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1 **Range use is related to free-range broiler chickens' behavioral responses during food and social**
2 **conditioned place preference tests**

3

4 Vitor Hugo Bessa Ferreira^{1,2}, Karine Germain³, Ludovic Calandreau^{2*}, Vanessa Guesdon^{1*}

5

6 ¹ Yncréa Hauts-de-France, ISA Lille, 48 bd Vauban 59046 Lille Cedex, France

7 ² INRAE, CNRS, IFCE, Université de Tours, Centre Val de Loire UMR Physiologie de la

8 Reproduction et des Comportements, 37380 Nouzilly, France

9 ³ INRAE, UE EASM, Le Magneraud, CS 40052, 17700 Surgères, France

10

11 ***Co-last authorship**

12

13 **Corresponding Authors:**

14 **Vanessa Guesdon and Vitor H B Ferreira**

15 **Email: vanessa.guesdon@yncrea.fr; vitor@zootecnista.com.br**

16

17 YNCREA HAUTS-DE-FRANCE, ISA Lille

18 48 Boulevard Vauban

19 Lille, France

20 PO Box : BP 41290

21 ZIP code: 59014 cedex

22 Phone : 33 (0)3 28 38 48 48

23

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29 **ABSTRACT**

30

31 Free-range broiler chickens usually show an uneven spatial utilization of an outdoor range. Due to
32 behavioral and cognitive between-individual differences, some animals may be driven to associate
33 food and conspecifics more strongly to the barn, causing them to be less prone to explore the range. In
34 this study, we aimed to understand how broiler chickens with different ranging levels (low- and high-
35 ranging chickens) would behave under conditioned place preference (CPP) test situations. We used
36 two cohorts conditioned to two natural rewarding stimuli: food and social companions. In a two-
37 chambered apparatus, one cohort (n = 31, 16 high-ranging, and 15 low-ranging chickens) was
38 conditioned to one chamber that always contained a cup with a food reward (mealworms), while the
39 cup in the other chamber was always empty. The same design was also used with the second cohort (n
40 = 31, 15 high-ranging and 16 low-ranging chickens), although instead of food, the reward was the
41 physical presence of two conspecifics. During the testing trials, the animals had access to both empty
42 chambers, and the time spent in each chamber was quantified. For the first day of the food CPP test,
43 both the high- and low-ranging chickens spent significantly more time in the conditioned chamber,
44 where they had previously found mealworms. During the following extinction days, the animals
45 showed a gradual loss of their learned preference, increasing their immobility in the apparatus. High-
46 ranging chickens were more immobile than low-ranging chickens, however, as their number of trials
47 without moving was significantly higher. Unexpectedly, during the first day of the social CPP test,
48 only high-ranging chickens showed a place preference. An overall place preference was observed only
49 on the second day, with no chamber preference during the extinction days. Our results suggest that
50 whether and how a stimulus-reward association occurs for free-range chickens may also be dependent
51 on individual differences and the nature of the reward (food or social). Since associative learning
52 occurs on a daily basis for farmed animals and the way individuals learn or value the reward varies,
53 this research advanced our knowledge of animal behavior and individual cognitive differences that can
54 be highly beneficial in improving animals' living conditions; this new understanding will allow for a
55 more individualized approach to rearing broiler chickens in outdoor systems.

56

57 **Keywords:** associative learning; cognition; conditioned place preference; free-range chicken; range
58 use.

59

60 **1. Introduction**

61

62 Broiler chickens in free-range systems usually spend their first month of life, from day one of
63 age, under highly controlled environments, i.e., the barn, where food, water, and companions are
64 present in a predictable and stable way (Campbell et al., 2018; Rodriguez-Aurrekoetxea et al., 2014).
65 At approximately 35 days of age, when the range is finally available, studies have shown that the
66 animals do not make use of the range equally, resulting in considerable variations in ranging behavior,
67 with anywhere from 15% to 87% of chickens going outdoors (Sosnowka-Czajka et al., 2007; Taylor et
68 al., 2017a). This low and spatially uneven use of the range is not only detrimental for flock welfare, since
69 it speeds up litter deterioration in the barn and increases the risk of parasitic infestation (Cravener et
70 al., 1992; De Jong et al., 2014), but it can also jeopardize the free-range idea that all the animals are
71 exhibiting a larger and fuller behavioral repertoire when using the outdoor range, since some animals
72 never go outside (Taylor et al., 2017b).

73 Aside from environmental influences that could potentially motivate chickens to go outside,
74 such as tree cover, time of day and season (Dawkins et al., 2003), within-group analyses have shown
75 that ranging behavior varies among animals, with some individuals showing consistently higher
76 ranging behavior than others (Taylor et al., 2017b). Variations in ranging behavior are also linked to
77 different behavioral and cognitive patterns, as the animals at the extremes of the ranging continuum
78 (low- and high-ranging chickens) present different coping styles and reactions to stress (Campbell et
79 al., 2016; Taylor et al., 2020), different attentional bias (Campbell et al., 2019b), different performance
80 during spatial memory tasks (Campbell et al., 2018; Ferreira et al., 2020a, 2019) and different
81 inhibitory control (Ferreira et al., 2020b). These results point towards a much more complex
82 interaction between coping styles and range use than initially anticipated by researchers. Like many
83 other species in the animal kingdom, chickens may have different personalities, motivations, and
84 cognitive styles (Garnham and Løvlie, 2018; Gosling, 2001; Marino, 2017).

85 Several studies have demonstrated relationships between learning and personality traits such
86 as exploration. More exploratory black-capped chickadees (*Poecile atricapillus*) were shown to be
87 faster learners of an acoustic operant discrimination task than less exploratory ones (Guillette et al.,
88 2009). Less explorative adult female red junglefowl, however, were faster to let go of a previous
89 association and learned a reverse task quicker than more explorative conspecifics; the opposite pattern
90 was found for red junglefowl chicks (Zidar et al., 2018a). Recently, we demonstrated that range use in
91 free-range chickens was also related to distinct learning and memory capacities, as chickens that
92 ranged less outperformed those who ranged more during spatial memory and inhibitory control tasks
93 (Ferreira et al., 2020a, 2020b, 2019). These contrasting findings shed light on the need for a better
94 understanding of the complex link between personality, learning, and memory (Dougherty and
95 Guillette, 2018).

96 In this work, we aimed to assess whether and how different ranging behavior patterns were
97 related to distinct associative learning capacities. To this end, free-range chickens with high and low
98 levels of ranging behaviors (high- and low-ranging, respectively) were subjected to a conditioned
99 place preference task (CPP), a standard method used to test an animal's ability to learn an association
100 between environmental stimuli and a reward (Hsu et al., 2002; Mathur et al., 2011; Tzschentke, 2007;
101 White and McDonald, 1993). We chose to conduct the CPP with two different naturally rewarding
102 stimuli, i.e., food and social companions. Using a two-chambered apparatus, the first cohort of high-
103 and low-ranging chickens was conditioned to one chamber where they were always presented a cup
104 with a food reward (mealworms), while the cup in the other chamber was always empty. The same
105 design was used with the second cohort, although the reward was the physical presence of two
106 conspecifics. During testing trials, the animals had access to both empty chambers, and the time spent
107 in each chamber was quantified. Since low-ranging chickens spent most of their time in the barn,
108 where food and conspecifics are present in a predictable way, our first hypothesis was that low-
109 ranging chickens would exhibit a stronger association than high-ranging chickens for both food and
110 social rewards. To further compare these two types of chickens, extinction trials (where conditioned
111 animals are repeatedly presented to the apparatus without any reward) were added to the testing phase
112 to assess how conditioned association persists in the face of a loss of reward. As the stimulus-reward

113 associations were initially expected to be stronger for low-ranging chickens, our second hypothesis
114 was that the conditioning of low-ranging chickens would be more resistant than that of high-ranging
115 chickens, resulting in delayed extinction for both food and social rewards.

116

117 **2. Methods**

118

119 **2.1. Ethical statement**

120

121 This study was conducted at the experimental unit UE 1206 EASM of INRAE, France. The
122 experiment was conducted under INRAE ethics committee approval (APAFIS # 17824-
123 2018112611585147 v4) in agreement with French legislation.

124

125 **2.2. Animals and housing**

126

127 The experiments were performed during two different years (February to May of 2018 and
128 2019) with two different flocks of broiler chickens housed and managed under the same conditions.
129 Flocks were composed of two hundred naked-neck (S757N) male broiler chickens (*Gallus gallus*
130 *domesticus*) reared from their first day of life in a free-range system with a stocking density of ten
131 individuals/m² in the barn (4 m x 5 m) and 0.42 individuals/m² in the outdoor range (27 m x 17.5 m).
132 Continuous artificial lighting (mean of 50.87 ± 29.88 lux in different parts of the barn) was provided
133 during the first three days after placement; then, from day 4 to day 14, it was gradually decreased until
134 there was a total use of natural lighting. The indoor ambient temperature was maintained at 28 °C
135 during the first week and decreased by 1 °C each week until it reached 23 °C when the birds were 38
136 days old. The chickens' weights were monitored at each diet change (starter, grower, and finisher
137 diets) and before slaughter (at 1, 28, 57 and 86 days of age for the 2018 flock, and 1, 28, 56 and 105
138 days of age for the 2019 flock). At 28 days of age, 120 individuals were randomly selected and
139 identified via a rectangular yellow plastic poncho around their necks containing unique acronyms for

140 easy identification (see Figure S1 in Supplementary Materials). The chickens had free access to the
141 range beginning at 36 days of age.

142

143 **2.3. Ranging behavior level and individual selection**

144

145 Measurements of chickens' ranging behavior levels followed the same procedures as
146 described in [Ferreira et al. \(2019\)](#). We performed seven interspaced scans per day at six different ages
147 (between 39 and 59 days of age; *2018*: D39, D42, D45, D53, D56, and D59; *2019*: D39, D42, D45,
148 D49, D52, and D55), totaling 42 scans, to determine the range location (inside the poultry house or
149 zones A, B, or C of the range) of identified individuals (chickens carrying a poncho). Since different
150 zones had different areas ($A = 0 - 4.5$ m, $B = 9 - 13.5$ m, $C = 13.5 - 27$ m from the poultry house), we
151 calculated an individual ranging distance index, considering a given chicken walked the equivalent of
152 a half-length of this zone plus the total length of the already crossed zones, in the case of zones B and
153 C.

154

155 **Ranging distance index** = number of times seen in zone A*2.25 + number of times seen in
156 zone B*9 + number of times seen in zone C*20.25

157

158 We then selected individuals based on their number of range visits and their ranging distance
159 index. Individuals with low values, indicating a low number of range visits and visits close to the barn,
160 and individuals with high values, indicating a high number of range visits and visits farther from the
161 barn, were selected and considered as low- and high-ranging chickens, respectively. Other studies have
162 shown these two variables are positively correlated with the time spent outdoors for both free-range
163 broiler chickens and laying hens ([Hartcher et al., 2016](#); [Taylor et al., 2017b, 2020](#)).

164

165 Thirty-one healthy individuals were selected for the food CPP (15 low-ranging and 16 high-
166 ranging chickens), and 31 healthy individuals were selected for the social CPP (16 low-ranging and 15
high-ranging chickens). Differences in range visits and distance indexes for these groups were verified

167 through non-parametric Mann-Whitney U tests; see Table 1a for results and Figure 1 for a graphical
168 illustration of distance index over the six observation days.

169

170

171 Figure 1 about here

172 Table 1 about here

173

174

175 **2.4. Food conditioned place preference**

176

177 For 12 consecutive days, from 82 to 93 days of age, following classification of ranging
178 behavior, 31 healthy individuals (16 high-ranging and 15 low-ranging chickens) were tested using the
179 food-conditioned place preference paradigm. These individuals were then divided into two subgroups,
180 equally balanced in the number of high-ranging and low-ranging chickens, to be tested throughout the
181 day (morning and afternoon). The first subgroup was caught in the morning and kept in crates (four
182 chickens per crate) placed in the test room without food for at least 1 hour before the beginning of the
183 task. As soon as all the chickens from this subgroup finished their trials (a maximum of three hours
184 between the first and last individual tested), they were released back into the barn. In the afternoon, we
185 proceeded in the same way with the second subgroup. Low- and high-ranging chickens were tested
186 alternatingly, and the testing order of the individuals, as well as the testing order of the subgroups, was
187 similar throughout the experiment.

188 The apparatus for the food CPP test was a wooden rectangular structure (l : 2 m, w : 0.6 m, h :
189 0.7 m) separated into two chambers of 100 cm long each that were separated by a central opaque wall
190 with a yellow plastic floor. The walls of the two chambers were covered with different patterns
191 (rectangular and circular). Identical black cups (height and depth varying according to different
192 phases) were placed at each end of the compartments (Figure 2a). One of these cups contained the
193 food reinforcement (five mealworms), depending on the side to be conditioned.

194

195 Figure 2 about here

196

197 **2.4.1. Habituation**

198

199 During the first two days of the experiment, each individual was placed in the middle of the
200 apparatus and had free access to the arena for two minutes twice a day (two trials). The time between
201 each trial was approximately 1h20, and the time spent in each chamber was recorded. Since the
202 animals showed an unconditioned preference for one of the chambers in this phase, we used a biased
203 version of the CPP in which individuals are conditioned to the chamber where they spent the least
204 amount of time (Tzschentke, 2007).

205

206 **2.4.2. Conditioning**

207

208 The conditioning phase occurred over four days following habituation. Each day, the animals
209 were placed individually in the arena (body and head parallel to the central wall) and had access to
210 only one chamber at a time (no free access to the two chambers) for two minutes. Approximately 1h20
211 later, the individuals were placed in the other chamber of the apparatus. One of the chambers had its
212 cup always filled with mealworms during conditioning (conditioned chamber), while the other
213 chamber had an empty cup (non-conditioned chamber). Both cups in the apparatus were 10 cm high
214 and 5 cm deep to allow for easy viewing of the inside of the cup. Some mealworms were also on the
215 ground near the cup to motivate individuals to inspect the cup. Animals that did not move and did not
216 visit the cups were excluded and no longer tested.

217

218

219 **2.4.3. Test**

220

221 Test trials took place for six days, and, as per habituation, individuals were placed in the
222 center of the arena and had free access to both sides for two two-minute trials (the cups were not filled

223 but still present to motivate individuals to explore). The cups were 15 cm high and 5 cm deep to
224 prevent animals from easily seeing the reward in the cup from its start position (middle of the arena)
225 and to motivate them to approach the cups one by one.

226 The objective of these test trials was first to check whether or not individuals did associate a
227 chamber with the reward, and second, to subject individuals to a process of extinguishing associative
228 learning that occurred during the previous phase (conditioning), thus assessing the strength of the
229 association in the face of a loss of reward. The variable recorded was the time spent in each of the
230 chambers. Animals that did not move for more than 50% of the test trials (6 test trials) were excluded
231 from statistical analyses.

232

233 **2.5. Social conditioned place preference**

234

235 Thirty-one healthy chickens (15 high-ranging and 16 low-ranging chickens) were tested on 16
236 consecutive days, from 82 to 97 days of age, on the social conditioned place preference task, which
237 followed the same procedure and used the same structure as the food-conditioned place preference. To
238 increase social motivation, the chickens waited in individual cages (*l*: 45 cm, *w*: 68 cm, *h*: 70 cm), i.e.,
239 with limited social contact, for at least 1 hour before the beginning of the task. During conditioning,
240 instead of food, tested individuals could approach two flockmates (reared in the same barn) through a
241 wire fence in one of the two chambers (conditioned chamber, Figure 2b). Flockmates did not
242 participate in the task, and each pair consisted of one high- and one low-ranging chicken. The pairs
243 were substituted for new pairs at the end of the first round of individual trials.

244 The non-conditioned chamber was always empty, and during test trials, no conspecifics were
245 present in the apparatus. The same inclusion criterion was applied and the same variables were
246 measured as in the food CPP.

247

248 **2.6. Statistics**

249

250 For both food and social CPP, a general linear model (ANOVA) with repeated measures was
251 performed. We first analyzed the first day of testing separately to verify if the time spent in the
252 chambers varied according to the presence or absence of the reward during the conditioning phase
253 (i.e., if the animals showed a conditioned place preference). Time spent in either chamber (mean of the
254 twice-daily trials) was included in the model as the within-subject factor and ranging level (low- or
255 high-ranging) was included as the between-subject factor.

256 Next, we analyzed the time spent in either chamber for the following days of testing to
257 determine the patterns of extinction between the two groups. Time and day were included as within-
258 subject factors, and ranging level (low- or high-ranging) was included as between-subject factor.
259 Greenhouse-Geisser corrections were applied when the assumptions of sphericity were violated. When
260 the main effects or interactions were significant, analyses were followed by Fisher's Least Significant
261 Difference (LSD) test. Finally, differences between the high- and low-ranging chickens regarding the
262 number of trials without moving during the food and social CPP were compared using a non-
263 parametric Mann-Whitney U test using a Monte Carlo simulation (data did not meet the criteria for
264 normality even after transformation).

265 During conditioning of the food CPP, eight individuals (5 low- and 3 high-ranging) did not
266 inspect the cups or did not eat the mealworms and were therefore excluded from the task. Four
267 individuals (3 low- and 1 high-ranging) were subsequently excluded from statistical analyses because
268 they exceeded our pre-set limit of 50% of test trials without moving (see Section 2.4.3). Therefore, the
269 number of individuals included in the food CPP analyses was 19 (8 low- and 11 high-ranging
270 chickens). Using the remaining dataset, differences in range visits and distance indexes were still
271 significant between the ranging groups (see Table 1b for results).

272 During the tests of the social CPP, inconsistency in the results of the first two testing days led
273 us to submit all individuals to two additional days of conditioning, after which we then proceeded to
274 perform six days of testing. Eleven individuals (5 low- and 6 high-ranging) were excluded as they
275 exceeded our pre-set limit of 50% of the test trials without moving (see Section 2.4.3). The number of
276 individuals included in the social CPP analysis, therefore, was 20 (11 low- and 9 high-ranging
277 chickens).

278 All statistical analyses were performed using IBM SPSS Statistics 21. Statistical significance
279 was accepted at $p \leq 0.05$. Data are presented as raw means \pm standard deviation (SD).

280

281 3. Results

282

283 3.1. Food conditioned place preference

284

285 During the first testing day, the chickens spent significantly more time in the conditioned
286 chamber compared to the non-conditioned chamber (76.49 ± 6.2 and 34.33 ± 6.27 for the conditioned
287 and non-conditioned chambers, respectively, effect of chamber: $F_{1, 17} = 13.70$, $p = 0.002$, $\eta_p^2 = 0.44$,
288 Figure 3a), independent of ranging level or ranging level x chamber interaction (effect of ranging
289 level: $F_{1, 17} = 0.007$, $p = 0.93$, $\eta_p^2 = 0$; effect of ranging level x chamber: $F_{1, 17} = 0.133$, $p = 0.71$, $\eta_p^2 =$
290 0.008).

291 Extinction trials confirmed that the chickens still preferred the conditioned chamber over the
292 non-conditioned chamber (effect of chamber: $F_{1, 17} = 12.49$, $p = 0.003$, $\eta_p^2 = 0.424$). As days passed,
293 however, the chickens stopped moving, which resulted in significantly less time spent in either
294 chamber (Greenhouse-Geisser corrected- $F_{3, 272, 55, 617} = 2.98$, $p = 0.035$, $\eta_p^2 = 0.149$, Figure 3b).

295 The time spent in either chamber also differed between ranging groups during the extinction
296 trials, as it was higher for low-ranging chickens compared to high-ranging chickens (48.17 ± 3.98 and
297 37.06 ± 3.40 for low- and high-ranging chickens, respectively; effect of ranging level: $F_{1, 17} = 4.495$, p
298 $= 0.049$, $\eta_p^2 = 0.209$).

299 No other significant effect was found (effect of days x ranging level: Greenhouse-Geisser
300 corrected- $F_{3, 272, 55, 617} = 0.453$, $p = 0.732$, $\eta_p^2 = 0.026$; chamber x days: Greenhouse-Geisser corrected-
301 $F_{3, 427, 58, 252} = 2.315$, $p = 0.077$, $\eta_p^2 = 0.120$; effect of days x ranging level x chamber: Greenhouse-
302 Geisser corrected- $F_{3, 427, 58, 252} = 1.337$, $p = 0.270$, $\eta_p^2 = 0.073$). Finally, Mann-Whitney U analysis on the
303 number of trials without moving confirmed that high-ranging chickens moved less frequently across
304 trials than the low-ranging chickens (trials without moving during the extinction phase: 3 ± 1.94 and
305 1.25 ± 1.75 for high- and low-ranging chickens, respectively; $U = 20$, $p = 0.048$, Figure 3c).

306

307 Figure 3 about here

308

309 **3.2. Social conditioned place preference**

310

311 For the first testing day after the social CPP, we found a significant interaction between
312 chamber and ranging level: only high-ranging chickens spent significantly more time in the
313 conditioned chamber. Furthermore, high-ranging chickens spent significantly more time in the
314 conditioned chamber than low-ranging chickens did (effect of chamber x ranging level x time: $F_{1, 18} =$
315 $7.8, p = 0.012, \eta_p^2 = 0.302$, Figure 4a). Neither the effects of chamber nor ranging level were
316 significant (effect of chamber $F_{1, 18} = 0.852, p = 0.368, \eta_p^2 = 0.045$; effect of ranging level: $F_{1, 18} =$
317 $0.693, p = 0.416, \eta_p^2 = 0.037$).

318

319 Figure 4 about here

320

321 All animals showed a preference for the conditioned chamber on the second testing day
322 (48.038 ± 8.47 and 19.376 ± 4.97 for the conditioned and non-conditioned chambers, respectively;
323 effect of chamber: $F_{1, 18} = 6.065, p = 0.024, \eta_p^2 = 0.252$, Figure 4b), independent of ranging level or
324 ranging level x chamber interaction (effect of ranging level: $F_{1, 18} = 1.292, p = 0.271, \eta_p^2 = 0.067$;
325 effect of ranging level x chamber: $F_{1, 18} = 0.683, p = 0.419, \eta_p^2 = 0.037$). The unexpected results from
326 the first day and the inconsistent results between the first two testing days led us to submit all the
327 individuals to two additional days of conditioning.

328 For the first testing day following supplementary conditioning, high-ranging chickens tended
329 to spend more time in the conditioned chamber than in the non-conditioned chamber (effect of
330 chamber x ranging level x time: $F_{1, 18} = 3.5, p = 0.078, \eta_p^2 = 0.163$; effect of chamber $F_{1, 18} = 1.068, p =$
331 $0.315, \eta_p^2 = 0.056$; effect of ranging level: $F_{1, 18} = 0.064, p = 0.804, \eta_p^2 = 0.004$) and a significant
332 conditioned chamber preference was seen only on the second day post-supplementary conditioning

333 (effect of chamber: $F_{1, 18} = 11.564$, $p = 0.003$, $\eta_p^2 = 0.391$; effect of ranging level: $F_{1, 18} = 0.096$, $p =$
334 0.760 , $\eta_p^2 = 0.005$; effect of ranging level x chamber: $F_{1, 18} = 0.561$, $p = 0.463$, $\eta_p^2 = 0.030$).

335 Extinction trials (from the third to the sixth days) did not show any significant main effect or
336 interaction (effect of days: Greenhouse-Geisser corrected- $F_{2.279, 49.447} = 1.468$, $p = 0.237$, $\eta_p^2 = 0.075$,
337 Figure 5; effect of chamber: $F_{1, 18} = 4.388$, $p = 0.051$, $\eta_p^2 = 0.196$; effect of ranging level: $F_{1, 18} = 0.516$,
338 $p = 0.482$, $\eta_p^2 = 0.028$; effect of days x ranging level: Greenhouse-Geisser corrected- $F_{2.279, 49.447} =$
339 0.399 , $p = 0.737$, $\eta_p^2 = 0.022$; effect of days x chamber: Greenhouse-Geisser corrected- $F_{2.509, 45.162} =$
340 2.367 , $p = 0.093$, $\eta_p^2 = 0.116$; effect of days x ranging level x chamber: Greenhouse-Geisser corrected-
341 $F_{2.509, 45.162} = 2.412$, $p = 0.089$, $\eta_p^2 = 0.118$). The number of trials without moving during the social CPP
342 did not differ between ranging groups (2 ± 2 and 2.66 ± 2.23 for low- and high-ranging chickens,
343 respectively; $U = 41$, $p = 0.53$).

344

345

346 Figure 5 about here

347

348 **4. Discussion**

349

350 Our results shed further light on the relationship between individual differences in behavior
351 and cognitive processes, specifically the relationship between ranging behavior and associative
352 learning. Learning and extinction of the associations between food/social companions and the test
353 chambers occurred differently depending on the ranging level of the chickens and the nature of the
354 reward (food or social). For the first testing day during the food CPP, both high- and low-ranging
355 chickens spent significantly more time in the conditioned chamber where they had previously found
356 mealworms. As expected, during the following extinction days, the animals showed a gradual loss of
357 their learned preference. However, high-ranging chickens were more immobile than low-ranging ones,
358 as their number of trials without moving was significantly higher. Unexpectedly, during the first day
359 of the social CPP test, only high-ranging chickens showed a place preference in comparison to the
360 low-ranging chickens. An overall place preference was perceived only on the second day; therefore,

361 we had to submit the broiler chickens to two additional conditioning days to standardize the possible
362 uneven association learning between the groups. Post-supplementary conditioning analyses confirmed
363 our earliest results, as the groups showed a similar pattern of behavior. During the extinction days of
364 social CPP, contrary to the food CPP, the preference for the conditioned chamber quickly faded and
365 was no longer significant.

366 The findings from the food CPP suggest that, like other species (de Jonge et al., 2008; Duarte
367 et al., 2014), chickens value food and can learn to associate environmental cues with a food reward.
368 However, the strength of this association may depend on individual differences in preferences. Since
369 individuals may value things differently, these preferences can affect how they cognitively respond to
370 their presence or, as is the case of our test, their absence (Sih and Del Giudice, 2012).

371 Even if low- and high-ranging chickens did not differ in their association strength on the first
372 day of the food CPP test, supplementary analyses suggested that high-ranging chickens differed from
373 low-ranging individuals by showing more immobility in the extinction phase. Here, high-ranging
374 chickens seemed to exhibit alternative behaviors in response to the same reinforcer. Alternative
375 behaviors such as immobility are known to progressively increase in rats submitted to extinction trials
376 in the Morris water maze escape paradigm (Huston et al., 2013). As foraging in the range requires
377 animals to move between different areas, the immobility of the high-ranging chickens may indicate
378 that these individuals learned more quickly that the apparatus no longer provided food, confirming the
379 different coping strategies between the high- and low-ranging chickens (Campbell et al., 2019a, 2016;
380 Taylor et al., 2020).

381 It is unlikely that these differences are linked to different levels of food motivation, as animals
382 that did not visit the cups or eat the mealworms during habituation were not included in subsequent
383 task phases. Under similar conditions, our previous studies on the relationship between free-range
384 chicken cognition and range use did not show any evidence of differences in food motivation between
385 low- and high-ranging chickens (Ferreira et al., 2020a, 2020b, 2019). Furthermore, the weight and
386 growth rates of both low- and high-ranging chickens were similar (see Table S1 in Supplementary
387 Material).

388 The social CPP presented unexpected, less straightforward results. As low-ranging chickens
389 prefer to stay in the barn where density is very high (10 individuals/m²), we predicted that, during the
390 tests, these chickens would spend more time where close proximity to flockmates was possible.
391 Conversely, the forced proximity in the small test chamber could become aversive to high-ranging
392 birds since the range is a low-density environment (0.42 individual/m²), which could cause them to
393 avoid the social conditioned chamber. Contrary to these predictions, during the first testing day, low-
394 ranging chickens did not seem to associate the presence of social companions with a preferred
395 chamber; however, high-ranging chickens did appear to make this association. A possible explanation
396 for these results may be linked to differences in individual sociability and coping styles: high-ranging
397 chickens may have searched for social proximity to cope with stress of the limited social contact
398 before testing. For other species, different degrees of sociability are also linked to different coping
399 styles and cognitive processing (Ferreira et al., 2018; Nawroth et al., 2017). However, contradicting
400 this hypothesis, we recently showed that, under similar conditions and using the same line of free-
401 ranging broiler chickens, low-ranging chickens had a higher inclination to be near conspecifics than
402 did high-ranging ones (Ferreira et al., 2020a). Additionally, Taylor et al. (2020) found that low-
403 ranging chickens had a greater physiological stress response to capture and confinement compared to
404 high-ranging chickens. Therefore, one could expect the low-ranging individuals to show a greater need
405 to cope through social proximity during our social CPP tests. An alternative explanation to why high-
406 ranging chickens showed a marked initial preference for the social conditioned chamber could be that
407 the presence of conspecifics in this chamber may have been interpreted as an indicator of foraging
408 opportunities. Fast and slow-exploring captive great tits (*Parus major*) have different patterns of
409 foraging behavior when tested in the presence of a tutor: while fast-exploring individuals readily
410 copied the behavior of the tutor and visited the feeders indicated by it, slow-exploring birds were less
411 flexible and did not change their behavior. The inverse pattern was seen when birds were tested
412 without the presence of a tutor (Marchetti and Drent, 2000). More studies on free-range chicken
413 sociability, social cognition, coping strategies and their relation to range use are needed to further
414 elucidate these questions.

415 The overall preference for the social conditioned chamber was observed later (second day) in
416 comparison to the food CPP, and even after additional conditioning, the extinction process during the
417 social CPP seemed to be much quicker than in the food CPP, as the chickens did not show a
418 preference for either chamber during extinction. There are two possible reasons (not mutually
419 exclusive) for these results: the first relies on the fact that, for animals in general, and particularly for
420 free-range broiler chickens that are selected for meat production and therefore eat frequently, food is a
421 better reward compared to the presence of social conspecifics, which results in stronger, more
422 immediate conditioning and slower extinction. Conversely, social reliance weakens as animals grow
423 older (McBride et al., 1969; Suarez and Gallup, 1983), which may have more heavily influenced the
424 quicker loss of the learned association than we had previously anticipated. As cognitive processes may
425 change during an individual's development (Zidar et al., 2018b), it would be interesting to repeat this
426 study with younger animals (before they are provided range access) to test how strong and consistent
427 the associative learning is for chicks and how it relates to range use.

428 Our results need to be interpreted with caution due to a high number of individuals that did not
429 move during the trials and were therefore excluded. Jones et al. (2012), using a three-chambered
430 apparatus to test sound CPP in domestic chicks, state that “in 40% of the post-conditioning sessions
431 there was no movement from the middle compartment of the chamber and 5 of the 32 tested chicks
432 failed to move from the middle compartment in all of their post- conditioning sessions”. Inactivity
433 during cognitive tests is therefore a common issue when studying domestic fowl (Campbell et al.,
434 2019a; Jones et al., 2012; Tahamtani et al., 2015). Non-moving individuals may be important and
435 informative concerning our hypothesis, but could not be assessed further as this inactivity may be
436 confounded with a lack of motivation to move, difficulties on learning the association or both. We
437 suggest future studies to take this behavioral response under consideration and increase the time of
438 habituation and conditioning, before proceeding to tests, in order to reduce all confounding variables
439 that can possibly influence chickens' behavior. Multiple association tests, with different arenas, could
440 also be an alternative to better assess the differences between different ranging patterns (Campbell et
441 al., 2019a).

442 Our findings reaffirm the importance of considering how memory processes are differently
443 affected and may show different nuances according to different behavioral types or coping strategies.
444 Associative learning occurs on a daily basis for farmed animals (during an association of food to
445 visual landmarks or during human-animal interactions, for example), but how individuals learn or
446 value the reward may not be similar as a result of individual differences.

447 Advancing our knowledge related to individual differences in the cognition of chickens can
448 help to identify the characteristics that affect range use. Because it provides a more comprehensive
449 understanding of an animal's ability to perceive and remember information related to its environment,
450 we can use this new knowledge to develop effective ways to stimulate birds to go out and benefit from
451 the advantages that range use offers. Over time, we expect a shift from the current 'one size fits all'
452 strategy to a more individual-based approach in the way chickens are reared, contributing to improved
453 farm animal welfare (Finkemeier et al., 2018; Richter and Hintze, 2018).

454

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459

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461

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466

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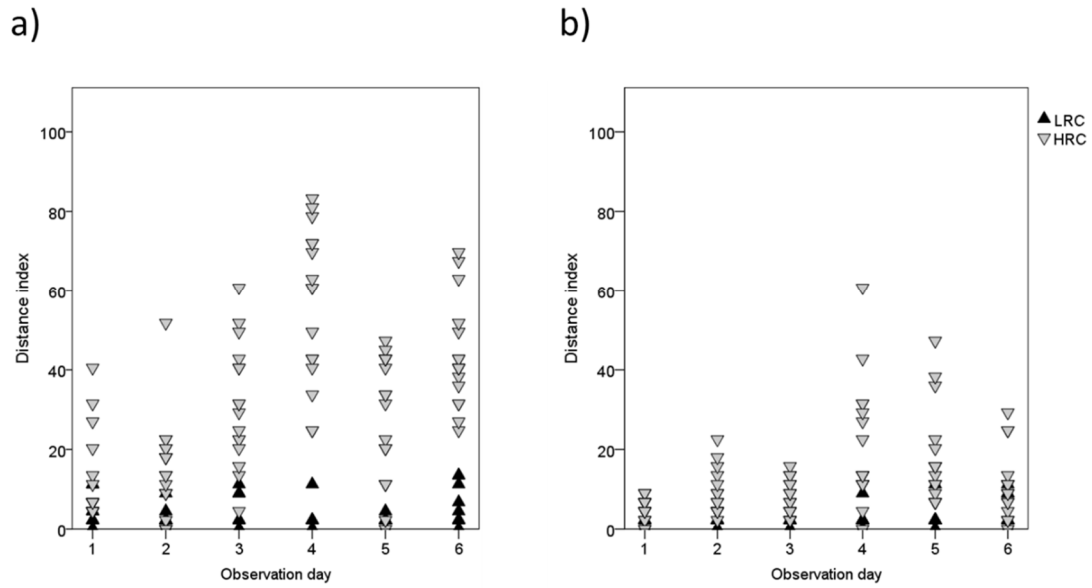


Figure 1: (a) Distance index of low- ($n = 15$) and high-ranging chickens ($n = 16$) over days of scan observations before the food CPP task. (b) Distance index of low- ($n = 16$) and high-ranging chickens ($n = 15$), LRC and HRC, respectively, over days of scan observations before the social CPP task.

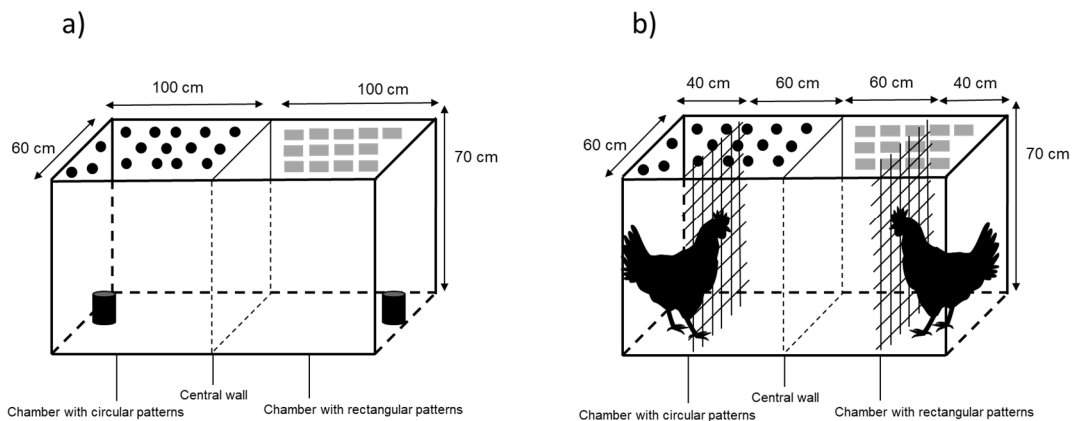


Figure 2: a) Schematic figure of the apparatus used for the food CPP task. Each chamber had different patterns on the wall. Cups were 10 cm high and 5 cm deep during conditioning and 15 cm high and 5 cm deep during the test. b) Schematic figure of the apparatus used for the social CPP task. During the conditioning phase, the extremes of each chamber were fenced to house two flockmate chickens (not tested). For both CPP tasks, the chickens were always placed near the central wall (conditioning) or in the center of the apparatus when the wall was not present (habituation and test).

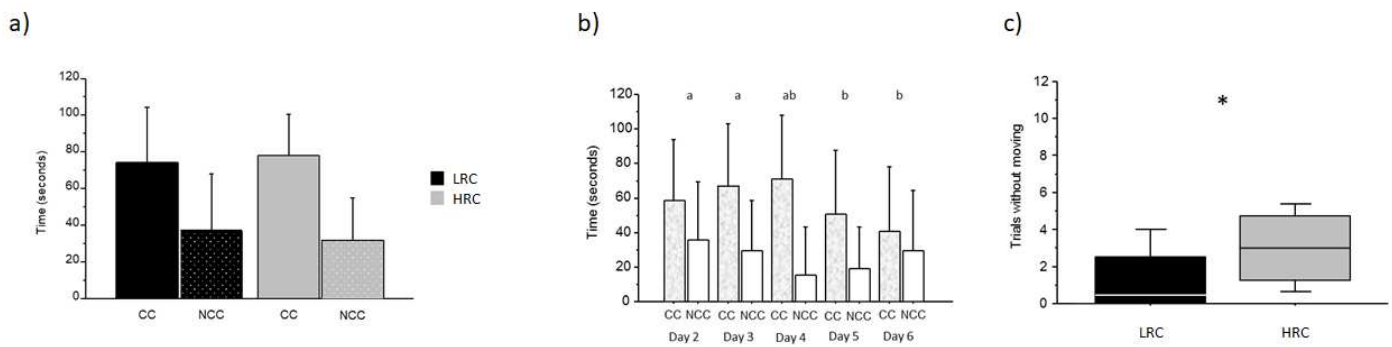


Figure 3: a) Time spent in either chamber (conditioned chamber, CC; non-conditioned chamber, NCC) on the first day of the test during the food-conditioned place preference. b) Time spent in each of the chambers (conditioned and non-conditioned) across extinction trials of the food-conditioned place preference. c) Trials without moving during the extinction phase for low- ($n = 8$) and high-ranging chickens ($n = 11$), LRC and HRC, respectively. Letters indicate significant differences in the time spent in either chamber between days. *: $p < 0.05$. Data are presented as raw means \pm SD (a and b) and median and percentile (c).

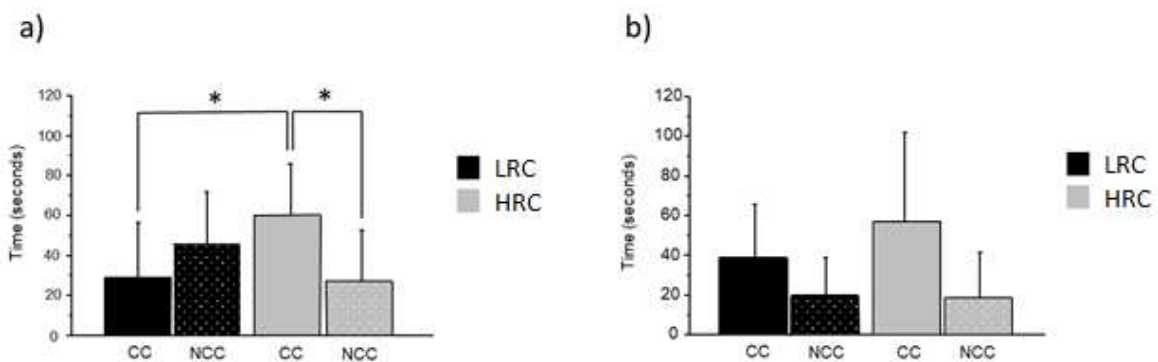


Figure 4: Time spent in either chamber (conditioned chamber, CC; non-conditioned chamber, NCC) for the first (a) and second testing days (b) during the social conditioned place preference for low- ($n = 11$) and high-ranging chickens ($n = 9$), LRC and HRC, respectively. *: $p < 0.05$. Data are presented as raw means \pm SD.

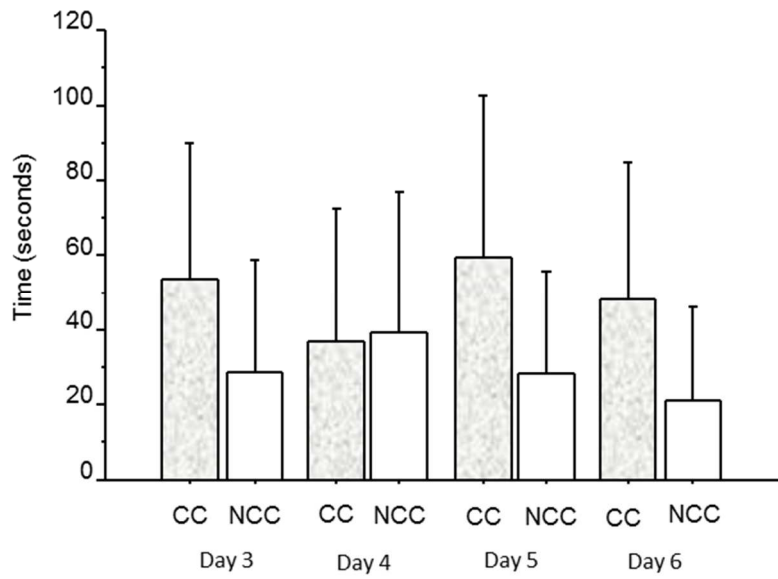


Figure 5: Time spent in either chamber (conditioned chamber, CC; non-conditioned chamber, NCC) across extinction trials during the social conditioned place preference for all tested chickens combined (n = 20, 11 low- and 9 high-ranging chickens). Data are presented as raw means \pm SD.

Table 1. (a) Number of range visits and distance indexes for selected chickens according to their ranging level: high-ranging (n = 16 and n = 15 for the food and social CPP, respectively) and low-ranging chickens (n = 15 and n = 16 for the food and social CPP, respectively), (b) Number of range visits and distance indexes for chickens included in the statistical analyses according to their ranging level: high-ranging (n = 11 and n = 9 for the food and social CPP, respectively) and low-ranging chickens (n = 8 and n = 11 for the food and social CPP, respectively). Data are presented as the means \pm SD.

a)	Year	Variables	High-ranging	Low-ranging	<i>p</i>
	2018 (Food CPP)	Range visits	18,25 \pm 1,8	4,06 \pm 1,57	U = 0, <i>p</i> < 0,001
		Distance index	187,31 \pm 44,71	13,65 \pm 7,81	U = 0, <i>p</i> < 0,001
	2019 (Social CPP)	Range visits	16,06 \pm 1,98	2,25 \pm 1,39	U = 0, <i>p</i> < 0,001
		Distance index	72,05 \pm 18,21	7,17 \pm 6,78	U = 0, <i>p</i> < 0,001
b)	Year	Variables	High-ranging	Low-ranging	<i>p</i>
	2018 (Food CPP)	Range visits	18,54 \pm 2	3,62 \pm 1,5	U = 0, <i>p</i> < 0,001
		Distance index	191,04 \pm 47,6	11,53 \pm 6,29	U = 0, <i>p</i> < 0,001
	2019 (Social CPP)	Range visits	15,55 \pm 1,94	2,45 \pm 1,43	U = 0, <i>p</i> < 0,001
		Distance index	73,25 \pm 18,24	7,97 \pm 7,48	U = 0, <i>p</i> < 0,001