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

A. R. Boyle,*† C. P. Ferris,* and N. E. O’Connell†¹

*Agri-Food and Biosciences Institute, Hillsborough, Co. Down, BT26 6DR, United Kingdom

†School of Biological Sciences, Medical Biology Centre, Queen’s University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, United Kingdom

ABSTRACT

Twenty-eight Holstein Friesian dairy heifers were assigned to 1 of 2 treatments after calving. These experimental heifers were introduced to an established group of resident cows either between 0600 and 0800 h (i.e., after morning milking, a.m.) or between 1600 and 1800 h (i.e., after evening milking, p.m.). The size of the resident group remained constant at 18 animals (12 multiparous cows and 6 primiparous cows). There were 5 replicate or resident groups in total, and 2 to 3 nonexperimental primiparous cows in each group were replaced by a.m. and by p.m. heifers as they calved. Fresh total mixed ration was provided daily between 1000 and 1030 h, and concentrate feed was offered in the milking parlor. The behavior of the experimental heifers was assessed over a 2-h period immediately after mixing into the resident group, and also after feed provision during the first month in the group. In addition, time spent lying was assessed each week for 1 mo using data loggers attached over 24-h periods. The lying behavior and location of the entire group was also assessed by direct observations during the 2-h period before evening milking on 2 consecutive days each week for 1 mo. The time spent feeding by experimental heifers was recorded automatically using computerized feeding gates. Milk production, milk cortisol concentrations, and changes in body condition and body weight were also assessed over the first month after calving. Heifers in the a.m. treatment spent longer in receipt of aggressive behaviors such as threats, butts, and chases immediately after mixing compared with those in the p.m. treatment. During the feeding periods, heifers in the a.m. treatment were observed feeding for longer, showed less pen exploration, and also received more butts. No significant treatment effects were shown on overall feed intake levels, milk yield, milk cortisol con-

centrations, body weight, or body condition score loss. However, feed intakes were higher in the a.m. treatment during the second week after mixing, and automated recordings showed that a.m. animals spent longer average periods of time feeding. No significant treatment differences were shown in overall time spent lying, with heifers in both treatments lying for less than 4 h during the first 24 h in the group. Observations of the entire group showed that p.m. heifers spent less time lying than resident animals or a.m. heifers. In conclusion, the reduction in received aggression and the lack of adverse effects on performance (milk production and weight and 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 since feeding on behavior immediately after mixing would be beneficial.

Key words: behavior, heifer, management, regrouping

INTRODUCTION

The regrouping of dairy cows can cause an increase in aggressive behavior, 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- to 2-h period after regrouping, with agonistic interactions almost doubling in the regrouped cows to 9.6 per cow/h compared with 4.9 per cow/h in the non-regrouped cows. Decreases in milk yields have also been observed as a result of introducing individual cows to a group, with daily yields declining from 43.4 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 (Wierenga, 1990; González 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; González et al., 2003).

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¹Corresponding author: niamh.oconnell@qub.ac.uk

Previous research suggests that 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 decrease 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 decrease levels of aggression. This agrees with data from sows showing decreased levels of aggression when regrouped in the evening rather than morning (Csermely and Wood-Gush, 1987).

The aim of the present 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.

MATERIALS 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 wk of age, with the exception of the period from April to September (inclusive) when they were at pasture. Experimental heifers were moved to the main dairy unit approximately 1 mo before 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) before 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 to 12 h. 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 mo).

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 1 of 6 Calan gates and animals were able to feed at any of the gates.

During the treatment period heifers were offered TMR (which comprised 60% concentrate and 40% forage) on an *ad libitum* basis. This was offered between 1000 and 1030 h. 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 parlor (0.5 kg in the morning and evening). After the calves had been removed, heifers were milked in a 50-point rotary parlor twice daily (at approximately 0600 and 1600 h).

Treatments and Experimental Groups

The present experiment included 2 treatments: a.m.: heifers (n = 14) added to the resident group after morning milking (between 0600 and 0800 h); and p.m.: heifers (n = 14) added to the resident group after evening milking (between 1600 and 1800 h).

Treatments were balanced for genetic merit (PTA for kilograms of fat + protein, kilograms of fat and kilograms of protein), sire, precalving BW and BCS. A total of 5 replicate or resident groups were used, with each group being established 5 d before the first experimental heifer was introduced. At establishment, each resident group contained 12 mature HF cows (average 149 DIM; range 2–9 lactations) and 6 nonexperimental HF heifers. The group size and cow:heifer ratio within the resident group was maintained throughout the study by removing a nonexperimental heifer from the group before each experimental heifer being added. The process of replacing all 6 nonexperimental animals with experimental animals was completed over an average period of 20 d for each replicate. In replicates 1 to 4, 3 nonexperimental heifers were replaced by animals in the a.m. treatment, and 3 by animals in the p.m. treatment. In replicate 5, 2 nonexperimental animals were replaced by animals in the a.m. treatment and 2 by animals in the p.m. treatment. Heifers were allocated to treatment at calving, and the order in which experimental animals were introduced to the resident groups alternated between treatments. All nonexperimental animals were replaced in 1 replicate group before moving on to the next.

In both treatments, heifers were introduced to the group approximately 30 min after all animals in the resident group had returned from milking. Nonexperimental heifers were removed from the resident group immediately before either the a.m. or p.m. milking (e.g., nonexperimental heifers were removed from the group before a.m. milking when a.m. heifers were being added to the group). In each resident group, no more than 1 experimental heifer was introduced on a particular day. The interval between successive heifers

Figure 1. Layout of cubicle accommodations where the 18 animals within each replicate were housed.

being introduced to the group ranged from 1 to 7 d; therefore, continual disruption to the social group occurred throughout the experimental period.

Measurements

Behavior After Mixing and Feeding. Each experimental heifer was observed for a continuous 2-h period immediately after joining the resident group. In addition, each heifer was observed during four 5-min periods (at 30-min intervals) during the 2-h period after

feeding on 1 day each week for 1 mo after introduction to the group.

Behavior was recorded by direct observation using a hand-held data recorder (Psion Organizer II, model LZ64; Noldus Information Technology, Wageningen, the Netherlands) using the ethogram shown in Table 1. The ethogram contained 4 different categories of behavior: the location of the animal in the pen (also measured as duration), the state of the animal (in terms of duration spent lying or standing), and behavioral events performed or received by the animal. The duration and frequency were recorded for all aggressive

Table 1. Ethogram of postmixing and postfeeding behaviors recorded

Behavior	Description
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/moving to a lying position
Standing	Standing up/moving to a standing position
Event	
Head-to-head contact	Contact between 2 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 toward or approaches another individual with her head down and then lunges without making contact
Chasing	When a cow actively moves toward 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 2 individuals
Allogrooming	Mutual grooming between 2 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 behavior
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)
Received event	
Receive butt	When the focal animal receives butting behavior
Receive avoid	When a nonfocal animal moves away from the focal animal
Receive threat	When the focal animal receives threatening behavior
Receive chase	When the focal animal receives chasing behavior
Receive shoulder	When the focal animal receives shouldering behavior
Receive nose	When the focal animals receives nosing behavior
Receive sniff	When the focal animal receives sniffing behavior

behaviors performed (i.e., head-to-head contact, butting, threatening, chasing, shouldering, and avoiding) and for all received behaviors. Only the duration of all other behavioral events was recorded.

Lying Behavior. Direct observations of lying and standing behavior, and of location of all animals in the group were performed. The group was scanned at 10-min intervals for 2 h (1300–1500 h) on 2 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 1). These observations finished approximately 1 h before p.m. milking.

Data loggers [Tinytag Plus, Re-Ed volt; Gemini dataloggers (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 parlor, and were secured using Vetrap bandage (An-

dover Healthcare Inc., Salisbury, MA). Lying behavior was monitored for 24 h after heifers were introduced to the group and also for one 24-h period per week in the following month. The data loggers were programmed to record whether the animal was lying or standing at 30-s intervals. Data loggers were validated on 4 occasions during each 24-h measurement period, with 2 lying/standing bouts being recorded by direct observations over a period of 2 h. Data indicating lying bouts of less than 2 min were regarded as anomalies and converted into standing periods (as suggested by Blackie et al., 2006).

Feeding Behavior. Feeding behavior of each heifer was recorded continuously from introduction to the group until d 28. Feeding behavior was recorded using the electronic Calan gate system (American Calan Inc., Northwood, NH), with each gate allowing access to a feed box (length: 120 cm, depth: 104 cm, width at top: 118 cm, and width at base: 63 cm) mounted on a weigh scale and linked to an automated cow identification system (Griffith Elder & Co. Ltd., 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 1 mo before calving. Data from the Calan gates were used to calculate individual DMI, number of meals per day, average duration of each meal, time spent eating, and intake rates. Any time 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 1 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 1 mo after introduction to the resident group. Samples were collected during the a.m. and p.m. milking of each sampling day, and these samples were assayed. Milk samples were refrigerated at 4°C after collection and defatted. Samples were defatted by centrifugation ($1,900 \times g$ for 20 min at 4°C) within 4 h 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). Samples were prepared as follows: 100 µL of skim 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 d 6 and d 35 after calving. Milk composition (fat, protein, and lactose) was measured weekly during the experiment, with samples taken during 2 successive milkings (a.m. and p.m.) and a weighted composition for the 24-h sample period subsequently calculated. Milk composition was estimated by UKAS accredited tests using a microscan model 605 (Foss Electric A/S, Hillerød, Denmark). The energy value of milk (MJ/kg) was calculated using the following equation $[(0.0384 \times \text{fat}) + (0.0223 \times \text{protein}) + (0.0199 \times \text{lactose}) - 0.108]$ (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/d).

BW and BCS. The BW and BCS (1–5, using increments of 0.25; Edmonson et al., 1989) of each heifer were recorded on a weekly basis for 1 mo after calving. Body condition scores were recorded by a single experienced technician. Animals were weighed automatically upon exiting the milking parlor. Body weight and BCS

loss were calculated by subtracting values recorded at 1 mo after calving from those recorded at d 1 after calving.

Statistical Analysis

Data were analyzed using Genstat 11.1 (Payne et al., 2008). The influence of treatment on behavior during the first 2 h after introduction to the group was analyzed by ANOVA, with the random effects being replicate and group within replicate, with group being a mean of animal behaviors within a treatment in each replicate. The influence of treatment on behavior after feeding was assessed by restricted maximum likelihood (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-h periods (recorded by data loggers), and on the average proportion of a.m., p.m., 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-h period and mean hours lying per bout (measured using data loggers) were analyzed 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 concentrations were analyzed by REML variance components analysis using a model that 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 behavior of heifers was analyzed 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 BW was analyzed by REML variance components analysis [fixed effects were time period (day of lactation or week), treatment, and treatment within day of lactation or week, and random effects were replicate, animal within replicate, and time period (day of lactation or week) within animal within replicate]. Analysis of variance (random effects were replicate and animal within replicate) was used to determine treatment effects on BW and BCS loss. For all REML analysis where time period has been used as a factor, main treatment effects and also interactions between treatment and time period are reported, but main effects of time period are

not reported. For all statistical models, residual values were plotted and visually assessed for normality. Some behaviors were performed too infrequently for statistical analysis and, therefore, results are not 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.

RESULTS

Behavior

Direct Observations After Mixing. The influence of treatment on the duration spent performing or receiving different behaviors or in different locations is presented in Table 2. Heifers in the a.m. treatment spent more time in receipt of threats ($P = 0.048$), butts ($P = 0.007$), and chases ($P = 0.033$) after mixing than those in the p.m. treatment. When analyzed on a frequency-per-minute basis, heifers in the a.m. treatment

received more butts compared with p.m. heifers [a.m.: 0.16 and p.m.: 0.05, standard error of the difference (SED): 0.013 min^{-1} , $P = 0.001$].

Direct Observations After Feeding. The behavior of heifers and behaviors directed toward heifers after feeding are presented in Table 3. Heifers in the a.m. treatment were observed feeding for longer periods ($P = 0.013$) and also spent more time in receipt of butts than those in the p.m. treatment ($P = 0.023$).

Significant interactions were observed between treatment and week in the duration spent exploring (SED = 2.279, $P = 0.038$; Figure 2), grooming (SED = 0.848, $P = 0.031$; Figure 3), and drinking (SED = 1.165, $P = 0.038$; Figure 4).

Lying Behavior. No significant treatment effect existed on time spent lying as recorded by data loggers ($P = 0.92$; Table 4). The average mean lying time was 7.5 h per 24-h period, and this decreased to <4 h per 24-h period after the initial mixing period.

In terms of lying behavior during evening (whole-group) observations, p.m. heifers spent a lower propor-

Table 2. Influence of treatment on the time spent performing and receiving different behaviors and in different locations and states during the 2-h period after mixing (% observation time)

Behavior	Treatment ¹		SED ²	P-value
	a.m.	p.m.		
Location				
Cubicle	25.30	33.40	9.510	0.44
Front passage	29.10	37.90	6.680	0.26
Rear/side passage	38.50	21.50	8.350	0.11
State				
Lying	6.90	5.70	7.790	0.89
Standing	93.10	94.30	7.790	0.89
Event				
Social, investigative	0.77	1.27	0.439	0.32
Social, cohesive	0.17	1.06	0.330	0.054
Allogrooming	0.04	0.24	0.155	0.26
Explore, general	13.90	14.10	1.140	0.86
Explore, feeder	1.66	1.57	0.418	0.85
Feeding	13.80	21.00	4.840	0.21
Ruminating	30.50	26.20	6.090	0.52
Motionless	28.10	21.00	5.850	0.29
Grooming	0.89	2.82	0.707	0.053
Drinking	0.94	1.05	0.240	0.66
Sniffing cow	0.40	0.35	0.119	0.67
Avoiding	0.11	0.04	0.060	0.28
Butting	0.14	0.20	0.114	0.62
Head-to-head contact	0.40	0.02	0.146	0.061
Received event				
Nose	0.12	0.16	0.051	0.57
Sniff	0.12	0.08	0.021	0.15
Threat	0.02	0.00	0.008	0.048
Shoulder	0.06	0.02	0.024	0.17
Butt	0.38	0.17	0.042	0.007
Chase	0.06	0.01	0.017	0.033

¹In the a.m. treatment, heifers were added to the resident group after morning (a.m.) milking and in the p.m. treatment, heifers were added to the resident group after evening (p.m.) milking.

²Standard error of the difference.

Figure 2. Interaction between treatment and week for exploratory behavior. AM = heifers were added to the resident group after morning (a.m.) milking; PM = heifers were added to the resident group after evening (p.m.) milking. Bars within the same time period with different letters differ significantly ($P < 0.05$).

tion of time lying than a.m. heifers or resident cows ($P = 0.002$). No significant difference was observed in the proportion of time spent lying between a.m. heifers and resident cows during this period. A significant interaction was found between treatment and week in the proportion of time that animals were located in the front passage (SED = 0.025, $P = 0.041$; Figure 5).

Figure 3. Interaction between treatment and week for grooming behavior. AM = heifers were added to the resident group after morning (a.m.) milking; PM = heifers were added to the resident group after evening (p.m.) milking. Bars within the same time period with different letters differ significantly ($P < 0.05$).

Feeding Behavior. Treatment had no significant effect on average daily feed intake levels during the first month after calving (a.m.: 14.3 and p.m.: 13.6, SED: 0.54 kg of DM, $P = 0.23$). However, during wk 2, heifers in the a.m. treatment had a significantly higher feed intake and a greater number of meals per day than

Table 3. Influence of treatment on the time spent performing and receiving different behaviors and in different locations and states after feeding (% observation time; behavior was recorded during four 5-min observations at 30-min intervals)

Behavior	Treatment ¹		SED ²	P-value
	a.m.	p.m.		
Location				
Cubicle	51.50	56.53	7.606	0.54
Front passage	44.15	36.67	5.648	0.32
Rear/side passage	3.91	6.27	3.232	0.48
State				
Lying	44.29	37.54	6.143	0.33
Standing	55.71	62.46	6.143	0.33
Event				
Social, investigative	0.12	0.16	0.050	0.41
Social, cohesive	0.07	0.24	0.063	0.055
Explore, feeder	4.43	4.77	2.650	0.93
Feeding	29.24	21.78	1.887	0.013
Ruminating	31.83	32.80	3.266	0.79
Motionless	23.86	24.75	2.684	0.85
Sniffing cow	0.03	0.08	0.042	0.29
Shouldering	0.05	0.08	0.044	0.57
Butting	0.61	0.65	0.186	0.66
Received event				
Nose	0.02	0.02	0.010	0.95
Sniff	0.01	0.00	0.005	0.29
Shoulder	0.06	0.07	0.021	0.60
Butt	0.29	0.17	0.032	0.023

¹In the a.m. treatment, heifers were added to the resident group after morning (a.m.) milking and in the p.m. treatment, heifers were added to the resident group after evening (p.m.) milking.

²Standard error of the difference.

Figure 4. Interaction between treatment and week for drinking behavior. AM = heifers were added to the resident group after morning (a.m.) milking; PM = heifers were added to the resident group after evening (p.m.) milking. Bars within the same time period with different letters differ significantly ($P < 0.05$).

Figure 5. Interaction between treatment and week for the proportion of animals located in front passage. AM = heifers were added to the resident group after morning (a.m.) milking; PM = heifers were added to the resident group after evening (p.m.) milking. Bars within the same time period with different letters differ significantly ($P < 0.05$).

those in the p.m. treatment (feed intake: a.m.: 14.4 and p.m.: 13.0, SED: 0.51 kg of DM, $P = 0.010$; meals per day: a.m.: 12.0 and p.m.: 10.5, SED: 0.72, $P = 0.048$). A tendency was also observed for the number of meals per day to be greater with heifers in the a.m. treatment when averaged over the month compared with the p.m. treatment (a.m.: 12.2 and p.m.: 11.2, SED: 0.52, $P = 0.052$). In addition, a significant treatment effect was observed on the total time spent eating each day, with a.m. heifers eating for significantly longer during the

first month after calving compared with p.m. heifers (a.m.: 171 and p.m.: 151, SED: 6.6 min/d, $P = 0.006$). This appeared to be due to significant treatment effects observed during wk 1 ($P = 0.007$) and wk 2 ($P = 0.011$). Treatment had no significant effect on the average duration of each meal (a.m.: 14.37 and p.m.: 14.69, SED: 1.100 min, $P = 0.77$) or eating rate (a.m.: 88.7 and p.m.: 94.8, SED: 5.64 g of DM/min, $P = 0.29$) during the first month after calving.

Table 4. Lying behavior parameters measured through automated data loggers over five 24-h periods or by direct observation (using scans of the whole group at 10-min intervals between 1300 and 1500 h)¹

Behavior	Treatment heifer ²		Resident cow	SED ³	P-value
	a.m.	p.m.			
Data loggers					
Total hours lying (h)	7.57	7.51		0.524	0.92
No. of lying bouts per day	14.46	12.73		0.954	0.15
Mean hours lying per bout	0.59	0.65		0.070	0.45
Direct observation (proportion of animals)					
Lying	0.45 ^b	0.34 ^a	0.42 ^b	0.022	0.002
Rear cubicles (facing wall)	0.30	0.25	0.21	0.044	0.15
Rear passage	0.02	0.04	0.02	0.009	0.22
Middle cubicles	0.22	0.18	0.19	0.030	0.47
Side passage	0.02	0.02	0.02	0.007	0.58
Front cubicles	0.14	0.19	0.22	0.044	0.18
Feed boxes	0.21	0.22	0.26	0.023	0.10

^{a,b}Mean values with different superscripts differ significantly ($P < 0.01$).

¹Data logger measurements were taken over five 24-h periods for treatment heifers only (postmixing and 1 d each week for the first 4 wk).

²In the a.m. treatment, heifers were added to the resident group after morning (a.m.) milking and in the p.m. treatment, heifers were added to the resident group after evening (p.m.) milking.

³Standard error of the difference.

Milk Cortisol

No significant difference existed between treatments in concentrations of milk cortisol recorded, with a.m. and p.m. heifers both exhibiting concentrations of 0.64 (SED = 0.144 ng/mL, $P = 0.99$), respectively.

Production Performance

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

BW and BCS Losses

No significant difference was found between treatments in total BW loss (a.m.: -16.9 and p.m.: -19.4, SED: 7.13 kg, $P = 0.73$) or total BCS loss (a.m.: -0.20 and p.m.: -0.14, SED: 0.073, $P = 0.47$). Also, no significant difference existed between treatments in average BW (a.m.: 516 and p.m.: 528, SED: 13.3 kg, $P = 0.39$) or average BCS (a.m.: 2.72 and p.m.: 2.75, SED: 0.063, $P = 0.70$) during the first month after calving.

DISCUSSION

Behavior After Mixing

The levels of aggression observed in the a.m. treatment (9.6 butts/h) are similar to the 7 to 10 agonistic encounters/h previously reported (Brakel and Leis, 1976; Knierim, 1999; Neisen et al., 2009). Heifers that encounter lower levels of aggression, such as in the p.m. treatment, 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 behavior 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 decrease levels of fighting due to lower levels of general social behavior, which possibly contributed to the lower levels of aggression in the p.m. treatment in the current study. 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 of introduction to the group was confounded with time 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 a.m. 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 since delivery of fresh feed affects aggression directed toward 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 behaviors 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.

Behavior After Feeding

Research by Kondo and Hurnik (1990) found that agonistic interactions primarily occurred during the 2-h period after regrouping, with aggression levels decreasing to premixing levels by 3 to 15 d after mixing (Hasegawa et al., 1997; Fernández et al., 2007). A similar pattern of behavior was observed between mixing and subsequent observations in this study; however, heifers in the a.m. 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. Previous research has shown that increased levels of feed-related aggression can occur during the 30- to 45-min period after the delivery of fresh feed or during the 2-h period on return from milking (Friend and Polan, 1974; Olofsson, 1999).

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

Table 5. Feeding behavior, production performance, body tissue, and cortisol level parameters of the experimental heifers during the first month after introduction to the group

Parameter	Treatment ¹		SED ²	P-value
	a.m.	p.m.		
Intake and feeding behavior				
Daily food intake (kg)	14.3	13.6	0.54	0.23
Meals per day	12.2	11.2	0.52	0.052
Daily eating time (min)	171	151	6.6	0.006
Milk production				
Milk yield (kg/d)	25.0	25.7	1.28	0.57
Fat (g/kg)	44.8	44.1	2.34	0.86
Protein (g/kg)	36.8	35.5	0.67	0.066
Lactose (g/kg)	45.9	46.2	0.58	0.53
Fat yield (kg/d)	1.12	1.13	0.075	0.79
Protein yield (kg/d)	0.90	0.92	0.044	0.64
Fat + protein yield (kg/d)	2.02	2.06	0.114	0.65
Milk energy content (MJ/kg)	3.34	3.35	0.095	0.81
Milk energy output (MJ/d)	83.09	85.94	4.719	0.51
Body tissue				
BW (kg)	516	528	13.3	0.39
BCS	2.72	2.75	0.063	0.70
BW loss (kg)	16.9	19.4	7.13	0.73
BCS loss	0.20	0.14	0.073	0.47
Stress hormone				
Milk cortisol (ng/mL)	0.64	0.64	0.144	0.99

¹In the a.m. treatment, heifers were added to the resident group after morning (a.m.) milking and in the p.m. treatment, heifers were added to the resident group after evening (p.m.) milking.

²Standard error of the difference.

Lying Behavior

Previous research has shown that integration of dairy heifers into a herd causes a significant decrease in lying time of heifers after mixing, with animals lying for 1.5 h per 12 h, compared with premixing lying times of over 7 h per day (Krohn and Konggaard, 1979; Knierim, 1999). It is pertinent to note that, in the current study, heifers in both treatments expressed this decrease in lying behavior after mixing, which is similar to Knierim's (1999) findings. Decreased 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). Animals in both treatments were lying for an average of 7.5 h per 24-h period, which is broadly in agreement with research by Singh et al. (1993), who recorded lying times in heifers of 8.39 h/d 6 wk after being mixed with adult cows.

Direct observations of lying behavior indicated decreased lying time by p.m. animals relative to a.m. and resident animals between 1300 and 1500 h. The reason for this is unclear, but it is possible that these animals used this opportunity to feed when other animals were lying. Although only observed during wk 4, this may also explain why these animals were located in the front passageway to a greater extent than other groups.

Feeding Behavior

Dry matter intake can have a significant effect on milk production and on BCS change (Grant and Albright, 1997). Although no significant overall difference existed in DMI between the 2 treatment groups in the current study, a.m. animals had higher intakes during wk 2, with these heifers also spending significantly more time feeding each day over the 1-mo observation period. This was also observed in direct observations after feeding, where a.m. heifers were observed feeding for significantly longer than p.m. animals. In fact, a.m. heifers consumed 1 meal per day more than p.m. 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 a.m. animals. It is also possible that introducing animals to a new group in a new environment during the p.m. 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 2 treatment groups.

Milk Cortisol

Treatment had no significant effect on the concentration of milk cortisol. In the current study, this may have occurred due to milk cortisol concentrations not being measured directly after mixing, meaning that we may have missed periods of peak stress. Indeed Verkerk et al. (1996) 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. Furthermore, behavioral factors such as standing and locomotion can affect cortisol concentrations (González et al., 2003) and this, in conjunction with the heifers being introduced into a new group, may have overridden any treatment effects.

CONCLUSIONS

Introducing heifers to the resident group after p.m. milking appeared to improve welfare during the initial mixing period by decreasing levels of aggression to which they were exposed. However, this treatment did not promote an increase in overall lying behavior. Heifers in the a.m. treatment had higher feed intakes during wk 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 decrease 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 p.m. 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 postfeeding.

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