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1	Lying behaviour of housed and outdoor-managed pregnant sheep
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ABSTRACT

12 Lying behaviour has been shown to be highly valuable in supporting the productivity and welfare of 13 cattle. The aim of this experiment was to investigate the effect of biological and physical factors on 14 the lying behaviour of sheep. Ninety-six Bluefaced Leicester x Welsh Mountain crossbred (Mule) 15 ewes managed to lamb indoors, and 80 Welsh Mountain (WM) ewes managed to lamb at grass were 16 used for the study. Acceleration values were collected for the two flocks from accelerometers fitted 17 vertically to the outside of the rear right leg and set to record at 1-min intervals for at least 14 d prior 18 to parturition. Ewes were simultaneously recorded using video equipment to identify lambing and to 19 verify predictions of lying (total lying time, mean lying bout duration and total number of lying bouts) 20 using data collected from 10 randomly selected ewes from the indoor flock on day -10 prior to 21 lambing. Linear regression was used to evaluate predicted behaviours with video footage. Predictions 22 of total lying time ($R^2 \ge 0.99$; P > 0.05 for slope = 1, intercept = 0), mean lying bout duration ($R^2 \ge 0.99$) 0.99; P > 0.05 for slope = 1, intercept = 0) and total number of lying bouts ($R^2 \ge 0.98$; P > 0.05 for 23 24 slope = 1, intercept = 0) were strongly associated with video footage (P < 0.001) demonstrating that a 25 1-min sampling interval provides reliable estimates of ewe lying behaviours. Measures of lying (mean 26 daily lying time, mean lying bout duration and mean daily lying bouts) were calculated for all ewes 27 using averages taken across days -10, -9 and -8 prior to lambing. Linear regression was used to test 28 for effects of independent variables (pregnancy scan result (single- or twin-bearing), ewe age, ewe 29 BCS, lambing ease, lamb sex and lamb birth weight) on each measure of lying. Significant 30 associations (P < 0.05) were found between measures of lying and pregnancy scan result, ewe age, 31 sex of singleton lambs and twin birth weight for housed, Mule ewes. Only ewe age and twin birth 32 weight were significantly associated (P < 0.05) with measures of lying for WM ewes managed at 33 grass. This information could help guide further research on sheep behaviour for management 34 purposes (e.g., to optimise stocking densities and welfare for pregnant ewes). Further work should 35 also consider evaluating measures of lying as proxies for imminent parturition. 36 Keywords

37 Sheep; Behaviour; Accelerometers; Pregnant; Lying behaviour; Lambing

1. INTRODUCTION

39 The use of precision sensors to monitor the behaviour and performance of livestock has been shown to be highly valuable, and significant research and development have been undertaken in support of 40 41 the management of cattle (Caja et al., 2016) and other intensively managed animals (Benjamin and 42 Yik, 2019; Li et al., 2020). Discrete, on-animal sensors are now providing previously unattainable 43 information, allowing for a better understanding of livestock behaviour for managing comfort (Molina 44 et al., 2020) and production disorders (Wagner et al., 2020) using sensors such as pedometers 45 (Edwards and Tozer, 2004) and accelerometers (Reiter et al., 2018). Further development of 46 integrated systems on farms will provide farmers with the information necessary to make management 47 decision at the level of the individual animal as well as at flock or herd level. Some sectors of the 48 livestock industry have seen a greater emphasis on the development of precision sensors and systems, 49 in-part because of the economic necessity and incentive to do so such as in the dairy industry (Rutten 50 et al., 2013). For example, the fertility management of cattle has developed greatly in recent years 51 with information such as feeding duration and activity used to support the optimum time for artificial 52 insemination (Mottram, 2016).

53 More recently, it has been demonstrated that the management of sheep could also be 54 supported by on-animal sensor technology. For example, accelerometers were used to measure the 55 activity of growing lambs showing promise for the early detection of behavioural changes associated 56 with parasitism (Ikurior et al., 2020). Research has also been undertaken to assess the feasibility of 57 recording other measures from sheep including attributes of movement for identifying gait anomalies 58 associated with lameness (Barwick et al., 2018), feeding behaviour (Giovanetti et al., 2017) and also 59 parturition (Fogarty et al., 2020). However, few studies have assessed the behaviour of sheep in 60 commercial settings. This will be needed to effectively differentiate optimal from suboptimal 61 performance. Lambing represents an important period in the production cycle for ewe comfort and 62 welfare but relatively little is known about the behaviour of pregnant sheep. Often, shepherds are in 63 increased contact with sheep during this time and this presents an opportunity to test the feasibility of 64 using well-established activity monitoring techniques. Lying behaviour has been shown to be a highly 65 valuable metric of behaviour in other species (Jensen, 2012; Blackie et al., 2011) and can be measured and monitored using accelerometers. These methods require low computational power and data
processing and have been shown to be highly valuable in identifying indicators to support the
management of other livestock (Ito et al., 2009). To date, no exploratory analyses on the lying
behaviour of pregnant sheep have been undertaken using accelerometers. This information could be
useful to better understand the behaviour of sheep particularly during times of increased stress (e.g.,
lambing) and provide information on the degree of variability within measures of lying that might be
present in a flock.

73 The aim of this experiment was to investigate the effect of biological and physical factors on 74 the lying behaviour of pregnant sheep. The objectives were 1.) to validate the use of leg-mounted 75 accelerometers for measures of lying behaviour 2.) explore whether commonly measured biological 76 and physical attributes of sheep around the time of lambing contribute to variability in measures of 77 lying behaviour. To achieve this, both indoor- and outdoor-lambing flocks were studied, representing 78 common UK sheep production systems. We hypothesised that variation in economically important 79 attributes of sheep (e.g., age, BCS and lambing ease), would partly explain variations in lying 80 behaviour during the final trimester of pregnancy.

2. MATERIALS & METHODS

82 The study was approved by Aberystwyth University Animal Welfare and Ethical Review Board on
83 18th December 2018.

84 2.1. Study farms

85 Data were collected at two different farms representing lowland (Farm A; Gogerddan farm, Aberystwyth University, Wales) and upland (Farm B; Llysfasi Farm, Coleg Cambria, Wales) 86 87 commercial lamb-producing enterprises between February and May 2019. Farm A lies approximately 88 40 m above sea level and is situated 6 km north-west of Aberystwyth, Ceredigion, Wales. The farm 89 comprises approximately 1,000 ewes of predominantly Welsh Mule-type sheep (Bluefaced Leicester 90 x Welsh Mountain (WM) crossbred ewes), with a small number of both Beulah and pure-bred Texel 91 ewes that are managed at grass throughout the year and housed in January to lamb between February 92 and March. Lambs are finished at grass. Farm B ranges between 200-300 m above sea level and is 93 situated 6 km south of Ruthin, Denbighshire, Wales and includes approximately 1,000 WM ewes and 94 160 Welsh crossbred ewes. The flock is managed at grass throughout the year with ewes lambing 95 between April and May. After weaning, lambs are finished at grass with a proportion finished on 96 silage and concentrate feed.

97 2.2. General ewe management

98 The first experiment took place at Farm A. Following an autumn mating period, ewes were pregnancy scanned on the 8th and 15th January 2019 and housed in two barns with capacity for 99 100 approximately 600 ewes. Ewes were penned according to their pregnancy status, with single, twin and 101 triplet-bearing ewes managed separately in straw-bedded pens measuring 8.8 m x 6.1 m. On average, 102 each pen contained approximately 40 ewes at any given point. Ewes had *ad-libitum* access to water 103 and a complete ration comprising of grass silage and a protein blend dispensed twice per day at 0800 104 and 1600 throughout their third trimester of pregnancy. Upon lambing, ewes were moved with their 105 offspring to an individual 1.5 m x 1.5 m pen situated around the inner perimeter of each barn. Both 106 ewes and lambs remained in these single pens for at least 24 h before being moved out to pasture or to 107 a separate larger pen. The second experiment took place at Farm B. All ewes were pregnancy scanned

on the 14th February 2019 and only those selected for the experiment were brought-in to in-bye fields
surrounding the farm buildings on the 9th March 2019 prior to lambing. For the purpose of the
experiment, both single and twin-bearing ewes were lambed as mixed groups in two fields (field A;
0.66 ha and field B; 0.87 ha) close to the farm buildings. Ewes were managed at grass throughout the
lambing period, had free access to water and were supplemented with *ad-libitum* access to a glucose
lick.

114 2.3. Ewe selection and data collection

115 2.3.1. Farm A - indoor lambing

116 Of the 600 Mule ewes that were initially due to lamb at Farm A, a group of 46 single-bearing and 52 twin-bearing ewes were selected and entered the experiment on the 25th and 26th February 117 118 2019. The age of each ewe was recorded (Cocquyt et al., 2005), and its body condition score (BCS = $\frac{1}{2}$ 119 1-5; Russel, 1984) and locomotion score (locomotion score 0-3; Angell et al., 2015) recorded by a 120 single observer. All study ewes had a locomotion score of 0. Ewes were clearly spray marked on each 121 side with a unique number for identification purposes and randomly allocated to one of six pens 122 allowing for 1.3 m² per ewe. To record the lying behaviour of the experimental ewes, HOBO Pendant 123 G accelerometers (Onset Computer Corporation, Bourne, MA) were fitted to the outside of the right-124 hind leg of each ewe according to a standard operating procedure (UBC Animal Welfare Program, 125 2013). The accelerometers were fitted vertically, such that the X-axis was pointing upward and the Z-126 axis pointing left towards the midplane of the ewe. Accelerometers were configured to sample at 1-127 min intervals. This sampling interval was selected as it has been previously used to record the lying 128 behaviour of dairy goats (Zobel et al., 2015) and is well-established for use in monitoring the lying 129 behaviour of cattle (Ledgerwood et al., 2010). This sampling interval also allowed for 14 d of 130 continuous data recording which meant that ewes could remain undisturbed other than for daily 131 management. Once accelerometers were fitted, each ewe was placed back into its allocated pen where 132 it remained until it gave birth. In order to visually verify predicted behaviours and the time of 133 lambing, cameras (5MP PoE Security Camera System, Reolink) were fitted to the end of each pen 134 containing focal ewes and were set to record throughout the entire experimental period. During the

135 night, the lights in the building remained on for management purposes. Upon lambing, ewes were 136 moved to individual pens and their accelerometer removed. At this point, measures were recorded for 137 each lamb, including lambing difficulty (0 = no assistance, 1 = slight assistance, 2 = severe assistance, 2138 3 = veterinary assistance) birth weight (kg) and sex. For ewes that had not given birth within the 139 initial 14 d observation period (n = 48), the original accelerometer was removed and replaced with a 140 second accelerometer which remained in place until the ewe had given birth. Upon removal, data were 141 downloaded from each accelerometer and saved in a spreadsheet program. Other than for specific 142 experimental procedures and data capture, all ewes were managed and monitored 24 h/d by farm shepherds. Data collection was complete by 22nd March 2019 at Farm A. Three single-bearing ewes 143 144 did not lamb during the trial leaving only pre-lambing variables for testing for these ewes. Behaviour 145 data for two twin-bearing ewes were not available due to the failure of accelerometers and a further 146 two did not give birth during the trial. This left a total of 46 single-bearing ewes (n = 43 for all 147 variables) and 50 twin-bearing ewes (n = 48 for all variables) available for analysis.

148 2.3.2. Farm B - outdoor lambing

Of the ewes brought-in to in-bye fields at Farm B on the 9th March 2019, 44 were single-149 150 bearing and 45 were twin-bearing WM ewes. At this point, the age of all ewes was determined, and 151 their body condition and locomotion scores recorded as described previously. For locomotion, three 152 ewes received a score of 1 and were treated for scald by farm staff. All other ewes were scored as 0. 153 Ewes were also fitted with an accelerometer and equally split between fields A (mean over 154 experiment = 40 sheep) and B (mean over experiment = 33 sheep). Six cameras (5MP PoE Security 155 Camera System, Reolink) were fitted around the boundary of field A to record as much information as 156 possible such as the time of lambing which may have been missed by the shepherd. Once ewes in 157 field A had given birth, they were replaced with ewes from field B in order to maximise the number 158 of ewes captured on video giving birth. Ewes were regularly monitored by a shepherd at least 3-times 159 daily between 06:30 h-08:30 h, 11:30 h-13:30 h and 16:00 h-18:00 h. All observations were made 160 from the boundary of the field and closer inspection was only undertaken when necessary. Shortly 161 after lambing, measures were recorded for each lamb as described for Farm A and if required, video

162 footage was used to verify the time of birth. At this point, accelerometers were removed, and data 163 downloaded. Accelerometers were replaced on ewes that had not given birth (n = 59) within the initial 164 14 d observation period before being returned to their respective field. Only seven ewes required any 165 level of birthing assistance in the flock monitored at Farm B, so this factor was not included in this 166 analysis. Data collection was complete by 1st May 2019 at Farm B. Data for three single-bearing ewes 167 were missing due to a fault with the accelerometers. One other single-bearing ewe did not lamb during 168 the trial and so analysis of post-birth data was not possible for this ewe. For twin-bearing ewes, six 169 accelerometers failed to function. This left a total of 41 single-bearing ewes (n = 40 for all variables) 170 and 39 twin-bearing ewes (n = 39 for all variables) available for analysis.

171 2.4. Data processing

172 Accelerometer data (g-force values) for each ewe were processed using R statistical software 173 (R Core Team, 2019) in a program adapted from that developed by UBC Animal Welfare Program 174 (UBC, 2013) based on the method of Ledgerwood et al. (2010). Guidance provided by Zobel et al. 175 (2015) for accelerometer cut-off values was used to determine whether the ewe was in a lying (or 176 standing) position and these values were incorporated into the program. Lying laterality was not 177 explored in this experiment as further work is required to determine suitable accelerometer thresholds 178 for sheep. Behaviours computed from the raw accelerometer data included lying time (min/d), lying 179 bouts (n/d) and lying bout duration (min/bout) using the X-axis data only. These behaviours were 180 calculated for each ewe, for each day that the accelerometer remained in place. It has been shown 181 previously that at least 3 d of behaviour data are required to obtain a reliable estimate of the behaviour 182 of cattle (Ito et al., 2009) and a similar method has been applied to dairy goats (Zobel et al., 2015). In 183 order to reliably estimate the behaviours of each ewe, the computed values for measures of lying were 184 standardised by averaging three days of data in the 10-d period prior to the day of lambing. These were days -10, -9 and -8 prior to lambing for each ewe as it was hypothesised that the behaviour of 185 186 ewes may be influenced by the onset of lambing (behaviour change within 24 hrs of birth) (Echeverri 187 et al., 1992; Fogarty et al., 2020). This procedure was repeated for all ewes at both farms. For ewes 188 that had not given birth in the initial 14 d observation period (Farm A = 48 sheep; Farm B = 59

sheep), two spreadsheets were saved, and the second spreadsheet processed for measures of lying for this experiment. For all ewes, the first day of accelerometer data was ignored, as were data recorded on the day of accelerometer changeover (if necessary). This was to allow for a 24 h settling-in period. A total of 10 ewes (n = 5 single-bearing, n = 5 twin-bearing) from Farm A were randomly selected to verify lying behaviours with recorded camera footage (24 h for each ewe).

194 2.5. Statistical analysis

To validate the accuracy of the accelerometer measurements, estimates of total lying time, total number of lying bouts and mean bout duration were compared with video footage taken on day -10 before lambing for 10 randomly selected ewes at Farm A (24 hrs each for 5 single- and 5 twinbearing ewes) using linear regression. Transitions between lying and standing were recorded on a per second basis and rounded to the nearest minute in a spreadsheet program for analysis with predicted behaviours. Regression slopes and intercepts were evaluated to see whether they differed significantly $(P \le 0.05)$ from 1 and 0 respectively.

202 Two of the three measures of lying were not normally distributed; therefore, median values 203 were used to assess all measures (lying time (h/d), lying bouts (n/d) and lying bout duration 204 (min/bout)) separately for both farms. The outcome variables "lying bouts per day" and "lying bout 205 duration" were log transformed to achieve data normality. Single variables were then tested for their 206 association with the 3 measures of lying using simple linear regression separately for both farms with 207 ewe within each farm as the observational unit. For Farm A, empty models were first tested to 208 estimate the random effect of pen (1-6), but the variation explained by pen was found to be non-209 significant (P > 0.05). Single variables associated with ewes at both farms included pregnancy scan result (single or twin), age $(1 \ge 5)$ and BCS (1-5). Variables associated with lambs at both farms 210 211 included lamb sex and lamb birth weight (kg). Only seven ewes required birthing assistance at Farm B 212 so lambing ease (0-3) was not analysed for this farm. No ewes at Farm A scored 3 for lambing 213 assistance. The statistical significance of a relationship was declared when the probability of the 214 regression slope differing from zero was ≤ 0.05 . All statistical analyses were undertaken in R (R Core 215 Team, 2019).

3. RESULTS

217 3.1. Associations between accelerometer predictions and video recordings

218 Results for linear regression models between predicted measures of lying and those verified by video

219 recordings for 10 sheep at Farm A are shown in Table 1. A strong linear relationship was found for all

- three measures of lying (P < 0.001) with coefficients of determination ≥ 0.99 , 0.99 and 0.98 for total
- 221 lying time (min/d), mean lying bout duration (min/bout) and total number of lying bouts (n/d)
- 222 respectively. With the accelerometer set to sample at 1-min intervals, accurate estimates of lying
- behaviours were obtained with each regression slope and intercept not differing significantly from 1
- **224** and 0 respectively (P > 0.05).

225 3.2. Measures of lying behaviour of housed and outdoor managed ewes

Ewes (n = 96) studied at Farm A (Figure 1A) spent a median duration of 13.1 h/d lying down ($25^{th} - 75^{th}$ percentile = 11.9 – 14.3 h/d), had a lying bout frequency of 26.8 bouts/d ($25^{th} - 75^{th}$ percentile = 23.3 – 31.1 bouts/d) and a median bout duration of 29.5 min/bout ($25^{th} - 75^{th}$ percentile = 25.2 – 33.6 min/bout). Ewes (n = 80) at Farm B (Figure 1B) spent a median duration of 11.7 h/d lying down ($25^{th} - 75^{th}$ percentile = 10.3 – 12.9 h/d), had a lying bout frequency of 19 bouts/d ($25^{th} - 75^{th}$ percentile = 23.1 15 – 23.8 bouts/d) and a median bout duration of 35.9 min/bout ($25^{th} - 75^{th}$ percentile = 30.8 – 45.4 bouts/d).

Univariate results for Farm A (Table 2) found that pregnancy scan result (foetal numbers) was significantly associated (P = 0.02) with the daily duration of lying of ewes. On average, twin-bearing ewes lay down 48.51 min/d longer than single-bearing ewes. However, no significant differences were found for frequency of lying bouts (P = 0.60) or lying bout duration (P = 0.46) between the groups.

There was no overall effect of age (P = 0.15) on the daily lying duration of ewes, but age was significantly associated with the number of daily lying bouts (P = 0.01) and the duration of each bout (P = 0.04). The duration of lying bouts for three-year old ewes was shorter, and the number of daily bouts was higher in this age group compared to the reference level (1-year-old ewes). 242 A significant association (P = 0.03) was found between the sex of singleton lambs and the daily lying time of ewes. Ewes carrying male lambs lay down for a shorter (-67.07 min) daily 243 244 duration. No associations were found between the sex of singletons and the other two measures of 245 lying. For twin-bearing ewes, no associations were found between the sex of lambs (male, female or 246 mixed-sexed groups) and any of the three measures of lying. Similarly, no associations were found 247 between the birth weight of singletons and any of the three measures of lying. However, of the twinbearing ewes, as twin birth weight increased there was a highly significant reduction (P < 0.001) in 248 249 the daily duration of lying (-43.07 min/d). This effect could not be accounted for by significant 250 changes to lying bout duration (P = 0.54) or lying bout frequency (P = 0.22). No other significant 251 associations were found between any of the other factors measured at Farm A and the three measures 252 of lying.

In contrast to ewes managed indoors at Farm A, no significant effect of pregnancy scan result was found for the three measures of lying behaviour for the ewes managed at the outdoor system at Farm B (Table 3). Furthermore, only the frequency of lying bouts was found to be associated with ewe age (P = 0.02). As with Farm A, no associations were found between ewe BCS and measures of lying at Farm B.

In contrast to the Mule ewes at Farm A, no significant effect was found for the sex of singleton lambs on the three measures of lying, but similarly, no effect of the sex of twin lambs was found. Effects of lamb birth weight on measures of lying were only found for twin-bearing ewes at Farm B. Increasing twin birth weight was associated with shorter lying bouts (P = 0.03) and a higher daily frequency of lying bouts (P < 0.01). Despite these associations, no significant relationship was found between twin birth weight and daily lying duration for WM ewes at Farm B.

4. DISCUSSION

The HOBO accelerometer showed accurate estimates of lying behaviour when verified against video 265 266 recordings of housed sheep. A 1-min sampling interval was sufficient in recognising 96% of lying 267 events in the 10 sheep used for verification at Farm A. Across the 10-test sheep, the average (\pm SD) 268 number of missed lying bouts was 1.5 ± 1.1 . All missed lying bouts were those that from the video 269 footage had a duration of between 30-59 s, which upon rounding were logged as a lying bout. 270 However, all lying bouts lasting < 1-minute were occasions where focal sheep were displaced, or a 271 disturbance had occurred where farm staff were operating. All lying events lasting \geq 1-min were 272 identified by the accelerometer in the verification group. Shortening the sampling interval to 30 s 273 would probably capture lying events lasting < 1-minute but this would also have the effect of halving 274 the number of days that data can be logged for. With this, we believe that a 1-min sampling interval, 275 providing 14 d of continuous data capture is sufficient and provides highly accurate estimations of 276 measures of lying of sheep as has been shown in studies of cattle (Ledgerwood et al., 2010; 277 Mattachini et al., 2013). Lying-side preference (laterality) has been found to vary considerably with 278 cattle and affected by factors such as pregnancy status and discomfort (Tucker et al., 2009). Laterality 279 was not explored in the current study. More work is required in examining the importance of lying 280 laterality in the study of sheep behaviour as has been done with cattle (Gibbons et al., 2012; Miller-281 Cushon et al., 2019) and goats (Zobel et al., 2015). If found to be useful, work will be needed to 282 accurately estimate measures of lying laterality in sheep, using appropriate thresholds from 283 accelerometer data.

284 This is the first time that measures of lying behaviour for both housed and outdoor-managed pregnant sheep have been recorded using this method. The results of the exploratory analyses provide 285 286 an objective insight into the daily lying times of pregnant ewes and show that significant variation 287 exists between individuals for measures of daily lying behaviour in each of the studied flocks. The 288 median daily lying duration, lying bout frequency and bout duration for housed sheep was 13.1 h/d, 289 26.8 bouts/d and 29.5 min/bout respectively. For the outdoor flock these values were 11.7 h/d, 19 290 bouts/d and 35.9 min/bout respectively. In a previous study, Arnold (1984) used direct visual 291 observations to measure the lying behaviour of sheep co-grazing with cattle and horses in a

292 Mediterranean environment. The average daily lying duration of sheep was 11.6 h per day which 293 broadly coincides with the results of the current study, particularly for the WM ewes at grass. Others 294 have assessed lying time as a proportion of total time using visual observations. A mean daily lying 295 proportion of 66% was found in a study examining the effect of pen size (mean = 0.75 m^2 per ewe) on 296 lying behaviour of pregnant, Norwegian Dala sheep (Bøe et al., 2006) and a lying proportion of 70% 297 in a study allowing for 1.5 m² per pregnant ewe (Jørgensen et al., 2009). In the current study, Mule ewes at Farm A spent 55% of their time lying (~1.3 m² per ewe) whereas WM ewes at Farm B spent 298 299 49% of their time lying which substantially differs from the studies noted. A low daily lying 300 proportion might have been expected for the housed flock at Farm A given the high level of staff 301 interaction and that lying space is more limited compared to when ewes are at grass. However, these 302 differences may also be explained in part by breed.

303 At Farm A, approximately 1.3 m² was available for each ewe which is within the guidelines 304 required for lowland, pregnant ewes (DEFRA, 2003). Little information is available on the expected 305 resting times of ewes in both housed and outdoor conditions, but some exploratory work has been 306 undertaken to assess the behaviour of pregnant ewes in relation to the amount floor space available in 307 housed conditions (Averós et al., 2014). It was found that ewes given a space allowance of 1 m^2 /ewe 308 travelled shorter distances but had greater levels of activity compared to ewes provided with either 2 309 or 3 m²/ewe. Although the lying bout duration of sheep at Farm B was longer than those of the housed sheep, they spent less time lying each day and had fewer daily lying bouts compared to the housed 310 311 flock. These sheep may have spent more of their time foraging throughout the paddocks and 312 responding to a greater variety of stimuli.

Some of the measured variables were shown to be significantly associated with measures of lying time in both systems. In the current study, housed sheep carrying twin-lambs had a greater daily lying duration compared to single-bearing ewes, but this was not found for the ewes managed at grass. This may have been linked to the combined weight of the developing foetuses. The average (\pm SD) combined twin birth weight of lambs born to Mule and WM ewes was 9.55 kg (\pm 1.26) and 7.34 kg (\pm 1.73) respectively. Proportionally however, the birth weight of single lambs relative to the combined weight of twin lambs was similar (65% and 64% for Mules and WM respectively). A better 320 comparison would be to assess birth weights with respect to maternal weights as has been undertaken 321 previously (Gardner et al., 2007), but these data were not available. It was also shown in that study 322 that the proportional increase in litter size relative to ewe weight was greater in Mules compared to 323 WM ewes, but it is also reported that hill breeds carry a significantly heavier litter proportional to 324 their own body weight (Dwyer and Lawrence, 2005). Both breed (e.g., ewe survival strategy) and 325 managerial factors (displacements and interventions by staff) may have contributed to the differences 326 seen between systems for daily lying time. In the housed system, although the lying time of twin-327 bearing ewes was not explained by significant changes to bout duration or the number of daily bouts, 328 these insignificant yet marginally higher figures for twin-bearing ewes likely led to higher lying 329 times. Over the course of 24 hrs, the lying time accrued amounted to significantly higher levels 330 compared to the single-bearing ewes. One hypothesis for this finding is that when sheep are managed 331 at higher densities (e.g., housed) and in close proximity, they may be more reactive to one another 332 especially when disturbed by factors external to their pen environment e.g., staff on foot or feed 333 wagon entering. This behaviour might be influenced by the inability of some to immediately see the 334 disturbance which may be different to the field environment where sheep can react more 335 independently (Sibbald et al., 2009). Anecdotally, the footage for the verification study demonstrated 336 just how receptive the housed ewes were to human movement and intervention, frequently moving as 337 entire groups in response to various stimuli.

Effects of age on measures of lying were found in both flocks although the relationships were 338 339 different. In the WM flock, only the frequency of lying bouts was associated with age with more bouts 340 undertaken in older ewes compared to younger ewes. In the housed ewes, both lying bout duration 341 and lying bout frequency were associated with age. The duration of lying bouts for three-year old 342 ewes were shorter with a greater number of daily lying bouts compared to 1-year old ewes. Parity 343 effects on lamb birth weight, litter weight and placenta weight have been reported previously with 344 significant increases in each with parities 1-3 (Dwyer et al., 2005). The authors also reported that 345 although lamb birth weights and litter weights were greater for Suffolk ewes compared to Blackface 346 ewes, litter weight expressed as a percentage of maternal weight was significantly greater for 347 Blackface ewes. Breed and environment may play a role in the behaviour of older ewes but given the

relatively few numbers available for inclusion in the higher age categories, further work would beneeded to fully evaluate these findings.

350 Interestingly, and for the housed sheep only, ewes carrying a male singleton lamb had 351 significantly lower daily lying times compared to ewes carrying female singleton lambs. Again, this 352 effect could not be fully explained by significant changes to the other two measures of lying and may 353 again be a cumulative effect of marginal reductions in lying bout duration as well as the number of 354 bouts. The average birth weight (\pm SD) of male and female singletons was 6.54 kg (\pm 0.99) and 5.98 355 kg (\pm 0.97) respectively and this may have explained the differences recorded in ewe lying time. It is 356 known that bearing male lambs can lead to increased labour duration and birthing difficulty (Dwyer, 357 2003) which can ultimately affect lamb survival. However, to our knowledge, little is known about 358 the prepartum behaviour of the ewe with respect to the sex of the lamb. This effect was not found for 359 twin-bearing ewes and it may be that for these ewes, the combined foetal weight is a more important 360 factor in defining measures of lying compared to lamb sex.

361 In both flocks, effects of increasing combined twin birth weights were found, but no effect of 362 singleton birth weight was found on any measure of lying. For housed ewes, daily lying times 363 decreased significantly with higher combined twin birth weights but neither lying bout duration or the 364 frequency of lying bouts were significantly affected. This may again relate to group behaviour in a 365 housed environment. Ewes with heavier twin-foetuses may only be losing marginal lying time per 366 lying bout compared to ewes with lighter twin-foetuses resulting in an overall significant reduction in 367 lying time with increasing twin-birth weight. One explanation may be that heavier twin foetuses lead 368 to greater lying discomfort during the final stages of pregnancy leading to increased time spent 369 standing. There are advantages to increased litter weights such as lamb survival, and other studies 370 have measured pre- and postnatal behaviours of several breeds and their associations with lamb 371 survival (Lynch et al., 1980; Rachlow and Bowyer, 1998) as well as biological factors (e.g., placental 372 efficiency) that may favour increased litter weight in hill breeds (Dwyer et al., 2003). For ewes at 373 grass, although there was no significant relationship between twin birth weight and daily lying time, 374 the duration of lying bouts decreased and the number of daily lying bouts increased significantly as

twin birth weight increased. This could again be a coping strategy for ewes with heavier twinfoetuses.

377 For housed ewes at Farm A, lambing ease was assessed, and it is perhaps unsurprising that 378 there were no significant associations between any of the three measures of lying and each level 379 within this factor. Measures of lying time were assessed more than a week prior to lambing and at this point at least, no differences in lying behaviours were detected that may be associated with difficult 380 381 parturition. It is likely that assessments would be required closer to the day of parturition and it would 382 be worthwhile to explore this as indicators of birthing difficulty would be useful for shepherds for 383 effective time management during this busy period in both housed and extensive systems. 384 In this work, measurement days were standardised (days -10, -9 and -8 prepartum) for 385 comparison between individuals and it may be the case that the associations found could change again 386 with proximity to birth (e.g., postural changes). This work could also be extended to other flocks to 387 further evaluate these predictions. It is also important to note that variables such as locomotion score 388 were not explored which could impact lying behaviour as has been shown with cattle (Thomsen et al., 389 2012; Weigele et al., 2018). Furthermore, exploring the impact of stocking density and breed on ewe 390 behaviour would also be useful. Further studies should also seek to identify whether one or more of 391 these measures of lying could be used as alternatives to more computationally intensive strategies to

392 identify parturition or ill health.

5. CONCLUSION
This is the first study that has explored the lying behaviour of housed and outdoor-managed pregnant
sheep using leg-mounted accelerometers. HOBO accelerometers accurately recorded measures of
lying time at a 1-min sampling interval showing wide variation between individual sheep. This
method can be recommended for recording flock lying behaviour over short durations. For Mule
sheep managed indoors, significant associations were found between measures of lying and pregnancy
scan result (single or twins carried), age of ewe, lamb sex (single) and the birth weight of twin lambs.
For WM sheep managed at grass, associations were only found for ewe age and twin lamb birth
weight. Further studies should seek to identify any further implications of these findings such as the
impact of stocking density on lying behaviour given pregnancy status. In addition, work should be
undertaken to assess whether simple measures of lying can be used to evaluate health status or to
predict imminent lambing which would be particularly useful for flock managers in extensive
systems.

DECLARATION OF COMPETING INTEREST

407 The authors declare no competing interest.

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	Behaviour	Intercept	SE	95% CI	P-value	Slope	SE	95%	P-value	\mathbb{R}^2
								CI		
]	Total lying (min/d)	0.23	8.39	-19.11 –	0.98	0.99	0.01	0.97 –	< 0.001	0.99
				19.58				1.02		
	Mean lying bout	0.005	0.17	-0.39 -	0.98	1	0.001	0.98 -	< 0.001	0.99
d	uration (min/bout)			0.40				1.02		
	Total lying bouts	0.58	1.92	-3.86 -	0.77	1.03	0.06	0.9 –	< 0.001	0.98
	(n/d)			5.01				1.16		

Table 1. Parameters of linear regression models for predicted measures of lying by HOBO accelerometers and behaviours recorded by video for 10 housed, Mule sheep at Farm A

						Daily	lying duration (min	Lying bout duration (log min/bout)			Frequency of lying bouts (log bouts/d)		
Variable	Level	Number of sheep	Coefficient	95% CI	P-value	Coefficient	95 % CI	P-value	Coefficient	95 % CI	P-value		
Pregnancy scan result	Single Twin	46 50	Reference 48.51	7.22 - 89.81	0.02	Reference 0.04	-0.06 - 0.13	0.46	Reference 0.03	-0.07 - 0.13	0.60		
Age	1 2	9 21	Reference -9.87	- -91.03 – 71.29	0.15	Reference -0.13	-0.31 - 0.05	0.04	Reference 0.12	- -0.07 – 0.30	0.01		
	3	32	-40.30	-117.16 - 36.56		-0.21	-0.380.03		0.15	-0.02 - 0.33			
	4	21	-38.63	-119.79 - 42.53		-0.18	-0.36 - 0.01		0.13	-0.06 - 0.31			
	≥5	13	-96.00	-184.347.67		-0.01	-0.21 - 0.19		-0.11	-0.31 - 0.09			
BCS	≤2 2.5	10 34	Reference 42.97	-31.77 - 117.70	0.54	Reference -0.01	- -0.19 – 0.16	0.90	Reference 0.07	-0.11 - 0.25	0.67		
	3	39	43.76	-29.88 - 117.40		-0.05	-0.22 - 0.12		0.11	-0.07 - 0.28			
	≥3.5	13	63.54	-23.85 - 150.92		-0.01	-0.21 - 0.19		0.08	-0.12 - 0.29			
Lambing ease single	0 1	26 11	Reference -51.15	-123.95 - 21.65	0.37	Reference 0.04	-0.15-0.23	0.74	Reference -0.10	- -0.29 – 0.09	0.47		
	2	7	-19.78	-105.97 - 66.40		-0.06	-0.29 - 0.16		0.03	-0.19 - 0.26			
Lambing ease twin (1)	0 1	31 12	Reference -28.02	- -98.94 – 42.90	0.71	Reference 0.02	- -0.14 – 0.17	0.96	Reference -0.05	- -0.21 – 0.10	0.79		
	2	5	4.92	-95.84 - 105.76		0.02	-0.20 - 0.24		-0.01	-0.23 - 0.21			
Lambing ease twin (2)	0 1	27 16	Reference 24.73	- -40.81 – 90.26	0.65	Reference 0.04	- -0.10 – 0.19	0.80	Reference -0.02	- -0.16 – 0.13	0.88		
	2	5	-17.87	-119.40 - 83.65		0.04	-0.18 - 0.26		-0.05	-0.28 - 0.17			
Lamb sex - single	Female Male	25 18	Reference -67.07	-127.476.68	0.03	Reference -0.06	-0.21 - 0.10	0.44	Reference -0.03	-0.18 - 0.12	0.70		
Lamb sex - twins	Female	15	Reference	-	0.48	Reference	-	0.21	Reference	-	0.60		

Table 2. Results of simple linear regression models for variables hypothesised to be associated with measures of lying time of pregnant, housed Mule ewes at Farm A

	Male	11	32.89	-51.07 - 116.86		0.12	-0.05 - 0.29		-0.08	-0.27 - 0.10	
	Mixed	21	-14.81	-86.32 - 56.70		-0.02	-0.16 - 0.13		-0.01	-0.16 - 0.15	
Birth weight single	-	43	5.32	-26.33 - 36.97	0.74	-0.03	-0.10 - 0.05	0.50	0.03	-0.05 - 0.11	0.44
Birth weight twins	-	48	-43.07	-66.1819.96	< 0.001	-0.02	-0.07 - 0.04	0.54	-0.03	-0.09 - 0.02	0.22

			Daily l	ying duration (min	/d)	Lying bout	duration (log mi	n/bout)	Frequency of lying bouts (log bouts/d)		
Variable	Level	Number of sheep	Coefficient	95% CI	<i>P</i> -value	Coefficient	95% CI	P-value	Coefficient	95% CI	P-value
Pregnancy scan result	Single	41	Reference	-	0.42	Reference	-	0.13	Reference	-	0.43
	Twin	39	-23.02	-80.14 - 34.10		-0.09	-0.21 - 0.03		0.06	-0.08 - 0.20	
Age	2	21	Reference	-	0.44	Reference	-	0.12	Reference	-	0.02
C	3	25	44.13	-30.77 – 119.04		-0.09	-0.250.06		0.16	-0.02 - 0.33	
	4	23	50.67	-27.22 – 128.56		-0.12	-0.28 - 0.04		0.21	0.02 - 0.39	
	5	11	68.62	-26.40 – 163.65		-0.24	-0.430.04		0.34	0.12 - 0.57	
BCS	2.5	9	Reference	-	0.28	Reference	-	0.99	Reference	-	0.84
	3	37	-77.75	-171.92 – 16.42		-0.01	-0.19 - 0.22		-0.14	-0.37 - 0.09	
	3.5	28	-42.99	-140.08 – 54.10		0.002	-0.21 - 0.21		-0.06	-0.30 - 0.18	
	4	6	-5.80	-139.34 – 127.74		-0.001	-0.29 - 0.29		-0.01	-0.34 - 0.32	
Lamb sex - single	Femal e	21	Reference	-	0.78	Reference	-	0.93	Reference	-	0.90
U	Male	19	11.15	-69.43 - 91.73		0.01	-0.15 - 0.17		0.01	-0.20 - 0.22	

Lamb sex - twins	Femal	10	Reference	-	0.56	Reference	-	0.36	Reference	-	0.17
	Male	10	-15.77	-138.99 – 107 46		-0.13	-0.40 - 0.13		0.11	-0.17 - 0.39	
	Mixe d	19	-55.97	-164.64 – 52.70		0.03	-0.20 - 0.26		-0.12	-0.36 - 0.13	
Birth weight single	-	40	19.04	-26.09 - 64.17	0.40	-0.001	-0.09 - 0.09	0.91	0.03	-0.08 - 0.15	0.57
Birth weight twins	-	39	23.12	-4.62 - 50.85	0.10	-0.07	-0.120.01	0.03	0.10	0.04 - 0.16	< 0.01

Table 3. Results of simple linear regression models for variables hypothesised to be associated with measures of lying time of pregnant, Welsh Mountain ewes managed at grass at Farm B



Figure 1. Frequency distribution of daily lying time (h/d; top panels), lying bout nequency (no.4) B middle panels) and lying bout frequency (n/d; bottom panels) for housed sheep at Farm A (A; n = 96) and sheep managed at grass at Farm B (B; n = 80). All measures of lying are averages taken on days -10, -9 and -8 prepartum for each ewe. Daily lying time shown in h/d for clarity.