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

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21 **Abstract**

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

39

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

41

42 **Introduction**

43 Transport has been shown to be stressful for cattle (Kent and Ewbank, 1983; Tarrant,
44 1990), and the detrimental effects increase with longer trips (Knowles, 1999;

45 Gonzalez et al., 2012); however, it is a common practice globally. Cattle may be
46 transported several times in their lifetime (Schwartzkopf-Genswein and Grandin,
47 2019) and some of the transport events are over long distances (≥ 400 km). Canadian
48 regulations allow for some of the longest transport durations globally (Government of
49 Canada, 2019); in 2019, amendments to the Canadian Humane Transport
50 Regulations under the Health of Animals Act, decreased the maximum transit time
51 from 48 to 36 h before requiring the unloading of animals at rest stations (facilities
52 equipped with pens that offer feed, water, and protection from the environment),
53 while increasing the minimum resting time from 5 h to 8 h (Government of Canada,
54 2019). Resting pens in Canada are usually dirt-floored, with minimal to no bedding,
55 and have one round feeder filled with hay and a water trough or waterer.

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

64 Previous research has found that dairy cattle prefer softer surfaces to lie down
65 (Manninen et al., 2002; Tucker and Weary, 2004; Wolfe et al., 2018), and when soft
66 surfaces are provided, they lie down for longer (Haley et al., 2001; Manninen et al.,
67 2002) and have more lying bouts (Haley et al., 2001; Tucker and Weary, 2004).

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

70 beef cattle are not only limited, but their results are contradictory (Keane et al., 2018).
71 Comparing cattle housed on straw bedding to those on concrete slats, Gygax et al.
72 (2007) and Rouha-Muelleder et al. (2012) observed that finishing bulls on European
73 beef farms did not differ in their daily lying duration, whereas Keane et al. (2017)
74 reported a higher proportion of heifers lying at any one time, and Hickey et al. (2003)
75 found that finishing beef steers lay down for longer when housed in bedded pens
76 compared to concrete slats.

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

85 **Methods**

86 ***General***

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

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

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

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

103 **Survey**

104 Load characteristics collected from the driver survey were number of animals being
105 transported by compartment, load sex (heifers, steers, or both) and estimated animal
106 weight ($[\text{total load weight} - \text{truck tare weight}] / \text{number of animals}$, kg). Number of
107 animals and sex were confirmed via visual inspection by POG. The survey also
108 gathered the following information regarding trip characteristics: source of the cattle
109 (assembly yard, auction market, farm, or feedlot), transport duration (h), duration the
110 truck was stationary (h), duration the truck was in motion ($h = \text{transport duration} -$
111 $\text{stationary duration}$), loading density (measured as an allometric coefficient (k -
112 $\text{value} = (\text{m}^2 / \text{animal}) / (\text{BW}^{2/3})$) as suggested by Petherick & Phillips, 2009), and number
113 of bales of straw used to bed the trailer at the point of origin. Additionally, rest station
114 characteristics were recorded upon arrival of each load. The variables recorded were
115 study site (1 or 2), temperature at unloading ($^{\circ}\text{C}$), space allowance in the resting pen
116 (measured as an allometric coefficient k -value $= (\text{m}^2 / \text{animal}) / (\text{BW}^{2/3})$), remaining
117 daylight hours upon arrival at the rest station, and time in resting pen since unloading

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

122 ***Study animals***

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

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

135 ***Housing and feeding***

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

138 At rest station 1, three pens measuring 15.5 × 9.5 m (length × width) each with a
139 concrete water trough (2.6 × 1.1 × 0.8 m; length × width × height) were used (Figure
140 1 A). At rest station 2, two pens measuring 14.5 × 10.5 m (length × width) with an

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

149 ***Treatments***

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

161 ***Behavioural observations***

162 All behavioural observations were done by the same researcher (POG). At rest
163 station 1, live behavioural observations were taken from an elevated platform (4.8 ×

164 3.8 m; length × width) positioned 2 m from the ground, located between pens (Figure
165 1 A). Similarly, at rest station 2 the observations were taken from an elevated
166 platform (3.0 × 2.9 m; length × width), positioned 5 m from the ground, located on the
167 side of the pen (Figure 1 B). Observation platforms at both rest stations allowed a full
168 view of treatment and control pens. Artificial lightning was used during the
169 observation periods after the sunset.

170 The observation period lasted 8 h and started between 0 and 52 min after the last
171 animal was unloaded (mean latency to begin observation was 26 min). The interval
172 to starting observations was needed to interview the truck drivers. The 8-h
173 observation period was selected because it is the minimum rest time required for
174 cattle that have been in transit for up to 36 h according to the amended Regulations
175 of the Health of Animals Act (Government of Canada, 2019). Each pen was observed
176 every ten minutes (48 observations/pen per 8-h observation period). Ten-minute
177 interval scan sampling has been validated as an accurate and precise method to
178 obtain unbiased estimates of the proportion of time feedlot cattle spend standing,
179 lying, and feeding (Mittlöhner et al., 2001). Behavioural observations were recorded
180 using instantaneous scan sampling that switched between pens every 5 min. During
181 each 5 min sampling period, a count of animals lying, eating, and drinking in a pen
182 were recorded on a tally sheet. An animal was recorded as lying down when its body
183 was in contact with the ground, regardless of whether the cattle were in sternal or
184 lateral recumbency. An animal was recorded as eating when it was standing at the
185 feeder or walking around the feeder and chewing hay. An animal was recorded as
186 drinking when it was at the water trough or water bowl, with the lips immersed in the
187 water, and neck movements indicating water ingestion.

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

191 ***Statistical analyses***

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

197 For all statistical analyses described below a significance level of 5% was used (i.e.,
198 $\alpha=5\%$).

199 *Linearity assumption for continuous variables*

200 The linearity assumption for continuous variables was examined graphically using
201 locally weighted regression (i.e., lowess curves) and by testing the statistical
202 significance of a quadratic term in a logistic regression model. A quadratic
203 relationship was modelled if the quadratic term was statistically significant and the
204 lowess curve revealed a quadratic relationship. If it was inappropriate to model a
205 linear or quadratic relationship, the variable was categorized into three quantiles.
206 The allometric coefficient (k -value) used to measure space allowance in the resting
207 pen while accounting for the size of the animals was categorized into three quantiles
208 for all the analyses: 'low' space allowance was for k -values 0.0463 - 0.0734 (2.08 -
209 3.29 m²/300 kg animal), 'moderate' space allowance was for k -values 0.0737 -
210 0.0823 (3.31 - 3.69 m²/300 kg animal) and 'high' space allowance was for k -values
211 0.0825 - 0.1829 (3.70 - 8.19 m²/300 kg animal). Truck's motion duration was

212 categorized into three quantiles for the drinking behaviour analysis: 'short' motion
213 duration was for trips 13:45 - 17:24 (hh:mm), 'moderate' motion duration was for trips
214 17:34 - 21:41 (hh:mm) and 'long' motion duration was for trips 22:28 - 24:26 (hh:mm).

215 *Correlations among independent variables*

216 Spearman or Phi correlation coefficients were used to estimate correlations between
217 independent variables depending on the type of variables being assessed (i.e.,
218 continuous or categorical). If two variables had an absolute correlation coefficient
219 equal or greater than 0.7 and both variables met the criteria to be included in the final
220 model (see Mixed Multivariable Logistic Regression Model section), then the variable
221 that made biological sense was included in the model. When both variables made
222 biological sense, two multivariable models were fitted, with each variable; information
223 criteria (Akaike's information criterion & Schwarz's Bayesian information criterion)
224 were used to determine the variable that provided the better model fit.

225 *Univariable mixed logistic regression models*

226 Univariable mixed logistic regression models using the "meqrlogit" command, with
227 random intercepts for load and group (bedded and non-bedded groups for each load)
228 to account for the hierarchical structure of the data, were fitted to assess associations
229 between the behaviours of interest (lying, eating, and drinking) and the following
230 independent variables: bedding treatment, sex, estimated animal weight, study site,
231 transport duration (h), truck's stationary duration (h), truck's duration in motion (h),
232 loading density, number of bales of straw used to bed the trailer at origin, ambient
233 temperature at unloading (°C), space allowance in the resting pen, daylight hours
234 while at the rest stations, and time in resting pen since unloading (h). A significance

235 level of 10% was used to determine which variables would be considered for
236 inclusion in the multivariable models (i.e., $\alpha=10\%$).

237 *Mixed multivariable logistic regression models*

238 Mixed multivariable logistic regression models with random intercepts for load and
239 group were fitted for the lying and eating behaviours, and a multivariable ordinary
240 logistic regression model was fitted for drinking behaviour. Using a manual backward
241 elimination approach, variables considered for inclusion in the model were retained if
242 they were statistically significant or acted as an explanatory antecedent or distorter
243 variable (i.e., confounder). A variable was considered a confounder if its removal
244 caused a 20% or greater change in the coefficient of a statistically significant variable
245 and it met the causal criteria (i.e., it was not an intervening variable). Source of cattle
246 was not included in these models due to the small number of loads per source. Two-
247 way interactions were then tested between bedding treatment and all the
248 independent variables examined. Interaction terms and their main effects were
249 retained in the model if statistically significant. The homoscedasticity and normality of
250 the best linear unbiased predictors (BLUPs) were evaluated graphically. Pearson
251 residuals were examined in all models to identify outliers. Variance partition
252 coefficients (VPC) were calculated, using the latent variable technique, for each
253 multi-level model to determine the amount of the total variance in the outcome that
254 could be attributed to the animal, group, and load levels (Dohoo et al., 2009).
255 If the variance components for a model were extremely small (e.g., $<1 \times 10^{-5}$), their
256 inclusion did not improve the fit of the model based on a likelihood ratio test, and the
257 model coefficients and p-values were not notably changed by their inclusion, or the
258 model could not converge with their inclusion, then an ordinary multivariable logistic
259 regression model that included robust standard errors that account for the

260 observations not being independent by group was fitted. For these models, Pearson
261 residuals were examined to identify potential outliers.

262 **Results**

263 A total of 14 loads, 922 animals (bedded=452; non-bedded=470), and 43,918 animal
264 observations (922 animals observed 48 times, except for one load that left after
265 observation 46 and two observations missed on different loads) were included in the
266 study. Space allowance in all pens was in agreement with the Beef Cattle Code of
267 Practice (Beef Cattle Code Development Committee, 2013) providing enough space
268 so that all animals were able to lie down at the same time and to easily navigate
269 around the pen. The behaviour of cattle, independent of bedding, was dynamic
270 through the 8-h observation period (Figure 2): most (51.9%) cattle ate during the first
271 hour after unloading, while 11.9% lay down, and 2.2% were observed drinking. The
272 behavioural priorities of the cattle changed during the second hour after arrival
273 (Figure 2): most (52.0%) laid down, while still 30.6% ate and 0.9% were observed
274 drinking. Between hours 2 and <6 their behaviour was somewhat stable (Figure 2):
275 about 60% of the cattle were lying, while approximately 20% were eating and roughly
276 1% were observed drinking. After 6 h since being unloaded, the proportion of cattle
277 lying increased to more than 75%, while the proportion of animals eating dropped to
278 <15%, while the proportion of animals drinking remained under 1% (Figure 2).

279 ***Descriptive statistics***

280 Descriptive statistics of continuous variables are summarized in Table 1. Most loads
281 transported steers (57.1%; n=8), followed by loads transporting heifers (28.6%; n=4)
282 and mixed loads (14.3%; n=2). Most loads had departed from feedlots (42.8%; n=6),

283 followed by farms (28.6%; n=4), auction markets (14.3% n=2), and assembly yards
284 (14.3%; n=2). Two loads of market weight cattle originated from the same feedlot and
285 the same pen and arrived at the rest station at the same time, however the drivers
286 were reluctant to split the loads for the study, so each truck load was assigned to
287 either a bedded or a non-bedded pen. Cattle were transported a median of 1,565.0
288 km from their point of departure to the rest station, with a mean (\pm SD) of 1,387.4 km
289 (\pm 241.50 km). Most loads (10, or 71%) were travelling east while the balance were
290 travelling west (four or 28%).

291 ***Multivariable mixed logistic regression model of lying behaviour***

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

299 Groups rested in non-bedded pens showed lower odds of lying as truck's motion
300 duration increased, whereas those rested in bedded pens had greater odds (Figure 3
301 A). The interaction between bedding treatment and time in the resting pen since
302 unloading showed that early in the observation period, the odds of observing cattle
303 lying were higher in the bedded groups, but both groups had an increase in the
304 probability of lying over time that plateaued at similar levels near the end of the 8-
305 hour observation period (Figure 3 B).

306 ***Multivariable mixed logistic regression model of eating behaviour***

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

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

322 The provision of bedding significantly increased the odds of observing cattle eating
323 for cattle rested at a moderate space allowance (Table 4). Cattle rested in bedded
324 pens with a low space allowance had significantly lower odds of being observed
325 eating compared to cattle rested in a moderate space allowance (Table 4). In
326 contrast, cattle rested in bedded pens with moderate space allowance had
327 significantly higher odds of being observed eating compared to cattle rested in pens
328 with high space allowance (Table 4).

329 ***Multivariable logistic regression model of drinking behaviour***

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

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

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

342 ***Diagnostics for all models***

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

346 **Discussion**

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

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

359 ***Lying behaviour***

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

368 Flint (2013) observed 81 loads of cattle at a commercial rest station and found no
369 association between transport duration and lying or eating behaviour. However, cattle
370 in Flint's study were only rested in dirt pens, which in light of our findings, suggests
371 that when bedding is provided, lying is prioritized with longer trips. Also, in our study,
372 transport duration was split into the truck's motion and stationary durations, allowing
373 us to explore in more detail the effect of each component of transit.

374 Pettiford et al. (2008) found that creatine kinase, a biomarker used to measure
375 muscle fatigue, increased steadily plateauing around 4 h into a 6-h road transport
376 journey, indicating that as transport duration increased, muscle fatigue also

377 increased due to cattle exerting effort to maintain balance during the trip or from
378 prolonged periods of standing. Our results showed that trailer's motion duration,
379 rather than transport duration, better explained cattle's behaviour at a rest station.
380 Kenny and Tarrant (1987) confined steers for 1 hour in a transport truck and
381 compared the physiological responses of the animals when the truck was either
382 stationary or in motion. They concluded that the motion of the vehicle was more
383 stressful than confinement itself (Kenny and Tarrant, 1987). In line with their findings,
384 we postulate that the motion of the trailer, rather than the transport duration, *per se*,
385 may increase cattle fatigue most significantly; cattle may exert more energy when
386 trying to maintain balance while the truck is moving than when it is stationary perhaps
387 due to trailer movement and the weight adjustments necessary to maintain stability.
388 The effect of time in pen since unloading on lying behaviour also depended on the
389 provision of bedding. Several studies have reported that lying is a priority behaviour
390 for cattle (Marti et al., 2017; Meléndez et al., 2020; Munksgaard et al., 2005;
391 Munksgaard & Simonsen, 1996). Lactating dairy cattle lie down sooner and for longer
392 periods of time after being forced to stand, even for a period of time as short as 4 h
393 (Norrington and Valros, 2016). When dairy cows were deprived from both, lying and
394 eating, their lying time increased compared to when they were not restricted (Metz,
395 1985; Munksgaard et al., 2005). In our study, cattle rested in bedded pens had
396 greater odds of lying in the early observation period, which agrees with the findings of
397 Tucker et al. (2018) who reported a shorter latency to lie down once cattle were able
398 to access a deep-bedded area (10 cm of straw over 30 cm of sand) after 4 h of lying
399 deprivation compared to non-deprived cows. Both groups, bedded and non-bedded,
400 had greater odds of lying compared to any other behaviour, as time in pen increased,
401 however the bedded groups showed greater odds of lying in the early observation

402 hours, compared to the non-bedded groups. We also observed that near the end of
403 our 8-h observation period, the odds of lying in both groups were lower and cattle
404 switched to other activities (e.g., eating, drinking, walking) instead of lying. The
405 decrease of lying activity started earlier in the bedded groups than in the non-bedded
406 ones, and the decrease was more pronounced in the bedded groups than in the non-
407 bedded ones. These results suggest that bedding not only elicits lying behaviour in
408 cattle, but may also improve their rest, as they were more likely to switch from lying
409 to other activities near the end of our observation period. Previous studies showed
410 that fatigued beef cattle chose to lie down rather than eat after 20-h (Marti et al.,
411 2017) and 36-h (Meléndez et al., 2020) road trips. Furthermore, dairy cattle chose to
412 spend the majority of their time lying down rather than eating after being deprived of
413 both (Metz, 1985; Munksgaard et al., 2005; Tucker et al., 2018). Munksgaard et al.
414 (2005) hypothesized that this behavioural exchange happens because cattle can
415 compensate eating deprivation by increasing their eating rate while they cannot
416 compensate for lying and rest, which must be recuperated in real-time.

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

421 We observed that estimated animal weight affected lying behaviour, independent of
422 bedding treatment. Our findings agree with previous research reporting that, in
423 general, heavy cattle spend more time lying compared to lighter cattle (Dikmen et al.,
424 2012). Flint (2013) described that heavier cattle might have higher energy reserves
425 compared to lighter cattle, leading them to lie down rather than eat while at rest
426 stations. Another potential explanation is that heavier cattle may experience more

427 fatigue while in transport, with more weight, more energy exertion might be needed to
428 maintain balance while in motion.

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

435 ***Eating behaviour***

436 The effect of motion duration on eating behaviour varied with provision of bedding;
437 bedded cattle showed little change in their odds of eating as truck's motion duration
438 increased, whereas non-bedded cattle showed a notable increase with longer motion
439 duration of the truck. This effect was opposite to the one observed on lying
440 behaviour. This finding suggests that cattle either lie down or eat while at a rest
441 station, which is not altogether surprising given that both are basic needs. After being
442 restricted from eating for long periods of time, cattle need to eat to replenish their
443 energy for basic biological processes as well as to replenish the energy required to
444 stand, maintain their balance during the trip, and possibly to move around to a
445 preferred space in the trailer. On the other hand, lying itself has also been found to
446 be an important behaviour for cattle as explained above (Marti et al., 2017; Meléndez
447 et al., 2020; Munksgaard et al., 2005). Our results agree with previous studies that
448 found a trade-off between eating and lying (Meléndez et al., 2020; Munksgaard et al.,
449 2005; Munksgaard & Simonsen, 1996).

450 The effect of time in pen since unloading on eating behaviour also varied with
451 provision of bedding. Again, this effect was opposite to the one observed for lying
452 behaviour; as time in pen increased, the odds of observing cattle eating were lower
453 for both groups, however groups rested with bedding showed an increase in the odds
454 of eating near the end of the observation period, whereas cattle without bedding
455 showed a steady decrease in the odds of eating, which suggests that cattle in non-
456 bedded pens were more likely to be lying down at the end of the observation period.
457 Flint (2013) observed cattle at a commercial rest station and reported that their
458 behaviour in the first 2 hours was significantly different than in the following 3 hours;
459 cattle ate before they lay down. The increase in the odds of eating near the end of
460 the observation period in bedded cattle, and together with the results from the lying
461 behaviour model, suggests that eating and lying behaviours are traded-off with one
462 another after long distance transport. Our results are similar to those of Knowles et
463 al. (1999) who transported groups of cattle by road for 14 - 31 h and rested them in
464 bedded pens after the trip. They reported a second bout of standing and eating about
465 8 h after arriving to their destination (Knowles et al., 1999). Their study (Knowles et
466 al., 1999) used market weight beef cattle, whereas our study had groups of young,
467 weaned calves, feeders, and market weight cattle. The diversity of weight and age
468 could explain the differences observed between studies.

469 Space allowance in the pen influenced eating when bedding was provided, and the
470 effect was complex and non-linear. Comparing cattle rested in bedded to those in
471 non-bedded pens, we found that the odds of observing eating behaviour were greater
472 only at a moderate space allowance. It is possible that cattle rested under these
473 conditions might have had a comfortable surface and enough space so as to
474 influence their motivation to eat, either by eating for longer periods of time or visiting

475 the feeder more often. With low space allowance it is possible that the feeder space
476 was limited, thus there were cattle constantly at the feeder and provision of bedding
477 had no effect on their behaviour. Cattle rested with high space allowance (more
478 room) might have had easier access to the feeder at the same time, thus they might
479 have satiated their hunger around the same time in bedded and non-bedded pens.
480 We also found that when cattle were rested in bedded pens the odds of observing
481 eating behaviour were greater at moderate space allowance compared to low and
482 high space allowances. These results suggest that cattle were more motivated to eat
483 in bedded pens at moderate space allowance, possibly because bedding provided a
484 more comfortable surface under this space allowance that allowed cattle to stay
485 standing at the feeder for longer periods. Non-bedded pens had no difference in
486 eating behaviour at different space allowances.

487 Results showed that estimated animal weight affected eating behaviour, independent
488 of bedding treatment. Previous research has found similar results, where heavier
489 cattle ate less frequently than lighter cattle (Dikmen et al., 2012; Flint, 2013). Heavy
490 cattle in our study were market weight cattle (>550 kg), all sourced directly from
491 feedlots, and were being transported to an abattoir. Given their point of origin, it is
492 possible that these cattle had ad libitum access to feed and water until the loading
493 time, whereas lighter cattle, sourced from auction markets, assembly yards, and
494 direct from farms, may have been off feed and water for longer prior to transport,
495 perhaps with their hunger exacerbated further for those weaned and sold through an
496 auction market. Another possible explanation is that lighter cattle are growing, and
497 their ruminal capacity is smaller, whereas market weight cattle have reached maturity
498 with a larger ruminal capacity; lighter cattle might need to eat more frequently than
499 heavy cattle to meet their nutritional requirements.

500 ***Drinking behaviour***

501 Bedding treatment did not affect drinking behaviour at the rest stations. This result
502 was not surprising given that drinking is much shorter in duration compared to lying
503 or eating, thus cattle would not need to trade-off drinking for lying or eating. Also,
504 because drinking is short in duration, it is more difficult to observe when using
505 instantaneous scan sampling at 10-min intervals. Mitlöhner et al. (2001) found that
506 10-min interval scan samplings was less precise for measuring drinking behaviour
507 when compared to continuous observation. Thus, there may be differences in the
508 drinking behaviour of cattle rested in bedded vs non-bedded pens, but the sampling
509 method used might have not been adequate to detect such differences.

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

518 Truck's motion duration affected drinking behaviour, the odds were greater when
519 short and moderate motion duration were compared to long duration. This result
520 might seem paradoxical, however with longer transport duration, cattle might feel
521 thirstier upon arrival, causing them to approach the water trough or bowl sooner and
522 drink faster, satiating their thirst early in the observation period and thus not returning
523 to drink later. Al-Ramamneh et al. (2012) observed goats and sheep using
524 continuous recordings of all the study animals from a subsample of the experimental

525 days and reported that water consumption per minute increased in goats and sheep
526 after water deprivation for 21 h and 42 h. As noted previously, depending on the
527 interval, scan sampling may be less than ideal for capturing short-duration
528 behaviours, such as drinking (Mitlöhner et al., 2001). However, without access to
529 more advanced technological options, scan sampling remains one of the most viable
530 methods for capturing such behaviors in field settings.

531 Drinking behaviour was also affected by time in pen since unloading. Cattle in rest
532 stations have previously been recorded drinking in the hours soon after arrival. Flint
533 (2013) observed a peak in drinking behaviour of cattle unloaded at a rest station
534 during the early minutes in the pen, decreasing as time elapsed, being close to zero
535 percent of animals drinking 1 h and 20 min after arrival. As mentioned previously, it is
536 possible that once cattle drank at the rest station, they satiated their thirst and spent
537 the balance of the observation time engaged in other activities.

538 Fourteen loads participated in this study, which is a much smaller sample size
539 compared to Flint's study (2013). This could be a limitation because of the potential
540 for type 2 errors. Conducting this study under commercial conditions prevented us
541 from measuring individual eating rates or using behavioural data loggers that would
542 yield individual data on lying and eating duration to compare differences in latency
543 and duration of the behaviours studied. As mentioned before, the method used to
544 record drinking behaviour is not the most accurate one for this type of behaviour,
545 thus a bolus or a flow meter would allow for higher accuracy data collection and
546 could be used in future studies to measure water consumption and record the
547 individual activity of animals at the waterer.

548 An additional limitation relates to correlations among certain predictor variables. For
549 example, in the Lying and Eating behaviour models, truck motion duration was

550 correlated with remaining daylight hours, and source of cattle was correlated with
551 estimated animal weight. To avoid multicollinearity, we included only one variable
552 from each correlated pair based on biological relevance and model fit. We
553 acknowledge, however, that this limits our ability to determine whether the
554 associations observed are driven by one variable or the correlated companion, and
555 this should be interpreted accordingly.

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

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569 **Competing interests statement**

570 The authors declare there are no competing interests.

571 **Author contributions**

572 **Paula Olivares Guzmán:** Conceptualization, data curation, formal analysis,
573 investigation, methodology, writing – original draft **David L. Pearl:** Conceptualization,
574 formal analysis, methodology, supervision, validation, visualization, writing – review &
575 editing **Karen S. Schwartzkopf-Genswein:** Conceptualization, funding acquisition,
576 methodology, project administration, supervision, validation, visualization, writing –
577 review & editing **T.M. Widowski:** Conceptualization, methodology, supervision,
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589 **Data availability statement**

590 None of the data were deposited in an official repository but are available from the
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706

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.

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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).

Figures

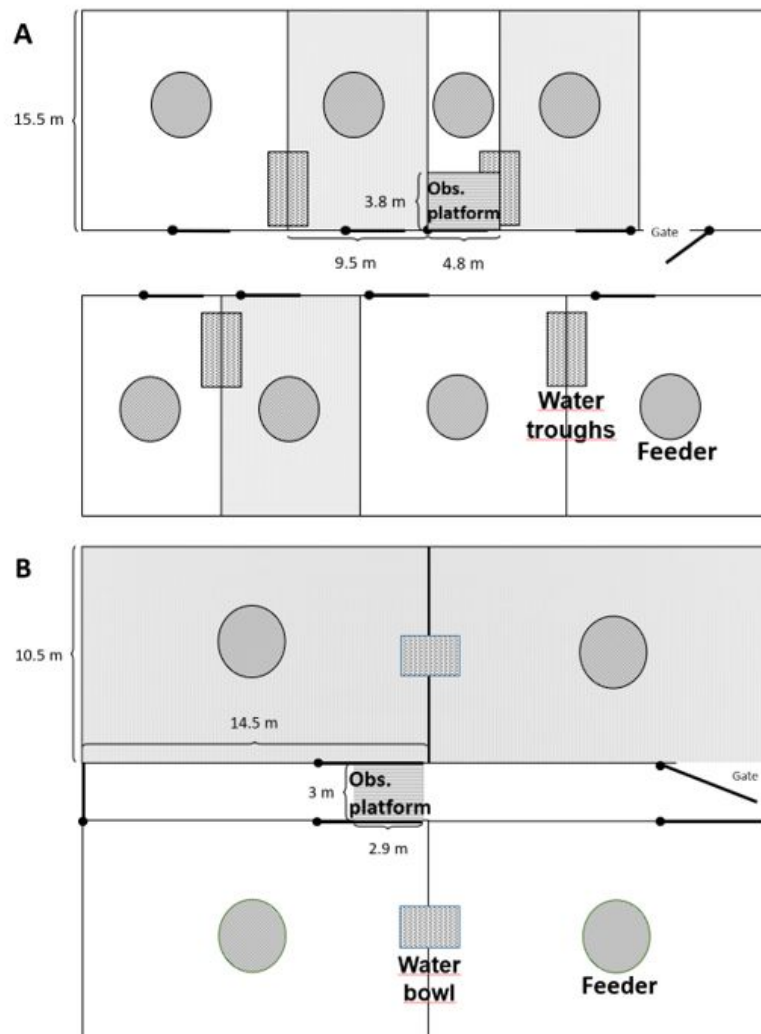



Fig. 1. Diagram of locations and measurements of pens used ( = pen used) for this study at two commercial rest stations.

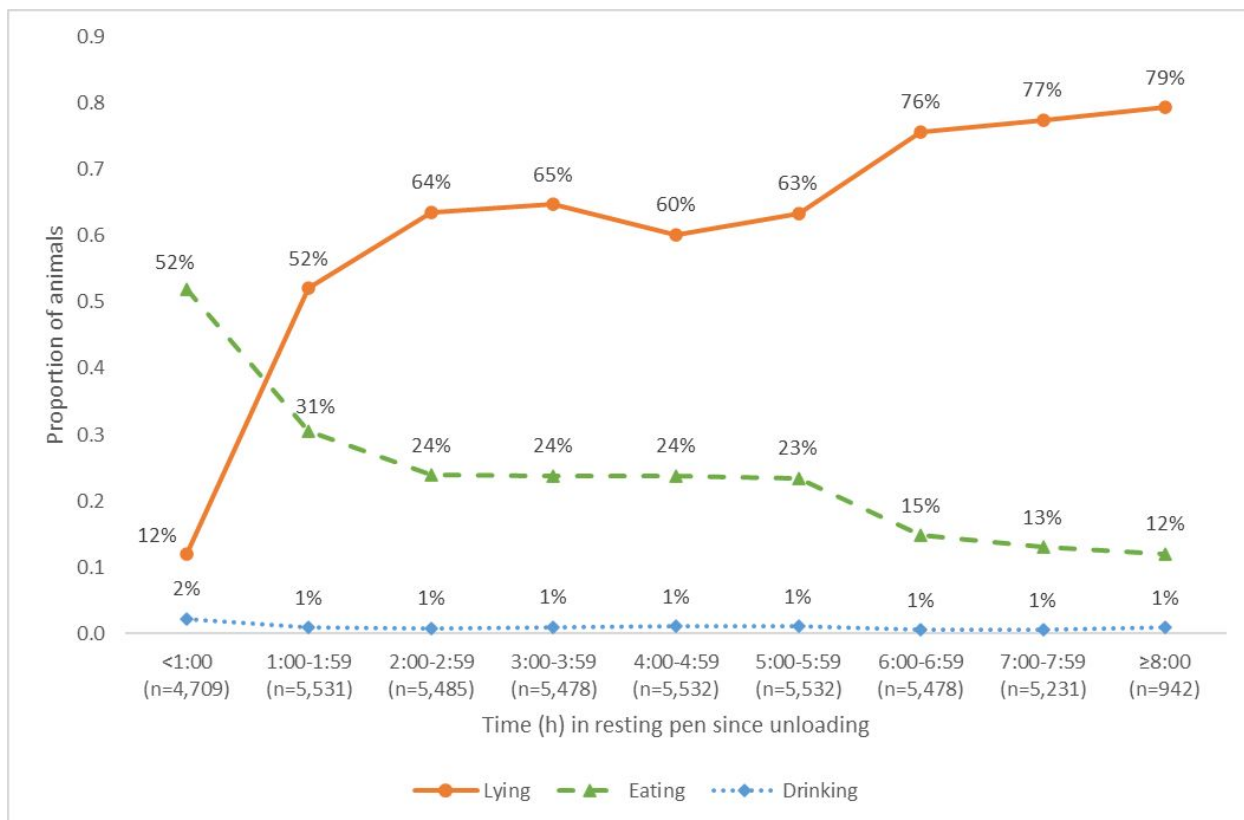


Fig. 2. Mean proportion of cattle lying, eating, and drinking in resting pens since unloading at two rest stations.

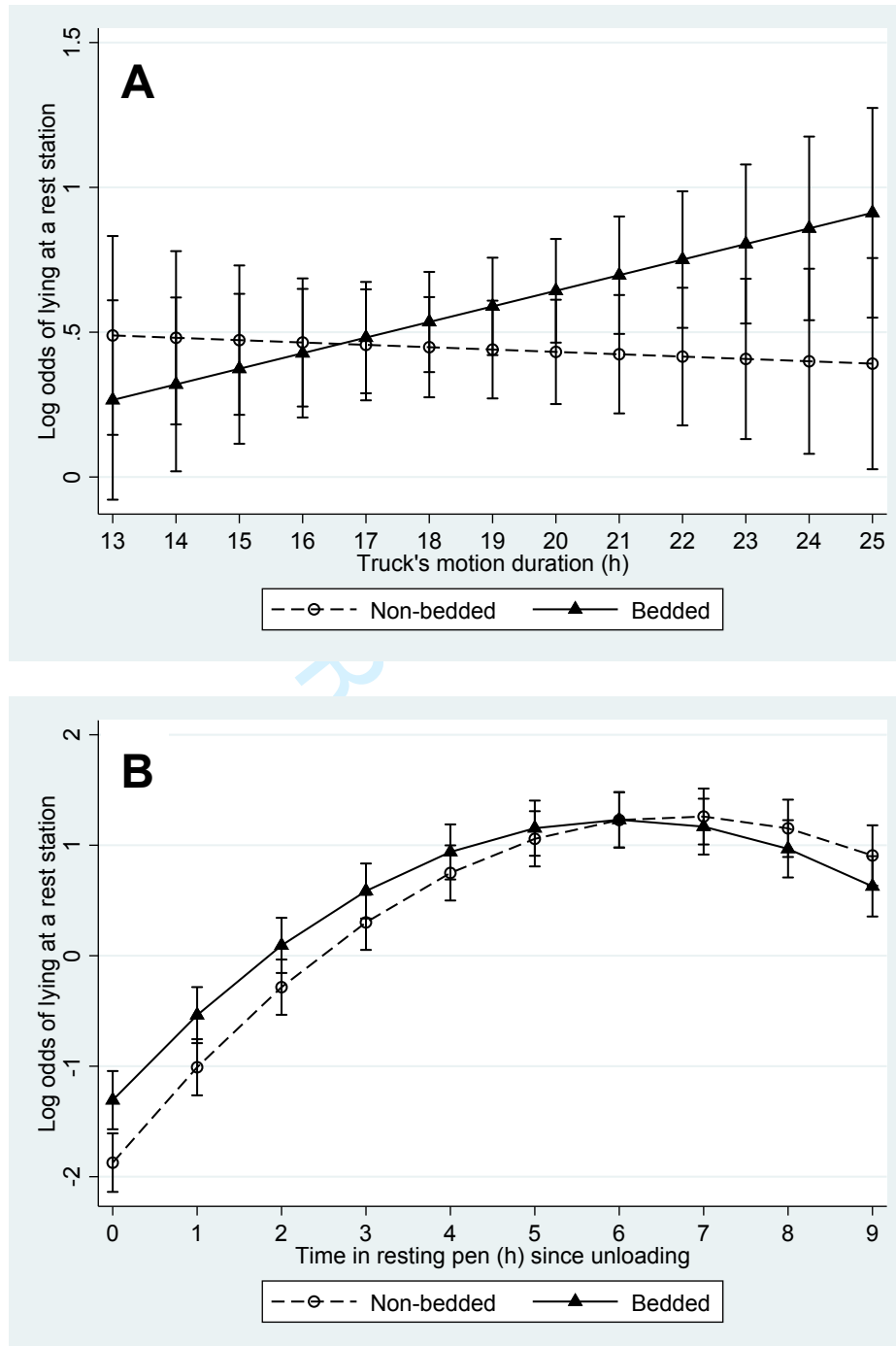


Fig. 3. The impact of (A) the duration of time the truck was in motion (h) and bedding treatment (non-bedded and bedded) and (B) time in the resting pen (h) since unloading and bedding treatment on the predicted log odds of observing cattle lying at two rest stations with 95% confidence intervals.

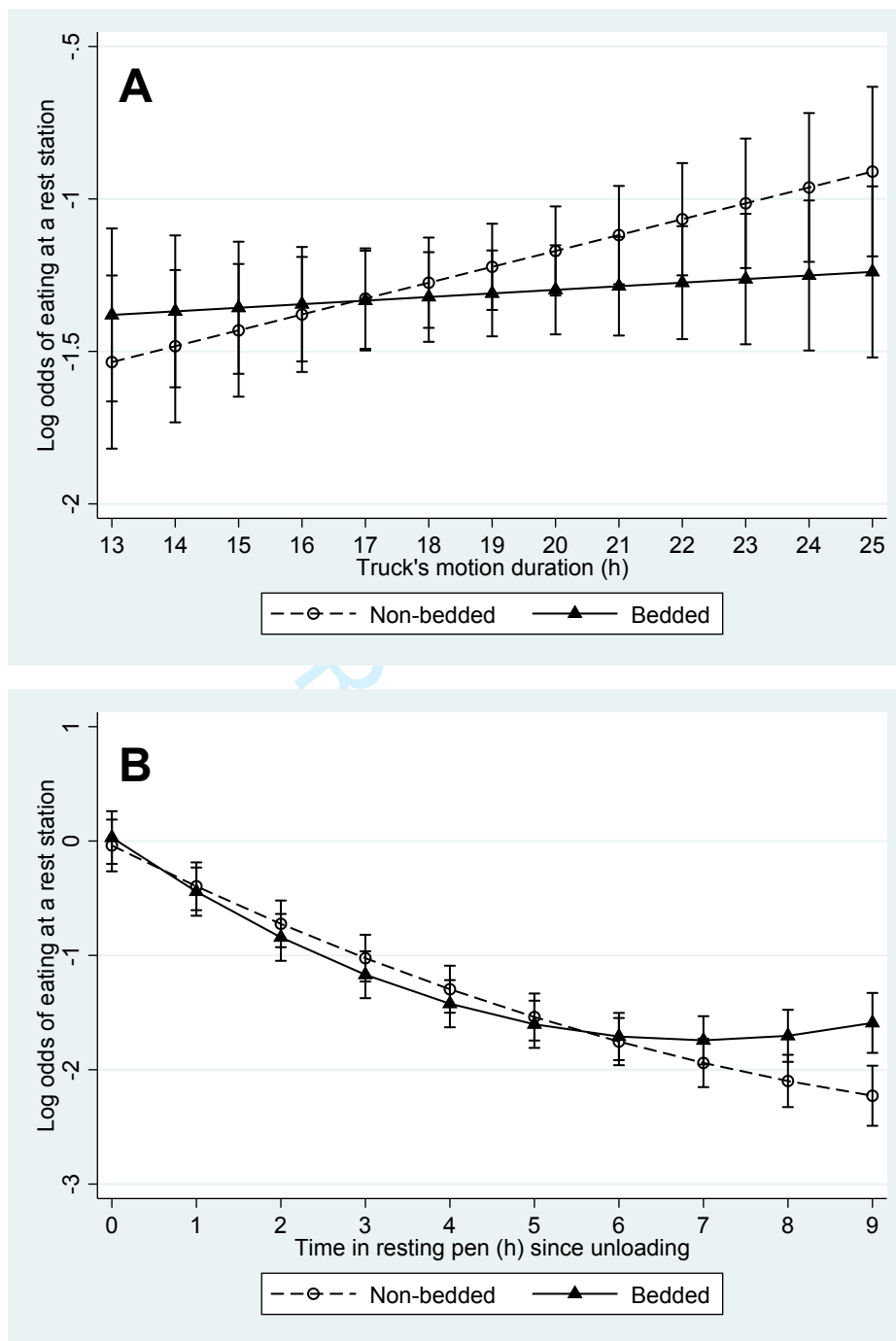


Fig. 4. The impact of (A) the duration of time the truck was in motion (h) and bedding treatment (non-bedded and bedded) and (B) time in the resting (h) pen since unloading and bedding treatment on the predicted log odds of observing cattle eating at two rest stations with 95% confidence intervals.