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Effect of straw bedding on cattle behaviour at rest stops during commercial long-distance transportation

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Abstract

Transport is a common and stressful practice for cattle, with longer trips potentially increasing negative effects. No studies have examined the impact of bedding on beef cattle behaviour at rest stations. This study aimed to 1) assess the effects of straw bedding, and 2) examine associations between load, trip, and animal-related characteristics with cattle behaviour at rest stations. Fourteen truckloads were split upon arrival; cattle were assigned to bedded ($n=452$) or non-bedded ($n=470$) pens. Numbers of animals lying, eating, and drinking were recorded every 10 min for 8 h. Two mixed logistic regression models (lying and eating) with random intercepts for load and truck compartment, and one ordinary logistic regression model (drinking), examined associations with bedding, load, trip, and rest station variables. Bedding affected lying and eating, but effects varied with motion duration and time in pen. Eating was influenced by space allowance, with different effects across categories. Weight influenced lying ($p=0.001$) and eating ($p<0.001$), independent of bedding. Drinking was associated with sex ($p=0.009$), motion duration (varied effect by category), and time in pen ($p<0.001$), but not bedding. Bedding increased the odds of lying sooner, and eating later, suggesting it may help reduce fatigue in cattle transported long distances.

Keywords: Cattle behaviour, Bedding, Rest stop, Transport, Multi-level model

Introduction

Transport has been shown to be stressful for cattle (Kent and Ewbank, 1983; Tarrant, 1990), and the detrimental effects increase with longer trips (Knowles, 1999;

Gonzalez et al., 2012); however, it is a common practice globally. Cattle may be transported several times in their lifetime (Schwartzkopf-Genswein and Grandin, 2019) and some of the transport events are over long distances (≥ 400 km). Canadian regulations allow for some of the longest transport durations globally (Government of Canada, 2019); in 2019, amendments to the Canadian Humane Transport Regulations under the Health of Animals Act, decreased the maximum transit time from 48 to 36 h before requiring the unloading of animals at rest stations (facilities equipped with pens that offer feed, water, and protection from the environment), while increasing the minimum resting time from 5 h to 8 h (Government of Canada, 2019). Resting pens in Canada are usually dirt-floored, with minimal to no bedding, and have one round feeder filled with hay and a water trough or waterer.

Providing bedding is a recommended best practice in the Beef Cattle Code of Practice (Beef Cattle Code Development Committee, 2013) but it is currently not considered a requirement, and commercial rest stations often do not provide it due to the added cost to the producer (additional labour required to clean/remove soiled bedding as well as having an appropriate disposal plan) and bedding has been identified as a risk factor for disease transmission (Ray et al., 2022). Several studies have reported that lying is a priority behaviour for cattle in general (Marti et al., 2017; Meléndez et al., 2020; Munksgaard et al., 2005; Munksgaard & Simonsen, 1996).

Previous research has found that dairy cattle prefer softer surfaces to lie down (Manninen et al., 2002; Tucker and Weary, 2004; Wolfe et al., 2018), and when soft surfaces are provided, they lie down for longer (Haley et al., 2001; Manninen et al., 2002) and have more lying bouts (Haley et al., 2001; Tucker and Weary, 2004).

However, research on the effects of bedding on cattle behaviour during rest stops is limited. Further, studies investigating the effects of bedding on lying behaviour of

beef cattle are not only limited, but their results are contradictory (Keane et al., 2018). Comparing cattle housed on straw bedding to those on concrete slats, Gygax et al. (2007) and Rouha-Muelleder et al. (2012) observed that finishing bulls on European beef farms did not differ in their daily lying duration, whereas Keane et al. (2017) reported a higher proportion of heifers lying at any one time, and Hickey et al. (2003) found that finishing beef steers lay down for longer when housed in bedded pens compared to concrete slats.

Evaluating the effect of bedding on cattle behaviour could provide insight into what conditions are most conducive for cattle rest while at the rest station. Thus, our research objectives were: 1) to determine the effects of straw bedding on the behaviour of cattle unloaded at rest stations, particularly lying behaviour; and 2) to determine the associations between load, trip, rest station, and animal-related characteristics and the behaviour of cattle unloaded at rest stations. We hypothesized that straw bedding would influence the cattle's motivation to lie down, and subsequently the performance of other behaviours such as eating and drinking.

Methods

General

All methods used in this study were approved by the Animal Care Committee (4202) and Research Ethics Board (19JN021) of the University of Guelph. This study took place at two commercially-operated livestock rest stations near Thunder Bay, ON, between May 23 to August 29, 2019, and October 30 to December 15, 2019.

Drivers asked to participate in the study were informed of the experimental conditions, which involved splitting the load into treated (bedded) and untreated (dirt) pens for the duration of the rest period, and completing a survey about their

professional experience, the characteristics of the animals they were hauling, their trailer, and the trip (e.g., transport duration) (Supplementary material). The full survey responses were used for another study (Olivares Guzmán, 2023) thus, only select information from the survey, relevant to this study, will be reported here.

The lead investigator (POG) had no prior knowledge about the load, trip, rest station, or animal characteristics of the load when she approached drivers to participate in this study. In cases where more than one truck was arriving on the same day, one truck was selected at random. If the driver declined to be part of the study, the driver of the next truck arriving at the rest station was approached.

Survey

Load characteristics collected from the driver survey were number of animals being transported by compartment, load sex (heifers, steers, or both) and estimated animal weight ($[\text{total load weight} - \text{truck tare weight}] / \text{number of animals}$, kg). Number of animals and sex were confirmed via visual inspection by POG. The survey also gathered the following information regarding trip characteristics: source of the cattle (assembly yard, auction market, farm, or feedlot), transport duration (h), duration the truck was stationary (h), duration the truck was in motion ($h = \text{transport duration} - \text{stationary duration}$), loading density (measured as an allometric coefficient ($k\text{-value} = (\text{m}^2/\text{animal}) / (\text{BW}^{2/3})$) as suggested by Petherick & Phillips, 2009), and number of bales of straw used to bed the trailer at the point of origin. Additionally, rest station characteristics were recorded upon arrival of each load. The variables recorded were study site (1 or 2), temperature at unloading ($^{\circ}\text{C}$), space allowance in the resting pen (measured as an allometric coefficient $k\text{-value} = (\text{m}^2/\text{animal}) / (\text{BW}^{2/3})$), remaining daylight hours upon arrival at the rest station, and time in resting pen since unloading

(h). We decided to include remaining daylight hours because loads arrived at different times of day (i.e., morning, afternoon, evening, and night) and to account for differences in daylight availability due to seasonal effects (i.e., longer daylight in the summer and shorter in the winter).

Study animals

Each load participating in the study (n=14) was divided into two groups of roughly the same number of animals, based on the trailer compartment where the animals were transported. Cattle in the belly and back compartments ('bottom' of the trailer) were considered one group, and those from the nose, deck, and doghouse ('top' of the trailer) comprised the other. The first two groups (first load divided into 'top' and 'bottom' groups) participating in the study were randomly assigned to a bedded or non-bedded pen, by flipping a coin, while the subsequent groups were alternated between treatment pens to balance the number of animals rested in bedded and non-bedded pens.

Under commercial conditions, steers and heifers were not rested in the same pen, thus, loads participating in the study transporting steers and heifers (n=2) rested their loads according to the sex of the animals rather than their location in the trailer.

Housing and feeding

Animals in the study were handled (loaded and unloaded) by the truck driver and the rest station personnel, as per usual rest station protocol.

At rest station 1, three pens measuring 15.5 × 9.5 m (length × width) each with a concrete water trough (2.6 × 1.1 × 0.8 m; length × width × height) were used (Figure 1 A). At rest station 2, two pens measuring 14.5 × 10.5 m (length × width) with an

automatic water bowl (1.0 × 0.6 × 0.7 m; length × width × height; Ritchie Industries Inc, Conrad, Iowa) were used (Figure 1 B). Each pen contained a circular metal hay feeder of 7.5 m circumference, with 18 slanted openings, each 0.4 m wide. Animals had ad libitum access to hay and water during their stay at the rest station. As per regular commercial operating conditions, each load was fed one round hay bale (60% timothy, 20% brome, and 20% alfalfa), weighing between 450 - 540 kg. Drivers decided the time the cattle would stay at the rest station, following the federal regulations at the time of the study (minimum of 5 h).

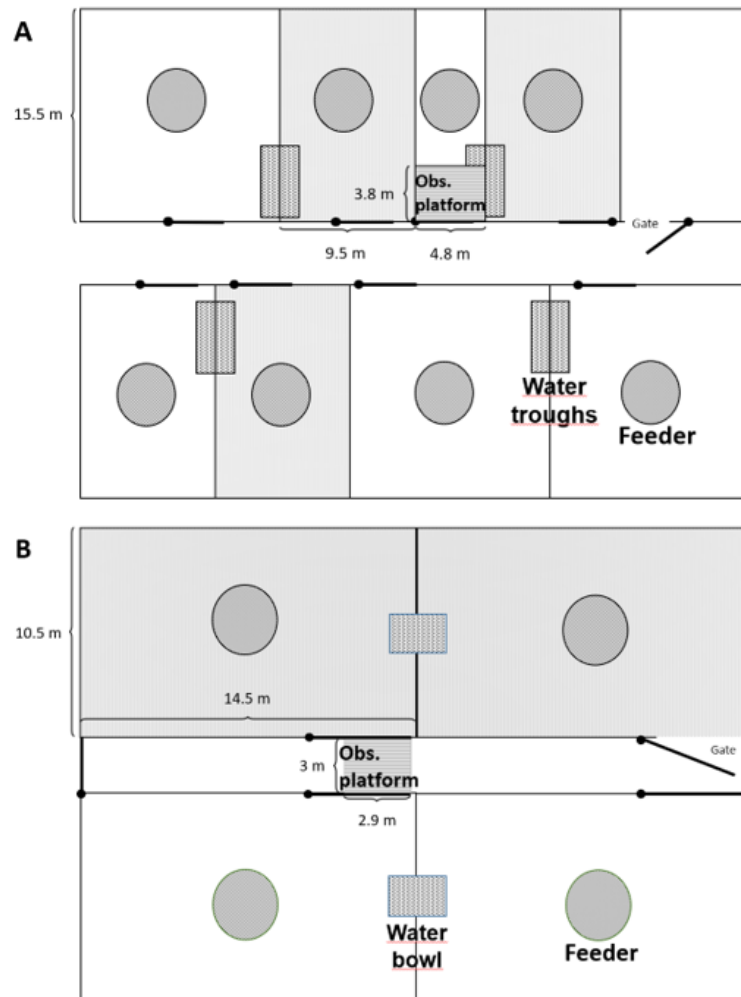


Fig. 1. Diagram of locations and measurements of pens used (■ = pen used) for this study at two commercial rest stations.

Treatments

Prior to the arrival of the first load, study pens were scraped by the rest station personnel, using a skid steer, and air dried for one day. Straw bedding was evenly added across the treatment pen by POG, to a depth of 14 cm (≈ 4.75 kg of straw / m^2). Bedding depth was confirmed using a custom-made plate meter. Bedding was added until the plate reached 14 cm at nine randomly selected locations within the pen, confirming that the bedding depth was evenly distributed throughout the pen. For the subsequent loads, any manure was cleaned from the pens prior to the arrival of the next load. In the bedded pens, all the remaining straw was fluffed using a pitchfork, and the straw was evenly distributed across the pen. Then, bedding depth was checked at nine randomly selected locations and, when needed, fresh straw was added to achieve the 14-cm depth.

Behavioural observations

All behavioural observations were done by the same researcher (POG). At rest station 1, live behavioural observations were taken from an elevated platform (4.8×3.8 m; length \times width) positioned 2 m from the ground, located between pens (Figure 1 A). Similarly, at rest station 2 the observations were taken from an elevated platform (3.0×2.9 m; length \times width), positioned 5 m from the ground, located on the side of the pen (Figure 1 B). Observation platforms at both rest stations allowed a full view of treatment and control pens. Artificial lightning was used during the observation periods after the sunset.

The observation period lasted 8 h and started between 0 and 52 min after the last animal was unloaded (mean latency to begin observation was 26 min). The interval to starting observations was needed to interview the truck drivers. The 8-h

observation period was selected because it is the minimum rest time required for cattle that have been in transit for up to 36 h according to the amended Regulations of the Health of Animals Act (Government of Canada, 2019). Each pen was observed every ten minutes (48 observations/pen per 8-h observation period). Ten-minute interval scan sampling has been validated as an accurate and precise method to obtain unbiased estimates of the proportion of time feedlot cattle spend standing, lying, and feeding (Mitlöhner et al., 2001). Behavioural observations were recorded using instantaneous scan sampling that switched between pens every 5 min. During each 5 min sampling period, a count of animals lying, eating, and drinking in a pen were recorded on a tally sheet. An animal was recorded as lying down when its body was in contact with the ground, regardless of whether the cattle were in sternal or lateral recumbency. An animal was recorded as eating when it was standing at the feeder or walking around the feeder and chewing hay. An animal was recorded as drinking when it was at the water trough or water bowl, with the lips immersed in the water, and neck movements indicating water ingestion.

To visually examine lying, eating, and drinking behaviour, we graphed the proportion of animals engaged in these activities at each observation point (mean number of animals at each point, divided by the mean number of animals per load).

Statistical analyses

The data were analyzed using STATA 15 statistical software (StataCorp LLC, College Station, TX). Descriptive statistics reported were mean, median, range, and standard deviation for continuous variables concerning animal and trip characteristics, and the rest station conditions upon arrival of the animals. For categorical variables, frequencies and proportions were reported.

For all statistical analyses described below a significance level of 5% was used (i.e., $\alpha=5\%$).

Linearity assumption for continuous variables

The linearity assumption for continuous variables was examined graphically using locally weighted regression (i.e., lowess curves) and by testing the statistical significance of a quadratic term in a logistic regression model. A quadratic relationship was modelled if the quadratic term was statistically significant and the lowess curve revealed a quadratic relationship. If it was inappropriate to model a linear or quadratic relationship, the variable was categorized into three quantiles. The allometric coefficient (*k*-value) used to measure space allowance in the resting pen while accounting for the size of the animals was categorized into three quantiles for all the analyses: 'low' space allowance was for *k*-values 0.0463 - 0.0734 (2.08 - 3.29 m²/300 kg animal), 'moderate' space allowance was for *k*-values 0.0737 - 0.0823 (3.31 - 3.69 m²/300 kg animal) and 'high' space allowance was for *k*-values 0.0825 - 0.1829 (3.70 - 8.19 m²/300 kg animal). Truck's motion duration was categorized into three quantiles for the drinking behaviour analysis: 'short' motion duration was for trips 13:45 - 17:24 (hh:mm), 'moderate' motion duration was for trips 17:34 - 21:41 (hh:mm) and 'long' motion duration was for trips 22:28 - 24:26 (hh:mm).

Correlations among independent variables

Spearman or Phi correlation coefficients were used to estimate correlations between independent variables depending on the type of variables being assessed (i.e., continuous or categorical). If two variables had an absolute correlation coefficient equal or greater than 0.7 and both variables met the criteria to be included in the final model (see Mixed Multivariable Logistic Regression Model section), then the variable

that made biological sense was included in the model. When both variables made biological sense, two multivariable models were fitted, with each variable; information criteria (Akaike's information criterion & Schwarz's Bayesian information criterion) were used to determine the variable that provided the better model fit.

Univariable mixed logistic regression models

Univariable mixed logistic regression models using the "meqrlogit" command, with random intercepts for load and group (bedded and non-bedded groups for each load) to account for the hierarchical structure of the data, were fitted to assess associations between the behaviours of interest (lying, eating, and drinking) and the following independent variables: bedding treatment, sex, estimated animal weight, study site, transport duration (h), truck's stationary duration (h), truck's duration in motion (h), loading density, number of bales of straw used to bed the trailer at origin, ambient temperature at unloading (°C), space allowance in the resting pen, daylight hours while at the rest stations, and time in resting pen since unloading (h). A significance level of 10% was used to determine which variables would be considered for inclusion in the multivariable models (i.e., $\alpha=10\%$).

Mixed multivariable logistic regression models

Mixed multivariable logistic regression models with random intercepts for load and group were fitted for the lying and eating behaviours, and a multivariable ordinary logistic regression model was fitted for drinking behaviour. Using a manual backward elimination approach, variables considered for inclusion in the model were retained if they were statistically significant or acted as an explanatory antecedent or distorter variable (i.e., confounder). A variable was considered a confounder if its removal caused a 20% or greater change in the coefficient of a statistically significant variable

and it met the causal criteria (i.e., it was not an intervening variable). Source of cattle was not included in these models due to the small number of loads per source. Two-way interactions were then tested between bedding treatment and all the independent variables examined. Interaction terms and their main effects were retained in the model if statistically significant. The homoscedasticity and normality of the best linear unbiased predictors (BLUPs) were evaluated graphically. Pearson residuals were examined in all models to identify outliers. Variance partition coefficients (VPC) were calculated, using the latent variable technique, for each multi-level model to determine the amount of the total variance in the outcome that could be attributed to the animal, group, and load levels (Dohoo et al., 2009). If the variance components for a model were extremely small (e.g., $<1 \times 10^{-5}$), their inclusion did not improve the fit of the model based on a likelihood ratio test, and the model coefficients and p-values were not notably changed by their inclusion, or the model could not converge with their inclusion, then an ordinary multivariable logistic regression model that included robust standard errors that account for the observations not being independent by group was fitted. For these models, Pearson residuals were examined to identify potential outliers.

Results

A total of 14 loads, 922 animals (bedded=452; non-bedded=470), and 43,918 animal observations (922 animals observed 48 times, except for one load that left after observation 46 and two observations missed on different loads) were included in the study. Space allowance in all pens was in agreement with the Beef Cattle Code of Practice (Beef Cattle Code Development Committee, 2013) providing enough space so that all animals were able to lie down at the same time and to easily navigate

around the pen. The behaviour of cattle, independent of bedding, was dynamic through the 8-h observation period (Figure 2): most (51.9%) cattle ate during the first hour after unloading, while 11.9% lay down, and 2.2% were observed drinking. The behavioural priorities of the cattle changed during the second hour after arrival (Figure 2): most (52.0%) laid down, while still 30.6% ate and 0.9% were observed drinking. Between hours 2 and <6 their behaviour was somewhat stable (Figure 2): about 60% of the cattle were lying, while approximately 20% were eating and roughly 1% were observed drinking. After 6 h since being unloaded, the proportion of cattle lying increased to more than 75%, while the proportion of animals eating dropped to <15%, while the proportion of animals drinking remained under 1% (Figure 2).

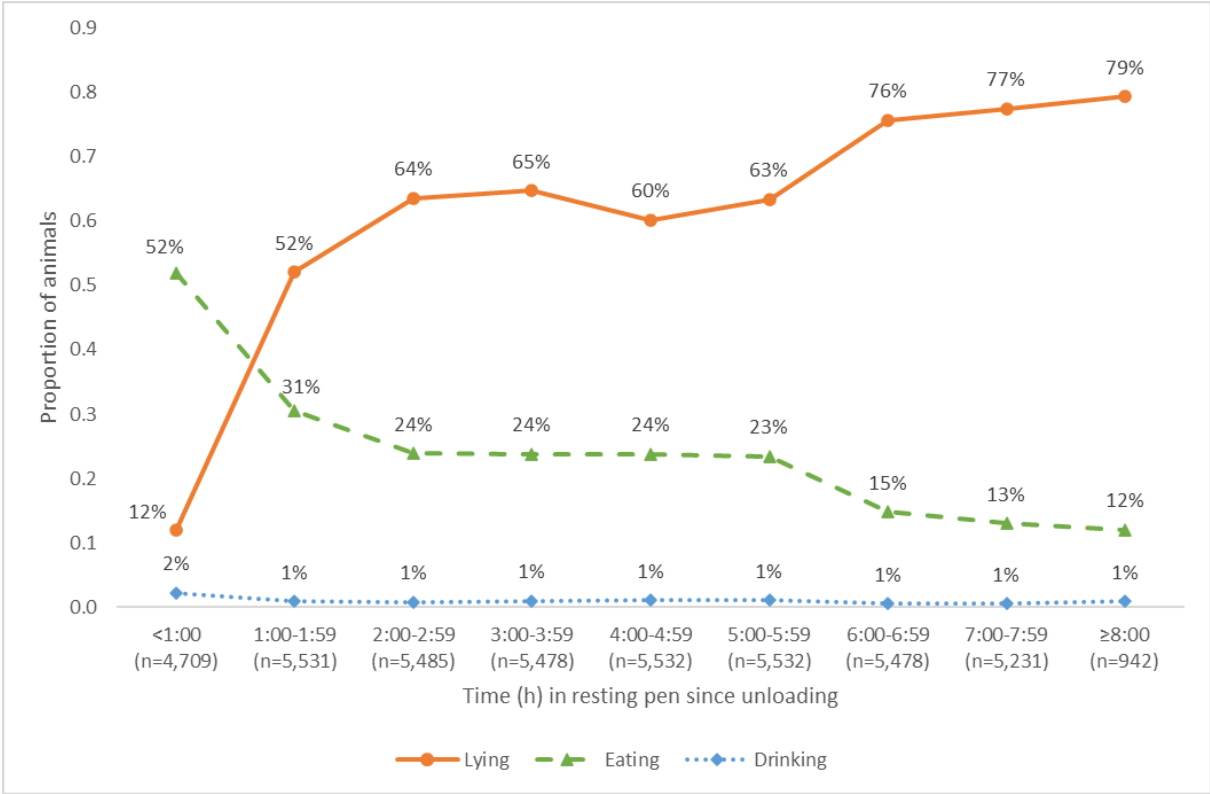


Fig. 2. Mean proportion of cattle lying, eating, and drinking in resting pens since unloading at two rest stations.

Descriptive statistics

Descriptive statistics of continuous variables are summarized in Table 1. Most loads transported steers (57.1%; n=8), followed by loads transporting heifers (28.6%; n=4) and mixed loads (14.3%; n=2). Most loads had departed from feedlots (42.8%; n=6), followed by farms (28.6%; n=4), auction markets (14.3% n=2), and assembly yards (14.3%; n=2). Two loads of market weight cattle originated from the same feedlot and the same pen and arrived at the rest station at the same time, however the drivers were reluctant to split the loads for the study, so each truck load was assigned to either a bedded or a non-bedded pen. Cattle were transported a median of 1,565.0 km from their point of departure to the rest station, with a mean (\pm SD) of 1,387.4 km (\pm 241.50 km). Most loads (10, or 71%) were travelling east while the balance were travelling west (four or 28%).

Multivariable mixed logistic regression model of lying behaviour

The following variables and interaction terms were included in the multivariable mixed logistic regression model of lying behaviour: estimated animal weight, bedding treatment, the truck's motion duration, an interaction between bedding treatment and truck's motion duration, time in pen since unloading and its quadratic term, and an interaction between bedding treatment and the linear component of time in pen since unloading (Table 2). The odds of observing cattle lying were significantly greater for every 100 kg the estimated animal weight increased (Table 2).

Groups rested in non-bedded pens showed lower odds of lying as truck's motion duration increased, whereas those rested in bedded pens had greater odds (Figure 3 A). The interaction between bedding treatment and time in the resting pen since unloading showed that early in the observation period, the odds of observing cattle lying were higher in the bedded groups, but both groups had an increase in the

probability of lying over time that plateaued at similar levels near the end of the 8-hour observation period (Figure 3 B).

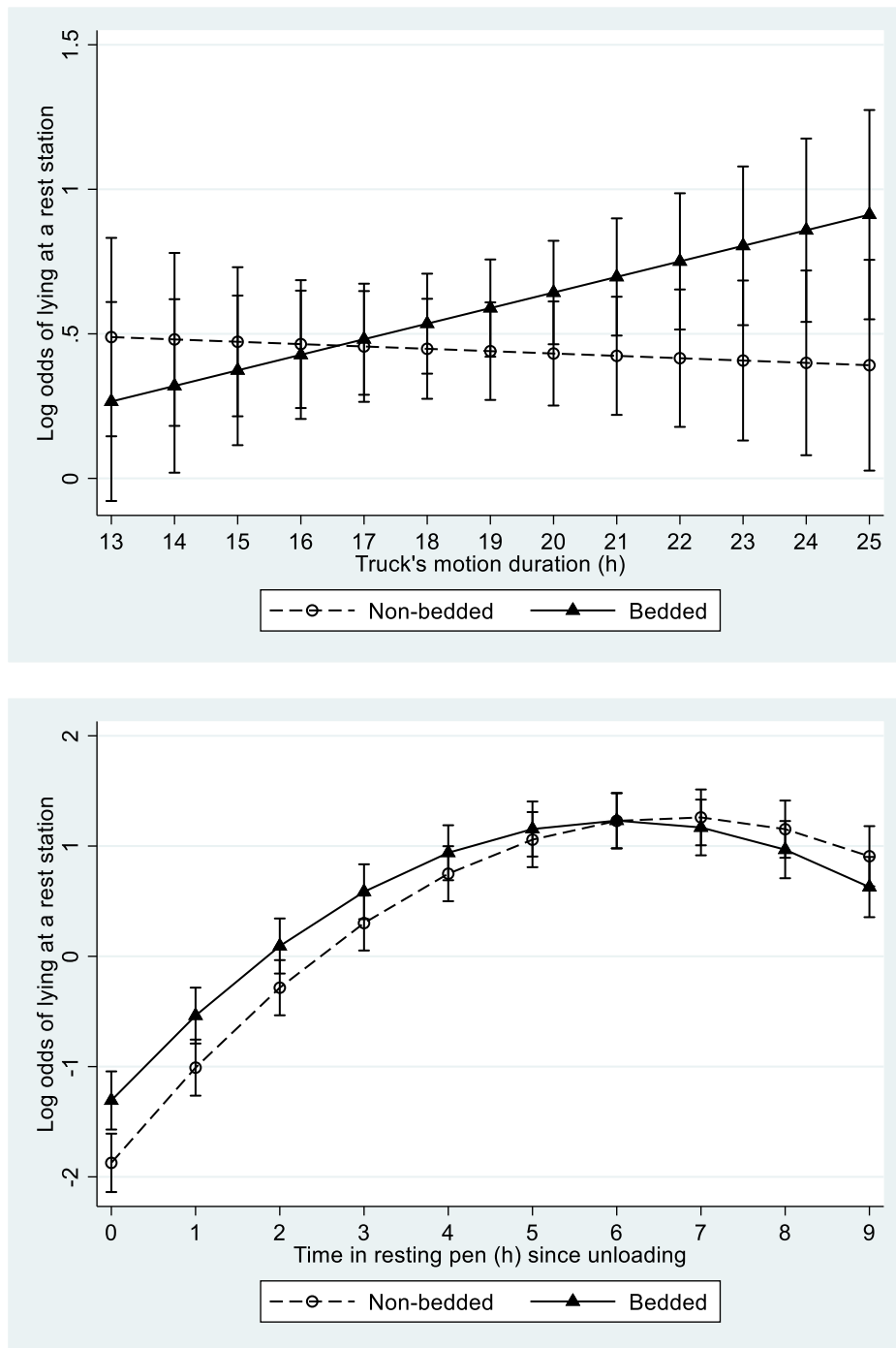


Fig. 3. The impact of (A) the duration of time the truck was in motion (h) and bedding treatment (non-bedded and bedded) and (B) time in the resting pen (h) since unloading and bedding treatment on the predicted log odds of observing cattle lying at two rest stations with 95% confidence intervals.

Multivariable mixed logistic regression model of eating behaviour

The following variables and interaction terms were included in the multivariable mixed logistic regression model of eating behaviour: estimated animal weight, bedding treatment, space allowance in resting pen (categorized), an interaction between bedding treatment and space allowance in resting pen, the truck's motion duration, the interaction between bedding treatment and truck's motion duration, time in pen since unloading and its quadratic term, and the interaction between bedding treatment and the quadratic term of time in pen since unloading (Table 3). The odds of observing cattle eating were significantly lower for every 100 kg the estimated animal weight increased (Table 3).

Groups rested in non-bedded pens showed notable greater odds of eating as motion duration increased, whereas those rested in bedded pens showed little change in the odds of eating as motion duration increased (Figure 4 A). The odds of observing cattle eating decreased over time and reached its nadir at similar levels, near the end of the observation period for both groups (Figure 4 B). However, differences between bedded and non-bedded groups were not constant over time (Figure 4 B).

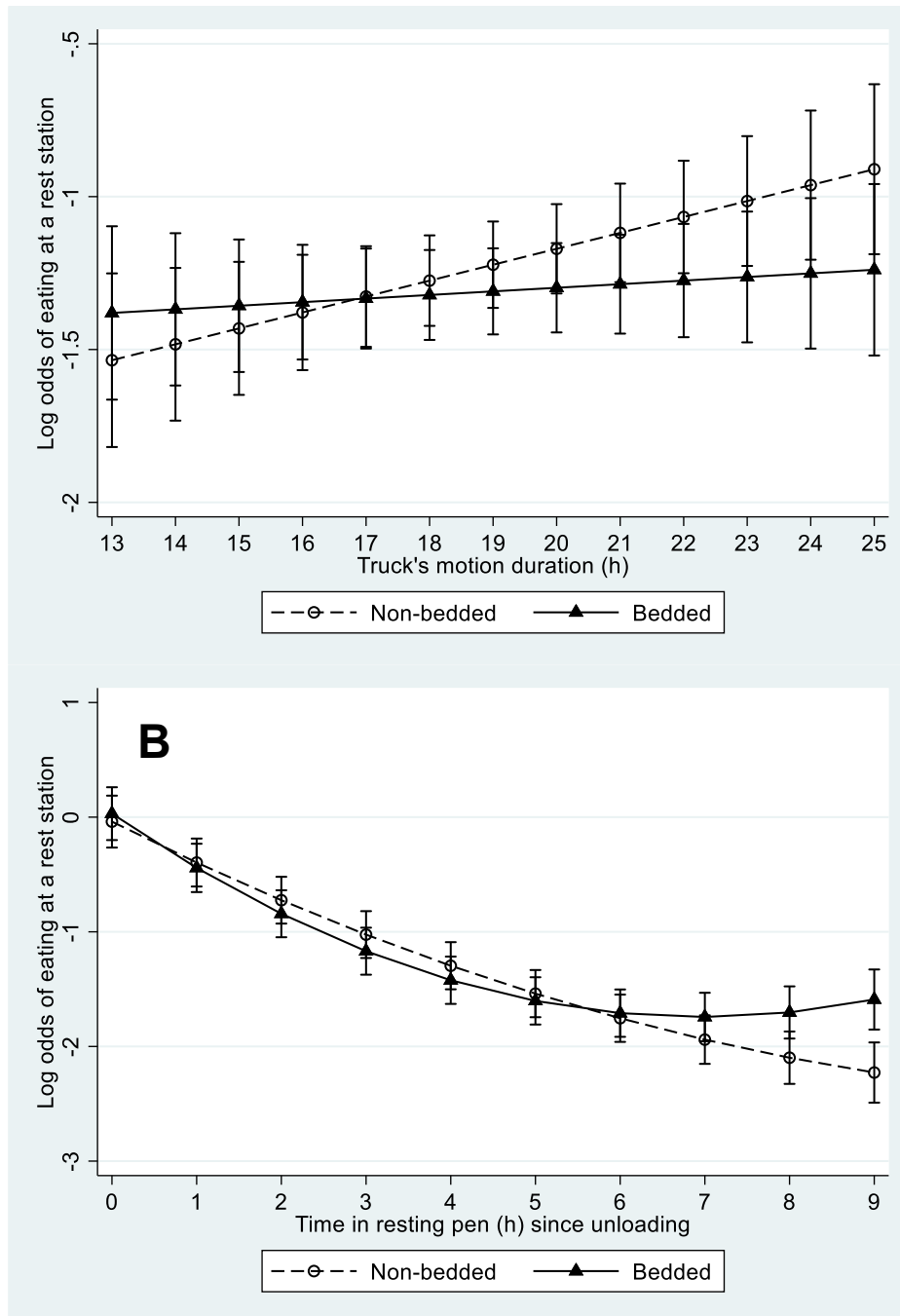


Fig. 4. The impact of (A) the duration of time the truck was in motion (h) and bedding treatment (non-bedded and bedded) and (B) time in the resting (h) pen since unloading and bedding treatment on the predicted log odds of observing cattle eating at two rest stations with 95% confidence intervals.

The provision of bedding significantly increased the odds of observing cattle eating for cattle rested at a moderate space allowance (Table 4). Cattle rested in bedded

pens with a low space allowance had significantly lower odds of being observed eating compared to cattle rested in a moderate space allowance (Table 4). In contrast, cattle rested in bedded pens with moderate space allowance had significantly higher odds of being observed eating compared to cattle rested in pens with high space allowance (Table 4).

Multivariable logistic regression model of drinking behaviour

The following variables were included in the multivariable logistic regression model of drinking behaviour: sex, bedding treatment, truck's motion duration (categorized), and time in pen since unloading (Table 5). Bedding treatment had no significant effect on drinking behaviour, but it was forced into the model because it was a key part of the main research question.

Loads transporting heifers were significantly more likely to be observed drinking at the rest station, than steers (Table 5). The odds of observing drinking behaviour were significantly lower with time in the resting pen since being unloaded (Table 5).

The odds of observing cattle drinking were significantly greater when transported for short and moderate compared to long periods of time (Table 5). Similarly, the odds were significantly greater when transported for short periods of time compared to moderate ones (Table 5).

Diagnostics for all models

BLUPs from the multivariable mixed logistic regression models of lying and eating behaviours fulfilled the assumption of homoscedasticity and normality. We did not identify any outliers in our models.

Discussion

To our knowledge, this is the first study to explore the effect of bedding on the behaviour of cattle unloaded for feed, water, and rest, at commercial rest stations in Canada. Our results supported our hypothesis in that provision of bedding affected cattle's motivation to lie down. With bedding, cattle were more likely to lay down sooner, while eating behaviour was similar for bedded and non-bedded groups during the same observation period.

Factors contributing to transport stress include the duration of the journey, time off feed and water, weather conditions, source of the cattle and space allowance (Fisher et al., 2009; Schwartzkopf-Genswein and Grandin, 2019). These factors were all examined in the present study, and we found that the effect of bedding significantly varies with space allowance in the resting pen, time since unloading, and the truck's motion duration, rather than transport duration.

Lying behaviour

The effect of the truck's motion duration on lying behaviour depended on provision of bedding. Our results showed that cattle's motivation to lie changed with the provision of bedding; cattle in bedded pens showed greater odds of lying as truck's motion duration increased, while cattle in non-bedded pens had lower odds of lying with longer truck's motion duration. This result could be explained by the preference of cattle to lie on softer surfaces (Manninen et al., 2002; Tucker and Weary, 2004; Wolfe et al., 2018), with bedding being influential in eliciting lying behaviour at rest stations.

Flint (2013) observed 81 loads of cattle at a commercial rest station and found no association between transport duration and lying or eating behaviour. However, cattle in Flint's study were only rested in dirt pens, which in light of our findings, suggests

that when bedding is provided, lying is prioritized with longer trips. Also, in our study, transport duration was split into the truck's motion and stationary durations, allowing us to explore in more detail the effect of each component of transit.

Pettiford et al. (2008) found that creatine kinase, a biomarker used to measure muscle fatigue, increased steadily plateauing around 4 h into a 6-h road transport journey, indicating that as transport duration increased, muscle fatigue also increased due to cattle exerting effort to maintain balance during the trip or from prolonged periods of standing. Our results showed that trailer's motion duration, rather than transport duration, better explained cattle's behaviour at a rest station.

Kenny and Tarrant (1987) confined steers for 1 hour in a transport truck and compared the physiological responses of the animals when the truck was either stationary or in motion. They concluded that the motion of the vehicle was more stressful than confinement itself (Kenny and Tarrant, 1987). In line with their findings, we postulate that the motion of the trailer, rather than the transport duration, *per se*, may increase cattle fatigue most significantly; cattle may exert more energy when trying to maintain balance while the truck is moving than when it is stationary perhaps due to trailer movement and the weight adjustments necessary to maintain stability.

The effect of time in pen since unloading on lying behaviour also depended on the provision of bedding. Several studies have reported that lying is a priority behaviour for cattle (Marti et al., 2017; Meléndez et al., 2020; Munksgaard et al., 2005; Munksgaard & Simonsen, 1996). Lactating dairy cattle lie down sooner and for longer periods of time after being forced to stand, even for a period of time as short as 4 h (Norrington and Valros, 2016). When dairy cows were deprived from both, lying and eating, their lying time increased compared to when they were not restricted (Metz, 1985; Munksgaard et al., 2005). In our study, cattle rested in bedded pens had

greater odds of lying in the early observation period, which agrees with the findings of Tucker et al. (2018) who reported a shorter latency to lie down once cattle were able to access a deep-bedded area (10 cm of straw over 30 cm of sand) after 4 h of lying deprivation compared to non-deprived cows. Both groups, bedded and non-bedded, had greater odds of lying compared to any other behaviour, as time in pen increased, however the bedded groups showed greater odds of lying in the early observation hours, compared to the non-bedded groups. We also observed that near the end of our 8-h observation period, the odds of lying in both groups were lower and cattle switched to other activities (e.g., eating, drinking, walking) instead of lying. The decrease of lying activity started earlier in the bedded groups than in the non-bedded ones, and the decrease was more pronounced in the bedded groups than in the non-bedded ones. These results suggest that bedding not only elicits lying behaviour in cattle, but may also improve their rest, as they were more likely to switch from lying to other activities near the end of our observation period. Previous studies showed that fatigued beef cattle chose to lie down rather than eat after 20-h (Marti et al., 2017) and 36-h (Meléndez et al., 2020) road trips. Furthermore, dairy cattle chose to spend the majority of their time lying down rather than eating after being deprived of both (Metz, 1985; Munksgaard et al., 2005; Tucker et al., 2018). Munksgaard et al. (2005) hypothesized that this behavioural exchange happens because cattle can compensate eating deprivation by increasing their eating rate while they cannot compensate for lying and rest, which must be recuperated in real-time.

It is important to note that in our study, cattle in bedded pens did not lie down sooner to engage in two activities simultaneously (i.e., lying to rest and simultaneously satisfy their hunger by eating the straw bedding); <1% of all animal observations had cattle eating while lying (47/43,918 animal observations; data not shown).

We observed that estimated animal weight affected lying behaviour, independent of bedding treatment. Our findings agree with previous research reporting that, in general, heavy cattle spend more time lying compared to lighter cattle (Dikmen et al., 2012). Flint (2013) described that heavier cattle might have higher energy reserves compared to lighter cattle, leading them to lie down rather than eat while at rest stations. Another potential explanation is that heavier cattle may experience more fatigue while in transport, with more weight, more energy exertion might be needed to maintain balance while in motion.

Long-distance transport for up to 36 h at a time may be considered by some to be exhausting for cattle. If long-distance transport is first and foremost physically demanding one might expect that cattle would lie down almost immediately after unloading, independent of bedding, however our results showed that even after 17 h in motion the provision of bedding influenced their motivation to lie down over other activities (e.g., eating).

Eating behaviour

The effect of motion duration on eating behaviour varied with provision of bedding; bedded cattle showed little change in their odds of eating as truck's motion duration increased, whereas non-bedded cattle showed a notable increase with longer motion duration of the truck. This effect was opposite to the one observed on lying behaviour. This finding suggests that cattle either lie down or eat while at a rest station, which is not altogether surprising given that both are basic needs. After being restricted from eating for long periods of time, cattle need to eat to replenish their energy for basic biological processes as well as to replenish the energy required to stand, maintain their balance during the trip, and possibly to move around to a

preferred space in the trailer. On the other hand, lying itself has also been found to be an important behaviour for cattle as explained above (Marti et al., 2017; Meléndez et al., 2020; Munksgaard et al., 2005). Our results agree with previous studies that found a trade-off between eating and lying (Meléndez et al., 2020; Munksgaard et al., 2005; Munksgaard & Simonsen, 1996).

The effect of time in pen since unloading on eating behaviour also varied with provision of bedding. Again, this effect was opposite to the one observed for lying behaviour; as time in pen increased, the odds of observing cattle eating were lower for both groups, however groups rested with bedding showed an increase in the odds of eating near the end of the observation period, whereas cattle without bedding showed a steady decrease in the odds of eating, which suggests that cattle in non-bedded pens were more likely to be lying down at the end of the observation period. Flint (2013) observed cattle at a commercial rest station and reported that their behaviour in the first 2 hours was significantly different than in the following 3 hours; cattle ate before they lay down. The increase in the odds of eating near the end of the observation period in bedded cattle, and together with the results from the lying behaviour model, suggests that eating and lying behaviours are traded-off with one another after long distance transport. Our results are similar to those of Knowles et al. (1999) who transported groups of cattle by road for 14 - 31 h and rested them in bedded pens after the trip. They reported a second bout of standing and eating about 8 h after arriving to their destination (Knowles et al., 1999). Their study (Knowles et al., 1999) used market weight beef cattle, whereas our study had groups of young, weaned calves, feeders, and market weight cattle. The diversity of weight and age could explain the differences observed between studies.

Space allowance in the pen influenced eating when bedding was provided, and the effect was complex and non-linear. Comparing cattle rested in bedded to those in non-bedded pens, we found that the odds of observing eating behaviour were greater only at a moderate space allowance. It is possible that cattle rested under these conditions might have had a comfortable surface and enough space so as to influence their motivation to eat, either by eating for longer periods of time or visiting the feeder more often. With low space allowance it is possible that the feeder space was limited, thus there were cattle constantly at the feeder and provision of bedding had no effect on their behaviour. Cattle rested with high space allowance (more room) might have had easier access to the feeder at the same time, thus they might have satiated their hunger around the same time in bedded and non-bedded pens. We also found that when cattle were rested in bedded pens the odds of observing eating behaviour were greater at moderate space allowance compared to low and high space allowances. These results suggest that cattle were more motivated to eat in bedded pens at moderate space allowance, possibly because bedding provided a more comfortable surface under this space allowance that allowed cattle to stay standing at the feeder for longer periods. Non-bedded pens had no difference in eating behaviour at different space allowances.

Results showed that estimated animal weight affected eating behaviour, independent of bedding treatment. Previous research has found similar results, where heavier cattle ate less frequently than lighter cattle (Dikmen et al., 2012; Flint, 2013). Heavy cattle in our study were market weight cattle (>550 kg), all sourced directly from feedlots, and were being transported to an abattoir. Given their point of origin, it is possible that these cattle had ad libitum access to feed and water until the loading time, whereas lighter cattle, sourced from auction markets, assembly yards, and

direct from farms, may have been off feed and water for longer prior to transport, perhaps with their hunger exacerbated further for those weaned and sold through an auction market. Another possible explanation is that lighter cattle are growing, and their ruminal capacity is smaller, whereas market weight cattle have reached maturity with a larger ruminal capacity; lighter cattle might need to eat more frequently than heavy cattle to meet their nutritional requirements.

Drinking behaviour

Bedding treatment did not affect drinking behaviour at the rest stations. This result was not surprising given that drinking is much shorter in duration compared to lying or eating, thus cattle would not need to trade-off drinking for lying or eating. Also, because drinking is short in duration, it is more difficult to observe when using instantaneous scan sampling at 10-min intervals. Mitlöhner et al. (2001) found that 10-min interval scan samplings was less precise for measuring drinking behaviour when compared to continuous observation. Thus, there may be differences in the drinking behaviour of cattle rested in bedded vs non-bedded pens, but the sampling method used might have not been adequate to detect such differences.

We found heifers were more likely to be observed drinking than steers. Source could potentially explain this result given that all loads carrying steers came from farms and feedlots (n=8/8) whereas loads of heifers came from auction markets or assembly yards (n=3/4; data not shown) and therefore we could not include both source and sex in the multivariable analysis. Cattle transported directly to farms or feedlots may have had access to water right up to loading whereas cattle departing from an auction market, or an assembly yard may not, and so may have increased time without drinking before beginning their journey.

Truck's motion duration affected drinking behaviour, the odds were greater when short and moderate motion duration were compared to long duration. This result might seem paradoxical, however with longer transport duration, cattle might feel thirstier upon arrival, causing them to approach the water trough or bowl sooner and drink faster, satiating their thirst early in the observation period and thus not returning to drink later. Al-Ramamneh et al. (2012) observed goats and sheep using continuous recordings of all the study animals from a subsample of the experimental days and reported that water consumption per minute increased in goats and sheep after water deprivation for 21 h and 42 h. As noted previously, depending on the interval, scan sampling may be less than ideal for capturing short-duration behaviours, such as drinking (Mitlöhner et al., 2001). However, without access to more advanced technological options, scan sampling remains one of the most viable methods for capturing such behaviors in field settings.

Drinking behaviour was also affected by time in pen since unloading. Cattle in rest stations have previously been recorded drinking in the hours soon after arrival. Flint (2013) observed a peak in drinking behaviour of cattle unloaded at a rest station during the early minutes in the pen, decreasing as time elapsed, being close to zero percent of animals drinking 1 h and 20 min after arrival. As mentioned previously, it is possible that once cattle drank at the rest station, they satiated their thirst and spent the balance of the observation time engaged in other activities.

Fourteen loads participated in this study, which is a much smaller sample size compared to Flint's study (2013). This could be a limitation because of the potential for type 2 errors. Conducting this study under commercial conditions prevented us from measuring individual eating rates or using behavioural data loggers that would yield individual data on lying and eating duration to compare differences in latency

and duration of the behaviours studied. As mentioned before, the method used to record drinking behaviour is not the most accurate one for this type of behaviour, thus a bolus or a flow meter would allow for higher accuracy data collection and could be used in future studies to measure water consumption and record the individual activity of animals at the waterer.

An additional limitation relates to correlations among certain predictor variables. For example, in the Lying and Eating behaviour models, truck motion duration was correlated with remaining daylight hours, and source of cattle was correlated with estimated animal weight. To avoid multicollinearity, we included only one variable from each correlated pair based on biological relevance and model fit. We acknowledge, however, that this limits our ability to determine whether the associations observed are driven by one variable or the correlated companion, and this should be interpreted accordingly.

Results from this study show that the effect of bedding on the behaviour of cattle at rest stations is complex, but not inconsequential. We found significant interactions between bedding and truck's motion duration and time in pen in the lying behaviour model. The eating behaviour model showed that bedding interacted with truck's motion duration, time in pen, and stocking density. We did not observe any effect of bedding on the drinking behaviour model, perhaps because of the time interval between observations.

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Competing interests statement

The authors declare there are no competing interests.

Author contributions

Paula Olivares Guzmán: Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft **David L. Pearl:** Conceptualization, formal analysis, methodology, supervision, validation, visualization, writing – review & editing **Karen S. Schwartzkopf-Genswein:** Conceptualization, funding acquisition, methodology, project administration, supervision, validation, visualization, writing – review & editing **T.M. Widowski:** Conceptualization, methodology, supervision, validation, visualization, writing – review & editing **Daniela M. Meléndez:** Methodology, validation, visualization, writing – review & editing **Sonia Marti:** Methodology, validation, visualization, writing – review & editing **Derek B. Haley:** Conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, visualization, writing – review & editing

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Data availability statement

None of the data were deposited in an official repository but are available from the authors upon reasonable request.

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Table 1

Continuous variables collected for loads (n=14) of cattle included in a study assessing the impact of providing straw bedding on cattle behaviour at two rest stations.

Variable	Min.	Median	Max.	Mean	SD
Trailer characteristics					
Number of bales of straw used to bed the trailer at point of departure	16	25	27	22.2	3.88
Loading density (m ² /animal)	0.5	1.07	2.2	1.11	0.423
Trip characteristics					
Distance travelled (km)	1012	1565.0	1785	1387.4	241.50
Transport duration (hh:mm)	23:30	25:36	32:42	27:43	03:27
Motion duration (hh:mm)	13:45	18:19	24:26	18:48	03:36
Stationary duration (hh:mm)	04:45	09:45	11:30	08:55	01:54
Rest station characteristics					
Space allowance in the resting pen (m ² /animal)	1.8	4.24	14.7	4.31	2.454
Space allowance in the resting pen (k-value)	0.046	0.0766	0.183	0.0835	0.03063
Temperature at unloading (°C)	-20	-0.5	20	1.3	12.59
Animal characteristics					
Animals in the truck	38	63.0	122	71.2	29.84
Estimated animal weight (kg)	215	433.0	731	453.9	191.42

Note: Space allowance was measured as an allometric coefficient $k\text{-value}=(\text{m}^2/\text{animal})/(\text{BW}^{2/3})$.

Table 2

Results of the mixed multivariable logistic regression model examining associations between lying behaviour observed at a rest station and load, trip, rest station, and animal characteristics at two rest stations.

Variable	Odds Ratio	95% C. I.	P-value
Estimated animal weight (100 kg)	1.20	1.08; 1.34	0.001
Bedding treatment	0.61 ^{*†}	0.27; 1.41	0.251
Truck's motion duration (h)	0.97 [*]	0.91; 1.03	0.281
Bedding treatment × truck's motion duration (h)	1.06 [*]	1.01; 1.11	0.009
Time (h) in pen since unloading	2.50 [†]	2.40; 2.61	<0.001
Time ² (h ²) in pen since unloading	0.93 [†]	0.93; 0.94	<0.001
Bedding treatment × time (h) in pen since unloading	0.90 [†]	0.88; 0.92	<0.001

Abbreviations: 95% C.I. = 95% Confidence interval.

* Should not be interpreted as odds ratios in isolation due to the interaction effect. See Figure 3 A for interpretation.

† Should not be interpreted as odds ratios in isolation due to the interaction effect. See Figure 3 B for interpretation.

Note: The variance component at the load level was 0.10 (95% C. I. 0.04 – 0.26) and at the group level was 0.03 (95% C. I. 0.01- 0.08). The variance partition coefficient estimated at the load, group, and animal-observation levels were 2.90%, 0.97%, and 96.13%, respectively.

Table 3

Results of the mixed multivariable logistic regression model examining associations between eating behaviour observed at a rest station and load, trip, rest station, and animal characteristics at two rest stations.

Variable	Odds Ratio	95% C. I.	P-value
Estimated animal weight (100 kg)	0.86	0.80; 0.93	<0.001
Bedding treatment	1.96 ^{*, †, ‡}	0.99; 3.84	0.051
Space allowance (<i>k</i> -value) in resting pen			
Low	Referent	Referent	Referent
Moderate	1.16 [*]	0.92; 1.47	0.219
High	1.13 [*]	0.89; 1.44	0.390
Bedding treatment × Space allowance (<i>k</i> -value) in resting pen			
Bedded × low	Referent	Referent	Referent
Bedded × moderate	1.11 [*]	0.79; 1.57	0.545
Bedded × high	0.74 [*]	0.55; 0.99	0.049
Truck's motion duration (h)	1.05 [†]	1.01; 1.10	0.012
Bedding treatment × truck's motion duration (h)	0.96 [†]	0.93; 0.99	0.008
Time (h) in pen since unloading	0.65 [‡]	0.62; 0.69	<0.001
Bedding treatment × time (h) in pen since unloading	0.93 [‡]	0.86; 1.01	0.097
Time ² (h ²) in pen since unloading	1.02 [‡]	1.01; 1.03	<0.001
Bedding treatment × time ² (h ²) in pen since unloading	1.02 [‡]	1.01; 1.03	0.002

Abbreviations: 95% C.I. = 95% Confidence interval.

* Should not be interpreted as odd ratios in isolation due to the interaction effect, see Table 4 for interpretation.

† Should not be interpreted as odds ratios in isolation due to the interaction effect, see Figure 4 A for interpretation.

‡ Should not be interpreted as odds ratios in isolation due to the interaction effect, see Figure 4 B for interpretation.

Note: 'Low' space allowance was for k -values between 0.0463 - 0.0734, 'moderate' space allowance was for k -values between 0.0737 – 0.0823, and 'high' space allowance was for k -values between 0.0825 – 0.1829.

Note: The variance component at the load level was 0.05 (95% C. I. 0.02 – 0.13) and at the group level was <0.01 (95% C. I. <0.01- 0.03). The variance partition coefficient estimated at the load, group, and animal-observation levels were 1.43%, 0.28%, and 98.29%, respectively.

Table 4

Contrast table* examining odds ratios, 95% CI and p-value of the interactions between bedding treatment and quantiles of space allowance in the resting pen on eating behaviour at two rest stations.

Space allowance in the resting pen	Bedding treatment	OR	95% CI	<i>P</i> -value
Low	Bedded vs Non-bedded	1.96	0.99; 3.84	0.051
Moderate	Bedded vs Non-bedded	2.18	1.26; 3.76	0.005
High	Bedded vs Non-bedded	1.46	0.81; 2.61	0.208
Moderate vs Low	Bedded	1.29	1.02; 1.64	0.036
Moderate vs High	Bedded	1.53	1.21; 1.94	<0.001
Low vs High	Bedded	1.18	0.96; 1.47	0.122
Low vs Moderate	Non-bedded	0.86	0.68; 1.09	0.219
Low vs High	Non-bedded	0.88	0.70; 1.12	0.309
Moderate vs High	Non-bedded	1.03	0.81; 1.29	0.831

Abbreviations: OR = Odds ratio; 95% C.I. = 95% Confidence interval.

* These contrasts are based on the full model presented in Table 3.

Note: 'Low' space allowance was for *k*-values between 0.0463 - 0.0734, 'moderate' space allowance was for *k*-values between 0.0737 – 0.0823, and 'high' space allowance was for *k*-values between 0.0825 – 0.1829.

Table 5

Results of the multivariable logistic regression model examining associations between drinking behaviour observed at a rest station and load, trip, rest station, and animal characteristics at two rest stations.

Variable	Odds Ratio	95% C. I.	P-value
Sex			
Steers	Referent	Referent	Referent
Heifers	1.36	1.08; 1.71	0.009
Bedding treatment	0.98	0.80; 1.20	0.854
Truck's motion duration			
Long	Referent	Referent	Referent
Moderate	1.51	1.19; 1.92	0.001
Short	1.93	1.52; 2.46	<0.001
Time (h) in pen since unloading	0.87	0.80; 0.94	<0.001

Abbreviations: OR = Odds ratio; 95% C.I. = 95% Confidence interval.

Note: Comparison between truck's 'Short' vs 'Moderate' motion duration (OR 1.28, 95% C.I. 1.00; 1.64, $p=0.05$)

Note: 'Short' motion duration was for trips between 13:45 - 17:24 (hh:mm), 'moderate' motion duration was for trips between 17:34 – 21:41 (hh:mm) and 'long' motion duration was for trips between 22:28 - 24:26 (hh:mm).