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1 Internal roosting location is associated with differential use of the

- 2 outdoor range by free-range laying hens
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25 Abstract

26	1.	In commercial free-range systems for laying hens popholes to the outdoor range are
27		often installed on one side of the house only. In multi-tier systems, it is possible that
28		some individuals fail to access the range due to internal barriers to movement.
29	2.	Five commercial multi-tier flocks from different units were studied. For each flock,
30		two different colour markers were used to distinguish 200 birds roosting near the
31		popholes (NP-Roost) and 200 birds roosting far from the popholes (FP-Roost) at
32		night. The following day, counts of marked birds on the range and inside the house
33		were performed.
34	3.	Significantly more NP-Roost birds were observed in all areas of the outdoor range
35		than FP-Roost birds the next day. Distance of FP area from the popholes was very
36		strongly positively correlated with effect size in the adjacent range area.
37	4.	Additionally, in the indoor area far from the popholes (FP) more FP-Roost birds
38		were observed the next day than NP-Roost birds. In the indoor area near to the
39		popholes (NP) more NP-Roost birds were observed the next day than FP-Roost
40		birds.
41	5.	These results suggest that roosting location is associated with differential range use
42		when popholes are only available on one side of the shed as birds that roosted far
43		from the popholes used the range less.
44		
45	Keyw	vords
16	Walfar	a Dehaviour Louinghong Multi tion Aviant Dange use Erectore

46 Welfare, Behaviour, Laying hens, Multi-tier, Aviary, Range use, Free range

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48 **1. Introduction**

49 Loose-housing systems for laying hens allow birds to move around the house freely, accessing various resources such as litter, feed, water, nestboxes, and in free-range systems, 50 51 the outdoor range. In both single and multi-tier (also known as aviary) systems the feed, 52 water and nestboxes are on one or more elevated tiers with litter and range access available 53 at ground level. Questions have been raised about the ability of birds to move throughout 54 these systems, particularly where multiple potential barriers to movement are present 55 (Stratmann et al., 2015; Ali et al., 2016). In all loose-housing systems birds have to 56 negotiate level changes (from tier to ground, or between tiers) to access resources, and some 57 housing configurations require birds to negotiate level changes just to move from one side of 58 the house to the other. Research has shown behavioural signs of hesitation and difficulty 59 negotiating the key level change between the slats (or first tier) and the litter (Pettersson et 60 al., 2017) and ramps between all levels of a multi-tier system were found to reduce falls and 61 collisions (Stratmann et al., 2015).

An additional potential barrier to free movement can arise if hens crowd together, creating
increased stocking densities in certain areas, particularly around resources (Collins *et al.*,
2011; Lentfer *et al.*, 2013) and walls (Newberry and Hall, 1990). Higher stocking densities
have been associated with reduced bird movement (Appleby *et al.*, 1989; Carmichael *et al.*,
1999).

67 At night, hens choose to roost on high perches when available (Olsson and Keeling, 2000) 68 and in multi-tier systems will fill the higher tiers (Odén et al., 2002; Ali et al., 2016). A 69 study of groups (mean group size of 588 birds) housed in a multi-tier system found that birds 70 that roosted in end areas of the pen stayed within that area during the following days more 71 than would be expected by chance and often roosted in the same place in the following night 72 (Odén et al., 2000). In a few small experimental studies hens have shown individual 73 differences in location use within commercial-style aviaries (Freire et al., 2003; Campbell et 74 al., 2016) although it is not clear whether this was due to capability of moving around, 75 individual preference or feather pecking by other birds. Home ranges and 'activity centres'

76 can be calculated for individual laying hens within commercial units (Leone and Estevez, 2008; Rodriguez-Aurrekoetxea and Estevez, 2016) suggesting that birds tend to use certain 77 78 areas more often. Interestingly birds that range more were found to have larger home ranges 79 and activity centres (Rodriguez-Aurrekoetxea and Estevez, 2016), possibly because of the 80 increased opportunity to travel further in the outdoor area. Inside the house having a small 81 home range may not be an issue as all key resources such as feed, water, nestboxes and litter are usually well distributed throughout. However, range access may prove difficult in this 82 83 case.

84 The proportion of birds using the range at a given time is often low (Pettersson *et al.*, 2016a) and research using RFID tracking technology on commercial farms has found that some 85 birds do not appear to use the popholes, and therefore the range, at all (8%: Richards et al., 86 2011; 29.5%: Gebhardt-Henrich et al., 2014). Some consumers believe free-range hens to 87 88 be happier and healthier than in other systems and access to the range to be the most 89 important factor for welfare (Pettersson et al., 2016b). Actual levels of range use in 90 commercial systems may therefore not meet consumer expectation. When popholes are 91 available only on one long side of the house, some birds will have to travel many metres to 92 access the range and in multi-tier systems a view of the popholes is blocked by the tiers 93 themselves for birds in most areas of the house. It is possible that hens may not even be 94 aware of the range area if they cannot see the popholes. If this was the case we would expect 95 birds that started off the day in an area where popholes were visible to be more likely to use the range than those that have to travel far to access that area. 96

97 The two aims of this study were to test our predictions that (i) birds that roost near to the 98 popholes will be more likely to use the range area the next day than those that roost far away 99 from the popholes and (ii) birds will stay near to their night-time roosting location the next 100 day.

101 2. Materials and Methods

102 The study used five commercial free-range multi-tier flocks with flock sizes of approximately 16,000 birds. Two flocks were on the same farm but housed in separate 103 104 buildings (see the table for flock and house information). All flocks had pre-existing colony 105 divisions within the house separating the flock into four colonies of approximately 4000 106 birds and popholes were fitted to one long side of the house only, evenly spaced. Birds were 107 not able to access other colonies areas inside the house although they could when out on the 108 range in all flocks except for flock 1 which had physical colony divisions on the range. Fig 1 109 shows the layout of multi-tier stacks within flocks 2-5. Flock 1 was slightly different as the 110 shed was divided into colonies in a 2x2 design. Nonetheless, the layout of stacks within the 111 studied colony remains the same as the other flocks. Although some houses closed the area 112 under the tiers off during the night, these were not closed off for flocks 1,3,4 and 5 during 113 the day. This meant that birds could walk along the litter from one side of the house to the 114 other. In flock 2 the area under the tiers was closed off for the first of the three observations 115 as the producer did not choose to open up this area until 11am. All flocks were allowed out 116 onto the range at 9 am. All flocks had 16-17 hours light.

117 The study involved a 2-day visit to each flock when the birds were 41-47 weeks of age.

118 These visits took place between late-August and mid-November 2016 and weather

119 conditions were similar for all flocks. House design and dimensions were recorded. Ethical

120 permission was obtained from the University of Bristol ethical committee prior to starting

121 the project.

122 --- SUGGESTED LOCATION FOR TABLE ---

123 **2.1. Marking birds**

In order to establish whether birds used all areas of the house and range, two samples of individual hens were marked during the night of Day 1 (between 9pm and 2am) when the lights were off and birds were roosting. Researchers used red light head torches when in the house to minimise bird disturbance. One colony was selected per flock and 400 birds 128 (approximately 10% of the colony) were marked. Two distinct areas were established – 'near' the popholes (NP) and 'far' from the popholes (FP) (see fig 1). On the tiers, 200 129 roosting birds from the NP area were marked green (NP-Roost) and 200 roosting birds from 130 the FP area were marked pink (FP-Roost). Birds were selected evenly across the areas, with 131 132 the highest tier unable to be sampled due to accessibility. All birds were generally in good 133 condition although any birds with visible problems such as bumblefoot were excluded. 134 Livestock marker crayons (Paintstik®, All-Weather®, USA) in fluorescent green and 135 fluorescent pink were used to colour one entire leg of each bird. The two colours plus 136 another (blue) were tested in a pilot trial and blue was found to be the least visible so was 137 not used. One researcher lifted each bird from its roosting position and held it steady while 138 another researcher marked the leg with the appropriate colour. Each bird was then placed 139 back in the same location, where they remained, with little indication of disturbance. This 140 process took about 30 seconds per bird.

141 **2.2.** Observations and counts

Marked birds were counted at three time-points the next day (approximately 90min apart) 142 between 10am and 1pm. Mean temperature, relative humidity and light levels were similar 143 144 in both NP and FP areas. Indoor counts were performed first at each time point, immediately 145 followed by range counts. Further counts were not performed; in part owing to time 146 constraints but also because the researcher's presence was likely to be disturbing the natural 147 distribution of the birds with every observation. When assessing range use the number of 148 NP-Roost and FP-Roost marked birds were counted in two areas of the range; adjacent and 149 non-adjacent. The adjacent area was divided from the non-adjacent area by visualising a line 150 as a continuation of the internal colony divisions (see fig 1). The observer had experience 151 counting ranging birds and walked methodically through the range areas counting both the 152 total number of birds outside, and those that were marked. Although only flock 1 had 153 physical colony divisions on the range we hypothesised that more marked birds would be 154 seen within the area adjacent to the studied colony popholes, hence dividing the range area

155 up visually for these counts. It was too difficult to see the legs of birds on the litter area of the house so these birds were not included in the counts. The researcher walked along the 156 edge of the stacks in the NP area of the house, counting all NP-Roost and FP-Roost marked 157 158 birds visible on the tiers in this section (back of top tier excluded). A head torch was used to 159 clearly see birds further back on the tiers. Not all birds were visible (e.g. within nestboxes 160 and on the back of the top tier) and these were not counted as it was deemed to be too 161 disruptive for the researcher to look in nestboxes or climb the tiers. The same was then 162 repeated for the FP area. It was not possible for the researcher to be blinded to the groups 163 marked but as the count measure is objective it is unlikely that bias occurred.

164 --- SUGGESTED LOCATION FOR FIGURE 1 ---

165 **2.3. Statistical analysis**

166 The multilevel statistics package MLwiN (Charlton et al. 2017) was used for the statistical analyses to accommodate the doubly repeated measures of measures within observation 167 168 number, within house. A separate multi-level model was produced for each area where 169 counts were taken – on the range (adjacent and non-adjacent) and in the house (NP and FP). 170 To look for potential differences in the numbers of NP-Roost and FP-Roost birds, the 171 difference between the two counts was calculated (FP-Roost minus NP-Roost) and used as 172 the outcome variable in these models. Differences in observation number was also tested 173 within these models as a fixed effect. The residuals from the models were checked to ensure 174 they met the assumptions of the model. Although the differences between counts were used 175 in the model, mean counts have been reported for clarity.

For observation 1 in flock 2, the area under the litter was blocked off. As this may have had
a large effect on the results and did not match the other flocks, this value has been replaced
with an estimated value. The estimation was produced by averaging the values for
observations 2 and 3.

180 To check for correlations between distance of FP area from a pophole and size of the effect 181 on range use, the number of FP-Roost birds counted on the range was subtracted from the 182 number of NP-Roost birds counted on the range for each flock. After checking for 183 normality, Pearson correlations were performed.

184

185 **3. Results**

- 186 **3.1.** Use of outdoor range areas
- 187 On average across all flocks and observations 7.34% of the whole flock (both marked and
- unmarked birds) were seen out on the range at a time with low variability (range of means
- between flocks: 6.03-8.98%, range of means across the three observations: 7.23-7.42). On
- average, 5.38% (SD=2.60) of marked birds were seen out on the range at a time.
- 191 Within the adjacent range area, significantly more NP-Roost birds (mean=16.20, SD=8.10)
- were counted than FP-Roost birds (5.33, SD=3.14) (parameter estimate: -9.733(SE: 2.553),
- 193 p<0.001). There was no significant effect of observation number. See fig 2a. Within the non-
- adjacent range area significantly more NP-Roost (11.47, SD=7.67) than FP-Roost birds
- 195 (4.00, SD=3.60) were also observed (parameter estimate: -6.200(SE: 2.293), p<0.001) with
- 196 no effect of observation number (Fig 2b).
- 197 Distance of FP area from the popholes was very strongly positively correlated with effect
- size (r=0.988, n=5, p=0.002). As this distance increased, the proportion of NP-Roost birds
- 199 relative to FP-Roost birds, seen on the range also increased. For the non-adjacent area of the
- 200 range this relationship did not reach significance (r=0.816, n=5, p=0.092).

201 --- SUGGESTED LOCATION FOR FIGURE 2 ---

202 **3.2.** Use of NP and FP areas in the house

- 203 Within the FP area of the house significantly more FP-Roost birds (15.86, SD=6.16) were
- counted than NP-Roost birds (6.93, SD=4.07) (parameter estimate:7.200 (SE:1.865),
- 205 p<0.001) (see Fig 3b). There were significant differences between observations with mean
- differences between FP-Roost and NP-Roost birds for observations 1-3 at 7.20(SD:2.588),
- 207 10.20(SD:5.02) and 3.40(SD:5.77) respectively.
- 208 In the NP area of the house significantly more NP-Roost (11.20, SD=4.04) birds than FP-
- 209 Roost birds (6.80, SD=2.12) were counted (parameter estimate: -3.133 (SE: 0.810), p<0.001)
- 210 (see Fig 3a). There was no effect of observation number.

211 --- SUGGESTED LOCATION FOR FIGURE 3 ---

212

213 **4. Discussion**

The results suggest that night time roosting location affects the next day's range use by

215 individual birds in free-range flocks. As most marked birds should be found in the adjacent

area of the range we expected counts from this area to be the most likely to show any

217 significant effects. The strongest results were seen in the adjacent area with more than twice

the number of birds that roosted near the popholes (NP-Roost birds) seen in this outside area

than birds that roosted far from the popholes (FP-Roost birds) across all three observations.

220 Significantly more NP birds than FP-Roost birds were also observed in the non-adjacent

area, suggesting that this effect of bird roosting location on range use exists even in less

accessible areas of the range.

These results suggest that by allowing access to outdoor areas on one side of a laying house only, some of the birds may have limited access to the outside compared with others simply because of their location in the house. The reasons why roosting far away from a pophole reduces the likelihood of using the range cannot be determined from this study, although there are a number of possible explanations. Hens appear to have individual home ranges in 228 commercial units (Rodriguez-Aurrekoetxea and Estevez, 2016) and this may limit their outdoor range use if a pophole is not available within this home range. It may also be the 229 230 case that it is important for popholes to be visible for a bird to choose to use the range area. 231 Another possible explanation is that the birds which roost in certain areas of the house may 232 have some other characteristics affecting their use of the range such as weight, or 233 behavioural traits such as fearfulness. However, this remains speculation at the moment and 234 has not been tested. The fact that the difference in numbers of birds from both roosting 235 locations was greater on the range when the distance to travel was greater suggests that the 236 results seen in this study are closely related to the distance of roosting location from 237 available popholes.

238 Our second objective was to test the prediction that hens stay in the vicinity of their roosting 239 location the next day. This study found that more FP-Roost birds than NP-Roost birds were 240 observed in the FP house area the next day and more NP-Roost than FP-Roost birds were observed in the NP house area. These results suggest that birds tend to stay in the same area 241 242 as they roosted the night before, with few birds coming from other areas of the house. This is 243 backed up by the results for the range areas, as birds with popholes near to their roosting 244 location (NP-Roost) birds ranged more. If hens are reluctant to travel far from their roosting 245 location the FP-Roost birds are less likely to make it to a pophole and out onto the range. 246 There was an effect of observation number in the FP area. Although the direction of the 247 effect remained consistent across all observations for more FP-Roost birds, the size of this 248 effect increased for the second observation and then decreased for the final observation 249 suggesting that the numbers of birds had begun to even out by observation three. However, 250 this preliminary study looked only at a few hours following pophole opening and so 251 information on bird movement over a longer period is essential to establish if this effect is 252 true.

This study was designed to provide the first evidence of a problem often discussed by
producers and scientists but lacking in scientific evidence – that some laying hens do not

access certain resources, particularly the outdoor range where popholes are limited. Very
little work has studied the effect of bird roosting location on movement in commercial flocks
although some data is available (Odén *et al.*, 2000) and this is the first to look at the effect
on multiple free-range commercial units.

259 As this was a preliminary study, further measures that may have helped to determine the 260 specific reasons behind the results such as measures of individual health and behaviour were 261 not taken but would be a valuable avenue for further investigation. Additionally, this study 262 only looked at one day and did not cover whether birds return to the same area to roost. This 263 was mostly due to limitations of the marking method as it was not designed to last longer 264 than a day or two. The results of this study did not find that marked bird numbers in each 265 location were affected by the time observed in most areas (within the scope of the study) but 266 it would be useful to know if this is a short or long-term effect. Due to practical and safety 267 reasons, it was not possible to mark birds from the highest tier. It is possible that this may 268 have influenced the results as birds that perch on the highest tier may have different 269 behavioural traits than the rest of the flock.

270 The marking method trialled in this study was found to be relatively successful. It was easy 271 to mark birds with two people and the colours chosen were very distinct. No negative effects 272 such as feather pecking by conspecifics were seen by the researchers during the study or 273 reported by the producers, likely because the legs of the birds were marked rather than the 274 plumage. While leg marks were easy to see on tiers, they were difficult to spot on crowded 275 ground areas such as the litter. For the purpose of this study this was not considered a major 276 issue as information about bird movement and location could still be collected from other 277 birds. The method may not be appropriate however if litter use is of particular interest. On 278 average, a slightly lower percentage of marked birds were seen ranging than the total 279 percentage of marked and unmarked birds ranging. As this was only a difference of 280 approximately 2% the ability of the researcher to identify marked birds on the range was

adequate. The method appears to be useful for marking groups of birds (rather than

282 individuals) on a short-term basis in commercial flocks.

To conclude, this study provides the first evidence that some laying hens within large freerange commercial units may have limited access to the outdoor range area if popholes are provided on one side of the house only. Efforts to improve the design of free-range units are often focused on the range area or pophole size and number. This research has highlighted a need to consider the placement of popholes in addition to these factors in order to provide access to all resources for all individuals in a commercial flock.

289

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293

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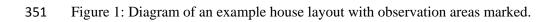
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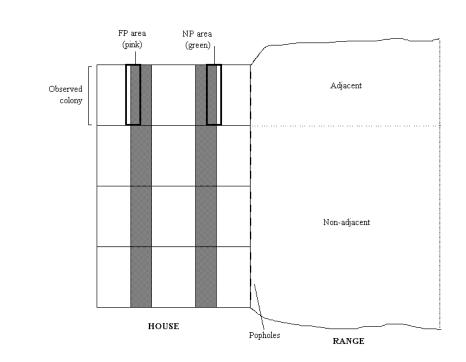
348 Table: Flock information

				Number	Light	Feeds per	Number	Pophole	Distance	Distance	No. of	Dint
				of tiers	schedule	day	of	size	of FP	of NP	tier stacks	Bird
Flock	Flock size	Genotype	System				popholes	(height x	area from	area from	to cross	age at visit
	5120					(studied	width)	popholes	popholes	(from FP		
							colony)	(cm)	(m)	(m)	to NP)	(weeks)
1	15837	Lohmann	Jansen	3	6:00-	5	6	46 x 230	9.65	4	2	12
1		Brown			22:00						2	42
2	16022	Lohmann	Dutchman	2	5:15-	5	5	50 - 272	12.10	3.7	2	41
2	16032	Brown	Natura Twin		21:40			50 x 272	12.10	5.7	2	41
2	16032	Bovan	Dutchman	2	6:00-	5	5	49 x 268 12.10	10.10	3.1	2	17
3		Brown	Natura Twin		21:00				12.10			47
	16032	Bovan	Dutchman	2	6:00-	5	5	5 50 x 270	12.80	3.35	2	47
4		Brown	Natura Twin		21:00							

		T . 1	Vencomatic	2		6	6					
5	16032	Lohmann Brown	Veranda		5:00-			51 x 205	9.80	2.6	2	44
		Brown	Aviary		22:00							







354 Figure 2: Mean counts of marked birds in the two range locations across the three

observations (error bars: +/- 2SE). 355

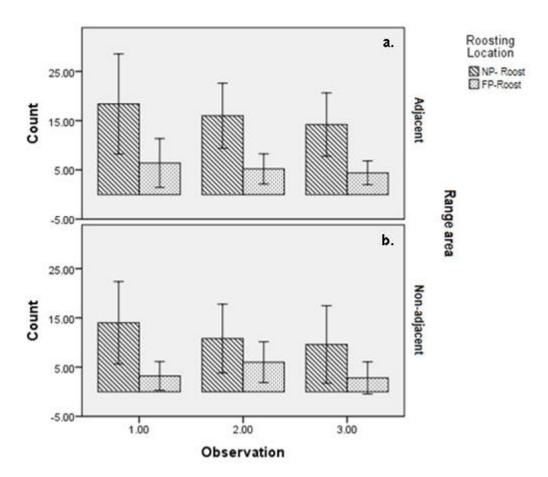


Figure 3: Mean counts of marked birds in the two indoor locations across the three
observations (error bars: +/- 2SE).

