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Vitor Hugo Bessa Ferreira, Karine Germain, Ludovic Calandreau, Vanessa Guesdon. Range use is related to free-range broiler chickens' behavioral responses during food and social conditioned place preference tests. *Applied Animal Behaviour Science*, 2020, 230, pp.1-8. 10.1016/j.applanim.2020.105083 . hal-03140110

**HAL Id: hal-03140110**

**<https://hal.inrae.fr/hal-03140110>**

Submitted on 22 Aug 2022

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

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## ABSTRACT

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

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

## 1. Introduction

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

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

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

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

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

## **2. Methods**

### **2.1. Ethical statement**

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

### **2.2. Animals and housing**

The experiments were performed during two different years (February to May of 2018 and 2019) with two different flocks of broiler chickens housed and managed under the same conditions. Flocks were composed of two hundred naked-neck (S757N) male broiler chickens (*Gallus gallus domesticus*) reared from their first day of life in a free-range system with a stocking density of ten individuals/m<sup>2</sup> in the barn (4 m x 5 m) and 0.42 individuals/m<sup>2</sup> in the outdoor range (27 m x 17.5 m). Continuous artificial lighting (mean of 50.87 ± 29.88 lux in different parts of the barn) was provided during the first three days after placement; then, from day 4 to day 14, it was gradually decreased until there was a total use of natural lighting. The indoor ambient temperature was maintained at 28 °C during the first week and decreased by 1 °C each week until it reached 23 °C when the birds were 38 days old. The chickens' weights were monitored at each diet change (starter, grower, and finisher diets) and before slaughter (at 1, 28, 57 and 86 days of age for the 2018 flock, and 1, 28, 56 and 105 days of age for the 2019 flock). At 28 days of age, 120 individuals were randomly selected and identified via a rectangular yellow plastic poncho around their necks containing unique acronyms for

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

### 2.3. Ranging behavior level and individual selection

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

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

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

Thirty-one healthy individuals were selected for the food CPP (15 low-ranging and 16 high-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

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

Figure 1 about here

Table 1 about here

## **2.4. Food conditioned place preference**

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

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



Figure 2 about here

#### **2.4.1. Habituation**

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

#### **2.4.2. Conditioning**

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

#### **2.4.3. Test**

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

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

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

## **2.5. Social conditioned place preference**

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

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

## **2.6. Statistics**

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

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

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

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

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

### 3. Results

#### 3.1. Food conditioned place preference

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

Extinction trials confirmed that the chickens still preferred the conditioned chamber over the non-conditioned chamber (effect of chamber:  $F_{1, 17} = 12.49$ ,  $p = 0.003$ ,  $\eta_p^2 = 0.424$ ). As days passed, however, the chickens stopped moving, which resulted in significantly less time spent in either chamber (Greenhouse-Geisser corrected- $F_{3, 272, 55.617} = 2.98$ ,  $p = 0.035$ ,  $\eta_p^2 = 0.149$ , Figure 3b).

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

No other significant effect was found (effect of days x ranging level: Greenhouse-Geisser corrected- $F_{3, 272, 55.617} = 0.453$ ,  $p = 0.732$ ,  $\eta_p^2 = 0.026$ ; chamber x days: Greenhouse-Geisser corrected- $F_{3, 427, 58.252} = 2.315$ ,  $p = 0.077$ ,  $\eta_p^2 = 0.120$ ; effect of days x ranging level x chamber: Greenhouse-Geisser corrected- $F_{3, 427, 58.252} = 1.337$ ,  $p = 0.270$ ,  $\eta_p^2 = 0.073$ ). Finally, Mann-Whitney U analysis on the number of trials without moving confirmed that high-ranging chickens moved less frequently across trials than the low-ranging chickens (trials without moving during the extinction phase:  $3 \pm 1.94$  and  $1.25 \pm 1.75$  for high- and low-ranging chickens, respectively;  $U = 20$ ,  $p = 0.048$ , Figure 3c).

Figure 3 about here

### 3.2. Social conditioned place preference

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

Figure 4 about here

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

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

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

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

Figure 5 about here

#### 4. Discussion

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

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

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

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

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

The social CPP presented unexpected, less straightforward results. As low-ranging chickens prefer to stay in the barn where density is very high (10 individuals/m<sup>2</sup>), we predicted that, during the tests, these chickens would spend more time where close proximity to flockmates was possible. Conversely, the forced proximity in the small test chamber could become aversive to high-ranging birds since the range is a low-density environment (0.42 individual/m<sup>2</sup>), which could cause them to avoid the social conditioned chamber. Contrary to these predictions, during the first testing day, low-ranging chickens did not seem to associate the presence of social companions with a preferred chamber; however, high-ranging chickens did appear to make this association. A possible explanation for these results may be linked to differences in individual sociability and coping styles: high-ranging chickens may have searched for social proximity to cope with stress of the limited social contact before testing. For other species, different degrees of sociability are also linked to different coping styles and cognitive processing (Ferreira et al., 2018; Nawroth et al., 2017). However, contradicting this hypothesis, we recently showed that, under similar conditions and using the same line of free-ranging broiler chickens, low-ranging chickens had a higher inclination to be near conspecifics than did high-ranging ones (Ferreira et al., 2020a). Additionally, Taylor et al. (2020) found that low-ranging chickens had a greater physiological stress response to capture and confinement compared to high-ranging chickens. Therefore, one could expect the low-ranging individuals to show a greater need to cope through social proximity during our social CPP tests. An alternative explanation to why high-ranging chickens showed a marked initial preference for the social conditioned chamber could be that the presence of conspecifics in this chamber may have been interpreted as an indicator of foraging opportunities. Fast and slow-exploring captive great tits (*Parus major*) have different patterns of foraging behavior when tested in the presence of a tutor: while fast-exploring individuals readily copied the behavior of the tutor and visited the feeders indicated by it, slow-exploring birds were less flexible and did not change their behavior. The inverse pattern was seen when birds were tested without the presence of a tutor (Marchetti and Drent, 2000). More studies on free-range chicken sociability, social cognition, coping strategies and their relation to range use are needed to further elucidate these questions.



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

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

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

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

## **Funding**

This experiment was funded by Yncréa Hauts-de-France and the French National Research Institute for Agriculture, Food, and Environment (INRAE).

## **Acknowledgments**

We are grateful to all members of the EASM of INRAE Magneraud unit, especially the animal caretakers, for their help beyond animal care, including in the construction of the test device and their practical advice. The authors are also grateful to Alice Ouvrier, Arthur Simoni, and Lorène Reiter for their practical assistance.

## **Bibliography**

Campbell, D., Talk, A., Loh, Z., Dyall, T., Lee, C., 2018. Spatial Cognition and Range Use in Free-

470 Range Laying Hens. *Animals* 8, 26. <https://doi.org/10.3390/ani8020026>

471 Campbell, D.L.M., Dickson, E.J., Lee, C., 2019a. Application of open field, tonic immobility, and  
 472 attention bias tests to hens with different ranging patterns. *PeerJ* 7, e8122.  
 473 <https://doi.org/10.7717/peerj.8122>

474 Campbell, D.L.M., Hinch, G.N., Downing, J.A., Lee, C., 2018. Early enrichment in free-range laying  
 475 hens: effects on ranging behaviour, welfare and response to stressors. *animal* 12, 575–584.  
 476 <https://doi.org/10.1017/S1751731117001859>

477 Campbell, D.L.M., Hinch, G.N., Downing, J.A., Lee, C., 2016. Fear and coping styles of outdoor-  
 478 preferring, moderate-outdoor and indoor-preferring free-range laying hens. *Appl. Anim. Behav.*  
 479 *Sci.* 185, 73–77. <https://doi.org/10.1016/j.applanim.2016.09.004>

480 Campbell, D.L.M., Taylor, P.S., Hernandez, C.E., Stewart, M., Belson, S., Lee, C., 2019b. An  
 481 attention bias test to assess anxiety states in laying hens. *PeerJ* 7, e7303.  
 482 <https://doi.org/10.7717/peerj.7303>

483 Cravener, T.L., Roush, W.B., Mashaly, M.M., 1992. Broiler production under varying population  
 484 densities. *Poult. Sci.* 71, 427–433. <https://doi.org/10.3382/ps.0710427>

485 Dawkins, M.S., Cook, P. a, Whittingham, M.J., Mansell, K. a, Harper, A.E., 2003. What makes free-  
 486 range broiler chickens range? In situ measurement of habitat preference. *Anim. Behav.* 66, 151–  
 487 160. <https://doi.org/http://dx.doi.org/10.1006/anbe.2003.2172>

488 De Jong, I.C., Gunnink, H., Van Harn, J., 2014. Wet litter not only induces footpad dermatitis but also  
 489 reduces overall welfare, technical performance, and carcass yield in broiler chickens. *J. Appl.*  
 490 *Poult. Res.* 23, 51–58. <https://doi.org/10.3382/japr.2013-00803>

491 de Jonge, F.H., Tilly, S.L., Baars, A.M., Spruijt, B.M., 2008. On the rewarding nature of appetitive  
 492 feeding behaviour in pigs (*Sus scrofa*): Do domesticated pigs contrafreeload? *Appl. Anim.*  
 493 *Behav. Sci.* 114, 359–372. <https://doi.org/10.1016/j.applanim.2008.03.006>

494 Dougherty, L.R., Guillette, L.M., 2018. Linking personality and cognition: a meta-analysis. *Philos.*  
 495 *Trans. R. Soc. B Biol. Sci.* 373, 20170282. <https://doi.org/10.1098/rstb.2017.0282>

496 Duarte, R.B.M., Patrono, E., Borges, A.C., César, A.A.S., Tomaz, C., Ventura, R., Gasbarri, A.,  
 497 Puglisi-Allegra, S., Barros, M., 2014. Consumption of a highly palatable food induces a lasting

498 place-conditioning memory in marmoset monkeys. *Behav. Processes* 107, 163–166.  
 499 <https://doi.org/10.1016/j.beproc.2014.08.021>

500 Ferreira, V.H.B., Barbarat, M., Lormant, F., Germain, K., Brachet, M., Løvlie, H., Calandreau, L.,  
 501 Guesdon, V., 2020a. Social motivation and the use of distal, but not local, featural cues are  
 502 related to ranging behavior in free-range chickens (*Gallus gallus domesticus*). *Anim. Cogn.*  
 503 <https://doi.org/10.1007/s10071-020-01389-w>

504 Ferreira, V.H.B., Peuteman, B., Lormant, F., Valençon, M., Germain, K., Brachet, M., Leterrier, C.,  
 505 Lansade, L., Calandreau, L., Guesdon, V., 2019. Relationship between ranging behavior and  
 506 spatial memory of free-range chickens. *Behav. Processes* 166, 103888.  
 507 <https://doi.org/10.1016/j.beproc.2019.103888>

508 Ferreira, V.H.B., Reiter, L., Germain, K., Calandreau, L., Guesdon, V., 2020b. Uninhibited chickens:  
 509 ranging behaviour impacts motor self-regulation in free-range broiler chickens ( *Gallus gallus*  
 510 *domesticus* ). *Biol. Lett.* 16, 20190721. <https://doi.org/10.1098/rsbl.2019.0721>

511 Ferreira, V.H.B., Silva, C.P.C. Da, Fonseca, E.D.P., Chagas, A.C.C.S. Das, Pinheiro, L.G.M.,  
 512 Almeida, R.N. De, Sousa, M.B.C. de, Silva, H.P.A. Da, Galvão-Coelho, N.L., Ferreira, R.G.,  
 513 2018. Hormonal correlates of behavioural profiles and coping strategies in captive capuchin  
 514 monkeys (*Sapajus libidinosus*). *Appl. Anim. Behav. Sci.* 207, 108–115.  
 515 <https://doi.org/10.1016/j.applanim.2018.07.002>

516 Finkemeier, M.-A., Langbein, J., Puppe, B., 2018. Personality research in mammalian farm animals:  
 517 Concepts, measures, and relationship to welfare. *Front. Vet. Sci.* 5, 131.  
 518 <https://doi.org/10.3389/FVETS.2018.00131>

519 Garnham, L., Løvlie, H., 2018. Sophisticated Fowl: The Complex Behaviour and Cognitive Skills of  
 520 Chickens and Red Junglefowl. *Behav. Sci. (Basel)*. 8, 13. <https://doi.org/10.3390/bs8010013>

521 Gosling, S.D., 2001. From mice to men: what can we learn about personality from animal research?  
 522 *Psychol. Bull.* 127, 45–86. <https://doi.org/10.1037/0033-2909.127.1.45>

523 Guillette, L.M., Reddon, A.R., Hurd, P.L., Sturdy, C.B., 2009. Exploration of a novel space is  
 524 associated with individual differences in learning speed in black-capped chickadees, *Parus*  
 525 *atricapillus*. *Behav. Processes* 82, 265–270. <https://doi.org/10.1016/j.beproc.2009.07.005>

526 Hartcher, K.M., Hickey, K.A., Hemsworth, P.H., Cronin, G.M., Wilkinson, S.J., Singh, M., 2016.  
 527 Relationships between range access as monitored by radio frequency identification technology,  
 528 fearfulness, and plumage damage in free-range laying hens. *animal* 10, 847–853.  
 529 <https://doi.org/10.1017/S1751731115002463>  
 530 Hsu, E.H., Schroeder, J.P., Packard, M.G., 2002. The amygdala mediates memory consolidation for an  
 531 amphetamine conditioned place preference. *Behav. Brain Res.* 129, 93–100.  
 532 [https://doi.org/10.1016/S0166-4328\(01\)00376-X](https://doi.org/10.1016/S0166-4328(01)00376-X)  
 533 Huston, J.P., Silva, M.A. d. S., Komorowski, M., Schulz, D., Topic, B., 2013. Animal models of  
 534 extinction-induced depression: Loss of reward and its consequences. *Neurosci. Biobehav. Rev.*  
 535 37, 2059–2070. <https://doi.org/10.1016/j.neubiorev.2013.02.016>  
 536 Jones, A.R., Bizo, L.A., Foster, T.M., 2012. Domestic hen chicks' conditioned place preferences for  
 537 sound. *Behav. Processes* 89, 30–35. <https://doi.org/10.1016/j.beproc.2011.10.007>  
 538 Marchetti, C., Drent, P.J., 2000. Individual differences in the use of social information in foraging by  
 539 captive great tits. *Anim. Behav.* 60, 131–140. <https://doi.org/10.1006/anbe.2000.1443>  
 540 Marino, L., 2017. Thinking chickens: a review of cognition, emotion, and behavior in the domestic  
 541 chicken. *Anim. Cogn.* 20, 127–147. <https://doi.org/10.1007/s10071-016-1064-4>  
 542 Mathur, P., Lau, B., Guo, S., 2011. Conditioned place preference behavior in zebrafish. *Nat. Protoc.* 6,  
 543 338–345. <https://doi.org/10.1038/nprot.2010.201>  
 544 McBride, G., Parer, I.P., Foenander, F., 1969. The Social Organization and Behaviour of the Feral  
 545 Domestic Fowl. *Anim. Behav. Monogr.* 2, 125–181. [https://doi.org/10.1016/S0066-1856\(69\)80003-8](https://doi.org/10.1016/S0066-1856(69)80003-8)  
 546 1856(69)80003-8  
 547 Nawroth, C., Prentice, P.M., McElligott, A.G., 2017. Individual personality differences in goats  
 548 predict their performance in visual learning and non-associative cognitive tasks. *Behav.*  
 549 *Processes* 134, 43–53. <https://doi.org/10.1016/j.beproc.2016.08.001>  
 550 Richter, S.H., Hintze, S., 2018. From the individual to the population – and back again? Emphasising  
 551 the role of the individual in animal welfare science. *Appl. Anim. Behav. Sci.*  
 552 <https://doi.org/10.1016/j.applanim.2018.12.012>  
 553 Rodriguez-Aurrekoetxea, A., Leone, E.H., Estevez, I., 2014. Environmental complexity and use of

554 space in slow growing free range chickens. *Appl. Anim. Behav. Sci.* 161, 86–94.  
555 <https://doi.org/10.1016/j.applanim.2014.09.014>

556 Sih, A., Del Giudice, M., 2012. Linking behavioural syndromes and cognition: A behavioural ecology  
557 perspective. *Philos. Trans. R. Soc. B Biol. Sci.* 367, 2762–2772.  
558 <https://doi.org/10.1098/rstb.2012.0216>

559 Sosnowka-Czajka, E., Skomorucha, I., Herbut, E., Muchacka, R., 2007. Effect of management system  
560 and flock size on the behaviour of broiler chickens. *Ann. Anim. Sci.* 7, 329–335.  
561 <https://doi.org/10.2478/aoas-2014-0063>

562 Suarez, S.D., Gallup, G.G., 1983. Social reinstatement and open-field testing in chickens. *Anim.*  
563 *Learn. Behav.* 11, 119–126. <https://doi.org/10.3758/BF03212318>

564 Tahamtani, F.M., Nordgreen, J., Nordquist, R.E., Janczak, A.M., 2015. Early Life in a Barren  
565 Environment Adversely Affects Spatial Cognition in Laying Hens (*Gallus gallus domesticus*).  
566 *Front. Vet. Sci.* 2, 1–12. <https://doi.org/10.3389/fvets.2015.00003>

567 Taylor, P., Hemsworth, P., Groves, P., Rault, J.-L., 2017a. Ranging Behaviour of Commercial Free-  
568 Range Broiler Chickens 1: Factors Related to Flock Variability. *Animals* 7, 54.  
569 <https://doi.org/10.3390/ani7070054>

570 Taylor, P., Hemsworth, P., Groves, P., Rault, J.-L., 2017b. Ranging Behaviour of Commercial Free-  
571 Range Broiler Chickens 2: Individual Variation. *Animals* 7, 55.  
572 <https://doi.org/10.3390/ani7070055>

573 Taylor, P.S., Hemsworth, P.H., Groves, P.J., Gebhardt-Henrich, S.G., Rault, J.-L., 2020. Frequent  
574 range visits further from the shed relate positively to free-range broiler chicken welfare. *animal*  
575 14, 138–149. <https://doi.org/10.1017/S1751731119001514>

576 Tzschentke, T.M., 2007. Measuring reward with the conditioned place preference (CPP) paradigm:  
577 Update of the last decade. *Addict. Biol.* 12, 227–462. <https://doi.org/10.1111/j.1369-1600.2007.00070.x>

578

579 White, N.M., McDonald, R.J., 1993. Acquisition of a spatial conditioned place preference is impaired  
580 by amygdala lesions and improved by fornix lesions. *Behav. Brain Res.* 55, 269–281.  
581 [https://doi.org/10.1016/0166-4328\(93\)90122-7](https://doi.org/10.1016/0166-4328(93)90122-7)

582 Zidar, J., Balogh, A., Favati, A., Jensen, P., Leimar, O., Sorato, E., Løvlie, H., 2018a. The relationship  
583 between learning speed and personality is age- and task-dependent in red junglefowl. *Behav.*  
584 *Ecol. Sociobiol.* 72, 168. <https://doi.org/10.1007/s00265-018-2579-2>  
585 Zidar, J., Balogh, A., Favati, A., Jensen, P., Leimar, O., Sorato, E., Løvlie, H., 2018b. The relationship  
586 between learning speed and personality is age- and task-dependent in red junglefowl. *Behav.*  
587 *Ecol. Sociobiol.* 72. <https://doi.org/10.1007/s00265-018-2579-2>  
588

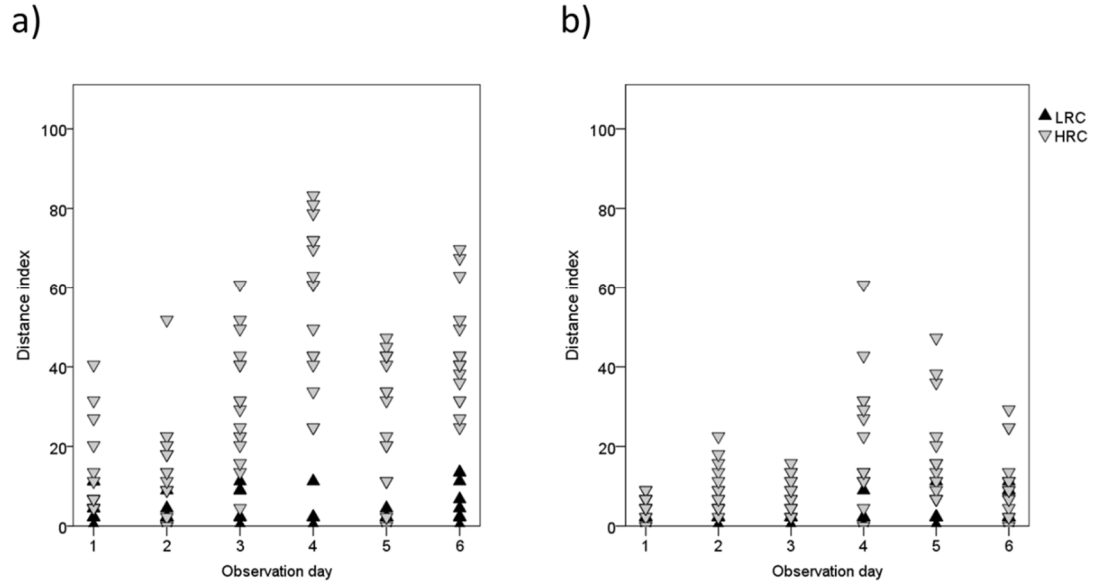


Figure 1: (a) Distance index of low- ( $n = 15$ ) and high-ranging chickens ( $n = 16$ ) over days of scan observations before the food CPP task. (b) Distance index of low- ( $n = 16$ ) and high-ranging chickens ( $n = 15$ ), LRC and HRC, respectively, over days of scan observations before the social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

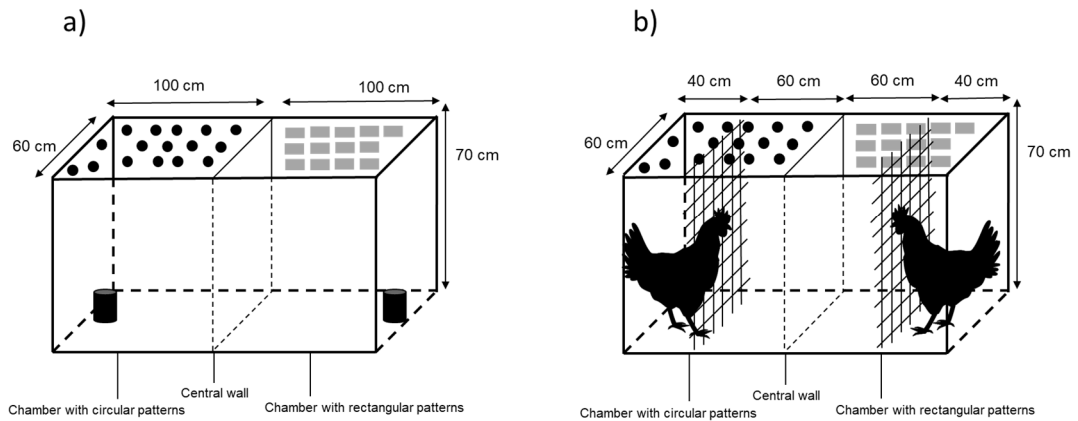


Figure 2: a) Schematic figure of the apparatus used for the food CPP task. Each chamber had different patterns on the wall. Cups were 10 cm high and 5 cm deep during conditioning and 15 cm high and 5 cm deep during the test. b) Schematic figure of the apparatus used for the social CPP task. During the 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).



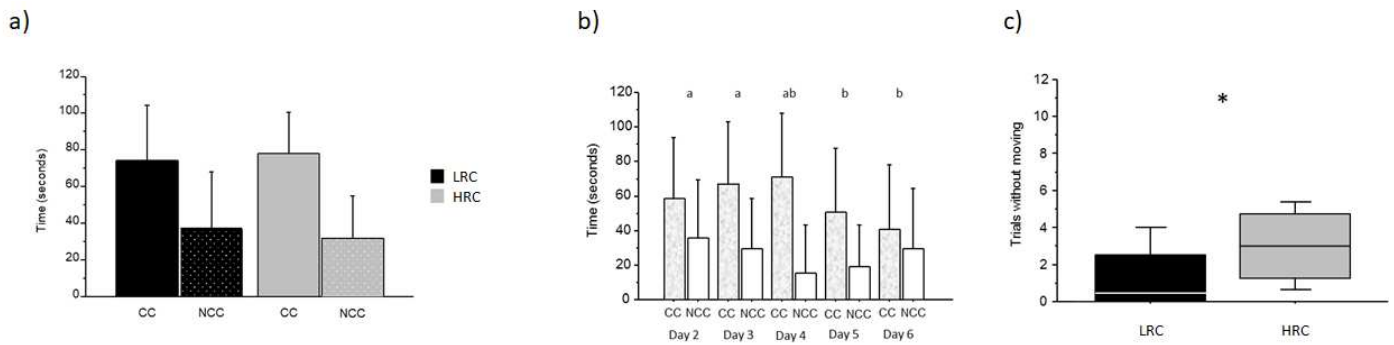


Figure 3: a) Time spent in either chamber (conditioned chamber, CC; non-conditioned chamber, NCC) on the first day of the test during the food-conditioned place preference. b) Time spent in each of the chambers (conditioned and non-conditioned) across extinction trials of the food-conditioned place preference. 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).

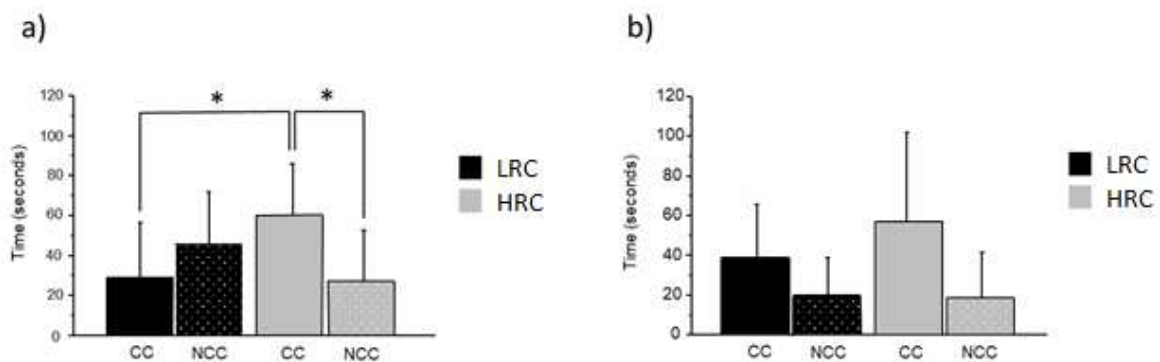


Figure 4: Time spent in either chamber (conditioned chamber, CC; non-conditioned chamber, NCC) for the first (a) and second testing days (b) during the social conditioned place preference for low- ( $n = 11$ ) and high-ranging chickens ( $n = 9$ ), LRC and HRC, respectively. \*:  $p < 0.05$ . Data are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

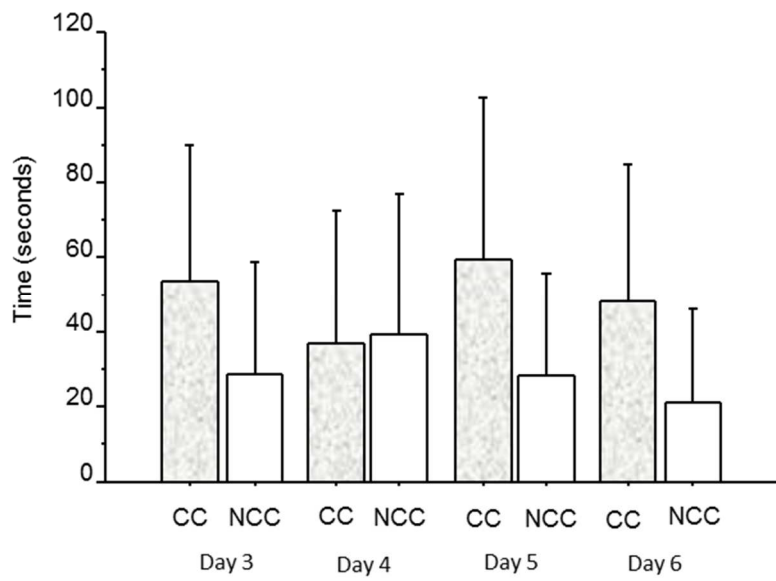


Figure 5: Time spent in either chamber (conditioned chamber, CC; non-conditioned chamber, NCC) across extinction trials during the social conditioned place preference for all tested chickens combined ( $n = 20$ , 11 low- and 9 high-ranging chickens). Data are presented as raw means  $\pm$  SD.

**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.

<b>a)</b>	<b>Year</b>	<b>Variables</b>	<b>High-ranging</b>	<b>Low-ranging</b>	<b><i>p</i></b>
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>b)</b>	<b>Year</b>	<b>Variables</b>	<b>High-ranging</b>	<b>Low-ranging</b>	<b><i>p</i></b>
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