	SG	DI			
Activity					
Social-investigative	0.16	0.22	0.062	0.94	0.388
Explore general	11.55	5.19	2.258	7.94	0.048
Explore-feeder	2.08	0.48	0.880	3.27	0.145
Feeding	38.00	24.90	7.390	3.15	0.150
Ruminating	23.60	27.40	7.590	0.25	0.643
Locomotion	2.63	5.79	0.979	10.43	0.032
Motionless	18.50	34.20	5.030	9.71	0.036
Grooming	1.40	0.62	0.321	5.84	0.073
Drinking	1.89	0.92	0.431	5.09	0.087
Location					
Cubicle	31.30	35.90	5.870	0.60	0.481
Front-passage	57.70	37.80	6.630	8.99	0.040
Rear/side passage	11.00	26.30	9.000	2.89	0.164
State					
Lying	14.20	10.50	7.250	0.25	0.641
Standing	85.80	89.50	7.250	0.25	0.641
Receive					
Butt	0.02	0.58	0.081	49.16	0.002

Table 13 Influence of treatment on the time spent performing and receiving different behaviours and in different locations and states during the two-hour period after cows in the Separate Group (SG) and Direct Introduction (DI) were introduced into the main resident group (% observation time)

Behaviour	Treatment		SED	$F_{(1,4)}$	P
	SG	DI			
Activity					
Social-investigative	1.33	0.22	0.462	5.80	0.074
Explore-general	15.80	5.20	1.510	49.11	0.002
Explore-feeder	0.34	0.48	0.331	0.20	0.678
Feeding	18.4	24.90	2.540	6.51	0.063
Ruminating	28.5	27.4	4.930	0.05	0.838
Locomotion	4.62	5.79	1.206	0.94	0.388
Motionless	28.20	34.20	4.980	1.47	0.292
Grooming	0.84	0.62	0.389	0.30	0.610
Drinking	1.17	0.92	0.293	0.75	0.436
Avoiding	0.03	0.12	0.030	8.95	0.040
Shouldering	0.04	0.03	0.012	0.45	0.540
Butting	0.21	0.06	0.077	3.81	0.123
Location					
Cubicle	59.60	35.90	5.030	22.25	0.009
Front-passage	27.50	37.80	1.870	30.21	0.005
Rear/side passage	12.80	26.30	6.520	4.28	0.107
State					
Lying	47.60	10.50	5.720	42.01	0.003
Standing	52.40	89.50	5.720	42.01	0.003
Receive					
Nose	0.04	0.27	0.095	5.97	0.071
Butt	0.09	0.58	0.108	20.73	0.010

Direct observations after feeding in resident group (weeks 3-6 of lactation)

During the 2-hour period after cows were offered fresh food (Table 14) there was a significant interaction between treatment and time in the time spent in locomotion (Figure 11) (Separate Group: 1.68, 2.12, 1.76, 0.83; Direct

Introduction: 2.62, 1.24, 4.07, 2.37, for weeks 3–6, respectively: SED 0.686, $F_{3,17}$ 4.87, P=0.013). Direct Introduction animals spent more time in receipt of butts (P<0.05) compared to animals in the Separate Group treatment. When analysed on a frequency/minute basis animals in the Direct Introduction treatment received significantly more butts (Separate Group, 0.03; Direct Introduction, 0.09; SED 0.019 min, $F_{1,3.9}$ 9.81, P=0.037).

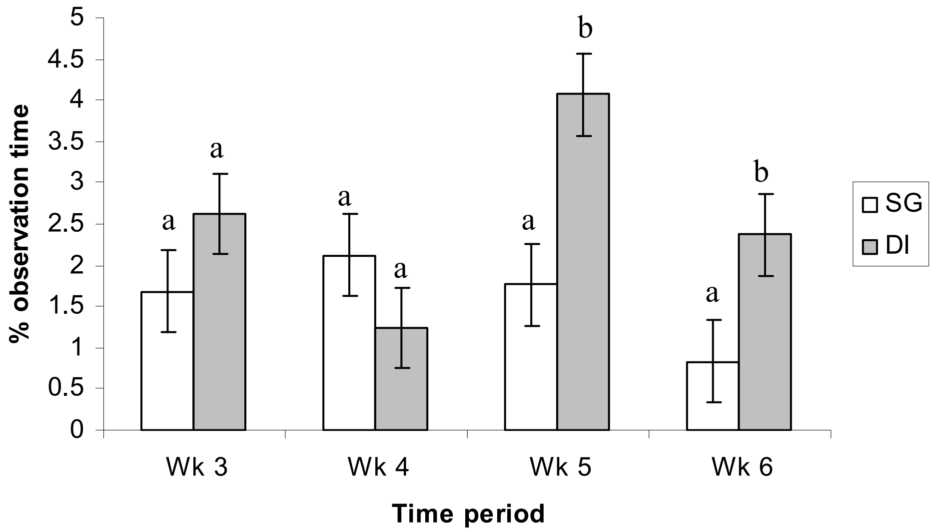

Figure 11 Interaction between treatment and week for locomotion (% observation time)

Table 14 Effect of treatment (Separate Group, SG; Direct Introduction, DI) on the time spent performing or receiving different behaviours and in different locations and states during the two-hour period after cows were offered access to fresh food during weeks 3-6 (% observation time)

Behaviour	Treatment		SED	F	P
	SG	DI			
Activity					
Explore-general	3.09	2.39	1.018	$F_{(1,4)}$ 0.45	0.539
Explore-feeder	0.06	0.26	0.175	$F_{(1,3.9)}$ 1.31	0.319
Feeding	57.48	62.15	6.477	$F_{(1,4)}$ 0.42	0.552
Ruminating	23.65	14.62	4.906	$F_{(1,4)}$ 3.23	0.148
Motionless	10.21	14.23	2.334	$F_{(1,3.9)}$ 2.95	0.162
Grooming	0.61	0.61	0.127	$F_{(1,3.9)}$ 0.03	0.875
Drinking	2.06	1.61	0.702	$F_{(1,3.9)}$ 0.41	0.556
Shouldering	0.08	0.08	0.024	$F_{(1,4)}$ 0.04	0.860
Butting	0.26	0.14	0.105	$F_{(1,4)}$ 1.18	0.338
Location					
Cubicle	33.07	25.52	6.821	$F_{(1,4)}$ 1.16	0.342
Front-passage	64.36	71.77	6.503	$F_{(1,4)}$ 1.30	0.318
State					
Lying	26.67	21.44	5.945	$F_{(1,4)}$ 0.69	0.452
Standing	73.32	78.56	5.945	$F_{(1,4)}$ 0.69	0.452
Receive					
Butt	0.14	0.40	0.094	$F_{(1,3.9)}$ 8.25	0.047

Lying behaviour

Treatment had no significant effect on lying behaviour during the first 24 hours after the Separate Group treatment were introduced into the ‘primiparous cow group’ and the Direct Introduction treatment were introduced into the resident group (Total hours lying: Separate Group, 5.84: Direct Introduction, 5.80: SED 0.723, $F_{1,4}$ 0.00, P=0.952; Number of lying bouts: Separate Group, 9.93: Direct Introduction, 10.80: SED 0.860, $F_{1,4}$ 1.02, P=0.371; Mean hours lying per bout:

Separate Group, 0.65: Direct Introduction, 0.62: SED 0.066, $F_{1,4}$ 0.19, P=0.688).

Treatment had no significant effect on lying behaviour as recorded by data loggers during weeks 3-6 of lactation (Total hours lying: Separate Group 9.78, Direct Introduction 9.95, SED 0.398, $F_{1,4}$ 0.19, P=0.682; Number of lying bouts: Separate Group 13.87, Direct Introduction 13.58, SED 0.887, $F_{1,4}$ 0.10, P=0.765; Mean hours lying per bout: Separate Group 0.76, Direct Introduction 0.82, SED 0.031, $F_{1,4}$ 3.08, P=0.154). The average mean lying time was 9.9 hours per 24 hour period. Also, treatment had no significant effect on lying behaviour as recorded by data loggers during weeks 1-6 of lactation (Total hours lying: Separate Group 9.43, Direct Introduction 9.42, SED 0.373, $F_{1,4.2}$ 0.00, P=0.996; Number of lying bouts: Separate Group 13.37, Direct Introduction 13.70, SED 0.959, $F_{1,4}$ 0.12, P=0.746; Mean hours lying per bout: Separate Group 0.76, Direct Introduction 0.77, SED 0.039, $F_{1,4}$ 0.01, P=0.920).

In terms of lying behaviour (weeks 3-6) during the two-hour observation period, no significant difference was found between animals in the two treatments and resident cows (P>0.1) (Table 15). Experimental cows in both treatment groups spent a higher proportion of time in the rear cubicles facing the wall compared to resident cows (P<0.01), while experimental cows in the Direct Introduction group spent a lower proportion of time in the middle cubicles compared to either the Separate Group treatment and resident cows (P<0.01).

Table 15 Effect of treatment (Separate Group (SG) and Direct Introduction (DI) on proportion of time animals spent in different locations and behaviours during weeks 3-6) after introduction of experimental animals to the resident group

	Treatment		Resident cows	SED	F	P
	SG	DI				
Direct observation (proportion of time animals observed lying or in locations)						
Lying	0.49	0.46	0.54	0.059	$F_{(2,8)}$ 0.98	0.416
Rear cubicles (facing wall)	0.22[a]	0.29[a]	0.12[b]	0.041	$F_{(2,8)}$ 9.62	0.007
Rear Passage	0.02	0.02	0.02	0.014	$F_{(2,5.4)}$ 0.17	0.845
Middle cubicles	0.20[a]	0.12[b]	0.22[a]	0.021	$F_{(2,8)}$ 13.42	0.003
Side passage	0.01	0.01	0.01	0.004	$F_{(2,7.7)}$ 0.06	0.943
Front cubicles	0.15	0.17	0.27	0.055	$F_{(2,8)}$ 2.87	0.115
Front passage	0.04	0.05	0.06	0.010	$F_{(2,6.1)}$ 2.09	0.204
Feeding at barrier	0.36	0.33	0.31	0.050	$F_{(2,8)}$ 0.56	0.593

Milk Production, Body Tissue, Milk Cortisol

Treatment had no significant effect on milk yield during weeks 3-6 post calving (Table 16). Also, treatment had no significant effect on milk yield during weeks 1-6 (Separate Group, 24.0: Direct Introduction, 22.6: SED 1.156 kg, $F_{1,24}$ 1.56, P=0.224).

Neither average live weight (Separate Group, 495; Direct Introduction, 504; SED 12.9 kg, $F_{1,24}$ 0.50) nor average body condition score (Separate Group, 2.45; Direct Introduction, 2.48; SED 0.048, $F_{1,23.8}$ 0.58) during weeks 3-6 post calving were significantly affected by treatment. Also, treatment had no significant effect on average live weight (Separate Group, 498; Direct Introduction, 506; SED 12.3 kg, $F_{1,23.9}$ 0.44) or average body condition score (Separate Group, 2.49; Direct Introduction, 2.52; SED 0.048, $F_{1,23.9}$ 0.19) during weeks 1-6 after calving. There was no significant difference between treatments in either live weight loss (Separate Group, -14; Direct Introduction, -12; SED 9.373 kg, $F_{1,20.2}$ 0.04) or condition score loss (Separate Group, -0.20; Direct Introduction, -0.11; SED 0.059, $F_{1,22.1}$ 2.28) between weeks 1 and 6.

There was no significant difference between treatments in mean milk cortisol levels recorded at weeks 3 and 6 post calving, with levels for the Separate Group and Direct Introduction treatments being 0.46 and 0.59 respectively (SED 0.101 ng/ml, $F_{1,4.2}$ 1.80, P=0.248) (Table 16). There was also no significant difference between treatments in the levels of milk cortisol recorded during weeks 1 and 2 post-calving (week 1: Separate Group, 0.74: Direct Introduction, 0.81: SED 0.229 ng/ml, $F_{1,3.9}$ 0.09; week 2: Separate Group, 0.43: Direct Introduction, 0.55: SED 0.131 ng/ml, $F_{1,3}$ 0.79).

Table 16 Production performance, body tissue and cortisol levels of the experimental heifers (Separate Group (SG) Direct Introduction (DI)) during weeks 3-6 after introduction to the resident group

Parameter	Treatment		S.E.D.	F	P
	SG	DI			
Milk Production					
Milk yield (kg/day)	24.9	23.2	1.227	$F_{(1,24)}$ 1.83	0.189
Fat (g/kg)	41.7	41.6	1.174	$F_{(1,23.9)}$ 0.03	0.866
Protein (g/kg)	33.0	33.1	0.505	$F_{(1,24)}$ 0.00	0.946
Lactose (g/kg)	47.1	47.1	0.346	$F_{(1,24)}$ 0.03	0.876
Fat yield (kg/day)	1.06	0.95	0.062	$F_{(1,24)}$ 3.08	0.092
Protein yield (kg/day)	0.83	0.76	0.042	$F_{(1,23.7)}$ 3.19	0.087
Fat + protein yield (kg/day)	1.89	1.71	0.101	$F_{(1,23.9)}$ 3.09	0.092
Milk energy content (MJ/kg)	3.16	3.16	0.047	$F_{(1,23.8)}$ 0.01	0.940
Milk energy output (MJ/day)	80.0	72.7	4.263	$F_{(1,23.9)}$ 3.01	0.096
Body tissue					
LWT (kg)	495	504	12.9	$F_{(1,24)}$ 0.50	0.485
Condition Score	2.45	2.48	0.048	$F_{(1,23.8)}$ 0.58	0.454
Stress hormone					
Milk cortisol (ng/ml)	0.46	0.59	0.101	$F_{(1,4.2)}$ 1.80	0.248

Milk cortisol value represents an average of values from weeks 3+6.

Discussion

Behaviour After Mixing and Feeding

It was expected that animals in the Separate Group would receive less aggression after introduction to the post-calving group than those in the direct introduction treatment. This assumption was based on the fact that the group size was much smaller, and the group was composed of primiparous cows only. This may have led to the Separate Group animals spending more time in the front passage, where the feed barrier was located, and showing increased exploratory behaviour. von Keyserlingk *et al.* (2008) found that on the day of regrouping animals were displaced significantly more in the feeding than in other areas, which is possibly why Direct Introduction animals were located less in the front passage. Boissy (1995) suggests that immobility or movement inhibition reflects fearfulness in animals. This suggests that Direct Introduction animals were more fearful in the current study. This may have been due to them receiving increased aggression from older cows. Indeed, previous research found that cattle enduring a stressful event exhibited fear related behaviours similar to the behaviour of Direct Introduction animals in the current study, in terms of greater variations in immobility and locomotion behaviour possibly reflecting agitated states (Muller and von Keyserlingk, 2006). In addition to the presence or absence of multiparous cows, a number of other factors may also have affected the behaviour of animals in the two treatment groups. For example, the increased group size in the Direct Introduction treatment resulted in a reduced floor space allowance per cow, and reduced feed barrier space per cow, both of which may have led to an increase in agonistic behaviours (Kondo *et al.,* 1989: Huzzey *et al.,* 2006).

Immediately following integration to the resident group, primiparous cows in the Separate Group treatment spent more time lying down and more time in the cubicles than Direct Introduction animals. It has been suggested that animals use cubicles as a refuge from aggression (Metz and Mekking, 1984; Potter and Broom, 1987), and it is possible that Separate Group animals use them for this purpose. An alternative explanation for the differences in time spent lying down was the differences between treatments in time post calving. Primiparous cows in the Direct Introduction treatment were introduced immediately after calving and therefore may have been less willing to lie down due to pain associated with parturition. Animals in the Separate Group were introduced two weeks after calving, and as such are likely to have significantly recovered from the calving process. Indeed, parturition is associated with considerable pain and stress and this is especially true for primiparous heifers as it takes them longer to calve (Doornbos *et al.,* 1984; Hydbring *et al.,* 1999; Wehrend *et al.,* 2005; Dobson *et al.,* 2008).

The significantly higher level of aggression received by Direct Introduction animals compared to the Separate Group animals following introduction to the resident group (7.2 vs 1.8 butts per hour) may have been related to the former animals spending less time in cubicles and more time in the front passage. These aggression levels broadly concur with Neisen *et al.* (2009) who found that when primiparous cows were introduced into a group of multiparous animals on their own they received 7.2 agonistic interactions per hour. The increased aggression that Direct Introduction animals received is likely to have contributed to the increased avoidance behaviour they exhibited, and it is likely that this behaviour was employed as a means of avoiding further aggressive encounters (Gibbons *et al.,* 2009). The increased exploratory behaviour performed by Separate Group animals may have reflected increased curiosity in their new environment, which may derive from the fact that they were receiving lower levels of aggression.

It is unclear why animals in the Direct Introduction treatment received increased aggression during the period after fresh food was offered (during weeks 3-6 of lactation) compared to those in the Separate Group treatment. Indeed the Direct Introduction animals had been housed with the resident group for a longer time than those in the Separate Group treatment, and it is known that social stabilisation usually occurs within a period of 1-2 weeks after regrouping (Kondo and Hurnik, 1990; Bøe and Faerevik, 2003). This effect on aggression could have occurred for a number of reasons. For example, the resident group may have first encountered the Direct Introduction animals at a time when they were particularly vulnerable following calving, and having learnt how to bully these animals, just continued to do so over the course of the study. Indeed, Lamb (1976) suggested that because cows were weakened and stressed by the onset of calving and lactation possibly makes them more susceptible to being bullied. Because animals in the Separate Group treatment had a longer recovery period prior to encountering the multiparous resident group animals may have strengthened them, and made them less susceptible to bullying. Alternatively, this increased level of aggression may have been associated with the interaction effect between treatment and time period on locomotion. Direct Introduction animals showed increased locomotion during weeks five and six post calving compared to the Separate Group animals. The Direct Introduction animals may have encountered a greater number of resident group animals because of increased movement around the pen and, as a result, become involved in more aggressive encounters (Friend and Polan, 1974; Langford *et al.,* 2011).

Lying and Location Observations

Although animals in the Separate Group treatment were in a less competitive environment than those in the Direct Introduction treatment, cows in both treatments displayed similar low levels of lying behaviour during the 24 hours after mixing into the post-calving group (i.e. 5.8 hours/day). It is possible that the stress and pain of calving limits the lying behaviour of heifers in standard cubicles during this period (Hydbring *et al.*, 1999). This may be exacerbated because they are calving for the first time, and that calving time tends to be longer in heifers than cows (Doornbos *et al.,* 1984; Berglund *et al.,* 1987; Wehrend *et al.,* 2005). Increased standing time may have an effect on subsequent lameness levels. The process of calving weakens the connective tissue of the hoof in primiparous cows, and this, coupled with prolonged standing on concrete, can lead to increases in susceptibility to lameness (Colam-Ainsworth *et al.,* 1989; Tarlton *et al.,* 2002). During weeks 3-6 of lactation both the Separate Group and Direct Introduction animals were observed lying for an average of 9.9 hours/day, with this similar to the 9.4 hours/day lying time observed by Chaplin *et al.* (2000) for primiparous cows under low levels of stress.

It is unclear why experimental heifers in both treatments preferred to utilise the cubicle row facing the wall more than cows in the resident group. It is possible that the experimental heifers simply preferred to face a wall. Schmisseur *et al.* (1966) found that some cows preferred to use certain stalls, and indeed that some had a specific preference for cubicles facing a wall. Alternatively, these cubicles were furthest away from the feed, and it is possible that lower social rank in experimental animals meant they were not able to gain access to more favourable cubicles (Friend and Polan, 1974; Singh *et al.,* 1993).

Milk Cortisol

Treatment had no significant effect on concentrations of milk cortisol, with the levels exhibited relatively similar to those recorded by Fukasawa *et al.* (2008) in cattle. It is worthy to note that during the first two weeks that the Separate Group cows were in the primiparous cow group, their milk cortisol levels did not differ from those of the Direct Introduction animals which had been mixed with the resident group. It is possible that the levels of stress experienced between treatments did not differ sufficiently to produce divergent hormonal responses. It should be noted that one disadvantage of using milk cortisol is that as samples were obtained during milking, any elevation in stress levels at other time points during the day may have been missed, e.g. stress associated with increased aggression after feeding (Verkerk *et al.,* 1996).

Production Performance

Housing primiparous cows separately for a two-week period after calving had no effect on any of the milk production parameters measured during the first six weeks after calving. O'Connell *et al.* (2008) also found no effect on milk yield when primiparous cows were housed in a separate pen for one week before entering the main milking group. In contrast, Østergaard *et al.* (2010) observed a positive milk production response when primiparous cows were housed separately (but with older cows) from the main herd for one month after calving. It must be noted that the study by Østergaard *et al.* (2010) assessed milk production in the majority of experimental animals until the end of their lactation, whereas experimental animals in the current study were only assessed for the first 6 weeks of lactation. In future studies, it may be beneficial to continue to monitor animals over their entire lactation to clearly identify if any long-term production benefits of post-calving regrouping regimes exist.

Conclusions

Retaining primiparous cows in a separate group for two weeks after calving led to these animals experiencing reduced levels of aggression after regrouping with the main herd and also subsequently after fresh food was offered. However, treatment appeared to have no effect on milk production and did not promote increased lying behaviour. In particular, it was expected that primiparous cows in the Separate Group treatment would lie for longer during the 24 hours after the initial mixing, but this was not the case. It is suggested that pain associated with calving may be linked with reduced lying behaviour of heifers in cubicles. Overall, the reduction in aggression received suggests that it is beneficial from a welfare perspective to retain primiparous cows in a separate group after calving.

Study 4

Does increasing the length of time primiparous cows stay in straw-bedded calving pens improve welfare and performance during the post calving period?

Abstract

Twenty-four primiparous Holstein Friesian dairy cows were assigned to one of two treatments after calving. Experimental animals were housed in a straw pen for a period of either 12-24 hours (Short Duration) or 36-48 hours (Long Duration) after calving. These experimental animals were then introduced to established 'resident' groups that contained 16 animals (10 multiparous cows and 6 primiparous cows). The behaviour of experimental animals was assessed over a 2-hour period immediately after mixing into the resident group. Experimental animals were also observed after feeding in the morning, and also during the afternoon, at intervals across the first month after calving. In addition, time spent lying was assessed after calving, after mixing and each week thereafter for one month using data loggers attached over 24-hour periods. Milk production and changes in body condition and live weight were assessed over the first month after calving. After mixing, animals in the Long Duration treatment spent a greater amount of time lying compared to those in the Short Duration treatment ($P<0.05$). Also, when time matched (i.e. observations starting in the 12-24 hour period after calving), animals in the Long Duration treatment (i.e. in straw pens) again spent significantly more time lying compared to those in the Short Duration treatment (in the resident group) ($P<0.01$). Primiparous cows in the Long Duration treatment exhibited more butting ($P<0.05$) and exploratory behaviour ($P<0.05$) after mixing than those in the Short Duration treatment. No significant treatment effects were shown for behaviours during post feeding/afternoon observations. Furthermore, no significant treatment effects were shown for milk yield, body weight or condition score loss. Overall, giving primiparous cows a 36-48 hour period in a straw pen after calving appears to improve welfare through promoting lying behaviour

in the immediate post-calving period and when introduced to the resident group. Furthermore, these animals performed more butting behaviour towards resident animals and it is suggested that this reflects greater ‘confidence’.

Introduction

The process of parturition can be a painful experience for most animals (Hydbring *et al.,* 1999; Dobson *et al.,* 2008). This painful experience may be exacerbated for primiparous cows as it takes them longer to calve (Doornbos *et al.,* 1984; Berglund *et al.,* 1987; Wehrend *et al.,* 2005). It is possible that lying behaviour may be adversely affected by pain associated with calving. Indeed, evidence shows that lying time in dairy cows can decrease during the days after calving, and that this is particularly evident with primiparous cows, where lying time can be 2.8 hours per day less than with multiparous cows (Bowe *et al.,* 2009). Reduced lying behaviour in the post-calving period may lead to subsequent lameness problems, particularly as tissues in the hoof appear to soften during this period (Colam-Ainsworth *et al.,* 1989; Tarlton *et al.,* 2002).

In many production systems primiparous cows are integrated with the main dairy herd within 24 hours of calving. The process of regrouping dairy heifers with multiparous cows can be particularly stressful, due to them being subject to bullying and aggression (Knierim, 1999; Gibbons *et al.,* 2009; Neisen *et al.,* 2009). It has been suggested that the problems dairy cows face may be compounded by the fact that they are still weak after calving (Lamb, 1976). In most cases, it may not be practically feasible for farmers to keep primiparous cows separate from the main dairy herd throughout lactation, or even for a prolonged period at the start of lactation. However, it is unclear if a moderately longer period in a straw pen to recover from calving prior to integration with the main herd in cubicle accommodation affects welfare and performance. The increased comfort and space for lying in calving pens may indeed encourage animals to lie down for longer and this may aid their recovery after calving (Phillips and Schofield, 1994; Tuyttens, 2005).

The aim of this study was to determine if allowing primiparous cows a slightly longer period in a straw-bedded calving pen (36-48 hours as opposed to 12-24 hours) leads to increased lying during the post-calving period and to improved welfare and performance following integration with a group containing mature cows.

Materials and Methods

Animals, Management and Housing

Twenty-four primiparous Holstein Friesian cows were used as experimental animals in this study. Following weaning at approximately seven weeks of age, these animals were housed in cubicle accommodation until March/April (a period of approximately two months) and were then turned out to grass until October. During this time the experimental animals were managed as part of a much larger group of nulliparous cattle. Following their second season at grass, animals were housed again in October until calving between January and March. Nulliparous cows were moved to the main dairy unit approximately one month prior to calving. They were moved in small batches according to expected calving date, and added to a dynamic group of nulliparous cows kept in cubicle housing. The size of this group ranged from 15-20 animals.

The experimental animals were moved to individual straw-bedded calving pens (5.9 x 3.3 m) prior to calving (based on predicted calving dates and daily assessments by experienced stockpersons). Discretionary calving assistance was given. Calves remained with their dams for 6-12 hours. All animals were housed individually with their calves during the calving and subsequent suckling periods.

The primiparous cows calved between January and April 2010. Primiparous cows in both treatment groups were introduced into their post calving 'resident' group between the hours of 06:30 and 07:00. The resident groups were housed in pens with 16 cubicles in 3 rows and solid concrete floors (see Figure 8). Out-of-parlour feeders were present in all pens but were not used. Concrete passageways were cleaned a minimum of 4 times daily using an automatic scraper, and feed was accessed via an open feed barrier. During the pre-calving period primiparous cows were offered a total mixed ration (TMR) *ad libitum* which comprised 100% forage (80% grass/silage, 20% chopped straw) with an additional 120 g/day of dry cow mineral. After calving, primiparous cows were offered TMR *ad libitum* that comprised 60% concentrate and 40% grass/silage. This was offered between 10.00 and 10.30 hours. Experimental animals also received 1 kg of concentrate in the milking parlour (0.5 kg in the morning and evening). Primiparous cows were milked in a 50-point rotary parlour twice per day (at approximately 06:00 and 16:00 hours).

Treatments and Experimental Groups

The influence of retaining primiparous cows in a straw pen for differing lengths of time after calving before being introduced to a group containing multiparous cows was assessed using two treatments and four replicates.

Treatments are described as follows:

Short Duration (SD) (n=12) - Primiparous cows were retained in a straw bedded pen for between 12 and 24 hours after calving before being introduced into the resident group.

Long Duration (LD) (n=12) - Primiparous cows were retained in a straw bedded pen for between 36 and 48 hours after calving before being introduced into the resident group.

Treatments were balanced for genetic merit (Predicted Transmitting Ability (PTA) for kg fat + protein, kg fat and kg protein), sire, and pre-calving body weight and body condition score. A total of four replicate or ‘resident’ groups of lactating cows were used, with each group being established 5 days before the first experimental animal was introduced (Figure 12). Each resident group contained 10 multiparous Holstein-Friesian (HF) cows (average 4.0 lactations) and 6 non-experimental HF primiparous cows. The group size and cow:heifer ratio in the resident group was maintained throughout the study by removing non-experimental primiparous cows from the group as new experimental primiparous cows were added. The process of replacing all 6 non-experimental animals with experimental animals was completed over an average period of 13 days. In each of the four replicates, three non-experimental primiparous cows were replaced by experimental animals in the Short Duration treatment, and three by experimental animals in the Long Duration treatment. The interval being successive animals introduced to the group ranged from 1-11 days, therefore there was continual disruption to the social group throughout the experimental period. During this period, the order in which experimental animals were introduced into the resident groups was balanced across treatments.

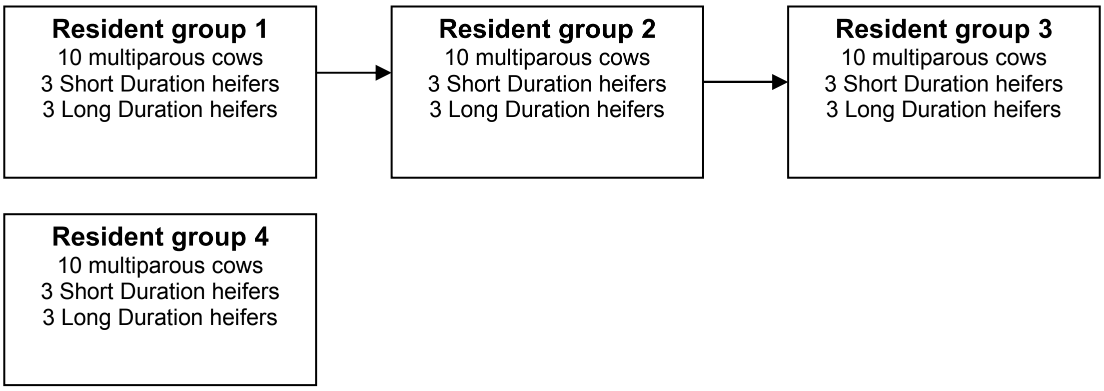

Figure 12 Structure of resident groups (Study 4)

Measurements

Behaviour after mixing and after feeding/afternoon periods

Each experimental primiparous cow was observed for a continuous 2-hour period immediately after joining the resident group. In addition, experimental animals were also observed during four 5-minute periods at 30-minute intervals during the 2-hour post-feeding period and also in the afternoon from 13:00 to 15:00 on days 2, 3 and 10, on another day in week 2 and on one day during weeks 3 and 4 after mixing into the resident group. Observations taken during the feeding and afternoon periods were combined for statistical analysis.

Behaviour was recorded by direct observation using a handheld data recorder (Psion Organiser II, Model LZ64, Noldus Information Technology, The Netherlands) using the ethogram shown in Table 17. The ethogram contained three mutually-exclusive categories of behaviour: the location of the animal in the pen (measured as a duration of time), and behaviours performed by the animal (“activity”) and received by the animal. The duration and frequency were recorded for all aggressive behaviours performed (i.e. shouldering and butting) and for all received behaviours. Only the duration of all other “activity” behaviours was recorded.

Table 17 Ethogram of post mixing and post feeding behaviours recorded

Behaviour	Description
Activity	
Butting	When the cow uses the front of her head to make vigorous contact with another cow
Shouldering	Displacement of an individual using the shoulder
Locomotion	All four legs moving and head not in contact with any substrate
Motionless	No legs moving and head not in contact with any substrate
Feeding	Head in the feed barrier and feeding
Explore-feeder	Nosing any part of the feed barrier
Explore-general	Nosing any substrate in pen including floors, walls and railings, but not the feeder or another cow
Social-investigative	Nosing another animal without displaying agonistic or cohesive behaviour
Ruminating	Regurgitating boluses of food
Drinking	Drinking at water trough
Grooming	Focal animal grooming itself
Location	
Cubicle	Focal animal located in cubicle
Front-passage	Focal animal located in front passageway
Rear/side Passage	Focal animal located in rear or side passageway
Received Behaviours	
Receive butt	When the focal animal receives butting behaviour
Receive avoid	When a non-focal animals moves away from the focal animal
Receive threat	When the focal animal receives threatening behaviour
Receive shoulder	When the focal animal receives shouldering behaviour

Lying behaviour

Lying behaviour using data loggers (DL) was monitored for the first 24 hours after experimental animals had been introduced to the resident group and also for a single 24-hour period each week thereafter for a period of 4 weeks. Lying behaviour was also measured in the Long Duration treatment for a 24-hour period that animals were in the straw pen. This measurement was taken 24 hours prior to the animals being introduced into the resident group. Calves were not present in the pen when these recordings were made. Please refer to Study 1 for experimental protocol.

Milk Production

Please refer to Study 1 for experimental protocol.

Body Weight and Condition Score

Please refer to Study 1 for experimental protocol.

Statistical Analysis

Data were analysed using Genstat 11.1 (Payne *et al.,* 2008). The influence of treatment on behaviour during the first 2 hours after introduction to the resident group was analysed by ANOVA. The random effects were replicate and group within replicate, and 'group' represented the mean of animal behaviours within a treatment and replicate. This model was also used to assess treatment effects on lying behaviour during the first 24 hours after mixing (recorded by data loggers). The influence of treatment on the behaviours during feeding/afternoon periods was assessed by REML Variance Components Analysis. A model which included time effects (fixed effects were time period (measurements were taken over 6 time periods), treatment and treatment within time period, and the random effects were replicate, group within replicate and time period within group within replicate) was used to assess treatment effects on social investigative, motionless, grooming, shouldering and butting behaviours. Due to low levels of behavioural data within the different time periods, it was not possible to run the full repeated measures model for remaining behavioural data. Therefore a REML model which excluded time effects was used to assess all other behaviours (fixed effect was treatment, random effects were replicate and group within replicate). This model was also used to assess the effect of treatment on the total hours spent lying (measured by data loggers) when the two treatments were time matched at day two post calving i.e. comparison of SD (24 hours after mixing into main group) with LD (24 hours while in the straw pen). Lying behaviour during weeks 1-4 (recorded by data loggers) was analysed using REML Variance Components Analysis (fixed effects were week, treatment and treatment within week, and random effects were replicate, group within replicate and week within group within replicate).

The influence of treatment on production performance, live weight and condition score was analysed using time period (day of lactation or week), treatment and treatment within time period as fixed effects, and replicate, animal within replicate, and time period (day of lactation or week) within animal within replicate as random effects. The effect of treatment on live weight and body condition score loss was analysed using treatment as a fixed effect and replicate and animal within replicate as random effects. For all REML analyses where time period/week has been used as a factor, main treatment effects and also interactions between treatment and time period will be reported, but main effects on time period will not be reported. For all statistical models, residual values were plotted and visually assessed for normality. Some behaviours were performed too infrequently for statistical analysis and therefore will not be presented. These included 'shouldering' and 'receiving shoulder' after mixing, and 'explore feeder', 'ruminating', the location 'cubicle', 'receiving avoid' and 'receiving threat' in the post-feeding/ afternoon observations.

Results

Behaviour

Behaviour after mixing

The influence of treatment on the duration of time spent performing or receiving different behaviours or in different locations is presented in Table 18. Primiparous cows in the Long Duration treatment performed exploratory and butting behaviour for a greater duration of time than those in the Short Duration treatment (P=0.029). Treatment effects on butting behaviour were also significant when analysed on a frequency/minute basis (Short Duration 0.00, Long Duration 0.02, SED 0.002/min, $F_{1,3}$ 49.56, P=0.006).

Table 18 Influence of treatment on the % time spent performing and receiving different behaviours and in different locations during the 2-hour period after mixing into the resident group (SD) - Short Duration, (LD) - Long Duration)

Behaviour	Treatment		SED	$F_{(1,3)}$	P
	SD	LD			
Activity					
Social-investigative	0.16	0.28	0.121	1.06	0.379
Explore-general	6.60	9.00	0.610	15.47	0.029
Explore-feeder	0.51	0.32	0.401	0.23	0.664
Feeding	13.70	17.00	7.36	0.20	0.686
Ruminating	30.20	27.70	3.880	0.39	0.575
Locomotion	5.52	4.59	0.777	1.43	0.318
Motionless	41.80	38.10	10.190	0.14	0.736
Grooming	0.53	1.12	0.316	3.46	0.160
Drinking	0.80	1.43	0.528	1.43	0.318
Butting	0.02	0.17	0.038	15.48	0.029
Location					
Cubicle	45.70	49.30	17.590	0.04	0.852
Front-passage	28.20	27.60	15.930	0.00	0.975
Rear/side passage	26.10	23.00	10.670	0.08	0.794
Receive					
Threat	0.07	0.07	0.035	0.00	0.965
Butt	0.19	0.22	0.063	0.23	0.667
Avoid	0.03	0.03	0.017	0.00	0.964

Behaviour after feeding

Table 19 shows the behaviours performed and received by primiparous cows during the post-feeding/afternoon periods. No significant difference was found between treatments for any of the behaviours recorded.

Table 19 Influence of treatment on the % time spent performing and receiving different behaviours and in different locations during the post-feeding/afternoon observations over the one month period (SD) - Short Duration, (LD) - Long Duration)

Behaviour	Treatment		SED	F	P
	SD	LD			
Activity					
Social-investigative	0.03	0.04	0.064	$F_{(1,3)}$ 1	0.391
Explore-general	3.04	2.54	0.583	$F_{(1,3)}$ 0.74	0.452
Feeding	34.60	35.50	3.600	$F_{(1,3)}$ 0.06	0.826
Locomotion	2.34	1.96	0.351	$F_{(1,3)}$ 1.21	0.352
Motionless	27.83	25.21	4.805	$F_{(1,3)}$ 0.44	0.555
Grooming	0.92	0.47	0.201	$F_{(1,3)}$ 7.72	0.069
Drinking	1.78	1.87	1.198	$F_{(1,3)}$ 0.01	0.941
Shouldering	0.02	0.05	0.018	$F_{(1,3)}$ 2.92	0.186
Butting	0.07	0.06	0.038	$F_{(1,3)}$ 0.27	0.642
Location					
Front-passage	43.37	42.89	4.254	$F_{(1,3)}$ 0.01	0.917
Rear/side passage	5.48	4.21	1.756	$F_{(1,3)}$ 0.52	0.521
Receive					
Shoulder	0.05	0.08	0.039	$F_{(1,3)}$ 0.62	0.488
Butt	0.29	0.26	0.052	$F_{(1,3)}$ 0.36	0.590

Lying behaviour (assessed using data loggers)

Treatment had a significant effect on the number of lying bouts and the total time spent lying within the 24-hour period after mixing (number of lying bouts: Short Duration 7.42, Long Duration 9.58, SED 0.441, $F_{1,3}$ 24.14, P=0.016; total hours lying: Short Duration 3.55, Long Duration 6.59, SED 0.852, $F_{1,3}$ 12.70, P=0.038). There was no significant treatment effect on mean hours lying per bout (Short Duration 0.53, Long Duration 0.68, SED 0.114, $F_{1,3}$ 1.65, P=0.289).

Treatment also had a significant effect on total hours lying on day 2 post-calving i.e. comparison of Short Duration treatment in the resident group with Long Duration treatment in the calving pen (Short Duration 2.9, Long Duration

10.7, SED 1.344, $F_{1,3.2}$ 33.62, P=0.008). No significant difference was found between treatments for lying behaviour during weeks 1 to 4 (number of lying bouts: Short Duration 13.98, Long Duration 12.50, SED 0.672, $F_{1,3}$ 5.11, P=0.107; total hours lying: Short Duration 8.12, Long Duration 8.33, SED 0.569, $F_{1,3}$ 0.15, P=0.727; mean hours lying per bout: Short Duration 0.62, Long Duration 0.68, SED 0.053, $F_{1,3}$ 1.21, P=0.351).

Production Performance

Treatment had no significant effect on milk production performance as shown in Table 20.

Live Weight and Body Condition Score Losses

No significant difference was found between treatments in total live weight loss (Short Duration -34.05, Long Duration -25.17, SED 7.870 kg, $F_{1,18}$ 1.27, P=0.274) or condition score loss (Short Duration 0.12, Long Duration 0.13, SED 0.064, $F_{1,14.9}$ 0.02, P=0.882) during the first month post calving. There was also no significant difference between treatments in average live weight (Short Duration 534, Long Duration 523, SED 20.160 kg, $F_{1,25.5}$ 0.24, P=0.628) or average body condition score (Short Duration 2.65, Long Duration 2.64, SED 0.118, $F_{1,19.1}$ 0.02, P=0.889) during the first month post calving.

Table 20 Production performance and body tissue levels of animals during the first month after calving ((SD) - Short Duration, (LD) - Long Duration)

Parameter	Treatment		SED	F	P
	SD	LD			
Milk Production					
Milk yield (kg/day)	26.7	27.2	1.623	$F_{(1,19)}$ 0.10	0.756
Fat (g/kg)	43.8	44.4	1.740	$F_{(1,19)}$ 0.08	0.781
Protein (g/kg)	34.5	34.9	1.044	$F_{(1,19)}$ 0.15	0.700
Lactose (g/kg)	46.9	46.0	0.455	$F_{(1,19.1)}$ 3.49	0.077
Fat yield (kg/day)	1.19	1.19	0.069	$F_{(1,18.7)}$ 0.00	0.949
Protein yield (kg/day)	0.93	0.92	0.049	$F_{(1,18.9)}$ 0.01	0.917
Fat + protein yield (kg/day)	2.12	2.11	0.113	$F_{(1,18.8)}$ 0.00	0.997
Milk energy content (MJ/kg)	3.28	3.29	0.079	$F_{(1,19)}$ 0.01	0.916
Milk energy output (MJ/day)	88.8	87.88	4.789	$F_{(1,18.8)}$ 0.03	0.876
Body tissue					
LWT (kg)	534	523	20.16	$F_{(1,25.5)}$ 0.24	0.628
Condition Score	2.65	2.64	0.118	$F_{(1,19.1)}$ 0.02	0.889
LWT loss (kg)	34.05	25.17	7.870	$F_{(1,18)}$ 1.27	0.274
Condition Score loss	0.12	0.13	0.064	$F_{(1,14.9)}$ 0.02	0.882

Discussion

Behaviour After Mixing

It has been suggested that after calving, dairy cows should be allowed a period of three days to fully recover and gain strength before being integrated with the main dairy herd (Lamb, 1976). Primiparous cows in the Long Duration treatment exhibited increased levels of exploratory and butting behaviour after introduction to the resident group. This may have been due to the fact that these animals were physically stronger, and therefore more willing to engage in exploratory behaviour and aggression at this time point. As the introduction of animals in both treatments coincided with the resident group returning from milking, it may be the case that animals in the Long Duration treatment were more ‘confident’ to compete for a place at the feed barrier, and therefore were involved in more agonistic interactions with other animals. Increases in agonistic interactions generally occur in the feeding area, in particular after milking or when fresh feed is delivered (Friend and Polan, 1974; Olofsson, 1999; Devries and von Keyserlingk, 2006).

Behaviour in the Post-feeding/Afternoon Observations

During these periods there appeared to be no difference in behaviours performed or received by experimental animals between the two treatments. Aggression levels were higher in these observations relative to those taken after mixing, and this could possibly have occurred as a result of increased feeding behaviour during this time period. This is supported by the fact that after social order has been formed in cattle, agonistic behaviour occurs primarily in the feeding area after feeding (McPhee *et al.,* 1962; Friend and Polan, 1974; Langford *et al.,* 2011).

Lying Behaviour

The results showed that animals in the Long Duration treatment spent greater time lying than animals in the Short Duration treatment at day 2 post calving. This finding was expected as dairy animals appear to lie for longer in straw yards compared to cubicles (Phillips and Schofield, 1994; Fregonesi and Leaver, 2002). This increased lying time may reflect increased physical comfort associated with lying on a softer surface (Norring *et al.,* 2008; Tuyttens, 2005). In addition, increased lying time may reflect comfort associated with not having to adapt lying positions to suit cubicle dimensions or design, which at this (possibly painful) stage after calving may induce difficulties in lying and standing (O’Connell *et al.,* 1992; Wierenga and Hopster, 1990; Phillips and Schofield, 1994). As previously mentioned, duration of labour is longer in primiparous than in multiparous cows (Doornbos *et al.,* 1984; Berglund *et al.,* 1987; Wehrend *et al.,* 2005), and increased comfort during the post-calving period may be particularly important in alleviating physical trauma and pain of parturition. It is important to note that there are a number of other factors, apart from physical comfort, which may have led to reduced lying time in the cubicle accommodation. For example, the stress of being regrouped with unfamiliar animals and the stocking rates typically used in cubicle accommodation can also adversely affect lying times (Krohn, 1978; Fregonesi *et al.,* 2007; von Keyserlingk *et al.,* 2008; Hill *et al.,* 2009).

The evidence would suggest that introducing animals too quickly after calving into cubicle housing may lead to substantially reduced lying times, and therefore that it may be beneficial to give them a short extended period on straw. From a welfare and lameness perspective, decreased lying time in general can have an adverse effect on lameness levels in cattle (Galindo and Broom, 2000). In freshly calved animals connective tissue in the hoof is weakened, and this exacerbates effects of standing on concrete on the development of sole ulcers (Colam-Ainsworth *et al.,* 1989; Tarlton *et al.,* 2002).

Treatment effects were also observed during the 24-hour period after animals were regrouped, with animals in the Long Duration treatment also lying for longer during this period. The mere process of regrouping animals appears to lead to reductions in lying behaviour (Krohn, 1978; Payne and Aikman, 2007; von Keyserlingk *et al.,* 2008). Obviously it is difficult to determine in the current study if differences in lying behaviour reflect different abilities to cope with regrouping, or relate to natural differences associated with different time periods post calving. When all observations from the first 4 weeks in the resident group were combined, there were no significant treatment effects on lying behaviour, with both Long and Short Duration treatments lying for an average of 8 hours per day. This is in agreement with findings by Singh *et al.* (1993) which show lying times of 8.39 hours per day with primiparous cows mixed with multiparous cows. The evidence suggests that the positive effects seen in lying behaviour in the Long Duration treatment are relatively short lived and are only beneficial in the post-calving and mixing periods.

Production Performance

No significant difference was found in milk yield between the two treatments in the current study. It is worth noting that there is very limited information in the published literature on the effects of extended recovery periods in straw pens on milk production in the post-calving period. O'Connell *et al.* (2008) found no significant difference in milk yield during the first month of lactation between primiparous cows that were retained in a straw pen for one week after calving and those added into an established group of resident cows within 24 hours of calving, which concurs with findings from the present study.

Conclusions

Retaining primiparous cows in a straw pen for a period of 36-48 hours rather than 12-24 hours after calving appeared to improve welfare through increasing the time spent lying in the post-calving and post-regrouping periods. These animals also appeared more ‘confident’ when integrated with a group containing mature cows by showing increased exploratory and aggressive behaviour.

GENERAL CONCLUSIONS

The time of day that primiparous cows are introduced to a group containing older cows influences the level of aggression to which they are exposed. It appears better to introduce them following evening rather than morning milking and this may be related to reduced general social activity. It is impossible to determine whether this effect was related to differences in interval from feeding, however the approach adopted in this study was chosen to mirror common commercial practice.

There also appeared to be benefits in mixing pre-calving animals with non-lactating cows rather than keeping them in a separate heifer group prior to calving. This approach appeared to produce more ‘confident’ animals that received reduced aggression. These findings are of significance to the agricultural sector, as despite being an issue of continued concern among farmers, the effect of mixing heifers with dry cows prior to calving on welfare and performance parameters does not appear to have been previously investigated in a replicated study.

Retaining animals in a ‘primiparous cow group’ after calving was also beneficial, as this extended period of two weeks allowed the animal to fully recover from the stress of calving in a more uniform group. Subsequently, when introduced into the main dairy herd these animals used cubicles more, which potentially reduced the risk of adverse encounters with resident animals, as indicated by results.

Finally, it is relevant to note that in the first three experiments, primiparous cows expressed very low lying times in the post-calving period, even in Study 3 when experimental animals were in the ‘primiparous cow group’. It emerged that when dairy animals were allowed a period of 36-48 hours in a straw pen after calving, they exhibited longer lying times during this period and also when introduced into the milking group. Therefore in summary, it is beneficial to allow primiparous cows a longer period in a straw-bedded pen, as it possibly aids their recovery from the stress and pain of calving.

The overall results from this project show that through the adoption of specific regrouping practices, the welfare of newly-calved primiparous cows can be improved. These improvements in welfare were evident in reductions in aggression after mixing and feeding and increases in total lying time. The treatments applied in all experiments appeared to have no effect on production performance parameters or body condition.

PUBLICATIONS TO DATE FROM THIS RESEARCH

Boyle, A.B., Ferris, C.P. and O'Connell, N.E. 2011. Are there benefits in introducing heifers to the main dairy herd in the evening rather than the morning? *Journal of Dairy Science* (submitted).

Boyle, A.B., Ferris, C.P. and O'Connell, N.E. 2011. Does housing nulliparous cows with multiparous animals prior to calving influence welfare and performance after calving? *Applied Animal Behaviour Science* (submitted)

Boyle, A.B., Ferris, C.P. and O'Connell, N.E. 2011. Should primiparous cows be housed separately from multiparous animals during the two-week period after calving? *Applied Animal Behaviour Science* (in preparation)

Boyle, A.B., Ferris, C.P. and O'Connell, N.E. 2011. Does increasing the length of time primiparous cows stay in straw calving pens improve welfare and performance during the post calving period? *Applied Animal Behaviour Science* (in preparation)

Boyle, A.B., Ferris, C.P., Kilpatrick, D.J. and O'Connell, N.E. 2010. The effect of time of day when dairy heifers are introduced to a group containing mature cows on welfare and performance. *Proceedings of the British Society of Animal Science and the Agricultural Research Forum*, Belfast, p. 96.

Boyle, A.B., Ferris, C.P. and O'Connell, N.E. 2011. The influence of housing dairy heifers with multiparous cows prior to calving on welfare and productivity during the post calving period. *Proceedings of the British Society of Animal Science and the Association of Veterinary Teaching and Research Work*, Nottingham, p. 94.

LITERATURE CITED

Abeni, F. and Bertoni, G. 2009. Main causes of poor welfare in intensively reared dairy cows. *Italian Journal of Animal Science* **8:** 45-66.

Annexstad, J. 1986. Raise replacements economically. *Dairy Herd Manage,* **23:** 20-22.

Barnett, J.L., Cronin, G.M., McCallum, T.H., Newman, E.A. and Hennessy, D.P. 1996. Effects of grouping unfamiliar adult pigs after dark, after treatment with amperozide and by using pens with stall, on aggression, skin lesions and plasma cortisol concentrations. *Applied Animal Behaviour Science,* **50:** 121-133.

Berglund, B., Philipsson, J. and Danell, O. 1987. External signs of preparation for calving and course of parturition in Swedish dairy cattle breeds. *Animal Reproduction Science,* **15:** 61-79.

Blackie, N., Scaife, J.R. and Bleach, E.C.L. 2006. Lying behaviour and activity of early lactation Holstein dairy cattle measured using an activity monitor. *Cattle Practice,* **14:** 139-142.

Bøe, K.E. and Faerevik, G. 2003. Grouping and social preferences in calves, heifers and cows. *Applied Animal Behaviour Science,* **80:** 175-190.

Boissy, A. 1995. Fear and fearfulness in animals. *The Quarterly Review of Biology.* **70:** 165-191.

Bouissou, M.F. 1975. Establishment of dominance-submission relationships in domestic cattle. III. Effect of social experience. Z. *Tierpsychol*. **38:** 419-435.

Bowe, S., Blackie, N. and Bleach, E.C.L. 2009. Changes in lying behaviour and activity during the peri-parturient period in primiparous and multiparous Holstein dairy cows. *Proceedings of the British Society of Animal Science,* pp 100.

Brakel, W.J. and Leis, R.A. 1976. Impact of social disorganization on behaviour, milk yield and body weight of dairy cows. *Journal of Dairy Science,* **59:** 716-721.

CAFRE. 2008. What is the true cost of rearing a dairy heifer. In: *Rearing your 2010 herd: feeding and managing dairy herd replacements for lifetime performance*. *College of Agriculture, Food and Rural Enterprise, Greenmount, Northern Ireland,* p. 51.

Chaplin, S.J., Ternent, H.E., Offer, J.E., Logue, D.N. and Knight, C.H. 2000. A comparison of hoof lesions and behaviour in pregnant and early lactation heifers at housing. *The Veterinary Journal,* **159:** 147-153.

Colam-Ainsworth, P., Lunn, G.A., Thomas, R.C. and Eddy, R.G. 1989. Behaviour of cows in cubicles and its possible relationship with laminitis in replacement dairy heifers. *Veterinary Record,* **125:** 573-575.

Csermely, D. and Wood-Gush, D.G.M. 1987. Some variables affecting aggression in grouped sows. *Proceedings of the International Symposium on Applied Ethology in Farm Animals,* 26-28.

DairyCo, 2008. Heifer Rearing: Technical Bulletin. *DairyCo.*

Daniels, K.J., Donkin, S.S., Eicher, S.D., Pajor, E.A. and Schutz, M.M. 2007. Prepartum milking of heifers influences future production and health. *Journal of Dairy Science,* **90:** 2293-2301.

DARD, 2008/09. Northern Ireland farm performance indicators. *Policy and Economics Division, Department of Agriculture and Rural Development for Northern Ireland.*

Dawson, L.E.R. and Carson, A.F. 2004. Management of the dairy heifer. *Cattle Practice,* **12:** 181-192.

Day, J.E.L., Kyriazakis, I. and Lawrence, A.B. 1995. The effect of food deprivation on the expression of foraging and exploratory behaviour in the growing pig. *Applied Animal Behaviour Science*, **42:** 193-206.

Devries, T.J. and von Keyserlingk, M.A.G. 2006. Feed stalls affect the social and feeding behaviour of lactating cows. *Journal of Dairy Science,* **89:** 3522-3531.

Dippel, S., Brinkmann, J. and Winckler, C. 2004. On-farm assessment of lying behaviour in dairy cattle. *Proceedings of the 38th International Congress of the ISE.*

Dobson, H., Smith, R.F., Bell, G.J.C., Leonard, D.M. and Richards, B. 2008. (Economic) Costs of difficult calvings (in the UK dairy herd): How vets can alleviate the negative impact. *Cattle Practice,* **16:** 80-85.

Doornbos, D.E., Bellows, R.A., Burfening, P.J. and Knapp, B.W. 1984. Effects of dam age, prepartum nutrition and duration of labor on productivity and postpartum reproduction in beef females. *Journal of Animal Science,* **59:** 1-10.

Drackley, J.K. 1999. Biology of dairy cows during the transition period: the final frontier. *Journal of Dairy Science,* **82:** 2259-2273.

Edmonson, A.J., Lean, I.H., Weaver, L.D., Farver, T. and Webster, G. 1989. A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science*, **72:** 68-78.

Fernandez, M.A., Alvarez, L. and Zarco, L. 2007. Regrouping in lactating goats increases aggression and decreases milk production. *Small Ruminant Research,* **70:** 228-232.

Fraser, T.H. and Broom, D.M. 1990. *Farm Animal Behaviour and Welfare,* 3rd ed. Bailliere Tindall, London.

Fregonesi, J.A. and Leaver, J.D. 2002. Influence of space allowance and milk yield level on behaviour, performance and health of dairy cows housed in straw yard and cubicle systems. *Livestock Production Science,* **78:** 245-257.

Fregonesi, J.A., Tucker, C.B. and Weary, D.M. 2007. Overstocking reduces lying time in dairy cows. *Journal of Dairy Science,* **90:** 3349-3354.

Friend, T.H. and Polan, C.E. 1974. Social rank, feeding behaviour, and free stall utilization by dairy cattle. *Journal of Dairy Science*, **57:** 1214-1220.

Fukasawa, M., Tsukada, H., Kosaka, T. and Yamada, A. 2008. Effect of lactation stage, season and parity on milk cortisol concentration in Holstein cows. *Livestock Science*, **13:** 280-284.

Galindo, F. and Broom, D.M. 2000. The relationships between social behaviour of dairy cows and the occurrence of lameness in three herds. *Research in Veterinary Science,* **69:** 75-79.

Galindo, F., Broom, D.M. and Jackson, P.G.G. 2000. A note on possible link between behaviour and the occurrence of lameness in dairy cows. *Applied Animal Behaviour Science,* **67:** 335-341.

Gibbons, J.M., Lawrence, A.B. and Haskell, M.J. 2009. Consistency of aggressive feeding behaviour in dairy cows. *Applied Animal Behaviour Science,* **121:** 1-7.

Goff, J.P. and Horst, R.L. 1997. Physiological changes at parturition and their relationship to metabolic disorders. *Journal of Dairy Science,* **80:** 1260-1268.

Gonzalez, M., Yabuta, A.K. and Galindo, F. 2003. Behaviour and adrenal activity of first parturition and multiparous cows under a competitive situation. *Applied Animal Behaviour Science,* **83:** 259-266.

Grant, R.J. and Albright, J.L. 1997. Dry matter intake as affected by cow grouping and behaviour. *Proceedings of the 58th Minnesota Nutritional Conference,* pp. 93-103.

Greter, A.M., Devries, T.J. and von Keyserlingk, M.A.G. 2008. Nutrient intake and feeding behaviour of growing dairy heifers: Effects of dietary dilution. *Journal of Dairy Science,* **91:** 2786-2795.

Hartmann, E., Christensen, J.W. and Keeling, L.J. 2009. Social interactions of unfamiliar horses during paired encounters: Effect of pre-exposure on aggression level and so risk injury. *Applied Animal Behaviour Science,* **121:** 214-221.

Hasegawa, N., Nishiwaki, A., Sugawara, K. and Ito, I. 1997. The effects of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behaviour and adrenocortical response. *Applied Animal Behaviour Science,* **51:** 15-27.

Heinrichs, A.J. 1993. Raising dairy replacements to meet the needs of the 21st century. *Journal of Dairy Science,* **76:** 3179-3187.

Hill, C.T., Krawczel, P.D., Dann, H.M., Ballard, C.S., Hovey, R.C., Falls, W.A. and Grant, R.J. 2009. Effect of stocking density on the short-term behavioural responses of dairy cows. *Applied Animal Behaviour Science,* **117:** 144-149.

Huzzey, J.M., Devries, T.J., Valois, P. and von Keyserlingk, M.A.G. 2006. Stocking density and feed barrier design affect the feeding and social behaviour of dairy cattle. *Journal of Dairy Science,* **89:** 126-133.

Hydbring, E., Madej, A., MacDonald, E., Drugge-Boholm, G., Berglund, B. and Olsson, K. 1999. Hormonal changes during parturition in heifers and goats are related to the phases and severity of labour. *Journal of Endocrinology,* **160:** 75-85.

Jensen, P. and Yngvesson, J. 1998. Aggression between unacquainted pigs-sequential assessment and effects of familiarity and weight. *Applied Animal Behaviour Science,* **58:** 49-61.

Johansson, B., Redbo, I. and Svennersten-Sjaunja, K. 1999. Effect of feeding before, during and after milking on dairy cow behaviour and the hormone cortisol. *Animal Science*, **68:** 597-604.

Knierim, U. 1999. The behaviour of heifers after single or group introduction to the dairy herd. *KBTL Schrift,* **382:** 115-120 (In German with English summary).

Kondo, S. and Hurnik, J.F. 1990. Stabilization of social hierarchy in dairy cows. *Applied Animal Behaviour Science,* **27:** 287-297.

Kondo, S., Sekine, J., Okubo, M. and Asahida, Y. 1989. The effect of group size and space allowance on the agonistic and spacing behaviour of cattle. *Applied Animal Behaviour Science,* **24:** 127-135.

Krohn, C.C. 1978. The effect of group change on behaviour and production performance in large dairy herds. *Proceedings of the first world congress of Ethology Applied Zootech,* **40:** E1-26.

Krohn, C.C. and Konggaard, S.P. 1979. Effects of isolating first-lactation cows from older cows. *Livestock Production Science,* **6:** 137-146.

Krohn, C.C. and Konggaard, S.P. 1982. Investigations concerning feed intake and social behaviour among group fed cows under loose housing conditions. *Beretning fra Stratens Hysdyrbrugs Forso,* **531:** 1-19.

Lamb, R.C. 1976. Relationship between cow behaviour patterns and management systems to reduce stress. *Journal of Dairy Science,* **59:** 1630-1636.

Langford, F.M., Rutherford, K.M.D., Sherwood, L., Jack, M.C., Lawrence, A.B. and Haskell, M.J. 2011. Behaviour of cows during and after peak feeding time on organic and conventional dairy farms in the United Kingdom. *Journal of Dairy Science,* **94:** 746-753.

Lawson, R. 1999. To develop improved strategies for the management of heifers after calving. Report prepared for the Milk Development Council, ADAS Bridgets.

Mayne, C.S., McCoy, M.A., Lennox, S.D., Mackey, D.R., Verner, M., Catney, D.C., McCaughey, W.J., Wylie, A.R.G., Kennedy, B.W. and Gordon, F.J. 2002. Fertility of dairy cows in Northern Ireland. *Veterinary Record,* **150:** 707-713.

McPhee, C.P., McBride, G. and James, J.W. 1962. Social behaviour of domestic animals III. Steers in small yards. *Animal Production,* **6:** 9-15.

Metz, J.H.M. and Mekking, P. 1984. Crowding phenomena in dairy cows as related to available idling space in cubicle housing system. *Applied Animal Behaviour Science*, **12:** 63-78.

Muller, R. and von Keyserlingk, M.A.G. 2006. Consistency of flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle. *Applied Animal Behaviour Science,* **99:** 193-204.

Nakanishi, Y., Kawamura, T., Goto, T. and Umetsu, R. 1993. Comparative aspects of behavioural activities of beef cows before and after introducing a stranger at night. *Journal Faculty of Agriculture Kyushu University,* **37:** 227-238.

Nakanishi, Y., Mutoh, Y., Umetsu, R., Masuda, Y. and Goto, I. 1991. Changes in social and spacing behaviour of Japanese black cattle after introducing a strange cow into a stable herd. *Journal Faculty of Agriculture Kyushu University*, **36:** 1-11.

Neisen, G., Wechsler, B. and Gygax, L. 2009. Effects of the introduction of single heifers or pairs of heifers into dairy-cow herds on the temporal and spatial associations of heifers and cows. *Applied Animal Behaviour Science,* **119:** 127-136.

Norring, M., Manninen, E., de Passille, A.M., Rushen, J., Munksgaard, L. and Saloniemi, H. 2008. Effects of sand and straw bedding on the lying behaviour, cleanliness, and hoof and hock injuries of dairy cows. *Journal of Dairy Science,* **91:** 570-576.

O'Connell, J.M., Giller, P.S. and Meaney, W.J. 1992. Factors affecting cubicle utilization by dairy cattle using stall frame and bedding manipulation experiments. *Applied Animal Behaviour Science,* **35:** 11-21.

O'Connell, N.E., Wicks, H.C.F., Carson, A.F. and McCoy, M.A. 2008. Influence of post-calving regrouping strategy on welfare and performance parameters in dairy heifers. *Applied Animal Behaviour Science,* **114:** 319-329.

Olofsson, J 1999. Competition for total mixed diets fed for *ad libitum* intake using one or four cows per feeding station. *Journal of Dairy Science,* **82:** 69-79.

Østergaard, S., Thomsen, P.T. and Burow, E. 2010. Separate housing for one month after calving improves production and health in primiparous cows but not in multiparous cows. *Journal of Dairy Science,* **93:** 3533-3541.

Patterson, D.C., Yan, T., Gordon, F.J. and Kilpatrick, D.J.. 1998. Effects of bacterial inoculation of unwilted and wilted grass silages. 2. Intake, performance and eating behaviour by dairy cattle. *Journal of Agricultural Science, Cambridge.* **131:** 113-119.

Payne, E.J. and Aikman, P.C. 2007. The effect of grouping early lactation dairy heifers solely with heifers or with multiparous cows on eating, ruminating and resting behaviour. *Proceedings of the British Society of Animal Science.*

Payne, R.W., Harding, S.A., Murray, D.A., Soutar, D.M., Baird, D.B., Welham, S.J. Kane, A.D., Gilmour, A.R., Thompson, R., Webster, R. and Tunnicliffe Wilson, G. 2008. *The Guide to GenStat Release 11.1 part 2: Statistics.* VSN International, Hemel Hempstead.

Phelps, A. 1992. Vastly superior first lactations when heifers fed separately. *Feedstuffs,* **64:** 11.

Phillips, C.J.C. and Rind, M.I. 2001. The effects on production and behaviour of mixing uniparous and multiparous cows. *Journal of Dairy Science,* **84:** 2424-2429.

Phillips, C.J.C. and Schofield, S.A. 1994. The effect of cubicle and straw yard housing on the behaviour, production and hoof health of dairy cows. *Animal Welfare,* **3:** 37-44.

Potter, M.J. and Broom, D.M. 1987. The behaviour and welfare of cows in relation to cubicle house design. In *Cattle housing systems, Lameness and Behaviour* (ed. H.K. Wierenga; D.J. Peterse) pp. 129-147.

Raussi, S., Boissy, A., Deval, E., Pradel, P., Kaihilahti, J. and Veissier, I. 2005. Does repeated regrouping alter the social behaviour of heifers? *Applied Animal Behaviour Science,* **93:** 1-12.

Sambraus, H.H. 1970. On social ranking in cattle. *Zeitschrift FR Tierzchtung Und Zchtun Gsbiologie,* **86:** 240.

Schein, M.W. and Fohrman, M.H. 1955. Social dominance relationships in a herd of dairy cattle. *British Journal of Animal Behaviour,* **3:** 45-55.

Schmisseur, W.E., Albright, J.L., Dillon, W.M., Kehrberg, E.W. and Morris, W.H.M. 1966. Animal behaviour responses to loose and free stall housing. *Journal of Dairy Science,* **49:** 102-104.

Singh, S.S., Ward, W.R., Lautenbach, K., Hughes, J.W. and Murray, R.D. 1993. Behaviour of first lactation and adult dairy cows while housed and at pasture and its relationship with sole lesions. *The Veterinary Record,* **133:** 469-474.

Sowerby, M.E. and Polan, C.E. 1978. Milk production response to shifting cows between Intraherd groups. *Journal of Dairy Science,* **61:** 455-460.

Stern, S. and Andresen, N. 2003. Performance, site preferences, foraging and excretory behaviour in relation to feed allowance of growing pigs on pasture. *Livestock Production Science*, **79**: 257-265.

Tarlton, J.F., Holah, D.E., Evans, K.M., Jones, S., Pearson, G.R. and Webster, A.J.F. 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. *The Veterinary Journal,* **163:** 196-204.

Tuyttens, F.A.M. 2005. The importance of straw for pig and cattle welfare: A review. *Applied Animal Behaviour Science,* **92:** 261-282.

Tyrrell, H.F. and Reid, J.Y. 1965. Prediction of the energy value of cows milk. *Journal of Dairy Science,* **48:** 1215-1223.

Uchida, E., Katoh, N. and Takahashi, K. 1993. Appearance of haptoglobin in serum from cows at parturition. *Journal of Veterinary Medical Science,* **55:** 893-894.

Van Putten, G. and Buré, R.G. 1997. Preparing gilts for group housing by increasing their social skills. *Applied Animal Behaviour Science,* **54:** 173-183.

Van Reenen, C.G., Van der Werf, J.T.N., Bruckmaier, R.M., Hopster, H., Engel, B., Noordhuizen, J.P.T.M. and Blokhuis, H.J. 2002. Individual differences in behavioural and physiological responsiveness of primiparous dairy cows to machine milking. *Journal of Dairy Science,* **85:** 2551-2561.

Verkerk, G.A., Phipps, A.M. and Matthews, L.R. 1996. Milk cortisol concentrations as an indicator of stress in lactating dairy cows. *Proceedings of the New Zealand Society of Animal Production,* **56:** 77-79.

Vieira, A.P., Guesdon, V., de Passille, A.M., von Keyserlingk, M.A.G. and Weary, D.M. 2008. Behavioural indicators of hunger in dairy calves. *Applied Animal Behaviour Science*, **109:** 180-189.

von Keyserlingk, M.A.G., Olenick, D. and Weary, D.M. 2008. Acute behavioural effects of regrouping dairy cows. *Journal of Dairy Science,* **91:** 1011-1016.

Wechsler, B. and Lea, S.E.G. 2007. Adaption by learning: Its significance for farm animal husbandry. *Applied Animal Behaviour Science*, **108:** 197-214.

Wehrend, A., Hofmann, E. and Bostedt, H. 2005. Investigations into the duration of expulsion and the separation of the afterbirth in suckler cows - a contribution to improve the monitoring of parturition. *Deutsche Tierarztliche Wochenschrift,* **112:** 19-24.

Wierenga, H.K. 1990. Social dominance in dairy cattle and the influences of housing and management. *Applied Animal Behaviour Science,* **27:** 201-229.

Wierenga, H.K. and Hopster, H. 1990. The significance of cubicles for the behaviour of dairy cows. *Applied Animal Behaviour Science,* **26:** 309-337.