Impact of paper bedding on lying behaviour and welfare in lactating dairy cows

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Abstract

Lying is a highly motivated behaviour in dairy cows. The level of comfort provided by the lying surface not only affects lying time, but can also affect several other aspects of welfare. We used a crossover design to compare shredded paper and sawdust bedding in relation to lying behaviour, activity, lameness, integument damage, cleanliness and productivity. Cubicles were bedded with a thin layer of bedding, which was replaced as necessary to retain its hygienic state. Twenty-eight lactating Holstein-Friesian dairy cows were divided into two balanced groups that experienced each bedding treatment for a 2 week period in opposing order. Paper bedding resulted in significantly less time spent lying down (paper: 45%, SD ± 6.7, sawdust: 48%, SD ± 7.3, P<0.01). Paper had a more beneficial effect on lameness development than sawdust did (lameness decreased for eight cows and increased for one cow whilst on paper, whereas it decreased for one cow and increased for ten cows whilst on sawdust, P<0.005). However, the magnitude of improvement in mobility score whilst on paper (0.5 points) was small compared to the deterioration seen whilst on sawdust (1-2 points). Bedding adhesion tended to be slightly increased during the paper bedding treatment (paper: 0.14 ± 0.16 SD, sawdust 0.03 ± 0.26 SD, P=0.09). No treatment effect was observed for lying frequency, the speed of transitions towards lying, pre-transition intention movements, or the risk of collisions during the transition (P>0.10). Furthermore, no significant effects on step count, damage to the integument, cleanliness or milk yields were observed

(P>0.10). Slips were too rare overall for analysis. These findings indicate that paper bedding and sawdust were mostly comparable in terms of impact on behaviour, welfare and productivity when provided on a short-term basis. Future trials are recommended to determine if treatment effects persist following prolonged exposure to paper bedding. Assessing the longitudinal development of lameness and the long-term impact of reduced lying time will be essential.

1. Introduction

Dairy cows are highly motivated to lie down and will work to remove barriers preventing them from doing so (Jensen et al., 2005). Therefore longer lying time is often equated with better welfare (Ferraz et al., 2020). However, the relationship between lying time and welfare-related parameters, such as lameness, is complex. For instance, lame cows may increase their lying time to mitigate pain experienced during locomotion (Chapinal et al., 2009; Kester et al., 2014; Sadiq et al., 2017). Conversely, they may compromise resting to avoid pain associated with the transition from standing to lying (Gomez and Cook, 2010; Solano et al., 2016).

Lying is also affected by the softness of the lying surface, which is determined by its base material and the bedding used. Soft surfaces are regarded as more favourable for lying, as evidenced by cows expressing a preference for straw-topped rubber mats over straw-topped concrete surfaces (Norring et al., 2010), and longer lying times being observed when softer surfaces are provided (Tucker et al., 2003; Norring et al., 2010; Johanssen et al., 2018). Such effects are likely due in part to soft materials offering increased cushioning and traction during transitions between standing and lying, enabling even cows with mobility issues to lie down without difficulty (Gomez and Cook, 2010). Apart from softness, lying is also stimulated by high bedding dry matter content (Fregonesi et al., 2007).

Bedding can also affect the development of hock lesions and bald patches. These often arise following prolonged contact with the lying surface, which increases the local pressure on the integument (Kester et al., 2014). As bedding is placed on top of the surface, it affects the amount of direct cow-surface contact and is thus likely to impact integument damage. The encumbered lying and rising motion of lame animals may also increase collisions with cubicle structures and therefore the risk of integument damage (Kester et al., 2014).

Paper may be an interesting bedding material for the dairy industry, as it is a readily available waste product and has a great capacity as an insulator and to absorb moisture. The latter property is of particular interest, as low absorption capacity may result in poor hygiene and thus a greater risk of udder or foot infections, dirtier cows and an increased slippage risk (Wolfe et al., 2018).

Previous research comparing paper bedding to sawdust indicates that cows lie down for significantly longer on the latter material (52 vs 61%, (O'Connell and Meaney, 1997). However, observations were restricted to night-time, when animals are more strongly motivated to lie down, and the study is therefore not necessarily representative for total lying behaviour. Additionally, each group of animals was exposed to one bedding material only, meaning that individual differences in lying behaviour may have affected the results. This is of specific concern for research on lying behaviour, as variation between individuals is known to be greater

than variation between farms (Ito et al., 2009). More recent research comparing these materials found no effects on lying behaviour, but was limited to the first day of exposure and conducted with heifers. The authors noted that effects of bedding substrates may be more pronounced in adult, heavier, animals (McBride and O'Connell, 2010). Therefore the impact of paper bedding on lying behaviour is difficult to predict based on previous studies.

The objective of the present study was to compare shredded paper bedding to the industry standard of sawdust in relation to lying, lameness, damage to the integument and cleanliness. Milk yields were measured as these can be affected by disruption of lying behaviour (Kull et al., 2019). Bedding treatments were applied as is common for sawdust in commercial practice: in a thin layer on top of a mattress and replaced when soiled. Differences in behaviour and welfare were expected to occur between the two bedding types due to differences in a combination of material characteristics like perceived softness as well as particle size and density, affecting the distribution of the material over the mattress surface and likelihood of dispersing onto slats. However, as both materials had a high dry matter content (>73%) this characteristic was not expected to contribute to the effects, as Wolfe et al. (2018) found no difference in lying behaviour on bedding types with relatively high dry matter contents (varying from 64-74%). The direction of treatment effects could not be predicted, as it was unknown how cows would perceive the two bedding types,

and because of the complex inter-relationship between lying and welfare indicators like lameness and integument damage.

2. Material and methods

2.1. Experimental design and animal management

Twenty-eight mid-to-late lactation Holstein-Friesian dairy cows were paired based on parity, mobility score, body weight, body condition score, days in milk and daily milk yield (Table 1). One cow in each pair was randomly assigned to one of two groups for the duration of the study. As this resulted in an unequal average start weight for the two groups, one of the pairs was switched over. This procedure was followed to create two experimental groups that were as similar as possible, although no direct comparisons would be made between the groups. Instead, a cross-over study design was used to ensure each group would experience both bedding treatments, but in the opposite order, so each cow could be used as her own control. Bedding treatments consisted of a two-week period with access to cubicles bedded with sawdust, and another two-week period with access to cubicles bedded with shredded paper. This setup was chosen because lying behaviour in free-stall systems is subject to great individual variation (Ito et al., 2009); Watters et al., 2013; Munksgaard et al. 2020). To minimize any effects of novelty during the experimental period, all cubicles were bedded up with paper 4 days before the start of the trial. Cubicles were then cleared completely and bedded up with the appropriate treatment at the start of the experiment.

Please insert Table 1 here

For the duration of the study, each group was loose-housed in separate adjacent pens of a barn. Each 131 m^2 pen contained 18 face-to-face cubicles (1.69 m x 1.09 m; length (from curb to brisket) x width). All cubicles had a curb height of 0.29 m and were fitted with partitions and a neck rail 1.2 m above the mattress, along with a 7 cm brisket board. Cubicles were fitted with mattresses (Cozy Cow Mattress, Teemore Engineering Ltd., Northern Ireland; 6 cm thick) that had been installed approximately 10 years before. Mattresses were topped with a small quantity of lime and an approximately 1 cm deep layer of either sawdust or shredded paper (shred size approximately 30 x 30mm). Experienced stockpeople followed standard procedures used on the test site to maintain a good hygienic state of the cubicles. When necessary, the hind part of the cubicles (approx. 46 cm) was brushed, followed by application of new lime and bedding material. Necessity of re-bedding was determined on a pen-level (i.e., all cubicles with one type of bedding were brushed and re-bedded at the same time, but independently of brushing and re-bedding the cubicles with the other type of bedding). Over the entire 4 week experimental period, paper bedding was replenished 11 times whereas sawdust was replenished 7 times. The total quantities used were 540 kg (sawdust) and 255 kg (paper). The percentage of dry matter in unused bedding of both types was determined weekly. Each week, three samples of each bedding type were oven dried at 60°C for 48 hours, and the evaporated weight was expressed as a percentage of

the undried sample weight. Data were averaged per bedding type per week. Paper bedding was markedly lighter per unit of volume than sawdust bedding, partly due to its greater dry matter percentage (93 vs 79%).

Water and feed were available ad libitum from self-filling water troughs and an open feed face (12.5 m), located at the side and front of the pens respectively. Fresh silage was provided between 10:00 h and 12:00 h daily. Cows were milked in-parlour twice per day between 05:00 and 07:00 h, and 15:00 and 17:00 h. The two slatted aisles bordering the cubicles were scraped manually by staff twice daily whilst cows were out for milking (2.03 m wide and 1.59 m wide; aisle adjacent to the solid feed bunk alley and aisle on opposite site of pen, respectively).

2.2 Measurements and data collection

2.2.1 Lying time, lying bouts and activity

The frequency and duration of lying bouts and the number of steps taken were recorded continuously throughout the experimental period using tri-axial accelerometers (IceQubes, IceRobotics Ltd., Edinburgh, Scotland, UK) attached to one hind leg. Data was reconfigured automatically into lying time, lying bouts and steps taken by the associated software (CowAlert, IceRobotics Ltd., Edinburgh, Scotland, UK). Daily data per cow was averaged for each 2 week period of the trial to investigate treatment effects.

2.2.2 Lying transitions and slips

Transitions from standing to lying and slips were analysed from video footage obtained every Friday of the experimental period between 10:00 and 18:00 h, using GoPro HERO5 Session cameras (GoPro Inc., US). On one occasion, the video recordings were not viable due to technical faults, and the video cameras were set up to record the following Saturday for the same time period. Cows were identified by their unique freeze-brand and/or corresponding collar ID tag.

In each recording period, the first lying bout per cow was assessed. The duration of intention movements, the time taken to complete the lying transition and the frequency of contact with the environment was recorded (Table 2). The occurrences of abnormal lying behaviours (see Zambelis et al. (2019)) and the number of attempts required to successfully complete the lying down motion were noted. Failed attempts were those where the cow descended onto one or both carpal joints before rising back onto hooves.

For each recording period, the first five minutes of each hour of footage was observed continuously and any slips or falls occurring along the open feed face; the passageways adjacent to and opposite the feed face; and within the cubicles were logged. The location, the severity (i.e. a momentary loss of balance vs descent onto carpal joints and/or hocks), and the bedding treatment were also noted.

All behavioural observations were carried out by a single trained observer. Intra-observer reliability was very high for video

scoring of the duration of intention movements (r_p=0.998, P<0.0001, 0.1 sec difference between sample means) and the lying transition (r_p=0.993, P<0.0001, 0.2 sec difference between sample means).

Please insert Table 2 here

2.2.3 Lameness

Lameness was evaluated at the start and end of each two week period, as cows exited the rotary milker and passed along a passageway. Assessment was performed by one experienced assessor using the mobility scoring system described in Table 3. The development of lameness over the two-week period was calculated by subtracting the start score from the end score.

Please insert Table 3 here

2.2.4 Cleanliness and integument scoring

Cleanliness and integument scoring was performed at the start and end of each two week period. Cows were approached within 2 metres and visually assessed. Although the assessment system was based on the Welfare Quality® assessment protocol for cattle (2009) several adjustments were made based on preliminary observations, to allow a more fine-grained scoring that was deemed more suitable for the current experiment (Table 4). The category of 'bedding adhesion' was specifically developed for this trial, and refers to the amount of bedding that adheres to the cow. The scores of the different body parts were averaged per cow per observation and score progression was calculated as previously described for

lameness (see 2.2.3). Scoring was carried out by two observers, who had assured inter-observer reliability by training together and discussing their outcomes.

Please insert Table 4 here

2.2.5 Milk yield

The daily milk yield (summed AM and PM yield) was recorded automatically in the milking parlour and recalculated in a daily average per cow per 2 week period.

2.3 Statistical analysis

Statistical analysis was carried out using R 3.4.2 (R Core Team, 2017). Results were analysed to assess the effect of bedding type on lying behaviour (% of time spent lying, number of lying bouts, duration of intention movements, duration of the lying down transition); the daily number of steps; the development of lameness scores; the development of cleanliness and integument scores; and milk yield. Paired tests were used in all cases (the pair being the same cow when housed on paper bedding and on sawdust): t-tests for data with normally distributed within-pair differences and Wilcoxon signed rank tests for the other variables. Slipping, abnormal lying behaviours, and additional lying attempts were rare or absent and therefore no statistical analysis was undertaken for these variables.

3. Results

3.1. Lying behaviour, locomotion and slips

Cows spent significantly less time lying down when paper was used than with sawdust (t_{27} = -3.0, P=0.006, Fig. 1). However, bedding type had no significant effect on the daily number of lying bouts (median [IQR] paper: 9.65 [8.00-11.67], sawdust: 10.35 [8.82-11.36], Wilcoxon signed rank test: P = 0.11).

Please insert Figure 1 here

No significant effect was found on the duration of intention movements; the duration of lying down; or the sum of the two when cows where housed on paper bedding compared to sawdust (13.0 ± 8.1 vs 13.0 ± 8.8 seconds, mean ± SD, t_{26} = -0.14, P = 0.88; 6.6 ± 2.4 vs 6.8 ± 2.6, t_{26} = -0.29, P = 0.78; 19.6 ± 9.1 vs 19.8 ± 9.4, t_{26} = -0.22, P = 0.83). No differences in the proportion of animals that made contact with the environment during the lying transition were observed between paper bedding and sawdust (median [IQR] paper: 1.00 [0.50-1.00], sawdust: 1.00 [0.50-1.00], Wilcoxon signed rank test: P = 0.94).

Bedding type did not significantly affect daily step count (1145.8 ± 221.6 vs 1130.5 ± 137.9, mean ± SD, paper bedding vs sawdust, respectively, t_{27} = 0.63, P = 0.53).

3.2. Lameness

Paper bedding had a significantly more favourable effect on mobility score development than sawdust bedding did (Wilcoxon signed rank test: P = 0.002, see Fig. 2). The median change in mobility score during each two-week period was 0 for each bedding

treatment (see Fig.2); indicating that the average cow did not show any change in mobility score on either bedding type. However, whilst bedded on paper the mobility score improved for eight cows and deteriorated for one cow (± 0.5 points), whereas on sawdust the mobility score improved for only one cow and deteriorated for 10 cows (± 1-2 points). Absolute values for individual animals at the different observation moments are displayed in the supplementary material (Supplementary Fig.S1), which also takes the order of the bedding materials into account.

Please insert Figure 2 here

3.3. Integument scores

3.3.1. Cleanliness and bedding adhesion

The change in cleanliness score was not found to be significantly affected by bedding type (median [IQR] paper: 0.17 [-0.17-0.50], sawdust: 0.00 [-0.33-0.21], Wilcoxon signed rank test: P = 0.11). During the paper bedding phase cows tended to show a greater increase in bedding adhesion than during the sawdust phase (0.14 ± 0.16 vs 0.03 ± 0.26 points, mean ± SD, paper bedding vs sawdust, respectively, t_{27}=1.75 P = 0.09).

3.3.2. Bald patches, skin lesions and swellings

Bedding type was not found to significantly affect the development of bald patches (-0.14 ± 0.35 vs -0.12 ± 0.23 points, mean ± SD, paper bedding vs sawdust, respectively, t_{27} = -0.30, P = 0.77); skin lesions (0.10 ± 0.28 vs 0.07 ± 0.20, t_{27} = 0.30, P = 0.76); or

swellings (median [IQR] paper: 0.00 [-0.14-0.00], sawdust: 0.00 [-0.04-0.00], Wilcoxon signed rank test: P = 0.55).

3.4. Milk yield

Daily milk yield was not significantly affected by bedding type (21.0 ± 4.0 vs 21.2 ± 3.8 kg , mean ± SD, paper bedding and sawdust, respectively, t_{27} = -0.20, P = 0.85).

4. Discussion

In this study we evaluated paper bedding against the industry standard of sawdust based on a selection of dairy cow welfare indicators. Compared to sawdust, paper bedding had a more beneficial effect on lameness development. This occurred despite the overall lying time being lower during the paper treatment. There was a weak tendency for a greater increase in bedding adhesion when paper was used. In contrast, no treatment effect was observed for the number of daily lying bouts, daily step count, duration of intention movements, speed of lying transitions, development of integument scores or milk yield.

The overall lying times and frequency of lying bouts observed in this study were in line with previous research (Mattachini et al., 2020; Munksgaard et al., 2020; Shepley et al., 2020). The slightly lower lying time on paper bedding as compared to sawdust is in general agreement with previous work (O'Connell and Meaney, 1997). However, the treatment effect was smaller in the current study than that found in the previous one (45 vs 48%

instead of 52 vs 61%). The experimental protocol of the older study (scan sampling over a period of 5 evenings) may have led to a less accurate and more time-bound estimate of lying behaviour than in the current study (in which behaviour was continuously recorded over two weeks). Our results are in contrast with a study using young heifers which found no effect on lying time (McBride and O'Connell, 2010). Young heifers' lying behaviour may be less affected by bedding than the heavier adult animals used in the present study. Alternatively, the greater bedding depth used for paper than for sawdust by McBride and O'Connell (2010) may have counteracted the effects of the materials themselves.

The relevance of the observed difference in lying time in terms of animal welfare is questionable, given that it was relatively small (45 vs 48%, equating to a 43 min/day difference), not accompanied by changes in the number of lying bouts or the speed with which cows lay down, and no indirect negative effects of reduced lying time were observed (i.e. milk yields were not reduced in the paper treatment and lameness was in fact positively affected). Additionally, visual inspection of daily lying times (Fig. 3) provided no evidence of compensatory lying when cows were moved from the paper to the sawdust. Previous research has shown that some cows display a compensatory increase in lying time when first moved from an unfavourable surface to a more comfortable one (Tucker et al., 2021). In our study no individuals showed heightened lying times specifically on the first days after being transferred from paper to sawdust. Therefore we saw no evidence of a recovery

356 period that would suggest the paper bedding was viewed as highly
357 aversive. When interpreting the differences in lying time in terms of
358 cow welfare it needs to be acknowledged that cows were only
359 exposed to the bedding treatments for a limited period of time.
360 More prolonged exposure may lead to more pronounced effects on
361 welfare, as changes may take more time to develop.

362 It was beyond the scope of the present study to irrevocably
363 determine which specific characteristic of the paper bedding caused
364 the lower lying time. However, certain reasons can likely be ruled
365 out based upon the observed effects. Neophobia seems an unlikely
366 explanation, as there was no consistent increase in lying time for
367 any of the individuals during the first days on paper that would
368 indicate ongoing habituation to the material (Fig. 3). Furthermore,
369 lower lying times persisted into the second week of the paper
370 treatment period. Similarly, it is unlikely that the lower lying time
371 was caused by a more rapid deterioration of bedding cleanliness, as
372 staff determined when paper and sawdust cubicles needed to be
373 cleaned and re-bedded separately, aiming for good hygienic
374 conditions in both treatments. The lack of effect on cow cleanliness
375 scores suggests that they were successful in achieving this. As no
376 effect of bedding on the speed of transitions or the number of lying
377 bouts was found, reluctance to transition towards lying on a certain
378 surface cannot explain the differences in lying time either. This
379 reluctance to transition can be exacerbated by surfaces that provide
380 minimal traction and a low shock absorption capacity, which lead to
381 more force being put on the carpal joints when lying or rising

(Campler et al., 2018). In the current study, the mattresses underneath the bedding material likely had a greater impact on the force exerted on the carpal joints than the thin layer of bedding used for both treatments. The lack of difference observed in the time taken to complete the transition from standing to lying, and in the proportion of transitions that involved collisions with cubicle partitions, suggests that both bedding types also provide comparable levels of traction. It may be that the reduced lying times were the effect of the cows' slight dislike of another material characteristic of the paper bedding. Alternatively, the improvement in mobility score for individual animals whilst on paper bedding (seen in both experimental periods, see Supplementary Fig.S1) may have reduced the motivation to lie down for these cows.

In contrast to previous research reporting no difference in lameness when paper and sawdust bedding was used (O'Connell and Meaney, 1997), paper bedding resulted in a more favourable development of lameness than sawdust did. It should be noted that the maximal degree of improvement in lameness whilst kept on paper bedding was small (0.5 points), and only occurred for eight of the 28 animals (see Supplementary Fig.S1). Nonetheless, this still meant that paper led to better results than sawdust, as on the latter we observed a deterioration of 1-2 points in 10 out of 28 cows. Because the majority of the cows showed no change in lameness on either bedding type the overall effect on lameness needs to be interpreted with caution. Given that lameness tends to develop slowly over time it would be worthwhile to investigate this

parameter in a longer-term study. However, in favour of the reliability of our short-term results, it needs to be remarked that due to our cross-over setup this study was protected from bias due to daily variation in testing conditions (e.g. a more slippery surface or a more pessimistic mood of the observer on a specific testing day), as at each point in time all cows in both treatments were scored in one session. Thus, these daily fluctuations in testing conditions were not confounded with the bedding treatment.

The exact mechanism by which paper bedding led to a more favourable development of lameness than sawdust bedding was unclear. Perhaps differences in the physical characteristics of the materials, or the way in which cows interacted with them affected their propensity to develop lameness. Increased lying time can be ruled out as an explanation because, as previously discussed, lying times were actually lower in the paper bedding treatment. This co-occurrence of decreased lameness and decreased lying time is in line with several previous studies (Chapinal et al., 2009; Olechnowicz and Jaskowski, 2011; Yunta et al., 2012; Watters et al., 2013), although this relationship does not occur consistently in all studies (Blackie and Maclaurin, 2019). It may be that the differences in dry matter content between the bedding materials affected pathogen propagation, but investigating this was beyond the scope of this study. Similarly, bedding treatments may have affected the amount of perching behaviour (i.e. standing with only the front feet in the stall). Perching increases the strain placed on structures within the hoof as a result of uneven weight distribution (Cook et al., 2004). A

difference in standing behaviour between treatments might explain the effects on lameness, but this remains to be evaluated.

Even though our cows spent 3% more of their time lying down when on the sawdust bedding than when on the paper bedding, this did not result in the development of more lesions or bald patches. This may be because the thin layers of bedding used in commercial practice (and replicated in this experiment) do little to protect cows from damage resulting from contact with the cubicle mattress surface (Weary and Taszkun, 2000), likely as a result of the litter being readily dispersed or displaced during stall usage. The age of the mattresses used in the study (10 years old) may also have contributed to the lack of treatment effect. Older mattresses are known to be less abrasive and more yielding than newer ones, and are therefore associated with less integument damage (Kester et al., 2014). Furthermore, abrasions may be more likely to occur during transitions to and from lying as a result of chaffing from mattresses and collisions with cubicle partitions (Kester et al., 2014). In the present study, neither the frequency of lying bouts or collisions were affected by bedding type, which could explain the lack of treatment effect on integument damage.

Alternatively, although small changes to the integument were observed over each treatment period, the duration of exposure (2 weeks) may have been too short to allow a significant accumulation of damage over time. Mowbray et al. (2003) report that skin damage became more noticeable after 3 weeks of

exposure to different bedding treatments. A longer-term experiment that could additionally take the severity of lesions into account could provide a more detailed picture of how lesions appeared, worsened and healed as a function of time and treatment.

In agreement with O'Connell and Meaney (1997), there was no treatment effect on the development of cows' cleanliness scores when bedded on shredded paper or sawdust. This is also in line with the aim of the experimental protocol to keep cubicles in a good hygienic state throughout. It needs to be noted that paper needed to be replenished 1.5 times as often as sawdust to achieve this, roughly in agreement with O'Connell and Meaney (1997). That maintaining sufficient cubicle hygiene with paper bedding is more labour-intensive may be a barrier to its implementation. There was a weak tendency for more paper bedding than sawdust to adhere to cows over the two-week period in the present study. The majority of the adhesion was localised at the belly or udder region and covered up to a palm-sized area in total. Due to the comparatively large particle size, sections of paper bedding were more distinct than sawdust which may lead to a reduced perception of cleanliness.

Milk yields were not affected in this study. These could have been affected if changes in lying time and/or lameness caused by the bedding resulted in problems with feed intake (Olechnowicz and Jaskowski, 2011; Yunta et al., 2012; Bran et al., 2019). The small changes in lying time and lameness status we observed were likely

484 insufficient to alter feed intake enough to affect yields during the
485 short duration of the study. However, such effects may become
486 more pronounced with longer exposure to paper bedding, which
487 would need to be confirmed in future research. Furthermore, it may
488 be necessary to evaluate the effect of paper bedding on mastitis
489 incidence in a large-scale trial. Although clinical mastitis was absent
490 in the current study and very rare in O'Connell and Meaney (1997),
491 neither trial had a sufficient sample size to estimate the true
492 occurrence of mastitis reliably.

493 In conclusion, paper bedding and sawdust were mostly
494 comparable in terms of impact on behaviour, welfare and
495 productivity when provided on a short-term basis. Exposure to
496 paper bedding resulted in a slight reduction in lying time, and a
497 more beneficial effect on lameness development. The exact
498 mechanisms by which these changes occurred remain to be
499 elucidated and the potential cumulative effects of a reduction in
500 lying time need to be evaluated. It should be noted that the
501 experimental periods were relatively short and included a limited
502 number of individuals. Longer and larger scale trials would be
503 helpful to identify long-term changes (e.g. lameness) and effects on
504 conditions with a relatively low occurrence (e.g. mastitis), along with
505 the impact any changes in lying behaviour may have on milk yield.

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Tables

Table 1

Characteristics of cows in each group (means ± SD). Body condition, mobility score and daily yield were determined ahead of the trial. Days in milk refers to the value at the start date of the trial.

	Group 1	Group 2
Body condition score[1]	2.5 ± 1.3	2.4 ± 2.0
Mobility score	2.4 ± 0.4	2.3 ± 0.4
Parity	2.6 ± 1.5	2.6 ± 1.5
Daily milk yield (kg)	31.1 ± 4.3	30.7 ± 5.3
Body weight (kg)	631 ± 62	622 ± 66
Days in milk	238 ± 16	237 ± 13

1 Adapted from Edmondson et al. (1989), scale 1-5 (increments of 0.25)

Table 2

Ethogram of lying behaviours, adapted from Zambelis et al. (2019).

Behaviour	Description
Intention movements	Cow repeatedly and continuously sniffs the lying surface, with possible sweeping movements of the head, without lying down. All four feet must be within the cubicle. If discontinued for more than 5 seconds, the behaviour is not scored as if belonging to the successful lying bout.
Lying down	The transition from standing to lying, from the descent of the first foreleg until the whole body is on the ground and stable.
Contact with environment	Cow comes into contact with dividers and/or neck rail during the lying-down motion.

633

634 **Table 3**

635 Scoring system used to assess lameness, adapted from

636 Manson and Leaver (1988).

Score	Description
1	Smooth walk, level back, no leg swing
1.5	Smooth walk, level back, slight leg swing
2	Leg swing, uneven walk, tracking up
2.5	Leg swing, uneven walk, not tracking up
3	Not tracking up, slight lameness visible
3.5	Obvious lameness not preventing normal behaviour
4	Obvious lameness, difficulty turning, normal behaviour affected
4.5	Severe lameness, behaviour substantially altered
5	Severe lameness, extreme difficulties walking, behaviour substantially altered

637

638 **Table 4**

639 Scoring system used for cleanliness and integument scoring,

640 adapted from the Welfare Quality® assessment protocol for cattle

641 (2009).

642

	Cleanliness	Bedding adhesion	Bald patch[1,2]	Lesion[3]	Swelling[4]
Lower hind legs[5]	**0:** No dirt/minor splashing **1:** 3D layer(s) of dirt < POH **2:** 3D layer(s) of dirt ≥ POH	**0:** Area < ½ POH[6] **1:** ½ - <1 POH **2:** ≥ POH	Count	Count	Count
Hind quarters[2]	**0:** No dirt/minor splashing **1:** 3D dirt < POH **2:** 3D dirt ≥ POH	**0:** Area < ½ POH **1:** ½ - <1 POH **2:** ≥ POH	Count	Count	Count
Rear	**0:** No dirt/minor splashing **1:** 3D dirt < POH **2:** 3D dirt ≥ POH	**0:** Area < ½ POH **1:** ½ - <1 POH **2:** ≥ POH	Count	Count	Count
Udder	**0:** No dirt/minor splashing **1:** 3D dirt < POH **2:** 3D dirt ≥ POH	**0:** Area < ½ POH **1:** ½ - <1 POH **2:** ≥ POH	*Not scored*	Count	Count
Belly	*Not scored*	**0:** Area < ½ POH **1:** ½ - <1 POH **2:** ≥ POH	*Not scored*	*Not scored*	*Not scored*
Carpal joint	*Not scored*	*Not scored*	Count	Count	Count

1 Only included if ≥ 2 cm ⌀

2 Defined as an area where the skin is clearly visible due to hair loss

3 Defined as damaged skin, as a result of either a scab or wound. Lesions may occur within bald patches

4 Defined as an abnormal enlargement of body tissue within a specific region

5 Left and right leg / hind quarters scored separately

6 POH: palm of hand

Figure captions

Fig. 1. Percentage of time spent lying during the two-week period with access to cubicles bedded with paper and sawdust. Medians are depicted by the bold horizontal lines, whereas means are indicated by the inverted triangle symbols.

Fig. 2. The change in mobility scores during the two-week period on each bedding type. Median values for paper bedding and sawdust are depicted by the bold line. Means are indicated by the

660 inverted triangles. The dashed line indicates a mobility score that
661 was the same at the start and end of a two week period.

662 Fig. 3. Percentage of time spent lying for each group on each
663 bedding material. Each symbol indicates an individual animal.

664 **Appendix A. Supplementary material**

665 Fig. S1. Mobility scores for individual animals at the start
666 and end of each two-week treatment period are shown. Cows were
667 assigned to one of two groups, scores for animals in each group are
668 displayed in separate panels. Treatments were applied in a cross-
669 over design, with each group exposed to each bedding type in the
670 opposite order. Each symbol indicates an individual animal. A
671 minimal offset has been added to each line to avoid overlap, in
672 actuality all mobility scores were either whole numbers or decimals
673 varying by 0.5 points.

674