Short-term increases in stocking density affect the lying and social behavior, but not the productivity, of lactating Holstein dairy cows

P. D. Krawczel,*† L. B. Klaiber,* R. E. Butzler,* L. M. Klaiber,* H. M. Dann,* C. S. Mooney,* and R. J. Grant*¹

*William H. Miner Agricultural Research Institute, Chazy, NY 12921

†Department of Animal Science, The University of Vermont, Burlington 05405

ABSTRACT

Reduced access to resources because of increased stocking density may have a detrimental effect on the behavior of the lactating dairy cow. The objective of this study was to determine the short-term responses in behavior, productivity, fecal cortisol metabolites, and udder and leg hygiene of lactating Holstein dairy cows housed at stocking densities of 100 (1 cow per freestall and headlock), 113, 131, and 142%. Multiparous cows ($n = 92$) and primiparous cows ($n = 44$) were assigned to 1 of 4 pens (34 cows per pen) in a 4-row freestall barn. Pens were balanced for parity, milk production, and days in milk. Stocking densities were imposed for 14 d using a 4×4 Latin square design. Time spent feeding and time spent ruminating were quantified by 24 h of direct observation of focal cows ($n = 12$ per pen) beginning at 0800 h on d 11 of each period. Data loggers recorded lying behavior (time and bouts) from the same focal cows per pen at 1-min intervals during the final 5 d of each period. Fecal cortisol metabolites were quantified from samples collected on d 13 and 14 of each period from the same focal cows. Displacements from the feed barrier were recorded on a pen basis after 9 milkings over the last 4 d of each period. Productivity was assessed on a pen basis from milk yield (recorded from d 10 to 14 of each period) and milk components (quantified from composite samples collected on d 12 of each period). Milk composition was further analyzed for milk fatty acid profiles, which were determined from a subset ($n = 6$ per pen) of the focal cows. Data were analyzed using the MIXED procedure of SAS, with the pen ($n = 4$ per treatment, except displacements where $n = 3$ per treatment) as the experimental unit. Feeding and ruminating (h/d) did not differ among treatments. Lying time was reduced at stocking densities of 131 and 142%, relative to 100 or 113%. Lying bouts were not affected by treatment. Stocking densities of 131 and 142% reduced the percentage of time cows spent

ruminating within a freestall relative to 100%. Displacements from the feed bunk increased linearly across treatments. Fecal cortisol metabolites, udder hygiene score, milk yields, milk composition, and milk fatty acids did not differ among treatments. Decreased lying time and increased aggression at the feed bunk suggest that an alteration of the time budgets of lactating dairy cows may occur at higher stocking densities, but it is unclear at what point these changes might have further biological consequences.

Key words: dairy cow, stocking density, behavior, productivity

INTRODUCTION

Overstocking of freestall barns is defined as housing more cows within a pen than the available number of stalls or providing less than the recommended 0.6 m of linear feeding space per cow, or both (Grant and Albright, 2001). Overstocking, or increased stocking density, is a common practice among dairy producers. At their maximal herd size, approximately 48% of freestall-based producers stocked their freestall facilities at least 110%, and 28.8% of producers were above a stocking density of 110% on average (USDA, 2010). In terms of feed bunk space, 67.9% of freestall-based producers provided less than 0.6 m at their maximal stocking density, and 58.6% provided less than 0.6 m on average (USDA, 2010). Growing evidence of the priority given to resting when cows were forced to choose between behaviors (Metz, 1985; Munksgaard et al., 2005) suggests that increased stocking densities may impede the ability of a cow to meet her daily time budget needs, which Grant and Albright (2001) are defined as 3 to 5 h/d of feeding, 10 to 14 h/d of lying in a freestall, and 7 to 10 h/d of ruminating.

The initial investigations of the effect of space allocation on the behavioral response of lactating dairy cows manipulated the stocking density at each resource independently (Friend et al., 1977). Resting time was unaffected until freestalls were stocked at 200%, and feeding was unaffected if more than 0.2 m of linear bunk space per cow was provided. More recently, in-

Received July 5, 2011.

Accepted April 6, 2012.

¹Corresponding author: pkrawcze@utk.edu

creasing the stocking density at one resource in absence of competition for the other has yielded similar results at lower stocking densities. Fregonesi et al. (2007) demonstrated a linear reduction in lying time as freestall stocking density increased from 100 to 150%. Huzzey et al. (2006) observed a linear reduction in feeding time as stocking density at the feed barrier (independent of feed barrier type) was increased from 75 to 300%. Linear increases in the number of displacements from the overstocked resource also were reported (Huzzey et al., 2006; Fregonesi et al., 2007). Increasing the stocking density of the freestalls and feed barrier concurrently has resulted in mixed responses. Lying, feeding, and ruminating behaviors were unaffected during a comparison of high (67% stocking density, relative to freestalls) and low (113% stocking density) space allocations (Fregonesi and Leaver, 2002). On the other hand, Hill et al. (2009) observed decreased resting time as stocking density increased from 100 to 142%, but found no effect on feeding time.

Little research has been done on the effect of stocking density on rumination. Batchelder (2000) reported a 10% reduction in the maximum percentage of cows within a pen ruminating at any given time as stocking density increased from 100 to 130% in a 4-row barn. Conversely, Fregonesi and Leaver (2002) observed no differences in total rumination time (approximately 8.6 h/d) or time spent lying and ruminating (approximately 7.5 h/d) as stocking density increased from 67 to 114% of freestalls. Both resident cows and newly introduced cows decreased rumination times after regrouping (by a maximum of 6% over 1 or 2 d, respectively) without a change in diet (Schirrmann et al., 2011). This suggests the potential for a social stress to alter a behavior typically related to diet composition. The potential consequence of altered rumination behavior was the shift in biohydrogenation of linoleic acid toward the production of *trans*-10, *cis*-12 CLA, which causes milk fat depression (Bauman et al., 2006). Furthermore, adequate buffering of the rumen for the maintenance of physiological pH was dependent on salivary buffer flow, which was estimated using the daily eating, ruminating, and resting times and the flow rate of saliva during those behaviors (Allen, 1997).

These behavioral changes may be related to growing evidence that increasing the stocking density alters the productivity and physiology of lactating dairy cows. Survey data suggested a positive relationship between stall availability and milk yield (Bach et al., 2008), which was similar to a previously reported relationship between lying time and milk yield (Grant, 2007). It is possible that increasing the stocking density may influence milk production as well as milk composition.

The blood corticoid response of cows to an ACTH challenge was associated with their access to feed (González et al., 2003), which indicates that the reduced or altered feed activity resulting from overcrowding may be stressful for cows. Additionally, cows had a greater corticoid response to an ACTH challenge when freestall availability was reduced to less than 0.5 freestalls per cow (Friend et al., 1979). Assessment of the stress response to increased stocking density using the concentration of fecal cortisol metabolites, which reflects the concentration of circulating cortisol 10 to 12 h earlier, eliminates the potentially confounding effect of blood sampling on cortisol concentrations (Möstl et al., 2002). To date, however, no evaluation of the effect of variable stocking density on the excretion of fecal cortisol metabolites has been reported.

Despite the potential for substantial effects of stocking density on the behavior, physiological responses, and productivity of lactating dairy cows, data on this subject are scarce within the range of stocking densities commonly observed on commercial dairy farms. Therefore, the objective of this study was to determine the short-term responses in behavior, productivity, fecal cortisol metabolites, and udder and leg hygiene of lactating Holstein dairy cows housed at stocking densities of 100 (1 cow per freestall and headlock), 113, 131, and 142%. Our hypothesis was that increased stocking density of freestalls and headlocks would alter the resting and feeding behavior, milk composition, stress response, and cleanliness of lactating dairy cows within a 4-row barn system during the 14-d treatment period.

MATERIALS AND METHODS

Animals, Management, and Housing

Ninety-two multiparous and 44 primiparous Holstein cows were assigned to 1 of 4 pens ($n = 34$ cows per pen) in a naturally ventilated, 4-row free-stall barn at the William H. Miner Agricultural Research Institute (Chazy, NY). The 4 groups were balanced for parity (2.4 ± 0.2 ; mean $\pm$ SE), DIM (176.9 ± 15.7), and milk production (51.3 ± 1.6 kg/d) at the beginning of the study. Cows were milked 3 times daily in a double-12 parallel parlor (Xpressway Parallel Stall System; Bou-Matic, Madison, WI). A TMR formulated for 40 kg of milk/d was delivered once daily when cows were removed for the 0430 h milking and was pushed up approximately 6 times daily (Table 1). Water troughs were located at both ends of each pen as well as in the return alley between the experimental pens and parlor in the same overall barn arrangement diagramed by Hill et al. (2009).

Table 1. Ingredient composition and mean analyzed chemical composition (dry matter basis) of TMR samples (n = 4) from each stocking density treatment

Item	Stocking density, ¹ %					Pooled SE
	All	100	113	131	142	
Ingredient, ² % of DM						
Haycrop silage	18.0	—	—	—	—	—
Conventional corn silage	18.8	—	—	—	—	—
Brown midrib corn silage	15.4	—	—	—	—	—
Wheat straw, chopped	1.7	—	—	—	—	—
Molasses	5.3	—	—	—	—	—
Whole cottonseed	2.3	—	—	—	—	—
Concentrate mix ³	38.4	—	—	—	—	—
Chemical composition						
DM, %		47.4	47.8	47.7	47.8	1.1
CP, %		18.0	18.8	18.2	17.6	0.4
Soluble protein, % CP		36.4	34.9	36.7	36.7	1.7
Neutral detergent CP, %		3.2	3.3	3.1	3.3	0.2
ADF, %		20.4	20.4	19.4	20.4	0.6
NDF, %		35.6	34.7	34.4	33.3	1.3
ADL, %		2.9	3.0	2.7	3.0	0.2
NSC, %		30.1	30.5	30.8	30.1	0.5
Starch, %		26.9	27.3	27.6	27.2	0.5
Sugar, %		3.2	3.2	3.2	2.9	0.3
Fat, %		4.5	4.6	4.4	4.6	0.2
Calcium, %		0.96	0.91	0.97	0.86	0.07
Phosphorus, %		0.37	0.36	0.36	0.38	0.01
NDF digestibility (24-h), % of NDF		54.4	53.2	53.6	52.6	0.8
DM digestibility (24-h), %		84.9	84.8	85.1	84.5	0.5

¹Stocking densities were 1.00, 1.13, 1.31, and 1.42 cows per freestall or headlock.

²Ingredients are common to all treatments.

³Composed of the following (% as fed): finely ground corn (42.55), soybean meal (11.15), canola (7.00), AminoPlus (Ag Processing Inc., Omaha, NE; 6.08), corn germ meal (6.08), distillers grains with solubles (4.79), blood meal (4.05), distillers dried grains with solubles (3.83), calcium carbonate (3.01), bakery product (2.65), fat mixer (2.05), sodium bicarbonate (1.62), rumen bypass fat (1.22), urea (1.02), salt (0.85), Optigen (Alltech, Nicholasville, KY; 0.61), magnesium oxide (0.50), Alimet (Novus International Inc., St. Charles, MO; 0.30), rumen-protected methionine (Cargill Inc., Minneapolis, MN; 0.12), Diamond V XPC (Diamond V Mills Inc., Cedar Rapids, IA; 0.11), ClariFly larvacide 0.67% (Central Life Sciences, Dallas, TX; 0.09), selenium 0.06% (0.09), 30,497 kIU of vitamin A/kg, 5,868 kIU of vitamin D/kg, 93,007 IU of vitamin E/kg, biotin 1% (0.02), and Rumensin80 G/LB (Elanco Animal Health, Greenfield, IN; 0.02); trace minerals: contained 0.02% Ca, 18.82% S, 153,737 mg of Zn/kg, 30,303 mg of Cu/kg, 136,364 mg of Mn/kg, 3,384 mg of Co/kg, and 3,030 mg of I/kg; organic trace minerals: contained 51,633 mg of Zn/kg, 17,959 mg of Cu/kg, 28,571 mg of Mn/kg, and 3,673 mg of Co/kg; organic selenium: contained 2,041 mg of Se/kg.

Each pen contained 34 head-to-head freestalls with an open-front lunge space (1.3 m wide, 2.6 m long, and 1.3 m high at the neck rail) and 47 headlocks (0.6 m wide; Jourdain Safety Headlock; Jourdain, Escrennes, France) mounted at a forward angle of 15° relative to a vertical plane. The surface of each stall was covered with a 1.7-m-long Animattress (Ani-mat Inc., Sherbrooke, QC, Canada). These mattresses were covered twice weekly with approximately 10 kg of kiln-dried sawdust. Soiled bedding was removed and clean sawdust was redistributed across the mattress at the beginning of each milking.

The sidewalls of the barn were 4.3 m high. Ventilation was regulated using curtains spanning 3.1 m and chimney vents controlled with thermostats and humidity sensors (Ventec Automatic System; Centre-Laitier, Notre-Dame du Nord, QC, Canada). Cross alleys at

the ends of each pen were 3.7 or 4.0 m wide × 5.2 m long; feed alleys and back alleys were 29.3 m long × 4.0 m wide and 29.3 m long × 3.1 m wide, respectively. Feed and back alleys were covered with rubber mats (Animat, Saint-Élie d'Orford, QC, Canada) and scraped every 2 h with an automatic scraper system (Houle, Drummondville, QC). Walking distance was less than 100 m from all pens to the parlor. Ambient temperature and humidity readings near the freestalls were monitored continuously using Hobo data loggers (Onset, Bourne, MA).

Research Design and Stocking Density Treatments

The 3 stocking density treatments (113, 131, and 142%) and the control (100%) were implemented using a 4 × 4 Latin square design with periods of 14 d. The

initial 9 d were used to acclimate the cows to the treatment. Data collection occurred during the remaining 5 d. The control treatment of 100% provided 1 stall and 1 headlock for each cow within the pen ($n = 34$). The control and treatment stocking densities were achieved by denying access to 0, 4, 8, or 10 stalls and 13, 17, 21, or 23 headlocks, respectively, in pens containing 34 stalls and 47 headlocks (Fregonesi et al., 2007; Hill et al., 2009). Eliminated stalls and headlocks were located at both ends of the freestalls and headlocks, which allowed all remaining stalls and headlocks to be centrally located.

Data Collection—Focal Cows

Eight multiparous and 4 primiparous cows of the 34 cows within each pen were selected to form the 4 focal groups. The focal groups were balanced by BW, DIM, and BCS. Within each group, the focal cows were selected to represent the range of BW because of the correlation between BW and social rank (Friend and Polan, 1974).

Data loggers (Gemini Dataloggers Ltd., Chichester, UK; O'Driscoll et al., 2008) monitored the time spent lying or standing and the number of standing bouts occurring during a 24-h period from d 10 to 14. The data loggers were attached to the outer side of one of the hind legs along the metatarsus bone of focal cows ($n = 12$) in each pen. The diurnal pattern of lying behavior was determined by summarizing the minutes of lying per hour, relative to 0000 h, over the 5 d of data collection.

Feeding time (h/d), total rumination time (h/d), posture (standing vs. lying) during rumination, and location (alley vs. stall) of rumination of the focal cows were measured during a 24-h cow watch conducted during d 11 of each period (Grant et al., 1990; Maekawa et al., 2002). Data were recorded by direct observation at 10-min intervals, which were highly correlated with continuous observation (Mitlöhner et al., 2001). Feeding behavior data were summarized on an hourly basis to determine the diurnal pattern of feed bunk attendance of the focal cows.

Hygiene was determined using the 4-point system described by Schreiner and Ruegg (2002). Because of the short duration of the data collection periods, the legs and udder of each cow were cleaned at the beginning of each period and given an initial hygiene score (udder and leg) by 2 trained observers. Two trained observers assessed the effect of treatment on hygiene score on d 14 of each period.

Fecal cortisol metabolites were assessed from grab samples collected from each focal cow after its return to the pen from the 0430 milking on d 13 and 14 (Tucker et

al., 2007). Approximately 200 g of the wet fecal samples were frozen at -20°C and then analyzed following the procedure detailed by Möstl et al. (2002).

Samples of unpreserved milk from a subgroup of 6 of the focal cows from each pen (4 multiparous and 2 primiparous cows) were analyzed for FA profile. Milk samples from each of 3 milkings on d 12 were frozen at -20°C until the analysis was conducted. To prepare samples for analysis, they were thawed and composited to reflect the proportion of the total milk yield collected during each milking. Milk fat was extracted and individual FA were identified and quantified following the procedures detailed by Lock et al. (2006).

Data Collection—Pen Level

Displacements from the feed bunk were recorded for 2 h following 9 milkings over the final 4 d. Sequential milkings were not recorded because of use of headlocks for routine farm management practices after the 0430 milking time. Data were captured by cameras (model 2531R, ULTRA Pro Series B/W 1/3-in. CCD DSP camera equipped with a 1/3-in. 5- to 50-mm DC F1.7 CS-mount lens; Central Alarm Systems Inc., Littleton, CO) mounted above the feed barrier and reviewed with digital video software (Omnicast version 4.1, Genetec Inc., Montreal, QC, Canada) using continuous sampling to determine the number of successful displacements from the feed bunk. A successful displacement was defined as physical contact between 2 cows resulting in the removal of one from a headlock (DeVries et al., 2004). Because of a camera failure, all data from 1 pen were lost for all 4 periods.

Dry matter intake per pen was calculated from the difference between the amount of TMR delivered and the amount refused daily from d 10 to 14. Refusal weights were recorded each morning immediately before delivery of fresh TMR, and a representative sample was collected. All composite samples were dried at 55°C , ground to 1 mm, and sent to a commercial feed testing laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) for analysis.

Milk yields were recorded electronically for each cow at each milking from d 10 to 14 (ProVantage Information Management System; Bou-Matic). Milk samples were collected during all 3 milkings on d 12 and analyzed for content of fat, true protein, lactose, and SCC (Dairy One Cooperative Inc., Ithaca, NY).

Statistical Analyses

Behavioral data and performance data (DMI and milk) were analyzed as a 4×4 Latin square design with fixed effects for period and treatment (stocking

density) and a random effect of pen (experimental unit) using the MIXED procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC). The pen was the experimental unit ($n = 4$ per treatment, except displacements where $n = 3$ per treatment), regardless of whether the data were collected from the focal cows or the entire pen of cows.

Post hoc analyses on variables with significant F -tests (lying time, rumination within a stall, leg hygiene, and displacements; $P \leq 0.05$) were conducted with 2 approaches. For lying time, rumination within a stall, and leg hygiene, means separation was conducted using Tukey's procedure, with significance declared at $P < 0.05$. Post hoc analysis of displacements was conducted using a simple regression model consisting of stocking density as the independent variable and number of displacements as the dependent variable. The analysis was conducted using the REG procedure of SAS.

RESULTS

Mean daily temperature within the pens was 12.3°C , with a range of 2.2°C to 31.5°C during the experiment. The mean daily relative humidity was 66.9%, with a range of 15.4 to 100.0%.

Lying time (h/d) decreased at stocking densities of 131 and 142% relative to 100 or 113% (Table 2; $P < 0.02$). However, lying bouts ($11.3 \pm 0.6/\text{d}$) and bout duration (67.1 ± 4.3 min/bout) were not affected by stocking density ($P > 0.50$). No changes were apparent in lying time per hour over a 24-h period among the treatments (Figure 1).

Feeding and rumination times (h/d) were unaffected by stocking density ($P > 0.50$; Table 3). However, the percentage of the total daily rumination occurring within a freestall was affected by stocking density ($P < 0.01$; Table 3). No changes were apparent in portion of focal cows at the feed bunk over a 24-h period among the treatments (Figure 1). The time spent outside the pen for milking (2.5 ± 0.2 h/d) did not differ among treatments ($P = 0.31$).

The initial udder hygiene scores did not differ among treatments after grooming on d 1 ($P = 0.72$; Figure 2). On d 14, mean udder hygiene scores were not affected by stocking density ($P = 0.99$; Figure 2). Initial leg hygiene scores did not differ among treatments after grooming ($P = 0.69$; Figure 2). However, on d 14, the leg hygiene scores differed between the 131 and 142% stocking densities ($P = 0.04$; Figure 2). The number of teats soiled with manure or bedding material (1.9 ± 0.1) was not affected by stocking density ($P = 0.18$).

The fecal cortisol metabolite, 11-oxoetiocholanolone, was unaffected by stocking density ($P = 0.21$; Figure 3). The secretion of *trans*-10, *cis*-12 CLA (0.06 ± 0.009 g/d) in milk fat was not affected by stocking density ($P = 0.32$; Table 4). No evidence of an alteration in the profile of milk FA was observed ($P > 0.10$; Table 4).

At the pen level, only social behavior was altered by stocking density. A linear increase in displacements from the headlocks occurred in response to increased stall and headlock stocking density ($y = 0.27x - 18.5$; $R^2 = 0.60$; $P < 0.01$; Figure 4). Productivity (DMI, milk yield, and milk composition) was unaffected by short-term changes in stocking density ($P > 0.30$; Table 5).

DISCUSSION

The observed decrease in lying time at the 2 higher stocking densities of freestalls and headlocks within this trial was consistent with previous reports when freestalls (Fregonesi et al., 2007) or headlocks and freestalls (Hill et al., 2009) were overstocked. However, the overall reduction in lying time (approximately 30 min at 131 or 142%) was less than the 1.4 h/d reduction at 133% (Fregonesi et al., 2007) or the 0.9 h/d reduction at 142% (Hill et al., 2009) observed previously. Collectively, in these recent stocking density trials (the current study; Fregonesi et al., 2007; Hill et al., 2009), reductions were observed in lying time at substantially lower stocking densities than reported by Friend et al. (1977), who did not observe a significant decrease in lying time until

Table 2. Mean lying behavior of focal cows from each pen in response to each treatment ($n = 4$ pens/treatment)

Variable	Stocking density, ¹ %				Pooled SE
	100	113	131	142	
Lying time, h/d	12.9 ^a	12.8 ^a	12.2 ^b	12.3 ^b	0.2
Lying bouts/d	11.7	11.5	11.4	10.7	0.6
Bout duration, ² min	67.0	67.4	64.8	69.3	4.3

^{a,b}Means with different superscripts differ ($P < 0.02$).

¹Stocking densities were 1.00, 1.13, 1.31, and 1.42 cows per stall and headlock.

²Bout duration equals the mean lying time divided by the mean number of bouts.

Figure 1. Behavior [mean minutes lying (A) and portion feeding (B) each hour] of the focal cows ($n = 12$) during a 24-h period within each treatment [100% stocking density (1 cow per freestall and headlock; solid line), 113% stocking density (dotted line), 131% stocking density (short dashed line), and 142% density stocking density (long dashed line); $n = 4$ pens/treatment]. Cows were fed beginning at 0430 h and milked beginning at 0430, 1230, and 2030 h.

the stall stocking density was 200%. This difference in lying response among studies may be explained by the experimental design (with the recent studies using a balanced Latin square, which increases the ability to separate means and prevents a carryover effect) to impose treatments, as opposed to the sequential, and nonreplicated, application of treatments used by Friend et al. (1977). Individual cows attempted to maintain

lying time when access to resting space was limited by decreasing the time spent feeding or engaging in social behavior (Munksgaard et al., 2005). Furthermore, increased access to freestalls through the manipulation of neck rail placement resulted in improved locomotion scores (Bernardi et al., 2009). These suggest the importance of access to freestalls extends beyond its use as a resting space. Future research on stocking

Table 3. Mean feeding and ruminating times and location of rumination activity of focal cows from each pen in response to treatment (n = 4 pens/treatment)

Variable	Stocking density, ¹ %				Pooled SE	P-value
	100	113	131	142		
Feeding, h/d	3.7	3.7	3.8	3.8	0.2	0.79
Total ruminating time, h/d	7.2	7.5	7.2	7.1	0.2	0.59
Ruminating within a stall, h/d	6.8	7.0	6.5	6.1	0.2	0.06
Rumination within a stall, % of total	95.1 ^a	93.7 ^{ab}	89.6 ^{bc}	87.3 ^c	2.4	<0.01

^{a-c}Means with different superscripts differ.
¹Stocking densities were 1.00, 1.13, 1.31, and 1.42 cows per freestall or headlock.

Figure 2. Least squares means of the udder (A) and leg (B) hygiene scores for focal cows (n = 12) at each stocking density (1.00, 1.13, 1.31, and 1.42 cows per stall and headlock; n = 4 pens per treatment). Udder hygiene did not differ on d 1 (gray bars; *P* = 0.72) or d 14 (black bars; *P* = 0.99). Leg hygiene did not differ on d 1, but a treatment effect was evident on d 14 (*P* = 0.04). Bars with asterisks (*) differ (*P* = 0.03).

density should examine the response of this variable. Finally, although changes in feeding behavior over different stages of lactation were established (DeVries et al., 2003), fewer data are available on the relationship between lying time and stage of lactation. The current trial was focused on midlactation cows, so additional research is needed to establish the lying response to variable stocking densities at different stages of lactation. The available data suggest a positive relationship between mean daily lying time and DIM (Bewley et al., 2010), which suggests the response to stocking density may differ across a lactation cycle.

We observed no effect of stocking density on feeding time. The literature on the relationship between stocking density and feeding time is mixed. Using the same stocking densities of headlocks and stalls as in the current trial, Hill et al. (2009) observed no effect on feeding time. Huzzey et al. (2006) reported a curvilinear response in feeding time (h/d) across feeding bunk stocking densities of 75 to 300%, or a minimum provi-

Figure 3. Least squares means of fecal cortisol metabolite (11-oxoetiocholanolone, ng/g) for the focal cows (n = 12) at each stocking density (1.00, 1.13, 1.31, and 1.42 cows per stall and headlock; n = 4 pens per treatment), which were unaffected by treatment (*P* = 0.21).

Figure 4. Least squares means of displacements from the feed bunk during the 2 h after milking for each pen of cows ($n = 9$) at each stocking density (1.00, 1.13, 1.31, and 1.42 cows per stall and headlock; $n = 3$ pens/treatment).

sion of 0.2 m of space per cow. Similarly, Friend et al. (1977) reported a significant reduction in feeding time only at 0.1 m of feed bunk space per cow relative the provision of 0.5 m per cow. The 2 studies (the current one and Hill et al., 2009) that did not record changes in feeding behavior in response to stocking density at the feed barrier provided a minimum space of 0.4 m per cow. The differences in the minimum space provided, or maximum stocking density imposed, may explain a por-

tion of the variation in the effect of stocking density at the feed bunk on feeding behavior. Additional factors, such as frequency of feed delivery and timing of feed delivery, may influence the effect of stocking density on feeding behavior, and these interactions should be evaluated in future studies. Furthermore, Huzzey et al. (2006) and Friend et al. (1977) increased only feed bunk stocking density, as opposed to increasing the stocking density at both the freestall and feed barrier in the current study and in the study by Hill et al. (2009). The interaction between these 2 resources has not been established. However, dairy cows prioritized resting over feeding when access to both was limited (Metz, 1985; Munksgaard et al., 2005), which suggests the potential that increasing the stocking density at both may limit the observable changes in feeding behavior.

Displacements from the feed bunk increased linearly in response to increased stocking density. Aggressive displacements at the feed manger have been the most consistently observed behavioral response to increased feed barrier stocking densities. DeVries et al. (2004) observed a 60% increase in aggression during feeding in response to a decrease from 1.0 to 0.5 m of feed bunk space per cow. Increased feed barrier stocking density from 75 to 300% resulted in a linear increase in displacements (Huzzey et al., 2006). Olofsson (1999) observed an almost 5-fold increase in displacements for 4 cows feeding from 1 feed bin relative to those feeding at a ratio of 1 cow per bin. Despite the consistency of

Table 4. Effect of stocking density on milk FA (g/d) of focal cows in each pen ($n = 4$ pens/treatment)

FA	Stocking density, ¹ %				SE	P-value
	100	113	131	142		
SFA	1,008.4	994.4	1,044.0	1,029.0	18.2	0.30
C14:0	163.4	160.8	169.3	166.8	2.73	0.24
C16:0	454.1	449.9	464.4	454.0	8.69	0.63
C18:0	167.1	168.0	179.7	176.4	7.60	0.15
MUFA <i>cis</i>	385.6	387.4	394.8	385.6	7.88	0.72
MUFA <i>trans</i>	75.0	58.3	62.1	60.0	8.16	0.51
C18:1 <i>trans</i>	74.8	58.1	62.0	59.8	8.16	0.51
C18:1 <i>trans</i> -4	0.25	0.23	0.26	0.27	0.008	0.11
C18:1 <i>trans</i> -5	0.46	0.18	0.21	0.21	0.13	0.48
C18:1 <i>trans</i> -6-8	4.94	4.78	5.08	5.04	0.083	0.10
C18:1 <i>trans</i> -9	5.21	4.52	4.73	4.72	0.31	0.51
C18:1 <i>trans</i> -10	12.62	10.96	12.12	11.08	0.91	0.27
C18:1 <i>trans</i> -11	18.91	20.25	21.26	20.08	0.89	0.30
C18:1 <i>trans</i> -12	7.01	6.59	6.99	7.13	0.18	0.23
PUFA	48.76	47.13	48.65	48.26	0.77	0.48
CLA ²	10.18	10.44	10.62	10.25	0.30	0.74
CLA <i>cis</i> -9, <i>trans</i> -11	8.79	9.11	9.14	8.88	0.26	0.72
CLA <i>trans</i> -10, <i>cis</i> -12	0.07	0.05	0.06	0.05	0.009	0.32
BCFA ³	23.05	22.53	23.78	22.94	0.51	0.33
<C16:0	398.2	387.4	411.4	409.2	6.77	0.14
>C16:0	706.5	690.6	716.1	700.9	15.86	0.71

¹Stocking densities were 1.00, 1.13, 1.31, and 1.42 cows per freestall or headlock.

²CLA = conjugated linoleic acid.

³BCFA = branched-chain FA.

Table 5. Mean effect of stocking density treatment on milk yield and composition from each treatment (n = 4 pens/treatment)

Variable	Stocking density, ¹ %				Pooled SE	P-value
	100	113	131	142		
DMI	26.1	26.5	26.6	26.5	1.5	0.94
Milk yield, kg/d	49.2	49.1	48.3	48.1	0.6	0.37
Milk composition						
Fat, %	3.51	3.46	3.42	3.47	0.04	0.51
True protein, %	3.11	3.14	3.11	3.12	0.02	0.39
Lactose, %	4.84	4.82	4.81	4.84	0.03	0.38
MUN, mg/dL	13.1	13.3	13.2	13.7	0.4	0.37

¹Stocking densities were 1.00, 1.13, 1.31, and 1.42 cows per stall and headlock.

this response, the biological consequences of increased aggressive interactions have not been established.

An effect of stocking density on the location of rumination activity was observed, although time spent ruminating (h/d) was unaffected. Similar to feeding time, a significant effect of stocking density on rumination time was not evident. In contrast, a comparison of the rumination behavior at a freestall stocking density of 100% versus 130% indicated a decrease in the mean and maximum percentages of cows ruminating at a given time (Batchelder, 2000). However, in another study the total rumination time and the total time spent ruminating while lying did not differ between stocking densities of 67 and 114% (Fregonesi and Leaver, 2002). A decrease in rumination time may place cows at a greater risk for ruminal acidosis because of a reduction in bicarbonate resulting from decreased saliva production (Cassida and Stokes, 1986). The biological consequences of posture during rumination (lying or standing) have not been established, and future research should address this issue.

Udder and leg hygiene were not affected in a meaningful way by stocking density. Furthermore, the mean hygiene scores were similar to those reported by Schreiner and Ruegg (2003). Overall, independent of stocking density, cows in the current trial were considered "clean" when using the scale described by Schreiner and Ruegg (2003), indicating a lower risk for subclinical mastitis. Other factors within the housing environment of the cow may play a greater role in the hygiene score of the cow than stocking density. Cows housed on sand bedding were cleaner than those housed in straw bedding (Norrington et al., 2008). However, a comparison of various stall bases indicated that cows housed on sand were dirtier than those with access to stalls with a mattress or waterbed base (Fulwider et al., 2007). This discrepancy may be attributed to differences in the frequency of bedding delivery between sand (1.9 times/wk) and the other bases (3.9 times/wk; Fulwider et al., 2007). Cows housed in pens cleaned by automatic alley

scrapers (as was the case in the current study) had lower udder hygiene scores than those cows housed in pens without alley scrapers (Magnusson et al., 2008).

Changes in lying, rumination, and aggressive behaviors during feeding in response to the stocking density treatments did not affect the stress response of the cows in this study, based on responses in fecal cortisol metabolites. Previously, denial of lying for 14 h/d resulting in a 5-h decrease in total daily lying time was associated with an increased cortisol response (Munksgaard and Simonsen, 1996). First, a stress response, quantified by increased concentrations of blood cortisol, was observed in several studies in which cows experienced reduced lying times because of overcrowding (Friend et al., 1979; González et al., 2003) or other means of denial of access to a resting space (Munksgaard and Simonsen, 1996). The severity of the loss of lying time in our study may have been insufficient to cause a cortisol response.

Data are limited that suggest milk fat percentage may be reduced by a greater stocking density (Hill et al., 2007). However, this milk fat depression was not observed in the current study, nor was the concentration of *trans*-10, *cis*-12 CLA in milk fat approaching the amount associated with milk fat depression (Bauman et al., 2006). The inability to reproduce this lowering effect on milk fat (Hill et al., 2007) suggests that stocking density may be a subclinical stressor. The concept of subclinical stress was detailed by Moberg (2000), who defined it as a stressor that depletes the biological resources of the animal without generating a detectable change in function. Future research on stocking density should evaluate its potential as a subclinical stressor in combination with management practices that have a demonstrated biological effect, for example, particle length and its role in milk fat depression (Grant et al., 1990) and stocking density.

Overall, the stocking density treatments imposed within this study had little effect on milk yield. This was inconsistent with the positive relationship between stall availability and milk yield (Bach et al., 2008),

the positive relationship between resting time and milk yield (Grant, 2007), and the increased milk yields resulting from sand bedding relative to other stall surfaces (Calamari et al., 2009). The lack of response in milk yields in the current study may be related to the duration of the treatments imposed and the limited behavioral response to increased stocking density. Ivan et al. (2005) observed significant treatment effects on milk yield in response to dietary treatments when using a crossover design with 14-d periods. However, changes in milk yield were evident only after 6 wk, in response to different bedding surfaces (Calamari et al., 2009). This discrepancy suggests that changes in the physical environment of a cow may require a longer duration before changes in productivity become apparent. An evaluation of freestall width that increased lying time (0.7 h/d) also did not result in an increase in milk yield over a 3-wk collection period (Tucker et al., 2007). Furthermore, the limited reduction in lying time, relative to previous stocking density research (Fregonesi et al., 2007; Hill et al., 2009), was a contributing factor to the lack of response in milk yield. An increased understanding of the relationships among behavioral changes, duration of treatment imposition, and biological costs is critical to furthering applied behavioral research.

CONCLUSIONS

These data suggest a decrease in lying time in response to stocking densities of stalls and headlocks above 113%. The percentage of rumination occurring in a freestall decreased at stocking densities of 131 and 142%, relative to 100%. As stocking density increased, displacements from the feed bunk increased linearly. The behavioral changes we observed in this short-term study in response to variable stocking density did not result in corresponding changes in productivity or other aspects of biological function.

ACKNOWLEDGMENTS

The authors are grateful for the assistance of the research staff and the farm staff of the William H. Miner Agricultural Research Institute. Funding was provided by the USDA-Agriculture and Food Research Initiative (Cooperative State Research, Education, and Extension Service Award No. 2008-35204-04584).

REFERENCES

- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80:1447–1462.
- Bach, A., N. Valls, A. Solans, and T. Torrent. 2008. Associations between nondietary factors and dairy herd performance. *J. Dairy Sci.* 91:3259–3267.
- Batchelder, T. L. 2000. The impact of head gates and overcrowding on production and behavior patterns of lactating dairy cows. Pages 325–330 in *Dairy Housing and Equipment Systems. Managing and Planning for Profitability*. Publ. 129. Nat. Resour., Agric., Eng. Serv., Camp Hill, PA.
- Bauman, D. E., I. H. Mather, R. J. Wall, and A. L. Lock. 2006. Major advances associated with the biosynthesis of milk. *J. Dairy Sci.* 89:1235–1243.
- Bernardi, F., J. Fregonosi, D. M. Veira, C. M. Winkler, M. A. G. von Keyserlingk, and D. M. Weary. 2009. The stall design paradox: Neck rails increase lameness but improve udder and stall hygiene. *J. Dairy Sci.* 92:3074–3080.
- Bewley, J. M., R. E. Boyce, J. Hockin, L. Munksgaard, S. D. Eicher, M. E. Einstein, and M. M. Schutz. 2010. Influence of milk yield, stage of lactation, and body condition on dairy cattle lying behaviour measured using an automated activity monitoring sensor. *J. Dairy Res.* 77:1–6.
- Calamari, L., F. Calegari, and L. Stafanini. 2009. Effect of different free stall surfaces on behavioural, productive and metabolic parameters in dairy cows. *Appl. Anim. Behav. Sci.* 120:9–17.
- Cassida, K. A., and M. R. Stokes. 1986. Eating and resting salivation in early lactation dairy cows. *J. Dairy Sci.* 69:1282–1292.
- DeVries, T. J., M. A. G. von Keyserlingk, and D. M. Weary. 2004. Effect of feeding space on the inter-cow distance, aggression, and feeding behavior of free-stall housed dairy cows. *J. Dairy Sci.* 87:1432–1438.
- DeVries, T. J., M. A. G. von Keyserlingk, D. M. Weary, and K. A. Beauchemin. 2003. Measuring the feeding behavior of lactating dairy cows in early to peak lactation. *J. Dairy Sci.* 86:3354–3361.
- Fregonesi, J. A., and J. D. Leaver. 2002. Influence of space allowance and milk yield level on behaviour, performance and health of dairy cows housed in straw yard and cubicle systems. *Livest. Prod. Sci.* 78:245–257.
- Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007. Overstocking reduces lying time in dairy cows. *J. Dairy Sci.* 90:3349–3354.
- Friend, T. H., F. C. Gwazdauskas, and C. E. Polan. 1979. Change in adrenal response from freestall competition. *J. Dairy Sci.* 62:768–771.
- Friend, T. H., and C. E. Polan. 1974. Social rank, feeding behavior, and free stall utilization by dairy cattle. *J. Dairy Sci.* 57:1214–1220.
- Friend, T. H., C. E. Polan, and M. L. McGilliard. 1977. Free stall and feed bunk requirements relative to behavior, production, and individual feed intake in dairy cows. *J. Dairy Sci.* 60:108–116.
- Fulwider, W. K., T. Grandin, D. J. Garrick, T. E. Engle, W. D. Lamm, N. L. Dalsted, and B. E. Rollin. 2007. Influence of free-stall base on tarsal joint lesions and hygiene in dairy cows. *J. Dairy Sci.* 90:3559–3566.
- González, M., A. K. Yabuta, and F. Galindo. 2003. Behaviour and adrenal activity of first parturition and multiparous cows under a competitive situation. *Appl. Anim. Behav. Sci.* 82:259–266.
- Grant, R. 2007. Taking advantage of natural behavior improves dairy cow performance. Pages 225–236 in *Proc. Western Dairy Manage. Conf.*, Reno, NV. Kansas State University, Manhattan.
- Grant, R. J., and J. L. Albright. 2001. Effect of animal grouping on feeding behavior and intake of dairy cattle. *J. Dairy Sci.* 84(E-Suppl.):E156–E163.
- Grant, R. J., V. F. Colenbrander, and D. R. Mertens. 1990. Milk fat depression in dairy cows: Role of silage particle size. *J. Dairy Sci.* 73:1834–1842.
- Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, W. A. Falls, and R. J. Grant. 2009. Effect of stocking density on the short-term behavioural responses of dairy cows. *Appl. Anim. Behav. Sci.* 117:144–149.
- Hill, C. T., P. D. Krawczel, H. M. Dann, C. S. Ballard, R. C. Hovey, and R. J. Grant. 2007. Effect of stocking density on the short-term behavior of dairy cows. *J. Dairy Sci.* 90(Suppl. 1):244. (Abstr.)

- Huzzey, J. M., T. J. DeVries, P. Valois, and M. A. G. von Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle. *J. Dairy Sci.* 89:126–133.
- Ivan, S. K., R. J. Grant, D. Weakley, and J. Beck. 2005. Comparison of a corn silage hybrid with high cell-wall content and digestibility with a hybrid of lower cell-wall content on performance of Holstein cows. *J. Dairy Sci.* 88:244–254.
- Lock, A. L., B. M. Teles, J. W. Perfield, D. E. Bauman, and L. A. Sinclair. 2006. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 reduces milk fat synthesis in lactating sheep. *J. Dairy Sci.* 89:1525–1532.
- Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002. Chewing activity, saliva production, and ruminal pH of primiparous and multiparous lactating dairy cows. *J. Dairy Sci.* 85:1176–1182.
- Magnusson, M., A. H. Herlin, and M. Ventorp. 2008. Short communication: Effect of alley floor cleanliness on free-stall and udder hygiene. *J. Dairy Sci.* 91:3927–3930.
- Metz, J. H. M. 1985. The reaction of cows to a short-term deprivation of lying. *Appl. Anim. Behav. Sci.* 13:301–307.
- Mitlöhner, F. M., J. L. Morrow-Tesch, S. C. Wilson, J. W. Dailey, and J. J. McGlone. 2001. Behavioral sampling techniques for feedlot cattle. *J. Anim. Sci.* 79:1189–1193.
- Moberg, G. P. 2000. Biological response to stress: Implications to animal welfare. Pages 1–21 in *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. G. P. Moberg and J. A. Mench, ed. CABI Publ., New York, NY.
- Möstl, E., J. L. Maggs, G. Schrötter, U. Besenfelder, and R. Palme. 2002. Measurement of cortisol metabolites in faeces of ruminants. *Vet. Res. Commun.* 26:127–139.
- Munksgaard, L., M. B. Jensen, L. J. Pedersen, S. W. Hansen, and L. Matthews. 2005. Quantifying behavioural priorities—Effects of time constraints on behaviour of dairy cows, *Bos taurus*. *Appl. Anim. Behav. Sci.* 92:3–14.
- Munksgaard, L., and H. B. Simonsen. 1996. Behavioral and pituitary adrenal-axis responses of dairy cows to social isolation and deprivation of lying down. *J. Anim. Sci.* 74:769–778.
- Norring, M., E. Manninen, A. M. Passillé, J. Rushen, L. Munksgaard, and H. Saloniemi. 2008. Effects of sand and straw bedding on the lying behavior, cleanliness, and hoof and hock injuries of dairy cows. *J. Dairy Sci.* 91:570–576.
- O'Driscoll, K., L. Boyle, and A. Hanlon. 2008. A brief note on the validation of a system for recording lying behaviour in dairy cows. *Appl. Anim. Behav. Sci.* 111:195–200.
- Olofsson, J. 1999. Competition for total mixed diets fed for ad libitum intake using one or four cows per feeding station. *J. Dairy Sci.* 82:69–79.
- Schirmann, K., N. Chapinal, D. M. Weary, W. Heuwieser, and M. A. G. von Keyserlingk. 2011. Short-term effects of regrouping on behavior of prepartum dairy cows. *J. Dairy Sci.* 94:2312–2319.
- Schreiner, D. A., and P. L. Ruegg. 2002. Effects of tail docking on milk quality and cow cleanliness. *J. Dairy Sci.* 85:2503–2511.
- Schreiner, D. A., and P. L. Ruegg. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. *J. Dairy Sci.* 86:3460–3465.
- Tucker, C. B., D. E. Dalley, J. L. K. Burke, and D. A. Clark. 2007. Milking cows once daily influences behavior and udder firmness at peak and mid lactation. *J. Dairy Sci.* 90:1692–1703.
- USDA. 2010. Facility characteristics and cow comfort on U.S. dairy operations, 2007. US Dept. Agric.-Anim. Plant Health Insp. Serv.-Vet. Serv., Cent. Epidemiol. Anim. Health, Fort Collins, CO.