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Dairy cow trade-off preference for two different lying qualities: lying surface vs. lying space. Shewbridge Carter. Providing good quality indoor lying areas for cows is important for cow comfort and welfare, however, it is unclear which lying quality, surface or space, is more important to cows. In a three-stage experiment, cows were given a choice of three lying surfaces 1) with a free-stall, 2) without a free-stall and 3) a trade-off between the most preferred surface with a free-stall and the two less preferred surfaces without. Of the 19 cows tested, 14 chose an open lying space (>60% lying time) over their preferred lying surface, suggesting cows value space over surface type when choosing where to lie down.

Dairy cow trade-off preference for two different lying qualities: lying surface vs. lying space

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ABSTRACT

Lying down is an important behavior for cows, contributing to their health and welfare. With dairy cows being housed for increasingly longer periods, if not all year-round, it is important to ensure dairy cow lying comfort is not compromised when housed. The study aim was to assess cow preference for two different qualities of lying area that appear to be important to cows, the surface type and an open lying space, to better understand how to optimize lying comfort for cows when housed. Twenty-four Holstein dairy cows were used during the study, which took place in Scotland from July to November 2018. The study consisted of 6 experimental periods, a total of 21-d each. Cows were tested four at a time and were individually housed in their own test pen. Each pen had three lying surfaces, sand (**SA**), mattress (**M**) and straw (**ST**) (2.4 m x 2.4 m each) with a free-stall in the middle of each, which could be removed. Cows were given access to one surface at a time (training period) with a free-stall for 2-d, then given a choice of all three surfaces for 2-d. When given the choice with free-stalls in position, cows spent, on average, the largest amount of their lying time on ST (46.6 ± 7.8%) followed by M (44.3 ± 12.4%). Free-stalls were then removed and the following day the training and choice phase was repeated, with cows, on average, lying on ST the most (64.4 ± 7.2%). Finally, a free-stall was re-fitted onto each cow's most preferred surface and the cows were given a choice between lying on their most preferred surface with a free-stall (**P1 + Free-stall**), or on their second and third preferred surface without a free-stall (**P2 + Open** and **P3 + Open**, respectively) for 3-d. During this final trade off stage, of the nineteen cows that data were available for, fourteen cows chose to give up the opportunity to lie down on their most preferred surface in order to have more space on P2 + Open and P3 + Open, three cows chose to lie down on P1 + Free-stall, and two cows made no clear choice. Overall, cows spent the largest amount of their total lying time on their second most preferred surface as an open lying space (65.7 ± 6.9%) compared to their

preferred surface with a free-stall (20.5 ± 5.9%) and their third preferred surface as an open lying space (13.8 ± 3.7%). The results indicate that when lying down, these dairy cows value an open lying space more than the lying surface.

Key Words: preference, behavior, welfare, housing

INTRODUCTION

Rest is important to animals, in order to conserve energy and allow for metabolic recoveries (Fraser, 1983), with cows mostly resting while lying down, as opposed to standing (Kilgour, 2012). Dairy cows deprived of lying down have been shown to prioritise the behavior over other deprived behaviors, such as feeding and socialising, and appear to work towards a set amount of time to lie down per day (Metz, 1985; Munksgaard et al., 2005). Cows deprived of lying are more likely to shift their weight and foot stomp, indicating discomfort (Cooper et al., 2007) and when deprived for 4hrs, are quicker to push a weighted pneumatic gate to gain access to a deep-bedded lying area, indicating a motivation for a comfortable lying area (Tucker et al., 2018).

With the majority of dairy cows experiencing indoor housing at some point throughout their lives, (99% of British cows and >99% of cows in the United States are housed for some period within each year (March et al., 2014; USDA, 2016)) and year-round housing growing in popularity (Haskell et al., 2006; Van den Pol-van Dasselaar et al., 2008), it is more relevant than ever to ensure housed environments are meeting the behavioral and welfare needs of cows. When cows are housed, free-stall housing is most common, with the free-stall design developing from original tie stall designs (Margerison, 2011). Knowing the importance of lying down for cows, much research has been done on cow lying preference for various free-stall modifications, such as stall size, (width and length: Tucker et al., 2004; neck rail placement: Tucker et al., 2005), surface type (Manninen et al., 2002; Tucker et al.,

2003), bedding type (Norring et al., 2010), and alternative stall design (Abade et al., 2015). Cow preference for the free-stall has also been tested against open lying spaces, such as pasture (Legrand et al., 2009; Charlton et al., 2011; Charlton et al., 2013; Motupalli et al., 2014), indoor open pack areas (Fregonesi et al., 2009) and outdoor open pack areas (Smid et al., 2019), however these studies have confounded the factors of surface type, size of total lying area and indoor vs. outdoor conditions. In general, cows prefer to lie on a soft surface, a stall with larger dimensions or an open lying surface, however it is unclear which of these is lying qualities is most important to cows and where the focus should be when improving cow lying comfort when housed.

In the current study, two different lying qualities that appear to be important to cows were selected as the focus: surface type and open space. The aim was to investigate the importance a cow puts on these two different aspects of a lying area. This was done by establishing their preference between three different lying surfaces, deep-bed sand (SA), rubber mattress (M) and deep-bed straw (ST), both with and without a free-stall on them. Once surface preference was established, the cows were given a choice to lie down on their most preferred surface with a free-stall or the lesser preferred surfaces with no free-stall. This presented the cow with a trade-off between surface type and open space when lying down to establish whether lying space or surface was more important to them. Based on these previous studies, we predicted that during the trade-off, cows would trade lying on their preferred surface with a free-stall and lie down for longer periods on either of the two less preferred surfaces without free-stalls, indicating a preference for an open lying area over a preferred lying surface.

MATERIALS AND METHODS

Ethical approval for this study was given by Scotland's Rural College (SRUC) Animal Ethics Committee (ED AE 12-2018) and the work was conducted under the authority of the UK Animal (Scientific Procedures) Act 1986 (Home Office, 1986).

Animals and management

The study was carried out at Crichton Farm, SRUC Dumfries, Scotland, United Kingdom, in an open-sided barn. Twenty-four pregnant Holstein dairy cows (3 primiparous and 21 multiparous) with an average lactation number of 2.75 (± 0.3, ± SEM) in mid to late lactation (271 ± 14.8 DIM; range 142 to 412 DIM), with a milk yield between 12.2 and 29.4 kg/d (mean 20.8 ± 0.79 kg/d) and weighed on average 728 kg (± 11.7 kg; range 643 – 847 kg) were selected for the study. Cows were selected based on milk yield <35 kg, with a body condition score (BCS) between 2.75 and 3.5 (mean 3 ± 0.03), as described by the Penn State method (Ferguson et al., 1994), and a lameness score (LS) no greater than 2, (mean 2 ± 0.1; 2 = imperfect locomotion but ability to move freely not diminished; Flower and Weary, 2006). BCS and LS were assessed by the same person (LSC) while cows walked across a concrete floor after their ~1500hr milking one week before cows came on trial.

The cows were allocated to 1 of 6 experimental periods according to their stage of lactation (n = 4 x 6), which were carried out from July 10th to November 12th, 2018 (study period 1: July 10th to July 30th; study period 2: July 31st to August 20th; study period 3: August 21st to September 10th; study period 4: September 11th to October 1st; study period 5: October 2nd to October 22nd; October 23rd to November 12th). Each experimental period lasted for a total of 21-d: 1-d set up, 6-d training and 2-d choice periods before a day to move and reset equipment, followed by another 6-d training and 2-d choice, finishing with a 3-d trade-off choice period (Figure 1).

Cows were individually penned while participating in the study, to ensure that during the free-choice periods, their choice was not influenced by the presence of other animals that might potentially be competing for the same resource or otherwise influence their choice of where to lie down. All cows were assigned to one pen for the duration of the study and had visual and tactile contact with a test cow in the adjacent pen.

Before the study, the cows in this herd had been housed indoors in a free-stall barn on mattresses, milked 3 times a day (0700, 1500 and 2200 h), with experience of straw pens during the pre-calving period, and had pasture access during the day from July to October or November, depending on weather conditions and harvesting schedules. This ensured that they had experience of lying on mattress-bedded free-stalls and in straw. One week before the start of a new experimental period, cows due to go on trial were housed together in a pen with a deep-bed sand area for lying, to allow them to experience sand as a lying surface. During this week, the cows were also brought down from three to two milkings a day, to better emulate common practice on British farms.

Performance and Lameness. Throughout the study, milk yield was recorded automatically at each milking for individual cows and was used to calculate average yield per cow for the duration of their time on the study.

BCS and LS were recorded when cows were on trial on day 1, 10 and 20 while cows were returning to trial pens after the morning milk, at approximately 0700 h. The average BCS and LS of each cow was calculated as the mean of these 3 scores.

After each milking, cows were automatically weighed while leaving the parlour. Data recorded after both morning and evening milkings were used to calculate the average weight for each cow for their duration on the study.

Experimental Design and Housing

Twice a day, at around 0600 h and 1830 h, the cows were collected and taken to the milking parlour (DeLaval 14:14 herringbone parlour) and milked after the main herd in the morning and before the main herd in the evening, to allow for a near 12hr:12hr split. No concentrates were fed during milking. Following the morning (approximately 0700 h) and evening (approximately 1900 h) milkings, the cows were returned to their pens and manually separated into their own pen. Cows experienced the lying treatments within the pens for approximately 11 h between morning and evening milking and 11.5 h between evening and morning milking. Eight security cameras (Viewlog, GeoVision Inc., Taiwan), two per pen, were set up to continuously record cow behavior within the pens and each cow wore an accelerometer (IceTag; Ice Robotics, Edinburgh, Scotland, UK) on their hind leg to automatically record lying behavior. Cows first entered the pens after a morning milking and, from there on, each experimental "day" began when the cows were put into their pens after the morning milking and ended with the start of morning milking the following day.

Pen Housing Layout. A 365m² area of an open-sided barn, separate from the barn where the main herd was housed, was divided into 4 pens to house each cow individually (6.0m x 15.2m) (Figure 2). Each pen had three different lying surfaces (20cm deep-bed sand (**SA**); rubber mattress bedded with sawdust (**M**) (Pasture Mat; Wilson Agri, Coleraine, Northern Ireland, UK); and 20cm deep-bed wheat straw (**ST**)) contained in wooden boxes, 2.4m x 2.4m x 0.2m in size, with each being able to have a free-stall and rounded plastic brisket board fitted or removed, depending on the experimental stage (Figure 3). Each surface had a 2.0m distance between one another and a 1.8m distance from the edge of the pen, to allow cow access from any side when free-stalls were removed, as well as discouraging a cow using multiple surfaces at the one time (Figure 2.).

A Latin square design was used to allocate the surfaces to the three locations in each pen, such that each surface occurred in each location at least once and that no cow had the same

168 layout of surfaces as her neighbour (Figure 2). This was to take into account order effect of
169 the training stages, whereby cows had access to one lying surface at a time and were
170 encouraged to lie down on each so that they had experience of all the lying surfaces.

171 After the second and fourth experimental periods, the location of surfaces within pens were
172 moved to account for location effect within the barn, as per another Latin square design
173 (Figure 4).

174 Pens provided individual ad libitum access to feed and water. An ad libitum total mixed
175 ration was provided daily at approximately 1000 h, with feed refusals being removed every
176 day before the fresh feed was provided. Water buckets were emptied and refilled every other
177 day. Pens were cleaned out once a day at approximately 0900 h, with lying surfaces being
178 tended to at this time i.e. sand flattened, rubber mattress re-covered with sawdust and fresh
179 straw topped up. Following the evening milking, lying surfaces were cleaned where
180 necessary.

181 ***Experimental Design***. Preference studies used as a measure of welfare run the risk of either
182 measuring a preference for the 'lesser of two evils' or indeed choosing the better of two good
183 options. An animal forced to take the less preferred option does not necessarily experience
184 good animal welfare (Duncan, 1992). By giving a choice of more than two options, the range
185 of choice is widened to help overcome these risks when interpreting the results. A preference
186 study with just two choices requires preference to be defined as >50% 'use' of one of the
187 options, with random choice being 50:50. Throughout the current study, for one lying option
188 to be preferred out of the three possible options, the percentage of total lying time for the
189 most preferred must at least be > 33⅓ %, however, we considered that cows showed a strong
190 preference when the percentage of lying time on any one surface was >60%, with the
191 maximum combined total for the remaining two surfaces being 40% of lying time.

Each experimental period was comprised of three stages (Stage 1: Free-stalls, Stage 2: Open Space and Stage 3: Trade-off), lasting a total of 20 days, not including the initial set up day (Refer to Figure 1.). Below is a description of the three stages for one cow:

Stage 1: Free-stalls – Free-stalls were fitted to each lying surface in an orientation that allowed companion cows to face one another when lying down. The introduction of the free-stall on the lying surfaces was to control lying posture and orientation, as a regular free-stall would.

A training period of 6-d consisted of the cow having access to one surface at a time for two consecutive days, with the other two surfaces blocked off using sheep pen hurdles. Training began with cows having access to the surface in their pen on the North East side of the barn, followed by the middle surface and lastly having access to the surfaces on the South West side of the barn. This allowed the cow to experience each of the three lying surfaces with a free-stall. A training protocol was in place for cows that did not understand that they could lie down in the free-stall. This consisted of training the cow to follow a bucket of concentrated pellets into the free-stall so that all four hooves were on the lying surface. Cows were rewarded here with pellets left in the lunge area, the bucket removed and the following behaviors observed. For cows that did not lie down within the first 10 minutes, or left the lying surface, this was repeated multiple times. All cows did lie down on each lying surface during this training period.

After the 6-day training period, all hurdles were removed and the cow was given free choice between all three lying surfaces with free-stalls on for two days.

Stage 2: Open Space - A day was taken in between Stage 1 and Stage 2 to remove all free-stalls and brisket boards from the pens, with the cows kept on free-stalls in the main herd barn overnight.

When the cow returned to the pen the following day, with all free-stall dividers and brisket boards removed, a training period equivalent to that in Stage 1 began, with the cow having access to one surface at a time, in the same order as previously, for two consecutive days, with the other two surfaces blocked off with sheep pen hurdles.

This allowed the cow to experience each of the lying surfaces without a free-stall and allowing the cow an opportunity to express a range of different lying postures and orientations that might be expressed in an open lying space.

After the 6-day training period, all sheep pen hurdles were removed and the cow was given free choice between all three lying surfaces for two days.

The video footage of the middle 24 hours of this choice period was analysed to determine the cow's most preferred lying surface (largest percentage of lying time, with a minimum threshold of 60%) to determine where to re-fit the free-stall in Stage 3. This was due to the time constraint between the end of Stage 2 and the need to re-fit a free-stall before the beginning of Stage 3. The full 48 hours of video footage was used for in the final analysis of Stage 2. It also showed that for most cows (but see below) the preference exhibited in the middles 24hr represented the choice over the whole period.

After viewing the middle 24 hours of video footage, three cows did not have a preferred lying surface, with no lying surface meeting the minimum threshold of 60% total lying time, and so the full 48 hour period was analysed for these cows. From this, a preferred lying surface was determined for two of the cows (>60%) and one cow did not meet the minimum threshold of 60% for one lying surface. The cow for which a preferred surface could not be determined was excluded from the statistical analysis for Stage 3.

Stage 3: Trade-off - The cow's most preferred lying surface, as determined in the previous stage, had a free-stall and brisket board refitted (P1 + Free-stall), with the two lesser preferred

surfaces left without (P2 + Open and P3 + Open, the second and third preferred surfaces, respectively). The cow then had free choice between these lying options for 3-d, giving her the choice between whether lying surface or space for lying down was more important to her.

Measurements

Behavioral Measures. Time in and out of the pen each day (i.e. at milking) was recorded from the video data to get a total time in pen per day for each cow. For each cow during the training periods, when there was no choice for lying location, the IceTag data was analysed to obtain lying bout start and end time. For choice periods, video data was used to obtain start time, end time and location of each lying bout for each cow. From this, the proportion of each day spent lying on each of the surfaces was calculated along with the frequency and duration of each lying bout for training and choice periods.

Weather Conditions. Weather conditions were recorded daily at 1000 h automatically throughout the study period using a Met Office weather station ~220m from the barn. Outdoor dry temperature (°C), rainfall (mm), wind speed (Beaufort Scale) and wind direction (on a 32 point scale with N = 1/32, E = 8, S = 16 and W = 24) was recorded.

Statistical Analysis

Training Stage 1 Free-stall on and Stage 2 Free-stall off. For the training period, the time spent lying down on each surface was analysed as a percentage of total time in the pen for the second day of training on each surface for Stage 1 and Stage 2, as all cows had been successfully trained after the first 24 hours. A general linear model was used to analyse lying behaviors during training (percentage of time spent lying, lying bout duration, and lying bout frequency). This model was created to test the effect of surface type, stage, order of training, and for a surface type x stage interaction, blocked by cow ID.

263 ***Choice Periods Stage 1, 2 and 3.*** During the choice period of Stage 1 and Stage 2, cow
264 preference for surface type was determined by analysing the percentage of total time lying on
265 each surface during the 2 days of choice for each stage, applying a mixed model using the
266 REML algorithm. The fixed-effects model was created to test the effect of surface type,
267 location of surface, and for a surface type x location interaction. Surface type x repetition,
268 surface type x pen location, and surface type x cow ID interactions were used as the random
269 model. When analysing Stage 2, the location of the surface and surface type x location of
270 surface interaction was dropped from the model when found to be non-significant.

271 The total percentage of time spent lying down was calculated for the training and choice
272 periods of Stage 1 and Stage 2. The data were normally distributed and a two-way analysis of
273 variance was used to test for a difference in time spent lying down between stages 1 vs. 2 and
274 training periods vs. choice periods, blocked by cow ID, and determined whether there was an
275 interaction between time spent lying down during stage 1 and 2 x training and choice periods.
276 Paired t-tests were used to determine whether there was a difference between a) lying bout
277 frequencies and b) lying bout durations between the choice periods of Stage 1 and Stage 2.

278 For Stage 3, lying time in minutes was analysed using a series of Wilcoxon matched pair sign
279 ranks test, paired for each cow, for a) P1 + Free-stall against P2 + Open and b) P1 + Free-stall
280 against P3 + Open, as the data were not normally distributed, even following a
281 transformation. Six cows had no preference between P2 and P3 and either one of the two
282 surfaces could have been preferred in principal. All possible combinations were considered
283 and the Wilcoxon test was applied 64 times ($2^6 = 64$) to each possible combination to test for
284 a) and b), with the mean values of these two series compiled.

285 Five cows in total were not included in the analysis for Stage 3; of these five cows, data from
286 four cows were lost due to the failure of video recording equipment. The fifth cow had not

made a clear choice, >60%, for one surface during Stage 2 (percentage of lying time on each surface during 48hrs of Stage 2 for cow 5 as per Figure 5 – SA: 0%; ST: 54.2%; M: 45.8%). These 5 cows are included in the analysis for Stage 1 and Stage 2, but not Stage 3.

To investigate whether cows that had a very high percentage of total lying time for one surface during Stage 2 (>80%; n=11) showed a similarly high percentage for one lying option in Stage 3, a Spearman's rank was performed on the lying option that had the largest percentage of total lying time for these eleven cows for Stage 2 and Stage 3.

Weather and Performance Factors. Weather factors and surface choice during choice periods for Stage 1, 2 and 3 were averaged per repetition and linear regressions, corrected using Bonferroni corrections, used to assess the effect of weather on choice. This weather data is summarised in Table 1.

Table 1. Summary of weather data, consisting of temperature (°C), rainfall (mm), wind speed (Beaufort Scale), and wind direction (32 point scale), averaged (mean ± SEM) and full range (Range) for the three day choice periods for Stage 1 (Free-stalls), Stage 2 (Open Space) and Stage 3 (Trade-off).

	Stage 1		Stage 2		Stage 3	
	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
Temperature (°C)	12.9 ± 1.78	4.6 - 16.6	13.6 ± 1.64	8 - 18.9	12.9 ± 1.3	9.1 - 16.2
Rainfall (mm)	5.2 ± 1.44	1.1 – 11.3	2.5 ± 1.56	0 – 9.5	6.8 ± 3.46	0.3 – 18.2
Wind Speed (Beaufort Scale)	1.6 ± 1.5	1 – 5.5	3.2 ± 0.6	1.5 – 5.5	3.1 ± 0.3	2.3 – 4
Wind Direction (32 point scale)	24.3 ± 1.9	22.5 - 32	20.8 ± 2	14 - 27	24.3 ± 1.8	18.3 – 27.3

Multiple regressions, corrected using Bonferroni corrections, were used to test for an effect of cow performance factors on surface choice during choice periods for Stage 1, 2 and 3. These factors were BCS, LS, DIM, days in calf, lactation number, weight and yield.

All data were analysed using GenStat (18th Edition, Lawes Agricultural Trust, Rothamsted, UK).

RESULTS

Training Stage 1 Free-stall on and Stage 2 Free-stall off

The average time spent lying (hr), percentage of total time spent lying (%), average lying bout duration (min) and average lying bout frequency for the second day of training for Stage 1 and Stage 2 is presented in Table 2.

There was no interaction between surface type x stage and no effect of order on percentage of time spent lying down, lying bout duration or lying bout frequency during the training periods, and these were therefore dropped from the models.

Overall, during the training periods, cows spent 1.1hrs longer lying down during Stage 2, without free-stalls, (W = 5.343; P = 0.022; *d.f.* = 1; 67.1 ± 0.9%) than during Stage 1, with free-stalls, (63.6 ± 1.2%). Surface type had no effect on percentage of time spent lying (W = 0.067; P = 0.967; *d.f.* = 2). Surface type had an effect on lying bout duration and lying bout frequency, with cows lying for longer but in fewer lying bouts on SA (lying bout duration: W = 12.975; P = 0.002; *d.f.* = 2; ST: 83 ± 3min; M: 91 ± 3.3min; SA: 100 ± 3.9min; lying bout frequency: W = 9.573; P = 0.01; *d.f.* = 2; ST: 10.7 ± 0.4; M: 10 ± 0.33; SA: 9.1 ± 0.32). There was no difference between Stage 1 and Stage 2 on either lying bout duration (W = 1.593; P = 0.209; *d.f.* = 1) or frequency (W = 0.335, P = 0.564; *d.f.* = 1).

Table 2. Summary, averaged for all cows, of lying time (h), percentage of total time spent lying (%), lying bout duration (min) and lying bout frequency (number) for the second day of training of Stage 1(Free-stalls; n=24) and Stage 2 (Open Space; n=24), when cows only had access to one lying surface at a time, for each lying surface and average (Average) for Stage 1 and Stage 2 (±SEM).

	Sand	Straw	Mattress	Average
Stage 1				
Lying Time (hr)	13.9 ± 0.47	13.14± 0.78	14.55 ± 0.42	13.86 ± 0.34
% of total time spent lying	62.5 ± 2.1	60.3 ± 3.6	65.7 ± 1.8	62.8 ± 1.7
Lying bout duration (min)	101.38 ± 6.56	75.42 ± 4.11	89.41 ± 3.61	88.74 ± 3.08
Lying bout frequency (number)	8.9 ± 0.5	10.6 ± 0.7	10 ± 0.3	9.8 ± 0.3
Stage 2				
Lying Time (hr)	14.93 ± 0.44	15.48 ± 0.21	14.46 ± 0.4	14.96 ± 0.21
% of total time spent lying	66.9 ± 1.9	69.2 ± 0.9	64.8 ± 1.7	66.9 ± 1.1
Lying bout duration (min)	98.64 ± 4.33	89.85 ± 3.94	92.65 ± 5.64	93.71 ± 2.7
Lying bout frequency (number)	9.4 ± 0.4	10.8 ± 0.4	10 ± 0.6	10.1 ± 0.3

Choice Periods Stage 1, 2 and 3

The average time spent lying (hr) per day, percentage of total time spent lying (%) and percentage of total lying time (%) for Stage 1, Stage 2 and Stage 3, averaged per cow, is presented in Table 3.

The percentage of total time spent lying on each surface during the choice periods of Stage 1, Stage 2 and Stage 3 are presented in Figure 5 for each individual cow.

During the 48-hr choice period for Stage 1, an interaction was found between surface type and surface location for ST and M (W = 11.93; P = 0.03; $d.d.f.$ = 42). Cows lay down longest on M when in the middle of the pen (North East: 18.6 ± 9.5%; middle: 46.1 ± 6.8%; South West: 25.8 ± 8.9%) but longest on ST when in the South West of the pen (North East: 17.6 ± 7.2; middle: 31 ± 8%; South West: 41 ± 9.1%).

346 Overall, cows lay down for >5 hrs longer on ST and M than on SA (W = 11.45; P = 0.02;
347 *d.d.f.* = 11; SA: 5.8 ± 1.5%; ST: 29.9 ± 4.9%; M: 30.2 ± 5.3%). This is due to some cows
348 having a strong preference for ST (9 cows >60% of lying time) and some for M (9 cows >
349 60% of lying time) as opposed cows splitting their time between ST and M (Figure 5).

350 During the Stage 2 48-hr choice period, no interaction was found between surface type x
351 surface location and the interaction was dropped from the model. A difference was found
352 between the percentage of time spent lying on the different surface types (W = 66.82; P =
353 0.027; *d.d.f.* = 2.1) with cows spending more time lying down on ST than on M or SA (SA: 9
354 ± 3.8%; ST: 45.8 ± 5.1%; M: 16.5 ± 4.7%). Cows were found to spend a greater percentage
355 of their time lying down during Stage 2 than Stage 1 (F = 22.16; P < 0.001; *d.f.* = 1; 71.3 ±
356 0.8% vs. 65.9 ± 1.3%). Percentage of time spent lying in the training periods were found to
357 be lower than during the choice periods for Stage 1 and 2 (F = 13.43; P < 0.001; *d.f.* = 1; 64.9
358 ± 1.1% vs. 68.6 ± 0.8%), a difference of <1hr. There was no interaction found between stage
359 1 and 2 x training and choice periods (F = 0.37; P = 0.543; *d.f.* = 1). There was no difference
360 in lying bout frequency between the choice periods of Stage 1 and Stage 2 (t = -1.1; P =
361 0.284; *d.f.* = 23; 20 ± 0.7 and 20.6 ± 0.6, respectively) and no difference in lying bout
362 duration between the choice periods of Stage 1 and Stage 2 (t = -1.42; P = 0.168; *d.f.* = 23;
363 90mins ± 3.1 and 94mins ± 2.5).

364 For the trade-off choice period of Stage 3, the 64 combinations of Wilcoxon tests were
365 averaged to get a mean p-value. The results showed that cows lay down on average for >6hr
366 longer on P2 + Open than P1 + Free-stall (W = 35.25; P = 0.023; P1: 13.9 ± 3.9%; P2: 44.8 ±
367 4.7%), expressing a strong preference to lie down on P2 + Open (65.7% of total lying time),
368 compared to lying on P1 + Free-stall (20.5%). There was no difference in lying times
369 between P1 + Free-stall and P3 + Open (W = 64.44; P = 0.730; P3: 9.5 ± 2.5%).

Table 3. Summary, averaged for all cows, of average lying time per day (h), average percentage of total time spent lying per day (%) and average percentage of total lying time per day on each lying option and an average daily total for all lying options combined (Daily Average) for the choice periods of Stage 1 (Free-stalls; n=24), Stage 2 (Open Space; n=24) and Stage 3 (Trade-off; n=19) (±SEM).

		Sand	Straw	Mattress	Daily Average
Stage 1	Lying time (hr)	1.29 ± 0.32	6.62 ± 1.09	6.65 ± 1.15	14.55 ± 0.27
	% of total time spent lying	5.8 ± 1.5	29.9 ± 4.9	30.2 ± 5.3	65.9 ± 1.3
	% of total lying time	9.1 ± 2.4	46.6 ± 7.8	44.3 ± 12.4	–
Stage 2	Lying time (hr)	1.9 ± 0.79	10.01 ± 1.14	3.64 ± 1.06	15.55 ± 0.28
	% of total time spent lying	9.0 ± 3.8	45.8 ± 5.1	16.5 ± 4.7	71.3 ± 3.7
	% of total lying time	12.4 ± 5.2	64.4 ± 7.2	23.2 ± 6.7	–
		P1 + Free-stall	P2 + Open	P3 + Open	Average
Stage 3	Lying time (hr)	3.02 ± 0.85	9.78 ± 1.02	2.08 ± 0.55	14.87 ± 0.22
	% of total time spent lying	13.9 ± 3.9	44.8 ± 4.7	9.5 ± 2.5	68.2 ± 1.0
	% of total lying time	20.5 ± 5.9	65.7 ± 6.9	13.8 ± 3.7	–

For cows that expressed a high percentage of lying time on one surface (>80%) during Stage 2 (n = 11), no correlation was found for the largest percentage of total lying time for one lying option between Stage 2 and Stage 3 ($\rho = 0.471$; $p = 0.144$). These cows had an average lying time on one surface of 97.3% during Stage 2, which dropped to an average of 73.3% during Stage 3.

Weather and Performance Factors

There was no effect of any weather factors on cow surface choice for Stage 1, 2 and 3. No cow performance effects on cow surface choice were found Stage 1 and Stage 2.

An interaction between number of lactations and cow choice during Stage 3 was the only cow performance effect at this stage ($P = 0.015$; 3 ± 0.3). Cows with fewer lactations (≤ 2.9) spent

386 a larger percentage of time lying on P2 + Open than higher lactation cows (57.8 ± 3.2% vs.
387 33.2 ± 6.6%, for below-average and above-average lactation number, respectively) but spent
388 a shorter percentage of time on P1 + Free-stall (4.9 ± 2.2% vs. 19.8 ± 6.2%) and P3 + Open
389 (5 ± 3% vs. 13.6 ± 3.6%).

390

391 **DISCUSSION**

392 The objective was to establish what aspect of a lying area was more important to a cow, the
393 surface type or an open space, using a preference trade-of. The majority of cows in this study
394 had a strong preference to lie down in an open space on a surface they had not shown a strong
395 preference for previously, suggesting that they were prepared to give up their preferred
396 surface in order to have an open space to lie in. There was no difference in lying time found
397 between P1 + Free-stall and P3 + Open, suggesting that lying in a free-stall on their preferred
398 surface is as favourable as lying on their least preferred surface. However, the difference in
399 lying time between the two least preferred options in Stage 3 (P1 + Free-stall and P3 + Open)
400 was ~1hr. Differences of this magnitude were significant in the analysis of Stage 1 and Stage
401 2 (n=24), but may not have been detectable in Stage 3 due to a smaller sample size (n=19).
402 There was no preference found during Stage 1 when free-stalls were on the surfaces, with
403 cows on average lying for a similar amount of time on both ST and M. However, Figure 6
404 illustrates that it is due to some cows choosing to spend the majority of their lying time on ST
405 and some on M, as opposed to most cows splitting their time between both surfaces. During
406 Stage 2, the majority of cows had a strong preference for ST.

407 As predicted for this current study, these cows did choose to trade their preferred surface type
408 for an open lying space. However, it has been suggested in other studies that cows are less
409 focused on the spatial constraints of a free-stall when deciding where to lie down and more so

410 on the free-stall surface. Studies investigating the effect of aspects of the free-stall structure,
411 such as stall length, width and neck rail placement, on lying time in cows (Tucker et al.,
412 2004; Tucker et al., 2005) tend to yield less definitive results compared to studies
413 investigating the effect of stall surface on cow lying preference (Tucker et al., 2003,
414 Manninen et al., 2002, Norring et al., 2010). This was further demonstrated in a study
415 whereby cows chose to lie in free-stalls over alternative free-stalls, with neck rails removed
416 and stall dividers replaced with a wooden board protruding from the deep-sand bedding,
417 eluding to an open space (Abade et al., 2015). However, the changes made to the total lying
418 space in these studies assessing use of 'adjusted' free-stalls are relatively small compared to
419 offering cows a true open lying area, as was done in the present study. Fregonesi et al. (2009)
420 found that cows had a preference for an open lying area, of the same total lying area and
421 same lying surface, over free-stalls, showing the value of a true open lying space to cows,
422 which is supported by the results in the current study.

423 Overall, in the current study, when cows had a choice, they generally preferred lying on
424 surfaces other than sand, which could be due to the overall lack of previous experience on
425 sand bedding compared to M and ST. When Manninen et al. (2002) gave four groups of
426 cows, with no previous experience of sand, a free choice of sand, straw and soft rubber mats
427 in free-stalls, the cows avoided the sand free-stalls, sometimes even choosing to lie in the
428 passageways to avoid them. In the Manninen et al. (2002) study, after the first two groups of
429 cows had refused to use the sand free-stalls at all, the second two groups were given a 3 day
430 forced period on each of the different beds before the choice period. These cows were found
431 to use the sand free-stalls more often, demonstrating the importance of resource exposure
432 before preference tests. Similarly, Tucker et al. (2003) found that cows previously housed on
433 deep-bedded sawdust free-stalls had a preference for sawdust over free-stalls with deep-
434 bedded sand or with rubber mattresses bedded with sawdust, but after a 2 day forced period

435 on each surface, two out of the twelve cows switched their preference to sand. In Tucker et
436 al., (2003) the rubber mattress free-stalls were the least preferred and were the surface the
437 cows had the least experience with prior to the study. It is worth noting that in the current
438 study, when cows had no choice in lying surface (in the training period), there was no
439 difference in total lying times between sand and the other surfaces, suggesting that although
440 these cows did not have a preference for sand, it was not sufficiently aversive when they had
441 no other lying option to cause animal welfare challenges associated with reduced lying times.

442 Cows lay down for ~1hr longer during Stage 2, when free-stalls were removed, compared to
443 Stage 1, when free-stalls were present, both when cows had no choice of surface (training
444 periods) and a choice of surface (choice periods). Using time spent lying down as an indicator
445 of comfort (Haley et al., 2000), this would suggest that cows find lying down in a free-stall
446 less comfortable than lying down in a more open space, even when given a choice of lying
447 surface types. Although these results are significant, we must consider whether a difference
448 of ~1hr is biologically significant. Studies have shown that cows have a daily lying time
449 ranging between 11.1hr – 12.5hr, depending on stage in lactation (Maselyne et al., 2017), and
450 heifers have an inelastic demand to lie down for between 12-13hrs (Jensen et al., 2005).
451 Lying times reported for all stages of this study are greater than 13hrs, suggesting that lying
452 comfort is not compromised in a free-stall during this study.

453 In the current study, during the training periods, when there was no choice of lying surface,
454 surface type had an effect on lying behavior, with cows on SA having the longest but least
455 frequent lying bouts. However, the special constraints of a free-stall did not have an effect on
456 lying behavior, with no difference of lying bout duration or frequency found between Stage 1
457 (free-stalls on) and Stage 2 (no free-stall). Longer but fewer lying bouts are generally
458 reported on harder lying surfaces, with cows more reluctant to stand up and lie down on
459 harder surfaces due to the discomfort experienced during the process (Herlin, 1997; Haley et

al., 2000; Haley et al., 2001; Tucker et al., 2003). This would indicate that the cows in this study found sand an uncomfortable surface on which to lie down on and from which to get back up from, which is supported by cow avoidance of SA during the choice periods. Additionally, lying space did not have an effect on this lying behavior, suggesting that the free-stall does not impede on the process of lying down and getting up for these cows. Contradictory to these results, studies have reported that compared to an open lying area (such as pasture or an open indoor lying area), when in a free-stall cows do exhibit longer but fewer lying bouts (Haley et al., 2000; Hernandez-Mendo et al., 2007) and that the free-stall is impeding the lying down and getting up motion. However, unlike the current study, those studies had a confounding factor of surface type, further suggesting that surface type can effect lying behavior, with lying bout duration and frequency an indicator of lying comfort, but only in terms of surface and not necessarily space.

An interaction was found between cow choice for lying surface and surface location within the pen during the Stage 1 choice period. As this interaction was only seen for Stage 1, it could be linked to the training protocol, whereby cows were always trained on the NE surface when first introduced into the pen. Taking into account their general avoidance of SA, and possible avoidance of the first surface they were trained on when they entered the pens, when these options are removed (see Figure 4) the locations of M and ST are for the majority in the middle and SW, respectively. Had the cows been given a couple of days to adjust to the pens before data collection began, this interaction may have been minimised.

Improved welfare aided simply by having control over one's environment, described as ‘agency’ (Wemelsfelder, 1997; Špinka, 2019), has been proposed as a reference point for welfare enhancement (Mellor, 2015; Mellor and Beausoleil, 2015; Mellor, 2016). It has been suggested that giving cows the ability to have choice within their environment, with even a perceived sense of control, may improve welfare (Motupalli et al., 2014; Webster, 2016;

485 Charlton and Rutter, 2017). In the current study, cows lay down longer when given a choice
486 of surfaces compared to during the training stages, when they only had access to one lying
487 surface at a time. Similarly, Legrand et al. (2009) found that when given the choice of indoor
488 free-stalls and pasture, cows spent longer lying down compared to when they were confined
489 to pasture alone. However, the difference in both studies was relatively small and highlights
490 that the full extent of the effects of offering animals choice over their environment is
491 unknown and requires further study.

492 ## CONCLUSIONS

493 On average, when a free-stall was refitted onto the cow's preferred surface and these cows
494 were presented with a trade-off between lying on their preferred surface or an open lying
495 space of less preferred surface, the majority of these cows chose the open lying space. These
496 cows made no choice between ST and M when a free-stall were present, however this was
497 most likely due to some cows having a strong preference for ST and some for M, as opposed
498 to most cows splitting their time evenly between the two surfaces. When the free-stall was
499 removed, the majority of cows had a strong preference for ST. These results suggest that
500 when choosing where to lie down, these cows valued an open lying space, without a free-
501 stall, over their preferred surface type. More work is needed to investigate cow motivation for
502 open lying space and the relationship between this and surface type.

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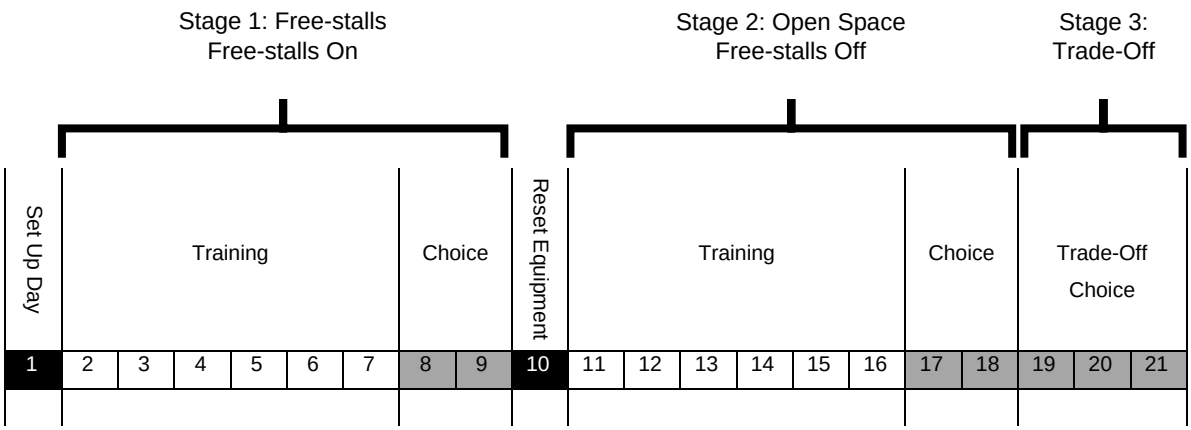

Figure 1. Representation of the 21-d experimental period, which was repeated 6 times using 4 cows. Days shaded in black represent equipment set up days when cows were not present in the pens. Days shaded in grey represent days when cows had a choice of all lying surfaces.

JDS.2020-18781: Figure 1.

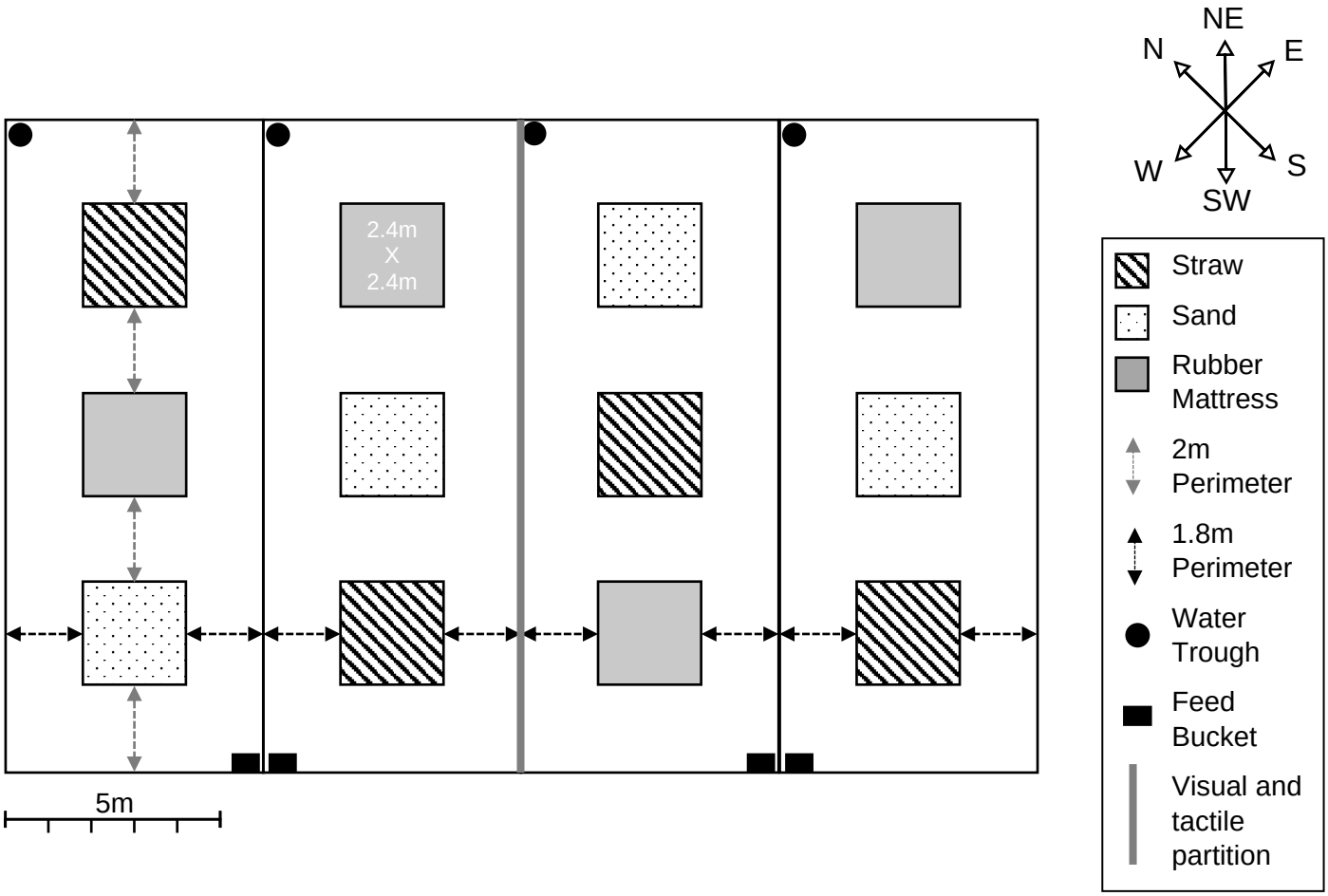

Figure 2. Plan of barn, divided into 4 pens with three lying surfaces in each, used for the experiment.

JDS.2020-18781: Figure 2.

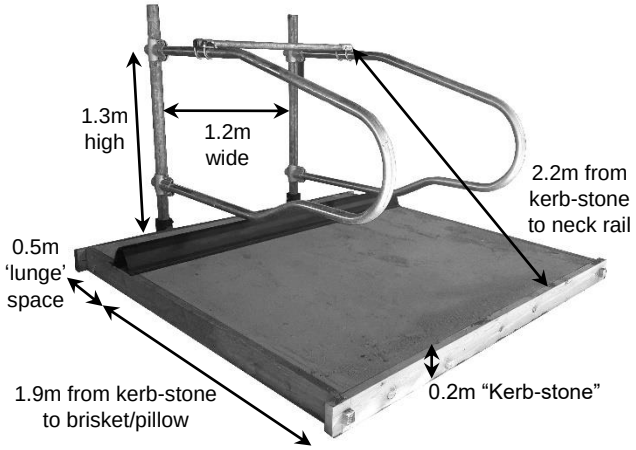

Figure 3. Photograph of free-stall design over deep-bedded sand surface with free-stall dimensions.

JDS.2020-18781: Figure 3.

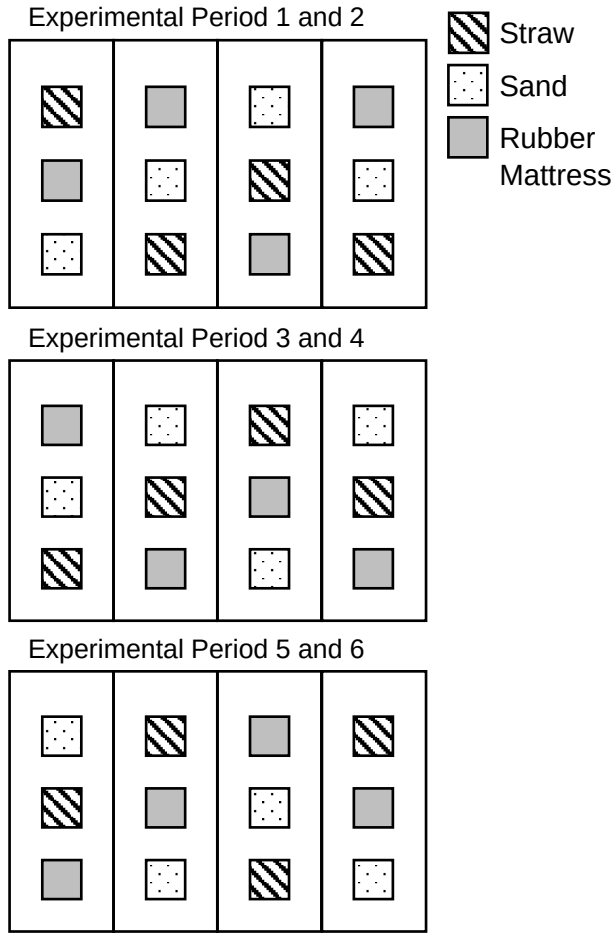

Figure 4. Plan of barn surface layout demonstrating the latin square design for changing surface location after every two experimental periods.

JDS.2020-18781: Figure 4.

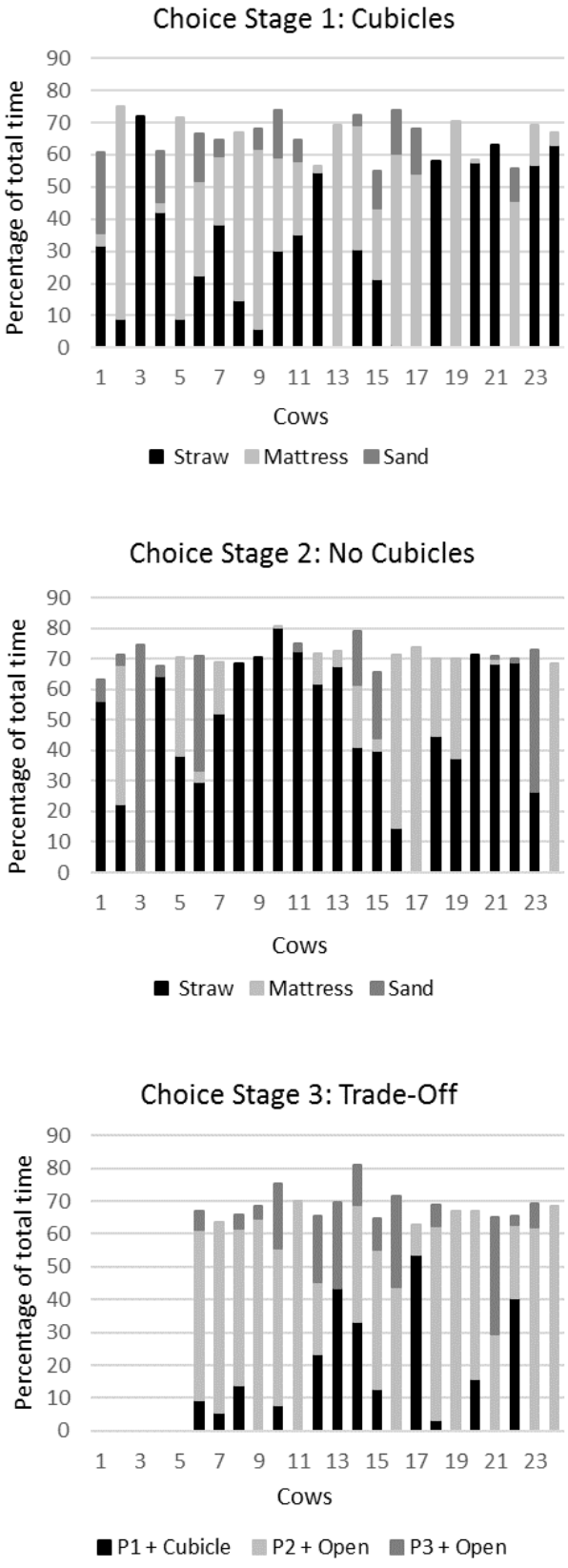

Figure 5. Percentage of total time spent lying on each surface during the choice periods of Stage 1 (Free-stalls; n=24), Stage 2 (Open Space; n=24) and Stage 3 (Trade-off; n=19) for each individual cow.

JDS.2020-18781: Figure 5.

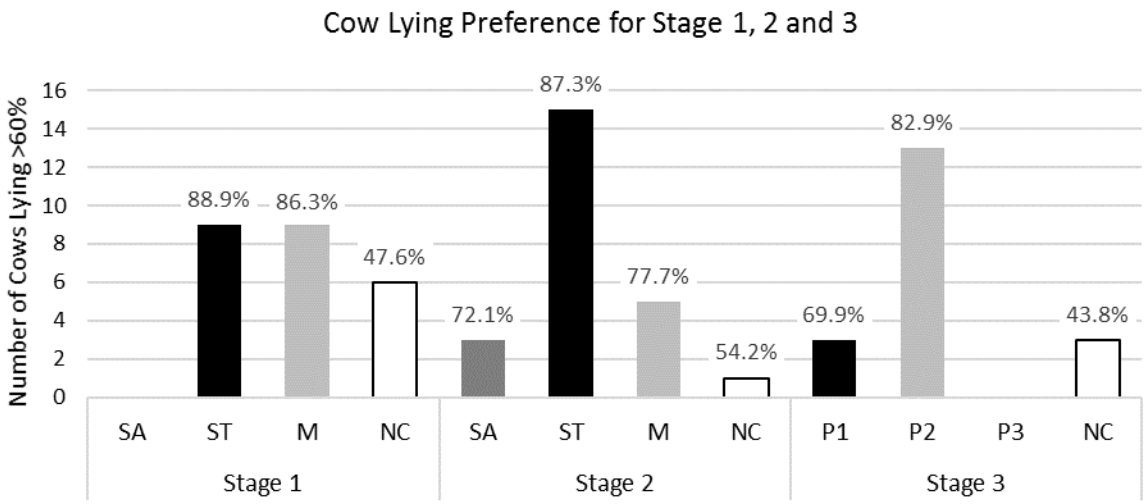

Figure 6. Number of cows that had a strong preference (>60% total lying time) on each lying option (SA = sand; ST = straw; M = mattress; P1 = P1 + Free-stall; P2 = P2 + Open; P3 = P3 + Open) with the average percentage of total lying time for that lying option and number of cows with no clear choice for just one surface (NC) with the average percentage of total lying time for the surface they lay the most on during Stage 1 (Free-stalls; n=24), Stage 2 (Open Space; n=24) and Stage 3 (Trade-off; n=19).

JDS.2020-18781: Figure 6.