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Research Paper

Behavioural responses of pasture based dairy cows to short term management in tie-stalls

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ABSTRACT

Dairy cows in experimental grazing herds are often confined for metabolic measurements. The objective of this study was to establish effects of transfer from pasture, to tie-stalls in a metabolism house, then back to pasture, on lying behaviour and locomotion score of lactating cows: Holstein-Friesian (H, n = 16), Jersey (J, n = 16) and H × J (HJ, n = 16). Cows were transferred to tie-stalls on d 1 for 12 days, and were offered freshly cut ryegrass according to herbage allowance (HERB) and genotype: J low = 14; J high = 17; H and HJ low = 16; and H and HJ high = 20 kg DM/d. Lying behaviour was recorded on four days: −2, −1 (Pre-confinement), 3 (Early confinement), 10, 11 (Late confinement), 13 and 14 (Post-confinement) relative to transfer (d 1) using data-loggers, and was also video-recorded during the first 15 h. Locomotion score was recorded on days −4, −3, 12 and 16. No effects of HERB on lying variables were observed during the first 15 h in confinement, but J cows made more lying intentions (21.0 vs. 12.2; $P < 0.05$) and tended ($P = 0.07$) to have a shorter latency to lie. Cows spent less ($P < 0.001$) time lying in early confinement (07:22:29 h/d) than on any of the other occasions (9:12:50 h/d). Cows had more ($P < 0.001$) and shorter ($P < 0.001$) lying bouts in confinement than while at pasture. Low HERB cows spent more time lying than high HERB cows (09:54:55 vs. 09:09:33 h/d; $P < 0.01$). J had higher locomotion scores than H (9.2 ± 0.2 vs.7.8 ± 0.2; $P < 0.001$), and tended ($P = 0.09$) to have higher scores than HJ (8.5 ± 0.2) cows. Locomotion scores were lowest pre confinement, highest at turnout (d 12), and intermediate after that at pasture (d 16) (7.6 ± 0.2, 9.3 ± 0.2 and 8.6 ± 0.3, respectively; $P < 0.01$). On transfer to the metabolism house cows showed disrupted patterns of lying although daily lying time returned to levels similar to pasture by late confinement. Confinement also resulted in a short-term deterioration in locomotory ability, which although improving, was still evident 4 days following the cows return to pasture with Jersey cows being more affected than the other genotypes. These findings suggest that longer adaptation periods and temporary release to loafing areas may improve both the validity of data collected and cow welfare.

1. Introduction

Dairy cows in experimental grazing herds are often confined for days or weeks in 'metabolism stalls' (Pratt and Holdaway, 1942) for precise and/or invasive measurements (e.g. feed/water intake, faeces/urine/tissue collection, gaseous emissions) where they are generally tied or restrained by the neck (i.e. 'tie-stalls') (Powell et al., 2007; Pinares-Patiño and Waghorn, 2014). There is growing consensus in pharmacological research that behavioural and physiological alterations resulting from experimental conditions imposed upon animals could affect the validity of the experimental measures under investigation (Würbel, 2001; Wolfer et al., 2004; Würbel and Garner, 2007). There is little research in the area with regard to dairy cows.

The transition from pasture to tie-stalls involves several abrupt changes to cows physical and social environment. The most obvious change is in the cows' ability to move around. In a study by Veissier et al. (2008), cows tethered for one day increased the time spent walking by 40% and distance covered by 50% when they were released in a test arena. Space restrictions and stall partitions may restrict lateral movement and inhibit lying behaviour (Tucker et al., 2004), as can a

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change in the surface underfoot (Tucker et al., 2003; Fregonesi et al., 2007; Tucker et al., 2009). Social behaviour is also limited to restricted contact with adjacent cows. Nevertheless, cows can readily adapt to new management systems (Jago and Kerrisk, 2011; O'Driscoll et al., 2011), hence, it is possible that although the changes associated with tie-stalls compared to pasture may pose initial challenges, cows can adapt to the tie-stall environment.

Lying is a high priority behaviour for cows (Munksgaard et al., 2005), is considered a key indicator of cow comfort (Overton et al., 2002; Tucker et al., 2009) and an acute change to either duration of, or patterns of lying behaviour are likely indicative of an environment or circumstance that is posing a challenge to the cow (Fregonesi and Leaver, 2001). Housed dairy animals have an inelastic demand for lying of 12–13 h per day (Jensen et al., 2005). There is no equivalent information for cows at pasture. However, it is possible that the duration required for cows lying at pasture is slightly reduced given the more comfortable underfoot conditions among other reasons as discussed by O'Driscoll et al. (2015). Indeed, cows in intensive grazing systems typically lie for 9–10 h per day (Olmos et al., 2009; O'Driscoll et al., 2010; O'Driscoll et al., 2015). Cows at pasture show a circadian pattern of lying (Arave and Albright, 1981; Driscoll et al., 2010, 2011; Driscoll et al., 2010, 2011) and, in general, long lying bouts are associated with a good level of comfort (Ito et al., 2014).

The lying down movement in dairy cows involves physical displacement of the cow of up to 300% of back length (longitudinal movement), and 180% of hip width (lateral movement) (Ceballos et al., 2004). The behaviour is carried out with a relatively defined sequence of movements, described by Jensen (1999). Cows may be unable to easily adapt their movements in a restricted area, particularly if they are large relative to the size of the cubicle/tie-stall. Moreover, cows impact the ground with considerable velocity (2.20 m/s; Ceballos et al., 2004) during the lying down movement, and thus inappropriately sized, designed or bedded stalls could cause injuries which could be compounded by the negative effects of a lack of exercise on limb health (Gustafson, 1993; Keil et al., 2006). Hence restrictions to movement with regard to lying and walking, combined with the potential for injuries when lying, mean that cows previously accustomed to management at pasture may show impaired locomotory ability after a period of confinement in tie-stalls.

The primary objective of this study was to determine whether dairy cows' lying behaviour and locomotion score are affected by a short period of confinement in tie-stalls following transfer from pasture. We hypothesised that cows would show disrupted lying behaviour on introduction to the tie-stalls until they adapted to lying down in close confinement. We also expected that cows would have a different pattern of lying behaviour in tie-stalls compared to pasture, due to space restrictions and the change in underfoot surface. As a consequence of these changes, we expected to see evidence of impaired locomotory ability once the cows were released from the tie-stalls 12 days later. We used three dairy cow genotypes (Holstein-Friesian (**H**), Jersey (**J**) and Holstein-Friesian × Jersey (**HJ**)), which differ considerably in mature body size. We expected that smaller cows would adapt more readily to the tie-stalls.

2. Material and methods

The experiment was undertaken between July and October 2010 at the 'Moorepark' research farm complex, part of the Teagasc Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (52°09′N; 8°16′W).

2.1. Animals and treatments

Animals were selected from a herd of 135 spring-calving, multiparous lactating pregnant cows which were part of a larger experiment investigating the effect of genotype (H, J and HJ) and stocking rate (high, medium or low) at pasture. The cows were blocked on genotype, parity number, calving date and pre-experimental milk yield and randomly assigned to one of three stocking rates (Thackaberry et al., 2011). Stocking rate was determined according to genotype body size. Thus the high, medium and low stocking rates were 3.0, 2.75 and 2.5 cows/ha respectively for H and HJ cows, and 3.25, 3.00 and 2.75 cows/ha for J cows. In 2010, cows from the high and low stocking rate treatments were used to evaluate in vivo digestibility capabilities of the three genotypes (Beecher et al., 2014) which necessitated housing the animals in tie-stalls in the metabolism house at Moorepark. Animal and management details are described in detail in Beecher et al. (2014). Briefly, four replicate groups of 12 cows from the original herd were blocked according to stocking rate (high and low), genotype (H, J or HJ), pre-experimental body weight (502 kg (SD = 72.8)), parity (3.5 (SD = 1.20)) and days in milk (182 d (SD = 26.4)). Each replicate group included four cows from each genotype, and within each genotype two cows from each stocking rate. Cows were offered a high or low herbage allowance (**HERB**) that reflected the stocking rate treatment that had been applied at pasture according to their genotype: J low = 14; J high = 17; H and HJ low = 16; and H and HJ high = 20 kg DM/d. The experiment was replicated over time, i.e., replicate groups went through the experimental procedure consecutively. The cows had no prior experience of tie-stalls, but they had experience of loose housing with cubicles every winter (60–90 days from approximately November to February). Indoor winter cubicles (2.13 m × 1.19 m) were bedded with rubber mats and allocated at a ratio of 1:1 (O'Driscoll et al., 2009).

2.2. Animal confinement and management

After an average 192 (SD = 30.8) days at pasture, cows were transferred to tie-stalls in the metabolism house after the morning milking (approximately 07:00 h), i.e., on d 1. The metabolism house had 12 tie-stalls (1.35 m long × 1.20 m wide) in which cows were tied or tethered individually by the neck before 12:00 h. The chain tethers were joined through a metal ring to a 1.10 m fixed vertical chain that allowed cows to freely move up and down (Fig. 1). The floor of the tie-stalls was covered with 3 cm deep rubber mats. Stall dividers were made from solid plastic (1.2 m high) (Fig. 1). Cows remained in the tie-stalls until d 12, at which point they were returned to pasture after the morning milking. Each tie-stall was provided with an automatic drinker and water was available ad libitum (Fig. 1). Cows were offered freshly cut grass (*Perennial ryegrass* L.) twice daily at 8:00 h and 14:00 h, at a high or low HERB that reflected the stocking rate treatment that had been applied at pasture. Herbage dry matter content was estimated daily by drying a daily herbage sample at 95 °C for 15 h. Cows were milked twice daily at 08:00 h and 16:00 h in the tie-stalls. Herbage chemical composition, animal production and digestibility results are

Fig. 1. Tie-stalls for metabolic measurements in dairy cows.

presented and discussed by Beecher et al. (2014) and will not be discussed further in this paper.

2.3. Measurements

2.3.1. Body size measurements

Cows were weighed one week before they were transferred to the tie-stalls. The height of the highest point of the cow's spine was estimated using photographs (Canon EOS 600D) taken while cows stood still in front of a measuring tape attached to a back wall of the weighing scale crate.

2.3.2. Lying behaviour during the first 15 h in the tie-stalls

The behaviour of cows in three of the four replicates was recorded continuously on d 1 from 14:00 h after all cows were tied securely in the tie-stalls. Recordings lasted until 05:00 h the following day (i.e. d 2) (15 h) using a time lapse video recorder (Mitsubishi HS-1024) connected to a multiplexer (Panasonic WJ-FS 216). Cameras (Sony CCTV camera WV-BP130/B) were positioned on the wall behind the tie-stalls in a slightly elevated position such that all four limbs of the cow were visible. Each camera covered 2 or 3 tie-stalls. The following parameters were observed: latency for the first intention to lie, number of lying intentions, number of lying interruptions, latency to first lie down, number of lying bouts, number of intentions per lying bout, and the number of interruptions per lying bout. A lying intention was defined as standing and sniffing the floor (moving the head from side to side with sweeping movements and the muzzle close to the ground; Jensen, 1999). A lying intention ended when the cow raised her head. A lying interruption was defined as bending a foreleg without subsequently lying down (adapted from Jensen 1999). The latency to sniff the floor/lie down were also recorded and were defined as the time from 14:00 h on d 1 to when the cow sniffed/lay down successfully for the first time. A single observer scored all video-recordings.

2.3.3. Daily lying behaviour as recorded by dataloggers

Daily lying behaviour was recorded continuously for 7 × 24 h periods which were grouped into four recording occasions: pre-confinement (Pre: d −2 and d −1); early confinement (Early: d 3); late confinement (Late: d 10 and d 11); and post-confinement (Post: d 13 and d 14). Lying time was recorded using dataloggers (Tinytag Plus, Re-Ed volt, Gemini Dataloggers (UK) Ltd., Chichester, UK). These were fitted below the hock of the right hind leg of each cow and secured using a Vetwrap™ bandage as per O'Driscoll et al. (2008). They were set to record whether the cow was standing or lying at 30 s intervals using a Windows based programme (Tinytag® Explorer, Gemini Dataloggers Ltd., Chichester, UK). This sampling interval is adequate to obtain an accurate representation of bovine standing and lying behaviour over a 24 h period (Mitlohner et al., 2001).

Data were filtered and adjusted in MS Excel prior to statistical analysis as described by O'Driscoll et al. (2008). The outcomes for analysis were total daily lying time, and the related variables lying bout duration, and the number of lying bouts in each treatment on each day.

2.3.4. Locomotion scoring

Locomotion scoring was carried out four times by the same trained observer, and grouped into three occasions: pre-confinement (Pre: d −4 and d −3), late confinement (Late: d 12) and post-confinement (Post: d 16). On each occasion cows were released individually after morning milking to a flat concrete surface, then scored as they walked past (lateral view) and then away (posterior view) from the observer. Five aspects of locomotion were scored (spine curvature, tracking, ab/adduction, speed and head bob) between 1 (perfect) and 5 (most impaired), using the system described by O'Driscoll et al. (2010). A single observer carried out locomotion scoring for the duration of the experiment. The five aspects were summed to give one score for each cow on each recording day, a higher score representing less favourable locomotion (total locomotion score).

2.4. Statistical analysis

Data were analysed using SAS V9.3 (SAS Institute, 2003). All data were screened for outliers prior to analysis using box plots (Proc Univariate). The cow was considered to be the experimental unit. Most analyses were carried out using mixed models (Proc Mixed). In all cases residuals were examined to verify normality and homogeneity of variances, and data were transformed if necessary. Degrees of freedom were estimated using Kenwood–Rogers adjustment. Model-fit was determined in all analyses by choosing models with the minimum finite-sample corrected Akaike Information Criteria (AIC). Differences in least squares means were investigated using the *t*-test, followed by Tukey's adjustment for multiple comparisons. Differences were considered significant at $P \leq 0.05$. Tendencies towards significance ($0.05 \leq P \leq 0.10$) are also presented. Data are presented as LSmeans ± s.e. Interactions were removed from the models if they did not tend to be significant ($P > 0.1$).

2.4.1. Lying behaviour during first 15 h in tie-stalls

Behaviour data recorded by video were analysed using general linear models (Proc Mixed). The models included the fixed effects of genotype (n = 3), HERB (n = 2), replicate (n = 4) and interactions.

2.4.2. Daily lying behaviour as recorded by the dataloggers

Daily lying time, lying bout duration and the number of lying bouts in each treatment were investigated using a mixed model approach (Proc Mixed). Lying bout duration was log transformed prior to analysis. The model included the fixed effects of genotype (n = 3), HERB (n = 2), recording occasion (n = 4; Pre, Early, Late or Post confinement), replicate (n = 4) and two way interactions. As measurements were taken on individual cows over several days, day was considered a repeated effect for analysis of total lying time and the number of lying bouts. Lying bout number, nested within day, was considered the repeated effect for lying bout duration. Date of recording was considered a random effect, as was "group of origin", to account for the group within which the cows were kept during each recording period. Specific hypotheses (differences in behaviour at pasture before and after confinement, and comparison between pasture and confinement) were investigated using the contrast statement.

The proportion of time in each hour that cows spent lying was also investigated. Prior to statistical analysis, the 120 stand/lie data points recorded by the dataloggers every hour were used to calculate the proportion of time each cow spent lying per hour on each experimental day. These arithmetic mean proportions were non-normal in distribution and could not be analysed in raw format. Thus a centred moving average was computed including recordings from all cows across all days, to create a standardized baseline. The moving average was calculated using m + 2 data points, where m = the 24 h per day. This value was then subtracted from the proportion of time spent lying during each actual hour on each experimental day, to obtain the residual, or deviation from the mean proportions of the time spent lying over all cows and days, for each hour of the day. Following this, the residuals were analysed using the Mixed procedure.

The model included fixed and random effects as before, with the addition of the fixed effect of hour of the day (1–24). The repeated measure was hour, nested within day and recording occasion. Interactions were also investigated. In order to aid visualization of the mean proportion of time spent lying within each treatment in each hour, graphs were constructed using arithmetic means.

2.4.3. Locomotion scoring

Total locomotion scores were analysed using the Mixed procedure. Scores recorded prior to confinement were averaged in order to obtain a single 'pre-confinement' value for each cow. Fixed effects were genotype, HERB, recording occasion, replicate, and interactions. Recording

Table 1
Effect of genotype on behaviours during the first 15 h after confinement in the tie stalls. H = Holstein Friesian, J = Jersey, HJ = Holstein-Jersey cross.

	Genotype				
	H	HJ	J	S.E	*P-value*
Latency					
Intention to lie (hh:mm:ss)	08:15:30	06:40:10	05:06:20	00:55:19	0.07
Successfully lie (hh:mm:ss)	10:01:40	09:43:49	07:55:48	00:46:52	0.16
No. lying intentions	11.9	12.5	21.0	2.7	< 0.05
No. lying interruptions	0.50	0.92	0.58	0.30	0.59
No. lying bouts	2.9	2.8	3.5	0.6	0.71
Intentions/lying bout	6.3	4.9	5.5	1.6	0.78
Interruptions/lying bout	0.36	0.42	0.13	0.18	0.51

occasion was the repeated measure. The individual aspects of the locomotion score (spine curvature, tracking up and ab/adduction) were also submitted to the Mixed procedure using the same model. The number of cows on each recording occasion that had a score of greater than 1 for speed was analysed using Fishers exact test. For analysis of speed, scores recorded prior to confinement were not averaged.

2.4.4. Body size measurements

The relationship between cow body size (body weight and spine height) and total locomotion scores, latency to lie down and daily lying times were examined using linear regression.

3. Results

3.1. Lying behaviour

3.1.1. Lying behaviour during first 15 h in the tie-stalls

Three J and one HJ cow (two cows from each HERB) did not lie down during the observation period. There was no interaction between HERB and genotype for any measure investigated. There was no effect of HERB, but there tended to be an effect of genotype on the latency for intention to lie ($P = 0.07$; Table 1). There was also an effect of genotype on the number of lying intentions ($P < 0.05$; Table 1), with J cows tending to have more intentions than HF cows ($P = 0.06$) and HJ cows ($P = 0.09$). However there was no effect of either genotype or HERB on the number of interruptions to lying, or the latency to first successfully lie down. Neither was there any effect of genotype or HERB on the total number of lying bouts, the number of intentions per lying bout, or the number of interruptions per lying bout (Table 1).

3.1.2. Daily lying behaviour as recorded by the dataloggers

Recording occasion had an effect on daily lying time ($P < 0.001$). Cows spent less time lying during early confinement than pre-confinement ($P < 0.001$), post confinement ($P < 0.001$), or than late confinement ($P = 0.001$; Fig. 2a). Indeed by late confinement, although daily lying time had increased to a level similar to pre-confinement, it was still lower than the total daily lying time post-confinement ($P < 0.05$). There was no difference in daily lying time at pasture pre or post confinement. However overall, time spent lying at pasture was longer than time spent lying in confinement ($P < 0.001$).

There was also an effect of HERB on lying time; cows on the low HERB treatment spent more time lying than cows on the high HERB treatment (09:54:55 ± 00:13:45 *vs.* 09:09:33 ± 00:13:47 h; $P < 0.01$).

Similar to daily lying time, there was an effect of recording occasion on both lying bout number and duration ($P < 0.001$ for both, Fig. 2b and c). Cows had more lying bouts per day, which were of shorter duration, while they were confined than while at pasture, with an increasing number of bouts of decreasing duration as time in confinement progressed (Fig. 2b and c). When considering only behaviour at pasture, lying bouts were also fewer in number and of longer duration, post-confinement than pre-confinement (Fig. 2b and c).

There was no effect of genotype or HERB on lying bout number or duration. However, there was an interaction between recording occasion and HERB on lying bout number ($P = 0.01$) and there tended to be for lying bout duration ($P = 0.09$). In general, cows on the low HERB treatment had longer, yet more frequent lying bouts while confined than at pasture.

3.1.3. Lying behaviour during the day

There was an effect of hour of the day, and recording occasion on the percentage of time per hour that cows spent lying, as well as an interaction between the two ($P < 0.001$ for all). There was no difference in the average time per hour spent lying pre- and post-confinement (43.3% and 44.4%, respectively). However, cows spent less time lying per hour during early confinement (30.7%) than during the other recording occasions ($P < 0.001$ for all), and less time per hour lying during late confinement (40.0%), than post-confinement ($P = 0.05$).

There was also an interaction between hour and recording occasion ($P < 0.001$; Fig. 3). In general, during the night (23:00–06:00 h) and the afternoon (12:00–16:00 h) cows spent more time per hour lying when they were at pasture compared to when they were in the tie-stalls, whereas during the early morning (08:00–10:00 h) and early evening (17:00–21:00 h) lying times per hour were similar between pasture and confinement.

3.2. Locomotion score

3.2.1. Overall locomotion score

Recording occasion had an effect on overall locomotion score ($P < 0.01$); score was lower (more favourable) pre-confinement (7.6 ± 0.2) than on d 12 (9.3 ± 0.2; $P < 0.01$), and on d 16 (8.6 ± 0.3; $P < 0.05$). There was no difference between scores on d 12 and d 16.

Genotype also had an effect on overall locomotion score ($P < 0.001$) throughout the study; H cows had lower scores (7.8 ± 0.2) than J (9.2 ± 0.2; $P < 0.001$) and tended to be lower than HJ cows (8.5 ± 0.2; $P = 0.09$). HJ cows in turn had lower scores than J cows ($P = 0.05$).

3.2.2. Individual aspects of locomotion score

During the study scores of greater than 1 for 'head-bob' were only recorded on four occasions, thus these data were not analysed statistically and will not be discussed further.

Overall, there was an effect of day on the number of cows that had a score of greater than 1 ($P < 0.001$) for speed. During the two pre-confinement days (d −3 and d −4), only one cow had a score of greater than 1 (Score = 2), and this occurred on d −3. All cows had a score of 1 on d −4. Data from d −3 only were subsequently compared with data from d 12 and d 16. A higher number of cows had a score of greater than 1 for speed on d 12 (Score 2, n = 18, Score 3, n = 1) than on d −3 ($P < 0.001$). Likewise on d 16 a higher number of cows had a score of greater than 1 (Score 2, n = 6) for speed than on d −3 ($P < 0.05$). Fewer cows had a score of greater than 1 on d 16 than on d 12 ($P < 0.01$). There was no effect ($P > 0.05$) of genotype or HERB on the number of cows that had a score of greater than 1.

There was no effect of recording occasion on tracking. However cows tended to have lower tracking scores when they were at pasture (2.1 ± 0.08), compared with immediately after confinement (d 12) (2.4 ± 0.08; $P = 0.1$). Moreover, there was an effect of genotype ($P < 0.001$); Holstein-Friesian cows had the lowest score (1.9 ± 0.1), which was lower than that of J cows (2.5 ± 0.1; $P < 0.001$). Tracking scores of HJ cows were intermediate (2.2 ± 0.1), which was higher than H cows ($P < 0.01$) and tended to be lower than J ($P = 0.08$).

There was also an effect of recording occasion ($P < 0.001$) on ab/adduction scores; similar to tracking, ab/adduction scores were lower

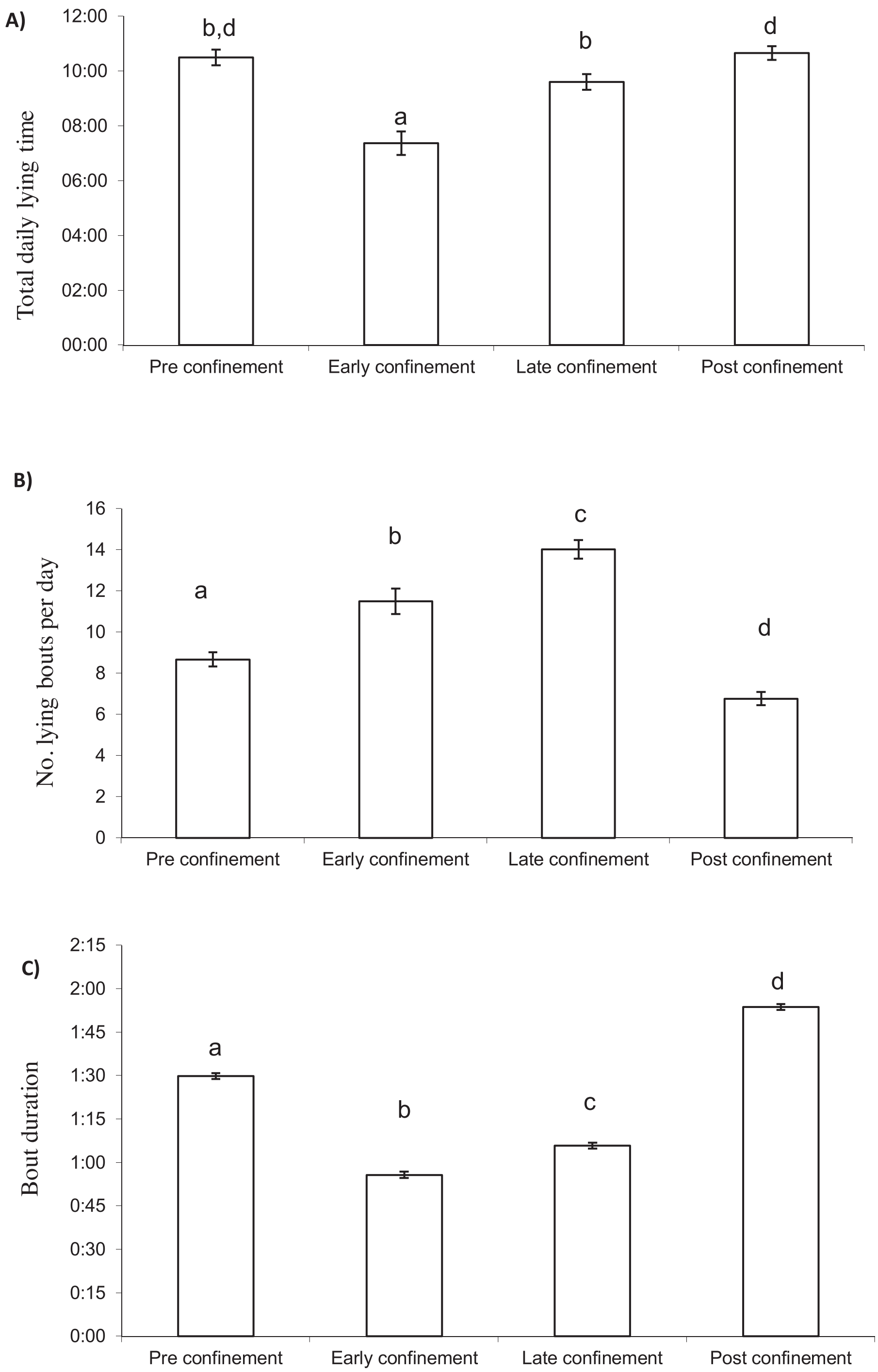

Fig 2. Daily lying time (A), number of lying bouts per day (B), and lying bout duration (C). Pre-confinement = d-2 and d-1; Early confinement = d 3, Late confinement = d 10 and d 11, and Post confinement = d 13 and d 14, relative to moving from pasture to the tie-stall accommodation. Cows were moved back to pasture from tie-stalls on day 12. Columns with different superscripts differ ($P < 0.05$).

pre-confinement (1.8 ± 0.1) than on d 12 (2.3 ± 0.1; $P < 0.001$) or d 16 (2.2 ± 0.1; $P < 0.01$), yet in this instance there was no difference between d 12 and d 16. Likewise, genotype also had an effect on ab/adduction scores ($P < 0.01$) with H cows having the scores lower (1.8 ± 0.1) than J (2.2 ± 0.1; $P = 0.01$) and H × J cows (2.3 ± 0.1; $P < 0.05$). There was no effect of genotype or day on spine curvature scores (2.1 ± 0.17).

3.3. *Effect of body size on lying behaviour and locomotion score*

There was an effect of genotype on cow bodyweight (H = 575.7 ± 9.36 kg; *J* = 433.7 ± 9.36 kg; and H*J* = 500.5 ± 9.36 kg) and spine height (H = 1.43 ± 0.45 m; *J* = 1.29 ± 0.41 m; and H*J* = 1.36 ± 0.20 m) ($P < 0.001$ for both). There were poor ($R^2 < 0.12$) correlations between cow bodyweight and spine height and total locomotion score, latency to lie down and daily lying times, independently of when they were taken (pre-, during or post-confinement in the tie-stalls).

4. Discussion

This study investigated whether confinement of pasture based cows in tie-stalls for a 12 day period would cause changes to lying behaviour and locomotory ability. As hypothesised, there was a significant reduction in daily lying time immediately upon transfer to the tie-stalls, irrespective of the genotype of the cow. While daily lying times

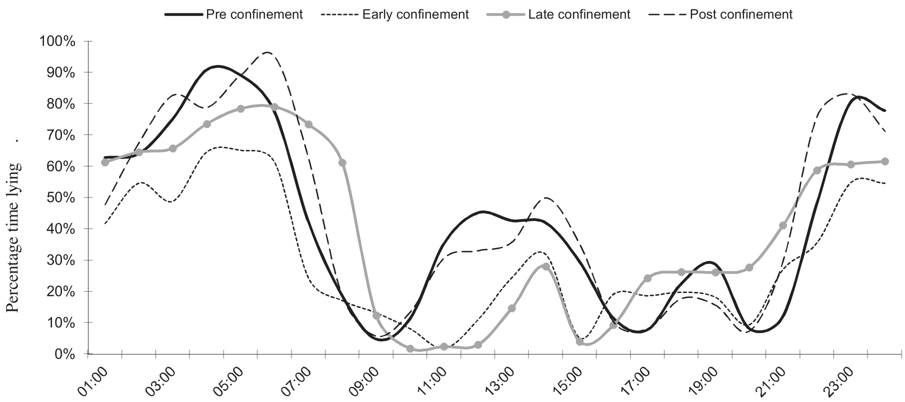

Fig. 3. Arithmetic mean of% time lying per hour prior to confinement in tie-stalls (Pre-confinement; recordings taken on days −2 and −1), 3 days after confinement (Early confinement), one week later (Late confinement; recordings taken on days 10 and 11), and when cows returned to pasture (Post-confinement; recordings taken on days 13 and 14). Days are relative to confinement in tie-stalls on d 1. The x-axis begins at 00:00, and each data point represents the proportion of time cows spent lying during the subsequent hour.

returned to a level similar to that at pasture 11 days after confinement, the related lying time variables such as lying bout duration and number recorded in the tie stalls were consistently different to values recorded at pasture. Confinement in the tie-stalls also resulted in a deterioration in locomotory ability, however improvements were observed 4 days after cows returned to pasture. Although there were differences in the locomotory ability of the genotypes involved in this study, there were no interactions between genotype, recording period, or herbage allowance.

4.1. Lying behaviour

Lying behaviour during the first day in the tie-stalls was recorded using video to identify subtle behaviours associated with lying, which are likely important in understanding cows' ability to adapt to lying down in close confinement (Jensen, 1999) but which are not discernible from automatically recorded data, i.e. dataloggers. Irrespective of genotype or herbage allowance, all cows showed disrupted lying behaviour on introduction to the tie-stalls. The mean lying time of 90 min for the first 15 h in the tie-stalls was considerably below the amount of time the cows had spent lying during the equivalent hours at pasture on the previous day (approximately 4 h less), and indeed four cows did not lie down at all during the observation period. During the first 15 h in the tie-stalls, only three lying bouts on average per cow were recorded. It is difficult to directly compare figures for lying bouts with other studies on cows in tie-stalls as the figures usually quoted are based on 24 h observations. Nevertheless, if we extrapolate the figures recorded over 15 h in the current study to a figure equivalent to 24 h it is clear that the number of lying bouts observed was considerably lower than the 6-12 daily lying bouts recorded for cows adapted to lying in tie-stalls in other studies (Jensen, 1999; Haley et al., 2001; Rushen et al., 2007). This suggests that in accordance with our hypothesis, the abrupt change to the new and both more restrictive and more uncomfortable environment posed a challenge to the cows during the first day in the tie-stalls.

Cooper et al. (2007) found that cows that were deprived of lying for only four hours performed more sniffing of the housing than cows that were not deprived. They also found correlations between sniffing the ground, and self-licking and weight shifting, and concluded that sniffing might be part of a complex of discomfort behaviours associated with lying deprivation (Cooper et al., 2007). Cows sniff the ground as a prelude to lying down, although at a low rate (0.38–0.71 sniffs/h; Cooper et al., 2007). Throughout the first 15 h in the stalls we observed high frequencies of this behaviour (15) during a period when cows only had three bouts of lying. These findings, combined with the low lying times observed on the third day of confinement, suggests that the act of lying down in the tie-stalls was initially problematic for the cows.

Nevertheless, there were some differences in behaviour during the first 15 h across the different breeds. There tended to be an effect of breed on the latency to the first lying intention, with Jersey cows having numerically the shortest and Holsteins the longest latency. Moreover, Jersey cows performed a greater number of lying intentions than the other breeds, and Holsteins the least. Although there was no effect of breed on the latency to lie down successfully, again, numerically Jersey cows had the shortest latency, and Holsteins the longest. These data suggest that the very act of initiating a lying down movement, whether the cow lay down successfully or not (i.e. made an 'intention to lie') was more inhibited in the larger breeds.

In general, cows spent less time lying while in the tie-stalls than while at pasture, although the time spent lying was within the normal range (9–11 h/d; Krohn and Munksgaard, 1993; Rushen et al., 2007; O'Driscoll et al., 2010), and it was only on d 3 that daily lying time was significantly below the normal range. Krohn and Munksgaard (1993) found that cows at pasture had a lower daily lying time than cows in tie-stalls which appears to be in conflict with our findings. However this may be because the cows were acclimatised to tie-stalls, whereas in our study the tie stalls represented a new environment. Cows in confinement systems generally lie down for longer than those at pasture, probably because they have fewer requirements for standing and walking, and minimal competition in the case of tie-stalls. Cows managed at pasture have grazing times ranging from 9 to 11 h per day (O'Driscoll et al., 2009; Prendiville et al., 2010; Enriquez-Hidalgo et al., 2014), which leaves proportionally less time for lying down. Thus, although during most of this study there was no difference in daily lying times between tie-stalls and pasture, if cows had been more accustomed to the stalls they may have had longer lying times than at pasture. In fact, daily lying time increased numerically even between day 10 and day 11, (data not shown) and thus if cows were confined for longer, daily lying time may have continued to increase.

Jensen (1999) found that heifers in tie-stalls for 3 days had a lower number of lying bouts per day than heifers tethered for 10 and 24 days. Similarly, in the current study the number of lying bouts per day increased as the confinement period progressed, from just over 11 on d 3 to approximately 14.5 by d 11. It is possible that as cows need time to learn to position the limbs comfortably, or maybe also need to find a suitable starting position to commence lying down while restrained around the neck (Jensen, 1999) that they minimize the number of changes between standing and lying in the early days of close confinement. As the time in confinement progressed, cows increased their daily lying times primarily by increasing the number of lying bouts per day. This meant that as the time in the tie-stalls progressed cows moved further away from the pattern of lying recorded at pasture (represented by a longer duration and lower number of lying bouts). Nevertheless it suggests that as the cows became more familiar with the tie-stalls, any reluctance to lie down decreased (Jensen, 1999).

In general, cows had shorter but more frequent lying bouts while in tie-stalls than at pasture. These results are in agreement with Olmos et al. (2009) who reported longer undisrupted lying times and fewer interruptions to lying when cows were at pasture compared to cows in a cubicle housing system. These differences reflect the discomfort experienced by cows lying in a more restricted area (Fregonesi and Leaver, 2002) and on a harder underfoot surface than pasture (Krohn and Munksgaard, 1993). Krohn and Munksgaard (1993) reported a longer duration of the lying down movement in cows in tie-stalls compared with cows at pasture.

The potential impact of differences in feeding behaviour and nutrition of cows at grass compared to cows in confinement on lying behaviour should not be discounted. For example, diets fed indoors may have a higher nutritional value (e.g. TMR) and will generally be associated with less foraging behaviour compared to a grazed grass diet (O'Connell et al., 1989; Hernandez-Mendo et al., 2007) due to the lower time required for feed searching and harvesting while indoors. It could be argued that this could mean shorter feeding and therefore potential for longer lying times of cows indoors. Nevertheless, while O'Connell et al. (1989) found shorter eating times of cows indoors in cubicles and on a grass silage diet, they had longer lying times while at pasture prior to confinement. However, Hernandez-Mendo et al. (2007) found that cows on pasture spend less time lying down than cows kept in cubicles. In the current study there was little dietary change for the cows between pasture and the tie-stalls where they were fed harvested grass. Hence aside from a potential reduction in the time taken to consume the grass the effect of nutrition on lying behaviour was likely to have been minimal and was more likely to have reflected discomfort.

Fisher et al. (2003) found that cows increase their total lying times while at pasture (by 3 h) after a transient period (5 h) of lying deprivation in an attempt to compensate for the lack of rest. In the current study, cows also appeared to compensate for the disruption to their lying behaviour when they returned to pasture after 12 days in tie-stalls. There was an immediate 50% reduction in the number of lying bouts compared to in the tie-stalls. Furthermore, the cows had both fewer and longer (14 min) lying bouts compared to when they were at pasture prior to confinement in the tie-stalls. Nevertheless, total daily lying time at pasture after confinement, did not differ from daily lying time pre-confinement, indicating that cows were likely not deprived of lying by the end of their time in the tie-stalls.

4.2. Locomotion score

In the present study, overall locomotion score was lowest (i.e. more favourable) prior to confinement, highest (i.e. less favourable) after release from confinement in the tie-stalls, and tended to decrease again after 4 days at pasture. There were similar effects on speed, tracking and ab/adduction scores. Furthermore, 12.5% and 10.4% of cows had at least one evaluated aspect of locomotion score > 3 (moderately or severely abnormal; O'Callaghan et al., 2003) on days 12 and 16, but none before confinement. Previous studies also reported that housed cows have a greater risk of poor locomotion compared to cows at pasture (Onyiro and Brotherstone, 2008; Olmos et al., 2009). Moreover, the cows were housed in a tie-stall system, which can increase the prevalence of limb and other lesions (Krohn and Munksgaard, 1993) which could interfere with locomotory ability; unfortunately this aspect was not evaluated in the present study.

4.3. Genotype

The hypothesis that smaller cows would adapt more readily to the tie-stalls was only partially confirmed. The lying behaviour characteristics observed during the first 15 h post-confinement suggest that Jersey cows reacted better to the transfer to the tie-stall than their counterparts. However, genotype had little effect on lying behaviour afterwards. This suggests that, after the initial transfer to the tie-stalls, it was the transition to confinement, rather than the size of the cow relative to the stall dimensions that caused the changes in behaviour. However locomotion score was affected by cow genotype, with overall, locomotion, tracking and ab/adduction scores lowest (i.e. most favourable) for H cows and highest (i.e. less favourable) for J cows. Our results disagree with Van Dorp et al. (2004) who reported that heavy and tall cows are genetically predisposed to the development of poor locomotion. Although the H cows in the present study were larger than J they were not as heavy or tall as the cows referred to by Van Dorp et al. (2004). Baird et al. (2009) suggested that there are natural breed differences in locomotory behaviour and that H cows can tolerate poorer claw health without it affecting their locomotion score. The locomotion score used in this experiment was developed for H cows (Gleeson et al., 2007), the possibility that the higher locomotion score observed for J and HJ cows was exclusively related to natural differences in walking between genotypes cannot be discarded.

4.4. Herbage allowance

Cows on the low HERB treatment spent more time lying than cows on the high HERB treatment, mainly during the night. It is likely that this is simply due to there being less herbage available to eat and therefore more time available for lying.

4.5. Implications of the findings for refinement of experimental procedures and cow welfare

Refinement of experimental procedures in animal studies should involve optimisation of the validity of the measures under evaluation, while minimising the time that the animals spend in experimental treatments. Changes to an animals' normal behaviour, whether in duration or pattern, are often the first response to a change in environment or situation (Wong and Candolin, 2014). In the current study there was an immediate, though short-term reduction in daily lying time and a more prolonged disruption in lying pattern during the 12 day period of confinement compared to while the cows were at pasture. These changes can be associated with concurrent or subsequent physiological responses; for instance, when cows were deprived of lying experimentally, they had reduced growth hormone concentration (Munksgaard and Løvendahl, 1993) and increased plasma cortisol concentration (Fisher et al., 2002). Such physiological data were not measured in this study and should be included in further work of this kind particularly in determining an optimum adaptation time. Nevertheless, it would appear that a longer period of adaptation may be required before the collection of metabolic or physiological (particularly if related to functioning of the HPA axis) data commences on cows transferred from pasture to metabolism housing in order to ensure validity of the data collected. Indeed Munksgaard and Simonsen (1996) found that more prolonged periods (23 days) of lying restriction did not result in sustained elevations of cortisol in cows. Nevertheless, changes in the duration of confinement to obtain valid data need to be balanced against implications for cow welfare. We found that cows' locomotory ability deteriorated during 12 days of close confinement and that this persisted for 4 days post-turnout. Temporary release of cows from tie-stalls for exercise/loafing and unrestricted lying on a comfortable surface during the experimental period could ameliorate locomotory disorders arising from close confinement. However, further research is required to better define the optimal intervals and scheduling of such releases.

5. Conclusions

The results confirm the hypothesis that, immediately upon transfer to tie-stalls, cows spend less time lying and show a different lying pattern compared to at pasture. After 11 days in the tie-stalls, however, daily lying time was similar to that recorded at pasture, although with a higher frequency of shorter lying bouts. Thus although cows may have experienced discomfort on initial transfer to the stalls and/or the change in their routine, they were able to adapt their behaviour so that daily lying time was similar to that at pasture. Twelve days of confinement in the tie-stalls resulted in a deterioration in locomotory ability. Although locomotion score 4 days later was more favourable than on the day of exiting the tie-stalls, it had not yet improved to pre-confinement scores. The Jersey cows had a faster adaptation to the transfer to the tie-stalls during the first 15 h of confinement but afterwards the lying behaviour of cows in tie-stalls was not greatly affected by genotype. Locomotion score was more favourable in Holstein-Friesian compared to Jersey cows. The changes in behaviour reported in this study should be considered in the refinement of experimental procedures involving close confinement of dairy cows to improve both the validity of data collected and cow welfare.

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