Effects of space allowance on the behavior and physiology of cattle temporarily managed on rubber mats

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ABSTRACT

Dairy cattle managed in some pasture-based systems, such as in New Zealand, are predominantly kept outdoors all year around but are taken off pasture for periods, especially in wet weather to avoid soil damage. The use of rubber matting for such stand-off practices is becoming more common to improve animal welfare, and our objective was to investigate the effects of different space allowances on cow behavior and physiology when managed temporarily on rubber mats during a weather-induced stand-off period. Thirty pregnant, nonlactating Holstein-Friesian dairy cows were divided into 6 groups of 5 and exposed to 6 treatments following a Williams designed 6×6 Latin square. The treatments consisted of 6 space allowances on a 24-mm rubber surface during a simulated weather-induced stand-off period: 3.0, 4.5, 6.0, 7.5, 9.0, and $10.5 \text{ m}^2/\text{cow}$. The stand-off period consisted of 18 h in the treatment pens followed by 6 h at pasture to allow for their daily feed intake (no feed was available during stand-off, following normal farm practice), for 3 consecutive days, with 6 d of recovery on pasture between treatments. When cows had more space available during the stand-off period, they spent more time lying on the rubber mats and less time lying on pasture during their daily 6-h feed break. Mean lying times (24 h, pasture and rubber mats combined) for the different space allowances were for $3.0 \text{ m}^2 = 7.5 \text{ h}$, $4.5 \text{ m}^2 = 10.2 \text{ h}$, $6.0 \text{ m}^2 = 11.9 \text{ h}$, $7.5 \text{ m}^2 = 12.4 \text{ h}$, and $10.5 \text{ m}^2 = 13.8 \text{ h}$. At 6.0 m^2 of space allowance per cow, the animals spent similar times lying per 24 h as when the cows were on recovery on pasture in between treatments (11.9 and 11.2 h, respectively). Aggressive interactions and nonaggressive lying disturbances were more frequent at lower space allowances (aggressive interactions decreased by 35% from 3.0 to $4.5 \text{ m}^2/\text{cow}$, with a slower decline thereafter). Cows were dirtier after the stand-off period, particularly at

lower space allowances. All cows had higher gait scores after the stand-off period; however, this change was unaffected by space allowance and very minor. Stride length, plasma cortisol, and body weight were all unaffected by the stand-off period and space allowance. The results suggest that to reduce aggressive behavior and maintain adequate lying times, dairy cattle managed temporarily on rubber matting for up to 18 h per day, without feed, should have a space allowance of at least 4.5 to 6.0 m^2 per cow.

Key words: aggressive behavior, lying time, rubber mat, space allowance

INTRODUCTION

In some pasture-based dairy systems, such as in New Zealand, cows are mainly kept outdoors all year around; however, in wet weather during winter they are sometimes removed from the pasture to a separate area to reduce soil damage. Depending on weather conditions, cows can be kept off pasture for up to 22 h per day for several consecutive days, and this practice can cause reduced health and welfare of animals when managed on hard or muddy surfaces (Fisher et al., 2003). Common surfaces for these stand-off practices include specially constructed wood-chip pads, concrete yards, small paddocks, gravel laneways, and, more recently, rubber matting on a concrete surface. We have previously shown that a well-maintained wood-chip surface and the addition of rubber matting onto concrete surfaces for stand-off purposes is beneficial for animal welfare compared with a concrete surface (Schütz and Cox, 2014); cows were cleaner and spent more time lying when they were stood off on wood chips, whereas cows on rubber mats had longer lying times and improved gait pattern compared with cows stood off on concrete. Indeed, lying behavior has been used as a measure of cow comfort in numerous other studies investigating different surface types (Fregonesi and Leaver, 2001; Fisher et al., 2003; Tucker et al., 2003;) and dairy cattle prefer and spend more time lying on soft, well-bedded (Tucker et al., 2003; Tucker and Weary, 2004; Drissler

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et al., 2005; Tucker et al., 2009; Schütz and Cox, 2014), and dry (Fregonesi et al., 2007b; Reich et al., 2010) surfaces. The provision of a suitable surface to rest on is crucial to maintain the health of dairy cattle (Singh et al., 1993; Leonard et al., 1996) and reduced lying times are associated with behavioral signs of frustration (Munksgaard and Simonsen, 1996; Munksgaard et al., 1999) and physiological stress (Fisher et al., 2002; Tucker et al., 2007).

Space allowance is an important part of farm animal welfare. It influences aggressive behavior (e.g., Wierenga, 1983; Kondo et al., 1989) and consequently access to resources, such as feed (e.g., DeVries and von Keyserlingk, 2006, 2009) and lying areas (e.g., Wierenga, 1983), as well as being related to opportunities to move freely and for exercise (Hurnik and Lewis, 1991). Space allowance also influences the level of behavioral synchrony in groups of animals (Nielsen et al., 1997; Færevik et al., 2008), which has been suggested as an indicator of positive welfare (Napolitano et al., 2009).

The practice of adding rubber mats onto concrete areas to improve animal welfare during stand-off purposes is becoming more common in New Zealand, and there is a need for science-based recommendations regarding space allowance for such practices to allow cows to maintain sufficient lying times. Overstocking of dairy cattle in free-stall systems results in reduced lying times and increased aggressive interactions (Leonard et al., 1996; Fregonesi and Leaver, 2002; Fregonesi et al., 2007a; Hill et al., 2009), but less is known regarding the effects of space allowance in loose-housed stand-off areas. Therefore, the aim of this project was to investigate the effects of different space allowances on cow behavior and physiology when managed temporarily on rubber mats during a weather-induced stand-off period.

MATERIALS AND METHODS

Animals and Treatments

All procedures involving animals were approved by the Ruakura Animal Ethics Committee under the New Zealand Animal Welfare Act 1999. Thirty pregnant, nonlactating Holstein-Friesian and Friesian-Jersey-cross cows were used in the study undertaken at the DairyNZ Lye Farm, near Hamilton, New Zealand (37°46'S, 175°18'E). The cows had a BW of 499 ± 55.5 kg, BCS of 4 ± 0.4 on a 10-point scale (Roche et al., 2004), and were 5 ± 1.5 yr old at the start of the study (mean $\pm$ SD for all preceding values). The cows were divided into 6 groups of 5 cows per group, balanced on BW. Groups were formed 5 d before the start of the study. Each of the groups of 5 animals was exposed to each of

the 6 treatments over a period of 6 wk (replicates) in a Williams Latin square arrangement, balanced for first-order carry-over effects. The treatments consisted of 6 different space allowances on a 24-mm rubber surface during a simulated weather-induced stand-off period: 3.0, 4.5, 6.0, 7.5, 9.0, and 10.5 m²/cow. The stand-off period consisted of 18 h in the treatment pens followed by 6 h at pasture to allow for their daily feed intake (0900 to 1500 h) per 24 h, for 3 consecutive days. This period was chosen as it is a common stand-off period during winter in the region. The stand-off period was followed by 6 d on pasture (recovery). These 2 periods were repeated 6 times until each group had experienced all treatments between May 19 and July 12, 2013 (southern hemisphere winter). A fresh sward of pasture (8 kg of DM per cow) and maize silage (3 kg of DM per cow) were offered to the cows each day at 0900 h who were grazed as one group during the time on pasture.

The treatment pens were formed by laying interlocking 24-mm rubber mats (Agrimat Kura, 1,190 $\times$ 850 $\times$ 24 mm, Numat Ltd., Auckland, New Zealand) onto an existing uncovered concrete laneway within the dairy yard complex at the farm. Commercially available steel farm gates (length: 4.8 m, height: 1 m, finished height was 1.2 m above rubber mat once the gates were mounted on posts) were used to divide the area into uncovered treatment pens that were located side by side, forming 6 differently sized pens in a row (15.0, 22.5, 30.0, 37.5, 45.0, and 52.5 m²). The cows had visual, auditory, and some tactile contact between groups at all times. The location of the treatment pens was randomized at the beginning of the study and again halfway through the study to avoid any location bias. No feed was available during the stand-off period; however, water was supplied at all times in circular 100-L plastic containers (diameter: 55 cm, height: 45 cm) following normal farm practice. The pens were cleaned daily each morning after use and drinking water renewed. The cows were habituated to the rubber mats before the commencement of the study by spending 4 consecutive nights (approximately 16 out of 24 h) as one group on the entire rubber surface (42.0 $\times$ 4.8 m) without gate partitions in place.

Environmental Conditions

Environmental conditions were recorded continuously throughout the trial. Air temperature (°C), relative humidity (%), and rainfall (mm) were recorded at 10-min intervals using a portable weather station (6163, Wireless Vantage Pro2 Plus, Davis Instruments, Harvard, CA) located in an unsheltered area within 20 m of the test area. The daily mean air temperature (24 h) was

9.7 ± 2.61°C and relative humidity was 91.0 ± 4.97% (mean ± SD). Rainfall was 5.5 ± 9.57 mm during the experimental period (mean ± SD of the daily total).

Lying Times

Lying and standing times were recorded continuously using Onset Pendant G data loggers (64k, Onset Computer Corporation, Bourne, MA) set to record the y- and z-axis at a 30-s interval. The data loggers were placed in a durable fabric pouch and attached on the lateral side of the hind leg above the metatarsophalangeal joint. The pouch was held in position by Velcro patches, one sewn to the pouch and the other glued (Kamar Adhesive, Kamar Products Inc., Zionsville, IN) to the leg of the cow. The pouch was further held in place by a strap around the leg of the cow. The data were downloaded using HOBOWare Pro software (Onset Corp., Pocasset, MA) and converted to daily summaries of lying behavior using SAS software (SAS Institute Inc., Cary, NC) code designed for this purpose (N. Chapinal, University of Guelph, Ontario, Canada, personal communication) and based on the work of Ledgerwood et al. (2010) correcting for single standing and lying events. The frequency and duration of lying bouts were determined directly from the data-logger files.

Aggressive Interactions and Lying Disturbances

Aggressive interactions were recorded during the 18-h stand-off period using an i-Watch 1/3”Sony Starlight Camera S2065H CCD (Sony, GangShan Town, Taiwan) with a Kenko varifocal lens (KVR06IDC, Tokina Co. Ltd., Tokyo, Japan) mounted on a 3-m pole.

Aggressive behavior was recorded in 2 ways. An aggressive interaction was defined as any rapid forceful contact between 2 cows, in which the head, shoulder,

or both parts of the aggressor simultaneously make physical contact with any part of the receiver (a reaction, such as a movement away from the impact or a skin flinch, is always seen from the receiver). We also recorded the number of lying displacements where a recumbent cow (no weight supported by any legs) stood up as a result of an interaction from another cow. This behavior was further divided into 2 categories: direct, which was when the lying disruption was caused by an aggressive contact (as defined above), or indirect, which was when the interaction was the result of a nonaggressive (not direct) contact. Multiple observers were used to collect the aggressive behavior information from the first and third nights of stand-off. The interobserver reliability for aggressive behavior, as measured by correlation, was 0.781.

Gait Score, Stride Length, and Hygiene Score

Gait score, stride length, and hygiene score were recorded immediately before and after the 3-d stand-off period when the cows were walking 10 m on a farm laneway on their way to and from the treatment pens. Gait score was recorded on a 5-point scale (Table 1). Stride length was calculated by counting the number of steps taken by the hind legs over the 10-m distance using a video camera (Sony Handycam Camcorder, DCR-SX65, Sony, Tokyo, Japan). Each cow was also given a hygiene score ranging from clean to very dirty (on a 5-point scale, Table 2).

Multiple observers were used to collect the behavioral information. Interobserver reliability for hygiene score, as measured by percentage agreement, was 77%. One person recorded the gait score; the intrareliability for that person, as measured by percentage agreement, was 93%. One person recorded stride length off video recordings; the intraobserver reliability for that person, as measured by correlation, was 0.941.

Table 1. Gait score used in the study (Thomsen et al., 2008)

Gait score	Description
1	Cow stands and walks normally. Places hooves with confidence, rear hooves placed where front hooves were.
2	Cow stands normally but arches back when starting to walk. Head is held lower and extended from the body. Gait is slightly abnormal.
3	Cow arches back both when standing still and when walking. Takes short steps with one or more legs.
4	Cow arches back both when standing still and when moving. Tries to reduce the weight on one or more hooves.
5	Cow has an arched back. Refuses to stand on a particular hoof or limps. Prefers to remain lying down or has great difficulty when trying to get up.

Table 2. Hygiene score used in the study

Hygiene score	Description
1	The animal is in general clean. Front udder, escutcheon, and hind limbs below the hip are clean. Stains are only on the hock points and tail provided adherent particles are minimal; no major fecal accumulation exists on the tail.
2	The animal is mildly dirty. Front of udder (teats and udder) is generally clean. Hocks are contaminated with adherent material on the back of leg. Hind limbs below the hip are overall clean but may have a few adherent particles. The escutcheon may have a few adherent particles, and clumps of fecal matter may be on the tail.
3	The animal is moderately dirty. Some dirt is on the front of the udder, including small clumps at rear of abdomen and some staining of lower abdomen. Hock points have small clumps of dirt and fecal matter adhering. The area on the hind limb below the hip is dirty and may have some clumps of fecal matter. The escutcheon and tail have some adherent material and clumps of fecal matter, and the tail is generally matted.
4	The animal is very dirty. The front of the udder and skin of the abdomen are very dirty, with dirt extending about a third of the distance up the abdomen. Hock points have clumps of dirt and fecal matter adhered to the lower hind legs. Hind limbs below the hip are very dirty, with material forming into clumps of fecal matter. Escutcheon has dirt and clumps of fecal matters adhering to backs of legs. Tail has much adherent material and clumps of fecal matter.
5	The animal is extremely dirty. As for score 4 but with big clumps of fecal matter hanging off the underside of the stomach.

Cortisol

Blood samples were taken before and after each stand-off period by coccygeal venipuncture into one plain evacuated tube (BD, Plymouth, UK) for analysis of concentrations of cortisol. Samples were kept at room temperature following collection and then centrifuged at $1,500 \times g$ ($\sim 3,000$ rpm) at room temperature for 10 min. Serum was aspirated, and aliquots were stored at -20°C until they were assayed. Samples were analyzed unextracted in duplicate using a commercially available kit (Siemens Coat-A-Count Cortisol RIA, Siemens Healthcare Diagnostics Inc., Los Angeles, CA).

BW

Body weight was recorded using weigh scales (Gallagher, Hamilton, New Zealand) as the cows left the treatment pens after the first and third nights of stand-off. This ensured comparative gut fill for both occasions.

Statistical Analysis

Data were analyzed using an ANOVA with random (block) effects for group, animals, replicate, and their interactions and treatment effects for space allowance, before and after the stand-off period, and their interaction. Cortisol and aggressive interactions were log-transformed; back-transformed means are presented. Before and after, comparisons were also made for each space allowance, using the pooled standard deviation from the ANOVA. Aggressive interactions were analyzed on group level because individuals could not be

identified from the video recordings. A large proportion of suspected aggressive behavior and lying displacements did not strictly follow our definitions because of the view being obscured by animals or camera angles (30 and 31% of observations of aggressive interactions and lying displacements, respectively). These observations were excluded from the analysis, and it is clear that we underestimated the total number of aggressive interactions (in particular in the treatment with space allowance of 3.0 m^2 per cow) and lying displacements. All statistical analyses were conducted using the statistical package GenStat, version 13.2 (VSN International, Hemel Hempstead, UK).

RESULTS

Lying Times and Lying Bouts

Space allowance influenced lying times during the stand-off period on rubber mats. Lying times during the stand-off period were longer when cows had more space available ($P < 0.001$, Figure 1). When cows were allowed their daily feed ration during 6 h on pasture in between stand-off periods (within a treatment), lying times were longer at lower space allowances ($P = 0.021$, Figure 1). Mean lying times (24 h, on pasture and rubber mats combined) for the different space allowances during the 3-d stand-off period were for $3.0 \text{ m}^2 = 7.5$ h, $4.5 \text{ m}^2 = 10.2$ h, $6.0 \text{ m}^2 = 11.9$ h, $7.5 \text{ m}^2 = 12.4$ h, and $10.5 \text{ m}^2 = 13.8$ h. At 6.0 m^2 of space allowance per cow, the animals spent similar times lying per 24 h as during recovery in between treatments (11.9 and 11.2 h, respectively, Figure 1).

Figure 1. Mean lying times of dairy cattle (n = 6 groups, 5 cows per group) with different space allowances during a 3-d stand-off period that consisted of 18 h on 24-mm rubber matting and 6 h on pasture per 24 h, and with 6 d on pasture in between treatments (recovery). The SE of the difference for the differences between space allowances was 0.27 h for 6 h on pasture, 0.67 h for 18 h on rubber matting, and 0.11 h for 24 h on pasture between treatments.

There was no effect of space allowance on lying times when cows were on pasture (recovery) for the 6 d between treatments ($P = 0.169$, Figure 1).

Space allowance influenced the number of lying bouts and bout duration when cows were on the rubber mats. Cows had fewer lying bouts at lower space allowances (Table 3), in particular in the 3.0-m² treatment. Mean lying-bout duration was longer when more space was available (Table 3). When cows were on the rubber mats the percentage of short lying bouts (less than 5 min) was for the different space allowances 3.0 m² = 4.2%, 4.5 m² = 2.0%, 6.0 m² = 2.0%, 7.5 m² = 2.3%, and 10.5 m² = 1.6% of total number of bouts (data were not analyzed).

When the cows were on pasture for 6 h in between stand-off exposures, lying bouts (number and duration) were unaffected by space allowance (Table 3).

Aggressive Interactions and Lying Disturbances

Aggressive interactions were more frequent at lower space allowances ($P = 0.006$, Figure 2), decreasing by 35% from 3.0 to 4.5 m² per cow, with a slower decline thereafter. Aggressive interactions between 2 upright animals were the most frequently observed aggressive behavior (Figure 2). Lying displacements were not common; nevertheless, nonaggressive (indirect) disturbances toward cows that were lying down were

Table 3. Number of lying bouts and bout duration [daily mean ± SE of the difference (SED)] of dairy cattle during a 3-d stand-off period that consisted of 18 h on 24-mm rubber matting with different space allowances (m² per cow) and 6 h on pasture per 24 h (n = 6 groups, 5 cows per group)

Item	Space allowance						P-value	SED
	3.0	4.5	6.0	7.5	9.0	10.5		
On rubber								
Number of lying bouts	3.7	4.7	5.8	6.2	6.3	6.1	<0.001	0.49
Duration of lying bouts (h)	1.03	1.35	1.35	1.33	1.38	1.48	0.002	0.08
On pasture								
Number of lying bouts	2.1	1.7	1.7	1.6	1.5	1.5	0.465	0.31
Duration of lying bouts (h)	0.32	0.27	0.32	0.27	0.23	0.28	0.384	0.03

Figure 2. Mean number of aggressive interactions and direct and indirect lying displacements of dairy cattle ($n = 6$ groups, 5 cows per group) with different space allowances when managed for 18 out of 24 h on 24-mm rubber matting during a 3-d stand-off period. Values are back-transformed means. The SE of the difference for the differences between space allowances was 0.50 per hour for aggressive interactions, 0.04 per hour for direct lying displacements, and 0.02 per hour for indirect lying displacements.

also more frequent when less space was available ($P = 0.006$, Figure 2), whereas direct aggressions toward recumbent animals were unaffected by space allowance ($P = 0.929$).

Gait Score and Stride Length

All cows had higher gait scores after the 3-d stand-off period than before (Table 4); however, the increase was very minor, and the gait patterns were never worse than slightly abnormal. This change in gait score was unaffected by space allowance (Table 4). Also, space allowance and change in gait score between before and after the stand-off period did not interact (Table 4). No cows became lame during the study. Similarly, stride length was unaffected by space allowance, change in stride length between before and after the stand-off period, or their interaction (Table 4).

Hygiene Score

Space allowance influenced the hygiene score during the 3-d stand-off period, and there was an interaction between space allowance and change in hygiene score during the stand-off period (Table 4). Cows were dirtier (higher hygiene scores) after the stand-off period at space allowances between 3.0 and 7.5 m² per cow (Table 4).

Cortisol and BW

Blood cortisol levels were unaffected by space allowance, the stand-off period, or their interaction (Table 4). This was also the case for BW.

DISCUSSION

Cows exposed to a 3-d stand-off period on rubber mats spent more time lying on the mats with increasing space allowance and less time lying on pasture, when they were allowed their daily feed ration, in between stand-off periods. Aggressive behavior and disturbances of recumbent cows were more frequent at lower space allowances. Cows were cleaner when more space was available, whereas gait pattern, cortisol in blood, and BW were unaffected by space allowance.

New Zealand dairy cattle (BW of about 500 kg) typically spend between 8 and 11 h per day resting when on pasture (Kendall et al., 2006; Tucker et al., 2008; Schütz et al., 2013; Schütz and Cox, 2014). The New Zealand dairy industry recommends a minimum of 8 out of 24 h of lying time for nonlactating dairy cattle, and such lying times were achieved with a space allowance of 4.5 m² in combination with 6 h of access to relatively dry pasture each day. However, the provision of more space in the present study increased lying times on rubber mats (both the number and duration of lying bouts increased with space allowance). For every 1.5-m² increase

Table 4. Mean gait score (scale 1 to 5), stride length (strides per meter), hygiene score (scale 1 to 5), plasma cortisol (nmol/L), and BW (kg) of dairy cattle before and after a 3-d stand-off period that consisted of 18 h on 24-mm rubber matting with different space allowances (m² per cow) and 6 h on pasture per 24 h (n = 6 groups, 5 cows per group)

Item	Space allowance	Before	After	<i>P</i> -value ¹				SED ¹	
				Within treatment before–after	Between treatment	Before–after	Treatment × before–after	Within treatment before–after	Between treatment
Gait score	3.0	1.2	1.3	0.387					
	4.5	1.2	1.3	0.096					
	6.0	1.3	1.4	0.373					
	7.5	1.2	1.3	0.164					
	9.0	1.2	1.3	0.193					
	10.5	1.2	1.3	0.660					
Overall					0.349	0.008	0.962	0.08	0.06
Stride length	3.0	1.3	1.3	0.589					
	4.5	1.3	1.3	0.485					
	6.0	1.3	1.2	0.038					
	7.5	1.2	1.3	0.132					
	9.0	1.3	1.3	0.352					
	10.5	1.3	1.3	0.629					
Overall					0.510	0.929	0.137	0.03	0.02
Hygiene score	3.0	2.2	3.7	<0.001					
	4.5	2.1	3.3	<0.001					
	6.0	2.1	3.1	<0.001					
	7.5	2.2	2.8	<0.001					
	9.0	2.3	2.4	0.252					
	10.5	2.2	2.4	0.108					
Overall					<0.001	<0.001	<0.001	0.14	0.12
Cortisol ²	3.0	17.6	17.8	0.951					
	4.5	17.5	22.8	0.071					
	6.0	19.4	20.6	0.695					
	7.5	20.9	23.4	0.433					
	9.0	20.4	21.6	0.695					
	10.5	20.7	21.2	0.860					
Overall					0.492	0.141	0.838	3.00	2.26
BW	3.0	529	529	0.810					
	4.5	531	530	0.892					
	6.0	528	528	0.522					
	7.5	534	536	0.111					
	9.0	534	532	0.101					
	10.5	533	532	0.892					
Overall					0.103	0.767	0.338	1.3	2.7

¹SE of the difference (SED) and *P*-values for within and between space-allowance treatments, before and after treatments, and their interaction are presented.

²Back-transformed means and SED.

in space allowance, lying times on the rubber mats increased by, on average, 1.4 h. Also, cows at lower space allowances spent more time lying on pasture when they were allowed their daily feed ration in between stand-off exposures (within treatment). These results indicate that cows that are not able to lie down for a sufficient amount of time during the stand-off period instead will try to compensate by lying down more when they are at pasture, a time where they ideally should be feeding. When the cows were stood off on 6.0 m² of flooring space per animal, they spent a similar amount of time lying (11.9 h) as when they were kept on pasture (6 × 24 h) in between stand-off treatments (11.2 out of 24 h). Space allowance did not influence lying times during this period, which suggests that all cows had sufficient

time to recover between treatment exposures and that carry-over effect was nonexistent between treatments. Our results suggest that cows temporarily stood off on 24-mm rubber matting, in combination with pasture access, should have a space allowance of at least 4.5 to 6.0 m² per cow to maintain sufficient resting times. These values are similar to those recommended for the bedded area in strawyard housing systems (5.0 and 6.0 m² per cow, in Fregonesi and Leaver, 2002). Interestingly, in a previous study where cows were repeatedly exposed to different surface types for 4 consecutive days at a space allowance of 4.9 m² per cow, lying times on the same 24-mm rubber mat as in this study were 1.2 h shorter, even though the space allowance was greater compared with in the present study when cows had

a space allowance of 4.5 m² per cow (7.3 and 8.5 h, respectively; Schütz and Cox, 2014). We speculate that other variables, such as lying conditions on pasture, weather conditions, duration of stand-off period, or the social dynamic within groups, contribute to differences between studies. Most of the literature to date investigating stocking density and space allowance concerns free-stall systems and overstocking of lying stalls. Our results are in general agreement with experimental studies showing that overstocking in free-stall systems reduce lying times (Fregonesi et al., 2007a; Krawczel et al., 2008, 2012; Hill et al., 2009); however, no relationship between stocking density and lying behavior has been found in some commercial on-farm research (Lombard et al., 2010; Charlton et al., 2014), thus highlighting the many factors that contribute to lying times on farm.

The shorter lying times (both total time and bout duration and frequency) associated with smaller space allowances are likely partly due to increased levels of aggressive interactions between animals and disturbances while lying. Indeed, we found that aggressive behavior was more frequent at lower space allowances, in particular when cows had a space allowance of 3.0 m² per cow. Also, nonaggressive disturbances of recumbent cows were more frequent at lower space allowances and were often a result of an aggressive interaction between 2 upright animals. This was accompanied by a greater percentage of short lying bouts in the smallest space allowance treatment (<5 min, data not analyzed). Dairy heifers were more aggressive and lying displacements more common when they had access to a 1.8-m² straw bedded area compared with 2.7 and 3.6 m² per animal (Nielsen et al., 1997). Others have found that overstocking in free-stall systems cause increased aggressive behavior (Fregonesi and Leaver, 2002); however, aggressive interactions did not differ when dairy cattle in a strawyard system had a bed space allowance of 9.0 and 4.5 m² per cow (Fregonesi and Leaver, 2002). In the latter study, the total space allowance was 13.5 and 6.7 m² per cow, respectively, and it is possible that both space allowances were sufficient for animals to maintain adequate lying times without having to compete for lying space, or alternatively by changing their diurnal lying patterns.

Lying surface (Lombard et al., 2010; Potterton et al., 2011) as well as overstocking and lying times (Leonard et al., 1996; Ito et al., 2010) are associated with leg health and lameness in dairy cattle. In the present study, the increase in gait score after the stand-off period was very minor and the gait scores were never worse than slightly abnormal. This could be due to the use of rubber matting as flooring surface instead of concrete because this has been shown to improve the leg health

and gait pattern of animals (Leonard et al., 1994; Vane-gas et al., 2006; Schütz and Cox, 2014). Also, cows had access to pasture each day, which is likely beneficial to leg health. Space allowance did not influence gait score or stride length, and the results suggest that whereas standing cows off on a 24-mm rubber mat for up to 18 h per day for 3 consecutive days might cause a slight discomfort to cows, it does not seem to increase the risk of leg injuries due to restricted space.

We have previously shown that cows stood off on rubber mats for 4 d were dirtier (higher hygiene scores) compared with cows kept on well-managed wood chips (Schütz and Cox, 2014). In the present study, cows were dirtier after the stand-off period, in particular when less space was available. Similar results were found by Fregonesi and Leaver (2002) where dairy cattle in a strawyard system were dirtier at lower space allowances and had lower milk lactose levels, an indicator of mastitis. Udder health is one of the main health issues of dairy cattle, and dirty surfaces are associated with a perceived increased risk of mastitis. However, most cows in New Zealand are normally nonlactating during a large part of winter, so the effect of hygiene is likely to be less important compared with when cows are lactating. It is, however, possible that rubber mats are not suitable to house lactating cows on, at least not at high stocking densities and without regular cleaning.

Short-term stand-off practices can alter blood cortisol levels (Schütz and Cox, 2014), fecal glucocorticoid metabolite concentration (Fisher et al., 2003), as well as the BW of cows (Fisher et al., 2003; Schütz and Cox, 2014). Stand-off practices in combination with high stocking density may be considered a potentially stressful situation due to a higher frequency of aggressive interactions and reduced lying times. However, this was not confirmed in the present study; blood cortisol levels were numerically higher after the stand-off period, in contrast with previous findings (Schütz and Cox, 2014), and space allowance did not influence cortisol levels or the BW of the animals. Overstocking of lying areas in a free-stall system for 7 d increased the cortisol response to an ACTH challenge (Friend et al., 1979); however, overstocking for a longer time (14 d) did not affect fecal cortisol metabolites in lactating dairy cattle (Krawczel et al., 2012) or plasma cortisol response to an ACTH challenge in nonlactating cattle (Huzzey et al., 2012). Huzzey et al. (2012) found a tendency for fecal glucocorticoid metabolites to be higher during overstocking, and the authors suggested that overall daily cortisol secretion might have been higher and that the lack of cortisol response to the ACTH challenge could be due to a physiological desensitization of the hypothalamic-pituitary-adrenal axis. It is unclear what the effects of space allowance are on the physiological stress response

of dairy cattle managed temporarily on rubber mats, and we encourage further research in this area.

CONCLUSIONS

Our results suggest that cows temporarily stood off on a 24-mm rubber mat, without feed and with pasture access, should have access to at least 4.5 to 6.0 m² of flooring surface per cow to minimize aggressive interactions and disturbances while lying and maintain adequate lying times. Cows with more space available spent more time lying on the rubber mats and less time lying on pasture in between stand-off exposures during the 3-d stand-off period. Cows with less space were more aggressive and disturbances of recumbent cows were more common. Cows were dirtier after the stand-off period, in particular at lower space allowances. Gait pattern (gait score and stride length), plasma cortisol, and BW were all unaffected by space allowance; however, gait scores were slightly higher after the 3-d stand-off period, indicative of a minor discomfort caused by the stand-off period.

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REFERENCES

- Charlton, G. L., D. B. Haley, J. Rushen, and A. M. de Passillé. 2014. Stocking density, milking duration, and lying times of lactating cows on Canadian freestall dairy farms. *J. Dairy Sci.* 97:2694–2700.
- DeVries, T. J., and M. A. G. von Keyserlingk. 2006. Feed stalls affect the social and feeding behavior of lactating dairy cows. *J. Dairy Sci.* 89:3522–3531.
- DeVries, T. J., and M. A. G. von Keyserlingk. 2009. Competition for feed affects the feeding behavior of growing dairy heifers. *J. Dairy Sci.* 92:3922–3929.
- Drissler, M., M. Gaworski, C. B. Tucker, and D. M. Weary. 2005. Freestall maintenance: Effects on lying behavior of dairy cattle. *J. Dairy Sci.* 88:2381–2387.
- Færevik, G., K. Tjentland, S. Løvik, I. L. Andersen, and K. E. Bøe. 2008. Resting pattern and social behaviour of dairy calves housed in pens with different sized lying areas. *Appl. Anim. Behav. Sci.* 114:54–64.
- Fisher, A. D., M. Stewart, G. A. Verkerk, C. J. Morrow, and L. R. Matthews. 2003. The effects of surface type on lying behaviour and stress responses of dairy cows during periodic weather-induced removal from pasture. *Appl. Anim. Behav. Sci.* 81:1–11.
- Fisher, A. D., G. A. Verkerk, C. J. Morrow, and L. R. Matthews. 2002. The effects of feed restriction and lying deprivation on pituitary-adrenal axis regulation in lactating cows. *Livest. Prod. Sci.* 73:255–263.
- Fregonesi, J. A., and D. J. Leaver. 2001. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. *Livest. Prod. Sci.* 68:205–216.
- Fregonesi, J. A., and D. J. Leaver. 2002. Influence of space allowance and milk yield level on behaviour, performance and health of dairy cows housed in strawyard and cubicle systems. *Livest. Prod. Sci.* 78:245–257.
- Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007a. Overstocking reduces lying time in dairy cows. *J. Dairy Sci.* 90:3349–3354.
- Fregonesi, J. A., D. M. Veira, M. A. G. von Keyserlingk, and D. M. Weary. 2007b. Effects of bedding quality on lying behavior of dairy cows. *J. Dairy Sci.* 90:5468–5472.
- Friend, T. H., F. C. Gwazdauskas, and C. E. Polan. 1979. Change in adrenal response from free stall competition. *J. Dairy Sci.* 62:768–771.
- 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.
- Hurnik, J. F., and N. J. Lewis. 1991. Use of body surface area to set minimum space allowances for confined pigs and cattle. *Can. J. Anim. Sci.* 71:577–580.
- Huzzey, J. M., D. V. Nydam, R. J. Grant, and T. R. Overton. 2012. The effects of overstocking Holstein dairy cattle during the dry period on cortisol secretion and energy metabolism. *J. Dairy Sci.* 95:4421–4433.
- Ito, K., M. A. G. von Keyserlingk, S. J. LeBlanc, and D. M. Weary. 2010. Lying behavior as an indicator of lameness in dairy cows. *J. Dairy Sci.* 93:3553–3560.
- Kendall, P. E., P. P. Nielsen, J. R. Webster, G. A. Verkerk, R. P. Littlejohn, and L. R. Matthews. 2006. The effects of providing shade to lactating dairy cows in a temperate climate. *Livest. Sci.* 103:148–157.
- Kondo, S., J. Sekine, M. Okubo, and Y. Asahida. 1989. The effect of group size and space allowance on the agonistic and spacing behavior of cattle. *Appl. Anim. Behav. Sci.* 24:127–135.
- Krawczel, P. D., C. T. Hill, H. M. Dann, and R. J. Grant. 2008. Short communication: Effect of stocking density on indices of cow comfort. *J. Dairy Sci.* 91:1903–1907.
- Krawczel, P. D., L. B. Klaiber, R. E. Butzler, L. M. Klaiber, H. M. Dann, C. S. Mooney, and R. J. Grant. 2012. Short-term increases in stocking density affect the lying and social behavior, but not the productivity, of lactating Holstein dairy cows. *J. Dairy Sci.* 95:4298–4308.
- Ledgerwood, D. N., C. Winckler, and C. B. Tucker. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. *J. Dairy Sci.* 93:5129–5139.
- Leonard, F. C., J. O'Connell, and K. O'Farrell. 1994. Effect of different housing conditions on behaviour and foot lesions in Friesian heifers. *Vet. Rec.* 134:490–494.
- Leonard, F. C., J. M. O'Connell, and K. J. O'Farrell. 1996. Effect of overcrowding on claw health in first-calved Friesian heifers. *Br. Vet. J.* 152:459–472.
- Lombard, J. E., C. B. Tucker, M. A. G. von Keyserlingk, C. A. Kopral, and D. M. Weary. 2010. Associations between cow hygiene, hock injuries, and free stall usage on US dairy farms. *J. Dairy Sci.* 93:4668–4676.
- Munksgaard, L., K. L. Ingvarsen, L. J. Pedersen, and V. K. M. Nielsen. 1999. Deprivation of lying down affects behaviour and pituitary-adrenal axis responses in young bulls. *Acta Agric. Scand. A. Anim. Sci.* 49:172–178.

- 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.
- Napolitano, F., U. Knierim, F. Grasso, and G. De Rosa. 2009. Positive indicators of cattle welfare and their applicability to on-farm protocols. *Ital. J. Anim. Sci.* 8(Suppl. 1):355–365.
- Nielsen, L. H., L. Mogensen, C. Krohn, J. Hindhede, and J. T. Sørensen. 1997. Resting and social behaviour of dairy heifers housed in slatted floor pens with different sized bedded lying areas. *Appl. Anim. Behav. Sci.* 54:307–316.
- Potterton, S. L., M. J. Green, J. Harris, K. M. Millar, H. R. Whay, and J. N. Huxley. 2011. Risk factors associated with hair loss, ulceration, and swelling at the hock in freestall-housed UK dairy herds. *J. Dairy Sci.* 94:2952–2963.
- Reich, L. J., D. M. Weary, D. M. Veira, and M. A. G. Von Keyserlingk. 2010. Effects of sawdust bedding dry matter on lying behavior of dairy cows: A dose-dependent response. *J. Dairy Sci.* 93:1561–1565.
- Roche, J. R., P. G. Dillon, C. R. Stockdale, L. H. Baumgard, and M. J. VanBaale. 2004. Relationships among international body condition scoring systems. *J. Dairy Sci.* 87:3076–3079.
- Schütz, K. E., and N. R. Cox. 2014. Effects of short-term repeated exposure to different flooring surfaces on the behavior and physiology of dairy cattle. *J. Dairy Sci.* 97:2753–2762.
- Schütz, K. E., N. R. Cox, K. A. Macdonald, J. R. Roche, G. A. Verkerk, A. R. Rogers, C. B. Tucker, L. R. Matthews, S. Meier, and J. R. Webster. 2013. Behavioral and physiological effects of a short-term feed restriction in lactating dairy cattle with different body condition scores at calving. *J. Dairy Sci.* 96:4465–4476.
- Singh, S. S., W. R. Ward, K. Lautenbach, J. W. Hughes, and R. D. Murray. 1993. Behaviour of first lactation and adult dairy cows while housed and at pasture and its relationship with sole lesions. *Vet. Rec.* 133:469–474.
- Thomsen, P. T., L. Munksgaard, and F. A. Tøgersen. 2008. Evaluation of a lameness scoring system for dairy cows. *J. Dairy Sci.* 91:119–126.
- Tucker, C. B., A. R. Rogers, and K. E. Schütz. 2008. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. *Appl. Anim. Behav. Sci.* 109:141–154.
- Tucker, C. B., A. R. Rogers, G. A. Verkerk, P. E. Kendall, J. R. Webster, and L. R. Matthews. 2007. Effects of shelter and body condition on the behaviour and physiology of dairy cattle in winter. *Appl. Anim. Behav. Sci.* 105:1–13.
- Tucker, C. B., and D. M. Weary. 2004. Bedding on geotextile mattresses: How much is needed to improve cow comfort? *J. Dairy Sci.* 87:2889–2895.
- Tucker, C. B., D. M. Weary, and D. Fraser. 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. *J. Dairy Sci.* 86:521–529.
- Tucker, C. B., D. M. Weary, M. A. G. von Keyserlingk, and K. A. Beauchemin. 2009. Cow comfort in tie-stalls: Increased depth of shavings or straw bedding increases lying time. *J. Dairy Sci.* 92:2684–2690.
- Vanegas, J., M. Overton, S. L. Berry, and W. M. Sischo. 2006. Effect of rubber flooring on claw health in lactating dairy cows housed in free-stall barns. *J. Dairy Sci.* 89:4251–4258.
- Wierenga, H. K. 1983. The influence of the space for walking and lying in a cubicle system on the behaviour of dairy cattle. Pages 171–180 in *Proc. Farm Anim. Hous. Welf. Seminar in the CEC Prog. Coord. Res. Anim. Welf.* Martinus Nijhoff, The Hague, the Netherlands.