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**Final dissertation**

**Investigation of the effects of domestication and artificial selection for  
productivity on ordinal-numerical cognitive abilities in three different  
breeds of chicks (*Gallus gallus*), with a focus on sex differences in  
Ross chicks**

*Indagine sugli effetti della domesticazione e della selezione artificiale per  
produttività sulle abilità cognitive ordinali-numeriche in tre razze differenti di  
pulcini (*Gallus gallus*), con un focus sulle differenze tra sessi nei pulcini Ross*

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## **ABSTRACT**

The process of animal domestication and its effects on animals' morphology, behaviour, and cognitive abilities have been the subject of extensive study. Domesticated animals typically reside in controlled and stable environments. This limited exposure to various environmental challenges, in addition to being subjected to some degree of artificial selection for productivity, might affect their cognitive abilities. One of the cognitive abilities potentially affected is ordinal ability, i.e., the ability to re-locate an item in a series of identical items based on its ordinal position. To investigate the effect of domestication on ordinal ability we compared three breeds of chicks (*Gallus gallus*), characterized by varying degrees of domestication and selection for productivity: Ross308, a commercial hybrid; *Gallina Padovana*, an ornamental domestic breed, and Red Junglefowl, domestic chickens' wild ancestor. For this purpose, chicks were trained to select the second container in a sequence of six identical containers arranged sagittally. They then underwent three different tests: sagittal, spatial, and fronto-parallel. The aim was to assess the extent to which the chicks relied on ordinal versus spatial information when making their choice. We compared the three breeds' performance in the ordinal test and, within the Ross308 breed, we compared the performance of males and females. Specifically, we expected non-domesticated breeds and those less strongly selected for productivity to exhibit superior performance. It was also examined whether there are sex differences in the performance of Ross308 chicks in the ordinal task. Previous research predominantly used male chickens for spatial and cognitive ability tests, making it impossible to draw conclusions about sex differences. In the three test conditions all breeds selected the numerically correct container significantly above chance level, demonstrating their ordinal abilities. In the fronto-parallel test, all breeds preferentially searched from the correct container from the left rather than from the right. Contrary to our expectations, all breeds performed well, showing no evidence that domestication and productivity selection affect ordinal abilities or lateralization. Spatial-numerical foraging abilities may remain adaptive in captivity, or selecting the second of six containers might not be challenging enough for all breeds, leading to a ceiling effect.

## INTRODUCTION

### **Domestication and artificial selection effects on animal behaviour and cognition**

The process of domestication begins when a small number of parent animals are separated from the wild species and are habituated to humans: this founder group changes over successive generations, at first in response to natural selection in the new environment within the human community, and only later to artificial selection for economic, cultural, and aesthetic reasons (Clutton-Brock, 1992). According to Clutton-Brock (1992) domestication is complete when the new population is permanently isolated from the wild species and its breeding, organization of territory, and food supply is under total human control.

In order to make domestication possible, it is also essential that the available species have social behavioural patterns compatible with those of humans. Hence, they should be gregarious, breed readily in captivity, have a wide home range and a short flight distance (Clutton-Brock, 1992). Other behavioural characteristics are a lack of fear of humans and docility (Andersson, 2000). One example of the process of domestication in action is that of the silver variety of the Red Fox *Vulpes vulpes*, described by Belyaev & Trut (Belyaev, 1979). They began by observing the behaviour of 6-8-week-old fox cubs. Those that were most responsive to being called, to being hand-fed, and handled were then chosen as breeding stock. After 15 years of selection for docile behaviour, the foxes would come when they were called and wagged their tails in greeting (Clutton-Brock, 1992). In conclusion, the factors involved in the process of domestication that differentiate domestication from the selection processes acting in the wild are basically three: relaxation of natural selection in regard to food and shelter seeking and predator avoidance; artificial selection for specific traits and against undesirable ones; unintentional selection of traits correlated either functionally or genetically to the selected ones (Andersson, 2000).

Domestication causes a genetic modification of the animal in relation to the wild ancestor. In terms of behaviour, it has been shown that there are no losses or additions of behaviours from the species' repertoires. Changes occurring during domestication are instead mostly quantitative rather than qualitative (Andersson,

2000). In the evolutionary approach to behaviour the concept of trade-off between costs and benefits of a certain response is central. The benefit of a certain response to natural stimuli is assumed to be higher for wild animals than for domestic animals, for which food and shelter already are provided. The optimal response intensity for the domestic animal would then be lower than the optimal response intensity for the wild animal (Andersson, 2000).

In a study by Andersson et al. (2001), the foraging behaviour of red jungle fowl (*Gallus gallus*), the ancestor of domestic fowl, was compared to that of domestic fowl. Wild-type fowl moved between food patches more often, aligning with optimal foraging theory, which suggests animals maximize long-term energy intake by periodically checking food options, while domestic fowl used a less energy-intensive strategy to obtain the same amount of food. The study suggests that domestication and reduced natural selection pressures led to less energy-costly foraging behaviour in domestic fowl (Andersson et al., 2001).

A recent literature review of 88 studies, undertaken by Ferreira et al. (2023), that investigated the domestication effects on animal cognitive performance, suggests that these effects remain unclear. Domesticated animals typically reside in controlled and stable environments. This limited exposure to various environmental challenges, compared to their wild counterparts, in addition to being subjected to some degree of artificial selection for productivity, might negatively affect their cognitive abilities (Ferreira et al., 2023).

### **Ordinal-numerical abilities and their adaptive function in animal species**

To successfully adapt to their natural environment and overcome the challenges it presents, animals rely on a wide range of cognitive abilities, among which ordinal-numerical abilities play a fundamental role. Numerical competence is the ability to represent, discriminate, and process numerical quantity information (Nieder, 2020). Numerical competence is of adaptive value. It enhances an animal's ability to survive by exploiting food sources, hunting prey, avoiding predation, navigating, and persisting in social interactions. It also plays a major role in successful reproduction. To make adaptive decisions animals exploit both numerical and non-numerical quantity (Nieder, 2020).

The German zoologist Otto Koehler was the first to study numerical abilities in various bird species in 1941 (Koehler, 1941). After his studies, for decades animals were still considered able to process numerical cues only when all other non-numerical cues such as odour, brightness, size, colour, shape, texture and similar were prevented, and only after extensive trainings. In the last three decades, however, growing evidence has shown various kinds of non-symbolic numerical competences in non-human species. These might be part of an ancient non-symbolic numerical system, shared across species and able to deal with numerical and quantity estimation. This system might have developed to better interact with the natural environment (Rugani & Regolin, 2021).

In particular, the ability to navigate is essential for animals that travel great distances. When finding their way, or searching for food sources during foraging, animals not only rely on numerical quantity (cardinal number) but also on numerical rank (ordinal number) (Nieder, 2020). Ordinality refers to the capability to identify a target element in a series of identical elements on the basis of its ordinal position (Rugani & Regolin, 2021). Ordinal abilities in an animal species were investigated by Chittka & Geiger (1995) in a study on honey bees, *Apis mellifera*. Bees were trained to find a feeder positioned between the third and fourth of four equally spaced and identical landmarks. In the tests, when the number of tents between hive and feeder was altered, bees landed at a shorter distance if they encountered more landmarks on their way to the feeder, whereas they flew farther if they encountered fewer landmarks. This behaviour suggests a basic form of counting, though since bees likely cannot transfer their counting to different objects, the behaviour should be considered as protocounting. The bees might use a kind of memory applied during normal foraging, that is, of the sequence of landmarks to be passed.

Numerous studies, from the early 1900s to the present day, have investigated the existence of ordinal-numerical abilities in several animal species. The ability to select an item based solely on its ordinal position within a sequence has been studied: in the crow (*Corvus americanus*) (Coburn & Yerkes, 1915); in rats (*Rattus norvegicus*) (Burt, 1916; Davis & Bradford, 1986; Suzuki & Kobayashi, 2000); in the Canary (*Serinus canaria*) (Pastore, 1961); in Honey bees (*Apis*

*mellifera*) (Chittka & Geiger, 1995); in domestic chicks (*Gallus gallus*) (Rugani et al., 2007); in fish species like the guppies (*Poecilia reticulata*) (Petrazzini et al., 2015); in the Rufous hummingbird (*Selasphorus rufus*), a wild nectarivore (Vámos et al., 2020). A second type of ordinal ability involves the comparison and ordering of numerical quantities, such as arranging them in ascending or descending order. This ability has been investigated in several species, including rhesus monkeys (*Macaca mulatta*) (Chen et al., 1997; Brannon & Terrace, 1998), brown capuchin monkeys (*Cebus apella*) (Judge et al., 2005), and a Grey parrot (*Psittacus erithacus*) (Pepperberg, 2012).

Another peculiar aspect that we should consider when studying ordinal-numerical abilities is the intrinsic association of numbers with space. Humans typically associate small numbers with the left and large numbers with the right on a mental number line (MNL) (Dehaene et al., 1993). A bias to start from the left in spatial orientation has also been observed in animals when they locate a target based on its ordinal position in a left-to-right sequence. This bias is linked to the simultaneous use of numerical and spatial information, which is processed mainly by the right hemisphere. For example, chicks show a leftward bias only when both numerical and spatial information are available. These results indicated that ordinal information is bilaterally represented in the cerebral hemispheres. Whenever both hemispheres process this information, the extra-activation of the right hemisphere takes place. This leads to the allocation of attention to the left hemisphere and thus produces a bias to “count” selectively from left to right (Rugani et al., 2010).

Ordinal-numerical abilities in young domestic chicks, and the impact of domestication and artificial selection on these abilities, were previously investigated in a study by Rugani et al. (2023). They explored the cognitive differences between two of the most commonly used broiler strains, Hybro and Ross308, by assessing their performance in an ordinal-numerical task. Ross308 chicks, which have intensively been selected for production traits, outperform Hybro chicks in terms of weight gain, final body mass, and feed conversion ratio. For the ordinal task chicks were trained to locate a food reward in the 4<sup>th</sup> container in a series of 10 identical and sagittally aligned containers. The chicks underwent

2 tests: one in the original sagittal orientation and a fronto-parallel test, where the series was rotated by 90° to evaluate the chicks' ability to transfer the learnt rule to a novel spatial orientation. In both tests Hybro chicks showed greater accuracy than Ross308 chicks. This results support the hypothesis that intense selection for productivity may influence cognitive abilities and behaviour (Rugani et al., 2023). This research provided a strong conceptual and methodological framework for the present study. This is why we decided to employ an ordinal task to examine the effects of domestication on the cognitive abilities of domestic chicks.

### **Sex differences in cognitive abilities in chicks (*Gallus gallus*)**

There are few studies investigating sex differences in cognitive abilities in chickens (*Gallus gallus*). Previous research predominantly used male chickens for spatial and cognitive ability tests, making it impossible to draw conclusions about sex differences. In a research by Regolin et al. (2005) on the working memory of chicks, the authors observed a tendency in females to prefer object characteristics in a delayed-response task where object- and position-specific cues were put in conflict (Regolin et al., 2005). Findings about sex differences in numerical discrimination and behaviour in domestic chicks were reported by Lemaire et al. (2021) in their study on the response of male and female domestic chicks to change in the number of imprinting objects. Male and female chicks were shown to behave differently when interacting with familiar and unfamiliar objects. In a familiar context, males tend to use numerical/quantity information to discriminate between two sets of familiar objects, while females focus on each familiar object individually. Instead, in a set composed of unfamiliar objects, males and females expressed similar behaviours and chose to approach the set containing fewer unfamiliar objects. The sex difference observed may derive from the natural history of wild birds. Males are more solitary, whereas females tend to live in strict hierarchies. Such organization may favour the prevalence of affiliative behaviours in females, as well as greater use of specific abilities such as transitive inference favouring individual recognition. Males might instead rely more on cues helping them to assess potential threats promptly, such as numerical cues (Lemaire et al., 2021).

### **Animal welfare: how can it be improved through research on cognition?**

Animal welfare is defined as the animal's physical and behavioural state in relation to its needs and expectations. Specifically, in regards to chickens, understanding their cognition is crucial for improving welfare, particularly in poultry production. Cognitive research can help address issues like uneven range use, feather pecking, and unfulfilled behavioural needs. It has been proved that providing environmental enrichment, like foraging materials, can reduce aggression and improve the welfare of broiler breeders (Ferreira et al., 2021).

In the wild, animals regularly solve problems to survive, requiring cognitive skills like navigation, tool use, and social cooperation. Many captive animal enrichment programs, however, fail to integrate these cognitive challenges. An "appropriate challenge" is a problem that may cause frustration but is solvable using the animal's cognitive and behavioural skills, and that allows the use of agency, namely animals' ability to explore and interact with their environment. Integrating cognitive challenges into enrichment programs can promote positive behavioural outcomes, ultimately improving animal welfare (Meehan & Mench, 2007). In conclusion, according to Duncan & Petherick (1991), animal welfare is primarily determined by the cognitive needs of the animal, and therefore meeting these cognitive needs can address both mental and physical well-being.

### **Chicks as subjects**

For this ordinal task we chose chicks (*Gallus gallus*) as subjects for several reasons. First, chickens are a precocial species, so they are able to move and be independent from the early days of life. Because of this feature, we can use them to study the influence of experience or, on the other hand, innate abilities on different aspects. To make this possible, the chick's experience in the early days of life is controlled and standardized for all subjects, so that differences in performance in tests on cognitive abilities can be traced uniquely to innate abilities. Another reason is that there are already numerous studies on cognitive abilities in chicks that can serve as an important basis. In particular, the presence of innate numerical abilities in chicks has already been demonstrated by previous researches, such as a study by Rugani et al. (2007) investigating the ability of 5-day-old domestic chicks (*Gallus gallus*) to identify a target in a series based on

its ordinal position (Rugani et al., 2007). Furthermore, we chose chicks as subjects for a comparative ordinal task because the wild relative of domestic chicks still exists in nature, as opposed to other domestic species such as cattle and horses. The domesticated chickens used for commercial meat and egg production today are descendants of the Red Junglefowl, which is found in Asia and was originally domesticated over 3,000 years ago (Al-Nasser et al., 2007). Due to the existence of different breeds of chickens, we can therefore study differences in cognitive abilities and other aspects that might derive from the type of selection each breed has undergone. In addition, we can investigate the effects of domestication on certain abilities by comparing domesticated animals to their wild non-domesticated relatives.

### **Hypothesis and aims of the present study**

To investigate the effect of domestication on ordinal ability three breeds of chicks were compared in this study, each characterized by varying degrees of domestication and selection for productivity: Ross308, a commercial hybrid; *Gallina Padovana*, a domesticated ornamental breed, and Red Junglefowl, domestic chickens' wild ancestor. They were trained to select the second container in a sequence of six identical containers arranged sagittally. Following training, in various test conditions, the three breeds' abilities were then investigated. The aim was to assess the extent to which the chicks relied on ordinal versus spatial information when making their choice. We compared the three breeds' performance in the ordinal test and, within the Ross308 breed, we compared the performance of males and females. Specifically, we expected breeds non-domesticated and those less strongly selected for productivity to exhibit superior performance. That is, we expected the Ross308 chicks to perform worse in the task due to their higher degree of domestication and selective breeding for productivity. Regarding the second research question it examines whether there are sex differences in the performance of Ross308 chicks in the ordinal task. Previous research predominantly used male chickens for spatial and cognitive ability tests, making it impossible to draw conclusions about sex differences. Overall, the aim of this study is to deepen our understanding of the effects of domestication on animals' cognitive abilities. Additionally, it seeks to

contribute to the body of knowledge regarding ordinal abilities in animals/chicks, with a particular focus on the ordinal task. Finally, the study investigated potential sex differences in these abilities.

## **METHODOLOGY**

### **Ethical approval**

All procedures used in the experiments included in this study were examined and approved by the Ethical Committee of the University of Padova (Organismo Preposto al Benessere Animale – OPBA; approval number 101010, 47/2024 as well as amendment n. 87703).

### **Subjects and housing**

Subjects were 35 chicks of the domesticated breed Ross308, 12 chicks of the domesticated breed *Gallina Padovana* and 15 Red Junglefowl chicks. All three breeds were tested at the department of General Psychology at the University of Padua, Italy. Ross308 are among the most widely used broiler strains, and as a result of artificial selection for productivity, they are superior to other strains in weight gain, final body mass, and feed conversion (Rugani et al., 2023). Regarding *Gallina Padovana*, the breed is known for its ornamental features and it is primarily bred for ornamental and egg-laying purposes. Compared to Red Junglefowl and Ross308, it is characterized by an intermediate degree of artificial selection for productivity. The Red Junglefowl, which is found in Asia, is the wild ancestor of the domesticated chickens used for commercial meat and egg production today (Al-Nasser et al., 2007).

The fertilized eggs of the domesticated breeds were obtained from a local commercial hatchery (Ross308: La Pellegrina, San Pietro in Gu, Padua, Italy; Gallina Padova: Istituto di Istruzione Superiore "Duca degli Abruzzi", Padova, Italia). For the Red Junglefowl, eggs were collected from natural nests in the game park "Oasi di Sant'Alessio", Sant'Alessio con Vialone, Pavia, Italy (Società Pavese di Ornitologia) and in the Horti of the Collegio Borromeo, Pavia. The chicks hatched after being incubated at a temperature of 37-38 °C and a humidity of 60% in the light. After hatching, when the chicks were only a few hours old,

they were transferred to standard metal home cages (28 cm × 32 cm × 40 cm). The floor of the cages was lined with absorbent paper towels. Fluorescent lamps (36 W) located 45 cm above the floor illuminated the cages. The light was on from 7 a.m. to 7 p.m., whereas in the remaining time light/dark-cycles were alternated every 2 to 3 hours. In the rearing room, temperature and humidity were between 28–31 °C and 68%, respectively. Both food (chick starter crumbles) and water were available *ad libitum*. In addition, on the first day, each chick received three mealworms (*Tenebrio molitor*) to habituate them to the food reward to be used in test. During the first three days chicks were housed singly, in the presence of five imprinting objects. Because they perceive them as social companions, chicks are highly motivated to reunite with the larger number of these imprinting objects when given the choice in a numerical discrimination task (Rugani et al., 2010), which was completed on day 3. After the numerical discrimination task chicks were housed in groups of 2-3 chicks.

### **Ordinal test**

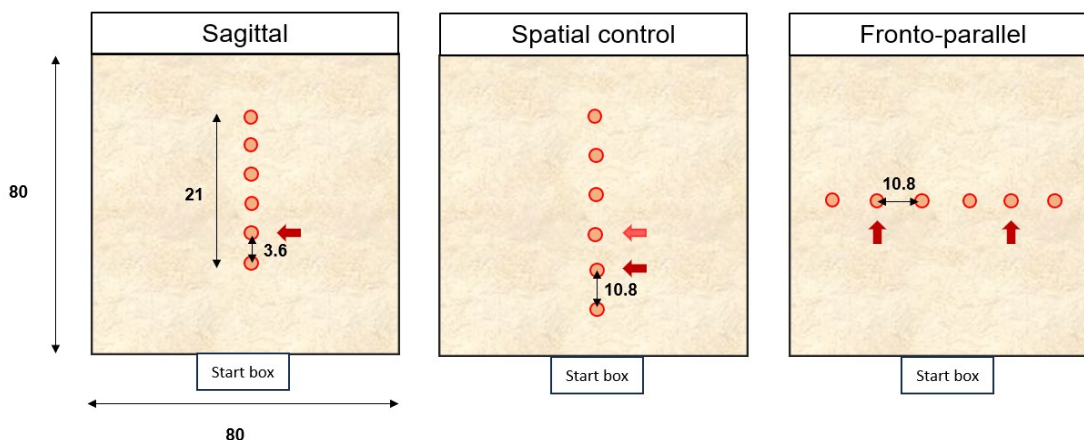
The ordinal test aimed to assess chicks' ability to remember an object in a sequence of identical objects based on its ordinal position. Chicks were trained to select the second out of six objects. In the test we verified whether chicks had successfully acquired that rule and whether they could transfer the rule also to situations in which spatial cues were not available and when the sequence was rotated. Training and testing took place on days 4 and 5.

### **Stimuli and apparatus**

The arena used for the ordinal task resembled that of Rugani et al. (2023). That is, it consisted of a square wooden arena (80 cm × 80 cm, 40 cm high) to which an external start box (15.5 cm × 15.5 cm, 10 cm high) was attached. Chicks could enter the arena via an opening (7 cm × 11 cm) that could be closed using a guillotine door. The floor of the arena was covered in wood shavings to recreate a natural foraging setting (Rugani et al., 2023).

During the test, six small red bottle caps (diameter: 3 cm, height: 0.8 cm) were positioned in the arena in an evenly spaced-out sequence. In both the baseline condition and the training phase, the distance from the centre of one cap to the

next was 3.6 cm, resulting in a total sequence length of 21 cm. At the start of each trial (training or test), all the caps were filled with wood shavings, rendering them visually indistinguishable. During the training and the sagittal test, the sequence of containers was centrally positioned in the arena, sagittally oriented relative to the chick in the start box (**Figure 1**). The centre of the first cap was located at 30.4 cm from the opening of the start box, while the last cap's centre was at 37.2 cm from the opposite end of the arena. In the spatial control test, the sequence was oriented sagittally, like in the sagittal test, but the distances between the containers were manipulated. Precisely, the third container was placed in the position in which the second (reinforced container) had previously been located. This was achieved by doubling the distance between the containers from 3.6 cm to 10.8 cm. In the fronto-parallel test, the sequence was rotated by 90° relative to the spatial control orientation, with an orthogonal distance of 38 cm between the sequence and the start box. The outermost containers were positioned at 15 cm from the left and right walls of the arena, respectively.



**Figure 1.** Overview of the experimental apparatus across three testing conditions: sagittal (left), spatial control (centre), and fronto-parallel (right), with key distances shown in cm. In the central figure the dark red arrow indicates the new position of the second (reinforced) container, whereas the light red arrow indicates its previous spatial position, now occupied by the third container.

## Experimental procedure

### Training and habituation

Chicks underwent a standardized training procedure, similar to that used by Rugani et al. (2023), in which all chicks completed the same number of trials, without implementing a success criterion (minimum number of correct trials)

which chicks needed to reach. Firstly, chicks underwent a habituation phase lasting approximately one minute, during which they were free to move around the arena. After this, the experimenter placed a mealworm in front of the start box. With the chick watching, a second mealworm was then placed on the second container. Once the chick had eaten both mealworms, the training started. The aim of the first training phase (Ph0) was to encourage chicks to actively and promptly approach the sequence. It consisted of a maximum of 20 and a minimum of 3 trials. A mealworm was placed on the second container so that it was fully visible. If the chick had not found the worm autonomously within 5 s, the experimenter indicated the location of the worm using a metal stick of approximately 20 cm, as chicks are highly attracted by shiny objects. Chicks proceeded to the next training phase after five consecutive successful trials, namely when they pecked at the second container within 5 s.

The subsequent training block consisted of the phases Ph1, Ph2, and Ph3, each composed of 5 trials. During these phases the duration of the trials until the use of the stick was varied, increasing from 15 s in Ph1 to 30 s in Ph2. It was again reduced to 15 s in Ph3. The visibility of the mealworm was also reduced, from 100% visibility to 50%, and then to 20% in the last two trials of Ph3. After Ph3, chicks were brought to their cages for a break of at least 20 min. The second training block consisted of phases Ph4–Ph8, during which the visibility of the mealworm was gradually reduced, as well as the use of the stick. Starting from Ph6 some trials had the mealworm fully hidden. In the remaining trials, the visibility was 20%. The stick was provided as a cue after 40 s. In Ph7 and Ph8 the mealworm was invisible for most of the trials, and the experimenter used the stick after 60 s, except for some trials in which no cue was provided. After the last training session, chicks were brought back to their cages for a break of at least 2 hours before the tests.

## **Test**

The test consisted of three conditions: first the sagittal test, then the spatial control test and, finally, the fronto-parallel test. Before each test, chicks underwent a pre-test, which consisted of five trials: in the first trial the visibility of the mealworm was 50%, in the next two it was 20%, and in the last two trials it

was non-visible. Between tests, chicks were brought back to their home cages for a break of 2h, which resulted in the last two tests being conducted on day 5 for some chicks. Each test consisted of 20 trials. Chicks had 60 s to peck at the second container and the stick was never used to indicate the position of the mealworm. The second container was baited, with a fully invisible mealworm, only in trials 4, 5, 7, 10, 13, 14, 16, and 19 (Rugani et al., 2007). This allowed us to control potential learning effects during the test as well as olfactory or visual cues. As in the training, trials in which chicks did not peck at one of the containers within 60s were considered null trials. After three consecutive null trials, the chick was brought back to the home cage for a break of at least one hour, which could be repeated up to three times before the chick was excluded. Only pecks at the interior of the container were considered valid.

### **Sagittal test**

During the sagittal test, the sequence of containers was arranged identically to the training phase. It was centrally positioned in the arena, sagittally oriented relative to the chick in the start box.

### **Spatial control test**

The spatial control test differed from the sagittal test only in the spacing of the containers. To assess whether chicks succeed in the sagittal condition by relying on absolute spatial cues (distance of the target container from the start box) or on its ordinal position, we positioned the third container in the spatial location previously occupied by the second, correct, one. This way, we could disentangle the use of spatial and ordinal cues: if chicks relied on spatial cues, they were expected to select the third container, whereas selecting the second container indicated reliance on ordinal information.

### **Fronto-parallel test**

In the fronto-parallel test, the sequence was rotated by 90° relative to the sagittal test. Given that this meant there were two second containers, the second from the left (2L) and the second from the right (2R), both 2L and 2R were baited with a mealworm in the reinforced trials. This allowed us to also assess chicks' potential bias to start searching from the left or the right and whether this bias differed across breeds (Rugani et al., 2007, 2023).

## **Behavioural coding**

All training and test sessions were video-recorded by a camera filming the arena from above. The software “Boris” (Behavioural Observation Research Interactive Software, Friard and Gamba) was later used for the scoring of the observed behaviours. Two types of events were coded: point events (no duration) and state events (duration). Among those the main dependent variable recorded was the number of the container chosen/pecked by the chick. As reference for the behavioural coding an ethogram was used, which defined several key behaviours. The "start of test" was marked by the first frame in which both of the chick's feet had left the start box and touched the ground in the arena. "Latency to choose" referred to the time interval from the start of the trial to when the chick made a choice or until 60 seconds had passed. A "correct choice" was defined as the chick pecking the correct element—second in the sagittal and spatial conditions, or second from the left or right in the fronto-parallel condition—before pecking any other element within the 60-second window, with only pecks inside the bottle cap being considered. Whereas an "incorrect choice" occurred when the chick pecked an incorrect element within the same time frame, again counting only pecks inside the bottle cap. “Scratching/digging” behaviour was identified when the chick rapidly moved its legs and claws, while remaining in the same spot with little or no ambulation, moving the wood shavings and often resulting in finding the worm or displacing bottle caps. The specific number of the element chosen was also recorded, as well as the final approach of the chick to the chosen element relative to the start box, categorized as front-left, front-right, back-left, or back-right.

## **Statistical analysis**

All statistical analyses were performed using RStudio (version 4.3.3; R Core Team, 2024). A Generalized Linear Mixed Model (GLMM) was employed to analyse the data. This model can be applied to a binary outcome (e.g., correct/incorrect), it accounts for random effects and utilizes a logit link function. In this study, two main statistical tools were employed to analyse group differences: the Analysis of Variance (ANOVA) and the emmeans package in R.

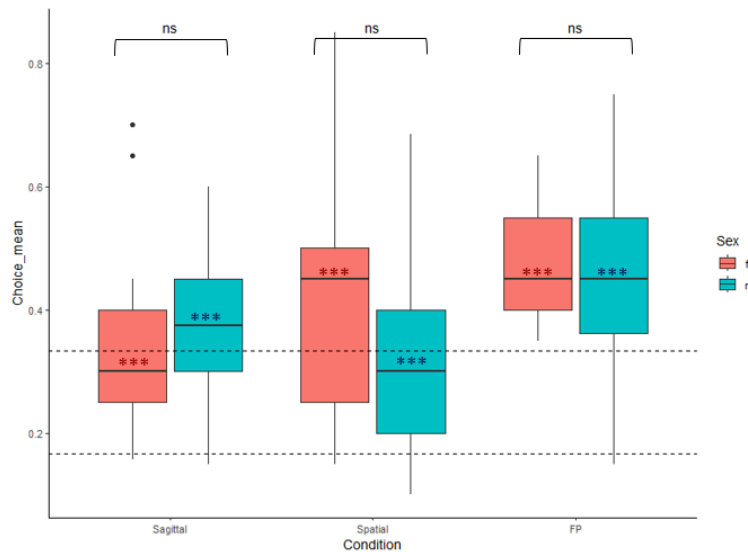
An ANOVA test was performed to compare the full and null models in R. The emmeans (Estimated Marginal Means) package was used to: extract the mean performance for each breed and sex; perform pairwise comparisons between levels of categorical variables (e.g., Ross308 vs. *Gallina Padovana*); control for multiple comparisons and therefore determine which differences were statistically significant. Graphical representations of the results were generated using the ggplot2 and survminer packages.

## RESULTS

For the sex comparison within the Ross308 breed, a subset of 13 females and 22 males was analysed. For the breed comparison, a random sample of 16 Ross308 chicks was compared against all 12 *Gallina Padovana* chicks and 15 Red Junglefowl chicks. For each test condition, we calculated and compared the mean proportion of correct choices for each group (**Figure 2**, **Figure 3**). The chance level—that is, the probability of a correct choice under random selection—was set at 1/6 (16.7%) in the sagittal and spatial tests, and at 1/3 (33.3%) in the fronto-parallel test, in which the number of correct alternatives was two (2L and 2R) out of six. A subject's performance was considered above chance if the observed proportion of correct choices significantly exceeded these baselines. In addition to accuracy measures, we analysed the distribution of choices across the six containers within each of the three test conditions and compared these choice patterns between breeds and sexes in Ross308. We also analysed the latency to make a choice, defined as the time (in seconds) taken to select a container from the moment the chick left the start box, with a possible range from 0 to 60 seconds. Mean latencies were compared across groups to explore potential differences in decision-making speed.

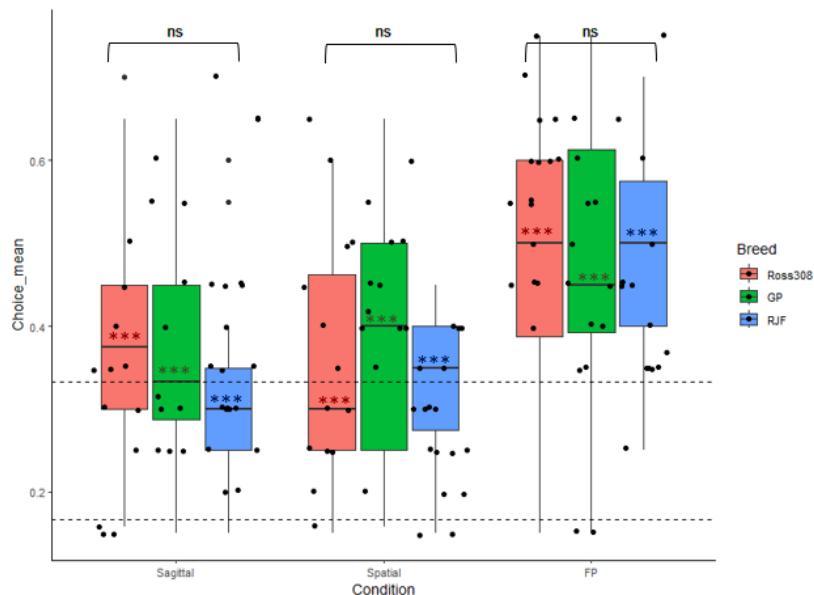
## Mean of correct choices

### Sex comparison



**Figure 2.** Graphical representation of the percentage of correct choices for each sex across the three experimental conditions. The horizontal grey lines represent the chance levels for the sagittal and spatial tests ( $y = 0.167$ ) and the fronto-parallel test ( $y = 0.333$ ); ns= not significant; \*\*\* indicates  $p < 0.001$ . Both male and female chicks selected the 2<sup>nd</sup> item significantly above chance level in all conditions ( $p \leq 0.0001$ ).

### Breed comparison

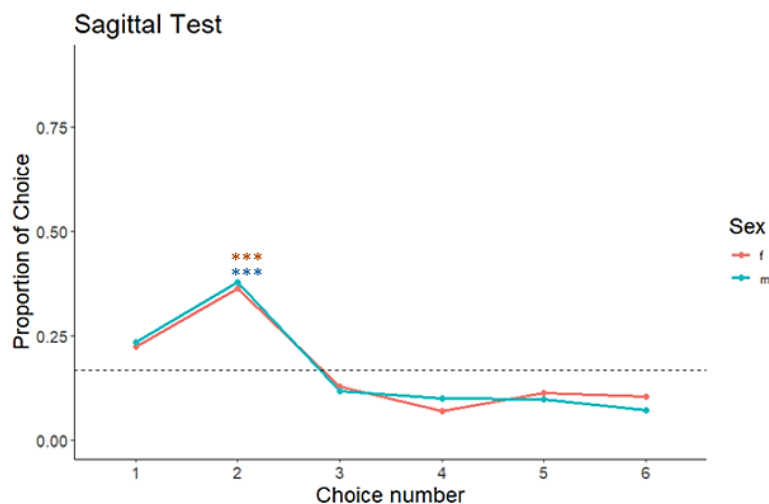


**Figure 3.** Graphical representation of the percentage of correct choices for each breed across the three experimental conditions. The horizontal grey lines represent the chance levels for the sagittal and spatial tests ( $y = 0.167$ ) and the fronto-parallel test ( $y = 0.333$ ); ns= not significant; \*\*\* indicates  $p < 0.001$ . Dots represent the mean proportion of correct choices for each subject. Ross308, *Gallina Padovana* and Red Jungle Fowl chicks all selected the 2<sup>nd</sup> item significantly above chance level in all conditions ( $p < 0.0001$ ).

## Sagittal test

### Sex comparison

In comparing the performance of female (F) and male (M) Ross308 chicks in the sagittal test, both groups performed significantly above chance level ( $p < 0.0001$ ) (**Figure 4**). Female chicks had a probability of correct choice of 0.361 (SE= 0.0372,  $z = 6.453$ ,  $p < 0.0001$ ), and male chicks had a probability of 0.369 (SE=0.0290,  $z = 8.609$ ,  $p < 0.0001$ ). The lower SE and the higher z ratio indicate that male chicks had a slightly more consistent performance than females. The comparison between the two groups revealed no statistically significant difference between female and males Ross308 chicks' performance in the sagittal test (OR= 0.967,  $z = -0.167$ ,  $p = 0.8676$ ).

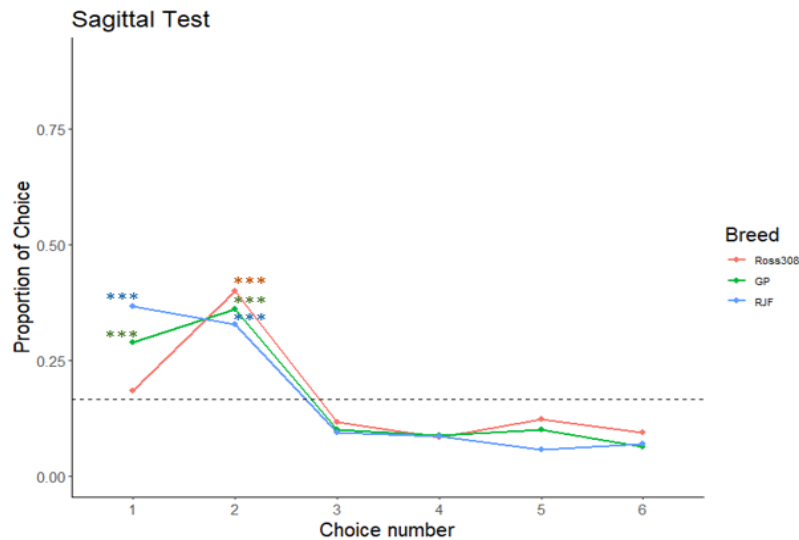


**Figure 4.** Graphical representation of the percentage of choices for each item in the sagittal test by male and female Ross308. The grey line represents the chance level ( $y = 0.167$ ); \*\*\* indicates  $p < 0.001$ . Both groups selected the 2<sup>nd</sup> item above the chance level ( $p < 0.0001$ ). The performance was similar for both male and female chicks.

### Breed comparison

In the sagittal condition all three breeds significantly chose the second container (**Figure 5**). The 1<sup>st</sup> container was also significantly chosen by *Gallina Padovana* ( $P = 0.281$ ,  $z = 3.647$ ,  $p = 0.0003$ ) and Red Jungle Fowl chicks ( $P = 0.362$ ,  $z = 6.584$ ,  $p < 0.0001$ ). Ross308 showed the highest estimated probability of choosing the correct item ( $P = 0.395$ , SE = 0.0340,  $z = 8.327$ ,  $p < 0.0001$ ), followed by *Gallina Padovana* ( $P = 0.350$ , SE = 0.0389,  $z = 5.794$ ,  $p < 0.0001$ ), and Red Jungle Fowl ( $P = 0.326$ , SE = 0.0338,  $z = 5.750$ ,  $p < 0.0001$ ). Pairwise comparisons indicated that Ross308 had higher odds compared to both *Gallina Padovana* (OR = 1.21,

SE = 0.269,  $z = 0.869$ ,  $p = 0.6600$ ) and Red Jungle Fowl (OR = 1.35, SE = 0.281,  $z = 1.437$ ,  $p = 0.3216$ ); however, these differences were not statistically significant. Similarly, *Gallina Padovana* exhibited slightly higher odds than Red Jungle Fowl (OR = 1.11, SE = 0.260,  $z = 0.456$ ,  $p = 0.8916$ ), but this difference was also non-significant. These results indicate that there is no significant breed effect on performance in the sagittal test.

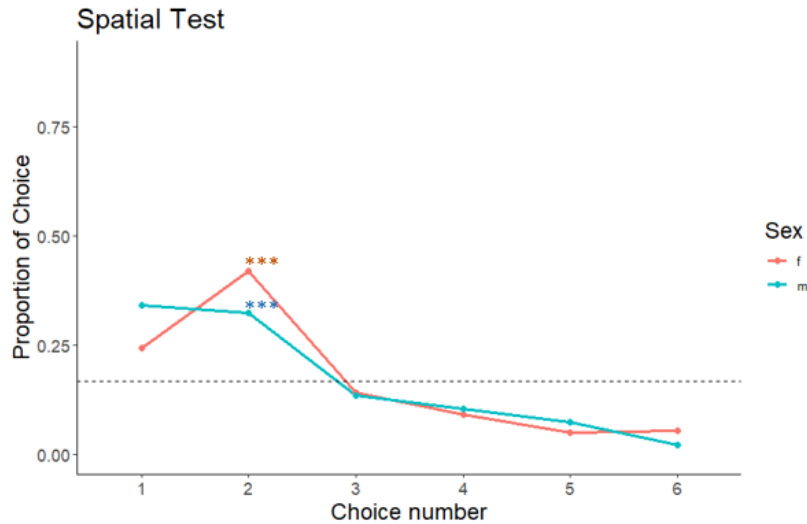


**Figure 5.** Graphical representation of the percentage of choices for each item in the sagittal test by Ross308, *Gallina Padovana* and Red Jungle Fowl chicks. The grey line represents the chance level ( $y = 0.167$ ); \*\*\* indicates  $p < 0.001$ . Each breed selected the 2<sup>nd</sup> item above the chance level ( $p < 0.0001$ ). The 1<sup>st</sup> container was also significantly chosen by *Gallina Padovana* ( $p = 0.0003$ ) and Red Jungle Fowl chicks ( $p < 0.0001$ ).

## Spatial control test

### Sex comparison

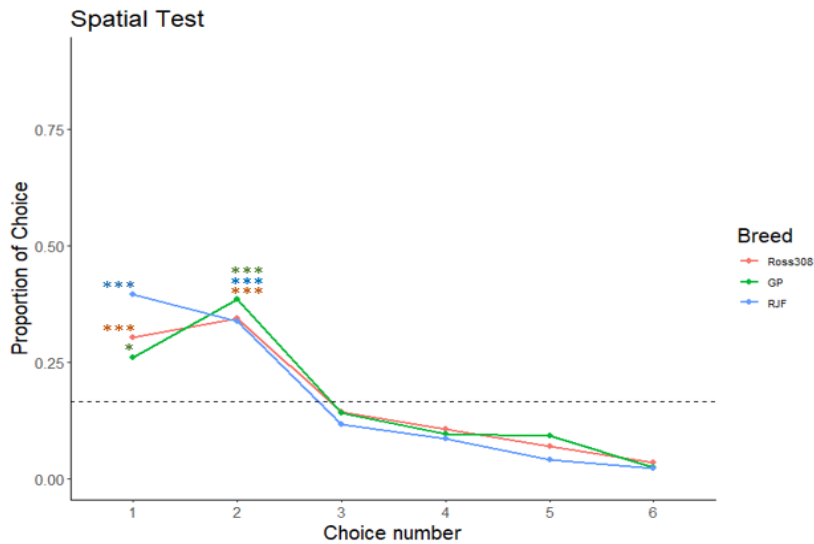
In comparing the performance of female (F) and male (M) Ross308 chicks in the spatial control test, both groups again demonstrated significant results, with  $p$ -values below 0.0001 for both sexes (**Figure 6**). Female chicks showed a probability of choosing the 2<sup>nd</sup> correct item of 0.411 (SE=0.0501,  $z = 6.031$ ,  $p < 0.0001$ ). In contrast, male chicks exhibited a probability of 0.315 (SE= 0.0350,  $z = 5.116$ ,  $p < 0.0001$ ). The comparison in performance between male and female Ross308 chicks revealed that females had higher odds of performing well in the spatial test compared to males (OR = 1.52, SE = 0.398,  $z = 1.592$ ,  $p = 0.1115$ ). This suggests that female chicks may have a modest advantage in spatial performance; however, the difference was not statistically significant.



**Figure 6.** Graphical representation of the percentage of choices for each item in the spatial test by male and female Ross308. The grey line represents the chance level ( $y = 0.167$ ); \*\*\* indicates  $p < 0.001$ . Both groups selected the 2<sup>nd</sup> item above the chance level, with females having a higher proportion of correct choices than males.

### Breed comparison

In the spatial condition, all three chick breeds performed significantly above chance in selecting the correct container (**Figure 7**). *Gallina Padovana* showed the highest estimated probability ( $P = 0.377$ ,  $SE = 0.0386$ ,  $z = 6.726$ ,  $p < 0.0001$ ), followed by Ross308 ( $P = 0.335$ ,  $SE = 0.0321$ ,  $z = 6.423$ ,  $p < 0.0001$ ), and Red Jungle Fowl ( $P = 0.321$ ,  $SE = 0.0333$ ,  $z = 5.617$ ,  $p < 0.0001$ ). Ross308 ( $P=0.280$ ,  $z= 3.497$ ,  $p=0.0005$ ) and Red Jungle Fowl ( $P=0.383$ ,  $z=6.014$ ,  $p<0.0001$ ) also significantly choose the 1<sup>st</sup> container. Pairwise contrasts revealed that *Gallina Padovana* had higher odds of performing well in the spatial condition compared to both Ross308 ( $OR = 1.196$ ,  $SE = 0.260$ ,  $z = -0.824$ ,  $p = 0.6884$ ) and Red Jungle Fowl ( $OR = 1.280$ ,  $SE = 0.286$ ,  $z = 1.106$ ,  $p = 0.5103$ ), although these differences were not statistically significant. Similarly, Ross308 showed slightly higher odds than Red Jungle Fowl ( $OR = 1.070$ ,  $SE = 0.224$ ,  $z = 0.324$ ,  $p = 0.9438$ ). According to these results, no significant breed effect was found in the spatial test condition.



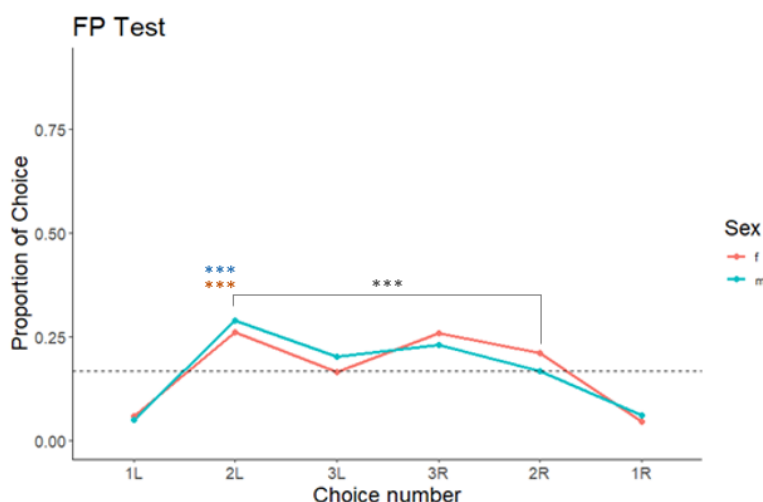
**Figure 7.** Graphical representation of the percentage of choices for each item in the spatial control test by Ross308, *Gallina Padovana* and Red Jungle Fowl chicks. The grey line represents the chance level ( $y = 0.167$ ); \* indicates  $p < 0.05$ , \*\*\* indicates  $p < 0.001$ . Each breed selected the 2<sup>nd</sup> item above the chance level ( $p < 0.0001$ ). The 1<sup>st</sup> container was also chosen above chance by the three breeds, with Red Jungle Fowl chicks choosing it on average more than the second one.

## Fronto-parallel test

### Sex comparison

In the fronto-parallel test both female and male Ross308 chicks performed significantly above chance (**Figure 8**), with females having a probability of correct choice of 0.472 (SE = 0.0361,  $z = 4.000$ ,  $p = 0.0001$ ) and males having a probability of 0.456 (SE = 0.0279,  $z = 4.575$ ,  $p < 0.0001$ ). A pairwise comparison showed that females had slightly higher odds of performing well in the fronto-parallel test compared to males (OR = 1.07, SE = 0.194,  $z = 0.359$ ,  $p = 0.7193$ ). However, this difference was not statistically significant, indicating that male and female Ross308 chicks performed similarly in this test condition.

In the fronto-parallel test, both male ( $P = 0.649$ , SE = 0.0582,  $z = 5.114$ ,  $p < 0.0001$ ) and female ( $P = 0.583$ , SE = 0.0792,  $z = 3.153$ ,  $p = 0.0016$ ) Ross308 chicks significantly choose the correct item on the left side of the sequence (2L), with no significant difference between males and females (OR=0.757, SE=0.313,  $z=1.004$ ,  $p=0.3156$ ).

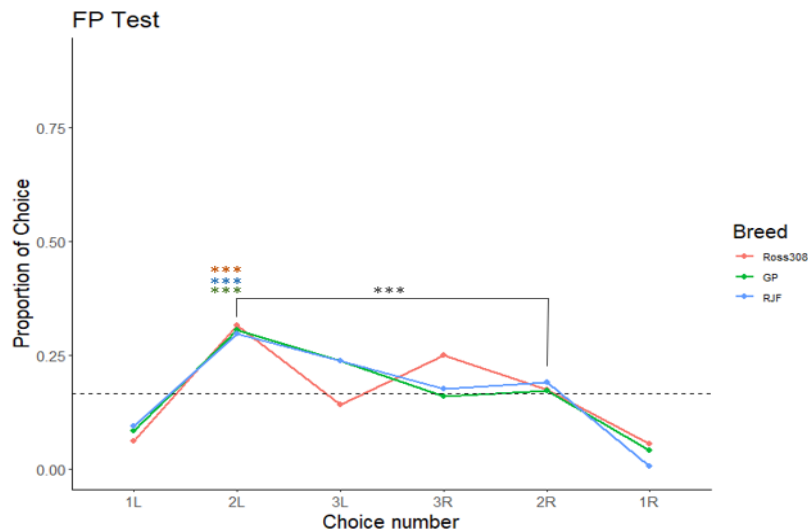


**Figure 8.** Graphical representation of the percentage of choices for each item in the fronto-parallel test by male and female Ross308. The grey line represents the chance level ( $y = 0.167$ ); \*\*\* indicates  $p < 0.001$ . Both groups selected the 2<sup>nd</sup> item from the left significantly above chance level ( $p \leq 0.0001$ ). The performance was similar for both male and female chicks.

### Breed comparison

The fronto-parallel test results indicate that all three breeds performed significantly above chance in choosing the correct container (**Figure 9**), as shown by their z-scores and p-values (Ross308:  $z = 4.478$ ,  $p < 0.0001$ ; *Gallina Padovana*:  $z = 3.592$ ,  $p = 0.0003$ ; Red Jungle Fowl:  $z = 4.324$ ,  $p < 0.0001$ ). The estimated probabilities of success were very similar across breeds: Ross308 at ( $P = 0.488$ ,  $SE = 0.0361$ ), Red Jungle Fowl at ( $P = 0.487$ ,  $SE = 0.0371$ ), *Gallina Padovana* slightly lower at ( $P = 0.476$ ,  $SE = 0.0416$ ). Furthermore, pairwise comparison showed no statistically significant advantage for any of the breeds. *Gallina Padovana* had slightly higher odds than Red Jungle Fowl ( $OR = 0.957$ ,  $SE = 0.213$ ,  $z = -0.198$ ,  $p = 0.9787$ ) and Ross308 ( $OR = 1.049$ ,  $SE = 0.231$ ,  $z = 0.218$ ,  $p = 0.9742$ ), while Red Jungle Fowl had similar odds compared to Ross308 ( $OR = 1.004$ ,  $SE = 0.208$ ,  $z = 0.019$ ,  $p = 0.9998$ ).

The analysis of the choices' side in the fronto-parallel test showed that all three Breeds chose the correct container on the left (2L) significantly more than the correct one on the right side (2R) (Ross308:  $P = 0.659$ ,  $SE = 0.0566$ ,  $z = 5.365$ ;  $p < 0.0001$ ; *Gallina Padovana*:  $P = 0.645$ ,  $SE = 0.0669$ ,  $z = 4.416$ ,  $p < 0.0001$ ; Red Jungle Fowl:  $P = 0.626$ ,  $SE = 0.0601$ ,  $z = 4.708$ ,  $p < 0.0001$ ). However, the pairwise contrasts did not reveal any statistically significant differences between the breeds.

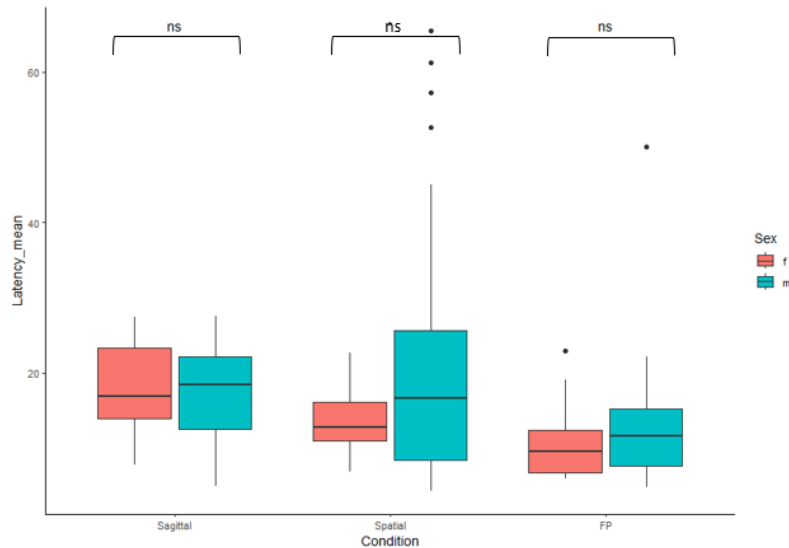


**Figure 9.** Graphical representation of the percentage of choices for each item in the fronto-parallel test by Ross308, *Gallina Padovana* and Red Jungle Fowl chicks. The grey line represents the chance level ( $y = 0.167$ ); \*\*\* indicates  $p < 0.001$ . Each breed selected the 2<sup>nd</sup> item from the left (2L) significantly above the chance level ( $p < 0.0001$ ).

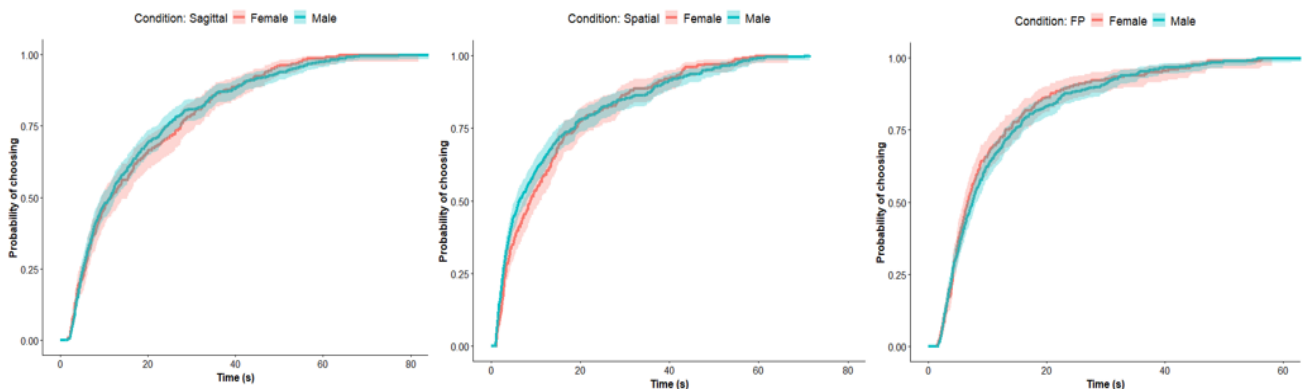
## Latency to choose

### Sex comparison

The analysis identified significant variations in mean latency times as a function of both task condition and sex (**Figure 10, Figure 11**). In females, the shortest latency was observed in the FP condition (M = 10.9 s), followed by the Spatial condition (M = 13.8 s), with the longest latency occurring in the Sagittal condition (M = 17.7 s). In contrast, males exhibited generally higher latencies across all conditions, with the lowest in the FP condition (M = 13.4 s), followed by the Sagittal condition (M = 17.3 s), and the highest in the Spatial condition (M = 23.4 s). These findings indicate that choice latency is influenced by both the experimental condition and the sex of the chicks, with the shortest times observed in the fronto-parallel condition compared to the spatial and sagittal ones. However, no significant sex differences were found in the three experimental conditions: sagittal ( $p = 0.9622$ ), spatial ( $p = 0.7523$ ), fronto-parallel ( $p = 0.5864$ ).



**Figure 10.** Graphical representation of the mean latency time required by the chick to select a container, measured in seconds. It represents the mean latency time for each sex across the three experimental conditions and it can range from 0 to 60 seconds (ns= the differences were not significant). Latency time decreased across all experimental conditions, with the shortest times observed in the fronto-parallel condition compared to the spatial and sagittal ones.

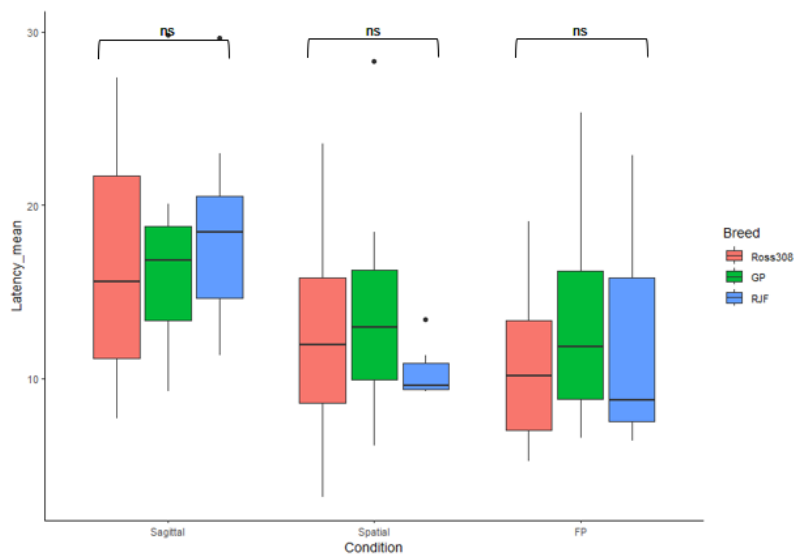


**Figure 11.** Plot of cumulative incidence illustrating the probability of making a choice over time (in seconds) for male and female Ross308 chicks under each test condition: sagittal, spatial, fronto-parallel.

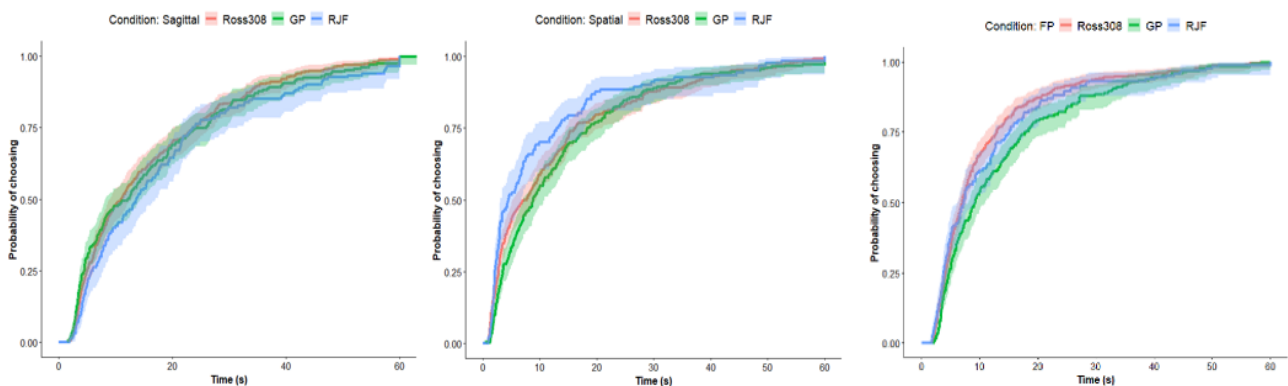
### Breed comparison

The data gathered on the latency of choice of the three breeds showed both condition-specific and breed-specific trends (**Figure 12**, **Figure 13**). The Sagittal test consistently produced the longest latencies, with Red Jungle Fowl showing the highest latency (18.5 s), followed by *Gallina Padovana* (16.7 s) and Ross308 (16.1 s). The Spatial test generally resulted in the shortest decision times, especially for Red Jungle Fowl (10.4 s), suggesting a quicker response in this condition. Regarding differences across breeds, Ross308 chose faster across all

conditions (10.8 s FP, 16.1 s Sagittal, 12.8 s Spatial), whereas Red Jungle Fowl had the slowest overall performance in the Sagittal test (18.5 s) but was the fastest in the Spatial test, showing variability based on the condition. *Gallina Padovana* exhibited relatively consistent latencies, slightly higher than Ross308 in all conditions (16.7 s Sagittal, 13.6 s Spatial, 13.8 s Fronto-parallel). However, no significant differences between Breeds were found in the three experimental conditions: sagittal ( $p=0.4988$ ), spatial ( $p=0.4277$ ), fronto-parallel( $p=0.3222$ ).



**Figure 12.** Graphical representation of the mean latency time required by the chick to select a container, measured in seconds. It represents the mean latency time for each breed across the three experimental conditions and it can range from 0 to 60 seconds (ns= the differences were not significant). Latency time decreased across all experimental conditions, with the shortest times observed in the fronto-parallel condition compared to the spatial and sagittal ones.



**Figure 13.** Plot of cumulative incidence illustrating the probability of making a choice over time (in seconds) for the three Breeds under each test condition: sagittal, spatial, fronto-parallel.

## **DISCUSSION**

### **Investigating sex differences in the performance of male and female Ross308 chicks**

Overall, male and female Ross308 chicks exhibited a similar performance in the ordinal task. Specifically, both sexes showed a similar proportion of correct choices in the sagittal and fronto-parallel test conditions, suggesting that they employ analogous cognitive strategies when processing ordinal information. In both tests, chicks were able to identify the second correct container in the sequence above chance level, indicating they had acquired the ordinal rule. Furthermore, both sexes' choice accuracy increased and the choice latency decreased in the fronto-parallel condition compared to the sagittal one, implying improved learning or task facilitation in the last condition. In the spatial test, during which ordinal and spatial cues were put in conflict for the first time, females had higher odds of performing well compared to males. Males may then rely more on spatial cues, while females may integrate object-specific and ordinal information more effectively. However, the differences found were not statistically significant. The lack of sex differences observed in the ordinal task is surprising if we examine it from the perspective of previous studies that have detected cognitive sex differences in chicks. For example, Lemaire et al. (2021) found that male chicks tend to use numerical/quantity information when interacting with familiar objects, whereas females tend to focus more on the individual objects. Such differences might have an adaptive function and derive from the natural social behaviour of the species: males are more solitary and may benefit from rapid threat assessment via numerical cues, while females, often integrated into social hierarchies, may rely more on transitive inference and individual recognition, which are advantageous skills for social navigation (Lemaire et al., 2021). Similarly, Regolin et al. (2005) reported that in a delayed-response task involving conflicting spatial and object-specific cues, female chicks showed a preference for object characteristics when choosing between two screens to locate a hidden goal. When, however, a cognitive task requires ordinal-spatial processing, no sex differences in processing might be present.

In conclusion, while both male and female Ross308 chicks demonstrate ordinal competence, sex differences in cognitive strategies might still exist on the basis of a different preference for spatial versus object-specific information. Future studies could test male and female chicks using more complex spatial-ordinal tasks to better detect potential sex differences in numerical and broader cognitive abilities.

### **Investigating the effects of domestication and selection for productivity on ordinal abilities in three chick breeds**

All breeds performed significantly above chance level in each test condition, demonstrating that wild and domesticated chicks (*Gallus gallus*) of various breeds are able to use ordinal abilities to succeed in an ordinal task. Therefore, these abilities may be fundamental skills that have been preserved throughout the processes of domestication and artificial selection. In the fronto-parallel test, all breeds showed a significant preference for the correct left-side container (2L), with no statistically significant differences in preference between breeds. A similar spatial bias has been observed in both humans and animal species in studies investigating the mental number line (MNL) (Rugani & Regolin, 2021). The MNL consists in the associations of small numbers with the left side and large numbers with the right side of the line. A bias to start from the left in spatial orientation has previously been observed in chicks in an ordinal task where they had to locate a target in a left-to-right oriented sequence (Rugani et al., 2011). This bias is linked to the simultaneous use of numerical and spatial information. Such information is processed mainly by the right hemisphere, which receives its visual input almost exclusively from the left eye in chicks (Rugani et al., 2011). Based on our findings, this bias seems to be preserved across different breeds and throughout the processes of domestication and artificial selection.

Regarding the latency to choose in the three test conditions, the sagittal test consistently produced the longest latencies across all breeds, indicating it may be the most challenging. Ross308 performed the quickest overall across all conditions, while Red Jungle Fowl exhibited a more variable response time, being fastest in the spatial test but slowest in the sagittal test. A longer latency to choose

might be linked to self-control, which can be defined as the ability to resist immediate gratification for a later benefit (Abeyesinghe et al., 2005). This ability was investigated in domestic fowl in a study by Abeyesinghe et al. (2005). In a two-key operant task, hens showed impulsive behaviour in the standard condition but were able to show self-control in the jackpot condition, when they had longer access to the food after the delay. For the authors, impulsive behaviour could be explained by temporal discounting, which is the perceived depreciation of reward value as a function of the uncertainty associated with delay (Abeyesinghe et al., 2005). Overvaluing more immediate outcomes may be an adaptive response in an uncertain environment, where obtaining delayed outcomes is uncertain because of interruptions such as predator attack, competition or food spoilage. The overall shorter latency to choose showed by chicks in the spatial test, in comparison to the sagittal test, could be explained by the use of this adaptive strategy in a new and unfamiliar condition, an environment in which they might perceive greater insecurity after being accustomed to the sagittal disposition. Another possible indicator of impulsive behavior is the frequent selection of the first container by certain breeds under specific test conditions. In the sagittal test both *Gallina Padovana* and Red Jungle Fowl significantly chose the first cap. Red Jungle Fowl chicks showed this tendency in the spatial test as well, with the mean proportion of choices directed to the first container exceeding that of the second in both the sagittal and spatial tests. While this pattern may suggest a lack of impulse control, an alternative explanation is that the chicks were employing a “working-up” strategy to locate the correct ordinal position in the sequence. The above chance percentage of errors on the cap that was the closest to the starting box may be the consequence of the chicks using one edge of the arena as a reference point and then mentally “working up” to find the correct ordinal position. This effect was previously observed in young domestic chicks performing an ordinal task (Rugani et al., 2007), in rats (Suzuki & Kobayashi, 2000), in rufous hummingbirds (Vámos et al., 2020).

We did not observe any significant differences in performance between the wild and domesticated breeds. This is in contrast with optimal foraging models, according to which in a domesticated setting, where the cost of a behaviour is

constant but the benefit is reduced, the optimal response intensity decreases (Andersson, 2000). According to this theory, relaxed natural selection pressures such as food scarcity and predation risk, could have led domesticated and highly selected breeds to perform worse than wild chicks. Domestication, in turn, should favour energy-efficient behaviours that maximize net gain. However, as noted by other authors, domestication does not lead to fundamental changes to the behavioural repertoire, but rather altered thresholds in response to stimuli. Possibly, the different breeds' thresholds are still highly similar in the ordinal task. Focusing on the differences in performance between the two domesticated breeds, we can assume effects of intense artificial selection for production on cognition. According to the resource allocation theory, animals distribute their energetic resources optimally between production and fitness (Beilharz et al., 1993). In wild-type fowl, such as the Red Jungle Fowl, this distribution balances cognitive, behavioural, and physiological traits to maximize survival and reproductive success. However, intense selection for traits like rapid weight gain in broilers leads to a reallocation of resources, potentially diminishing investment in cognitive or behavioural flexibility. In a study by Höhne et al. (2023), high-performing hens engaged in less contrafreeloading (performing foraging behaviour although food is freely available) and foraging-related behaviour than low-performing hens. These findings support the hypothesis that high-producing hens allocate more energy to reproduction and less to foraging, likely due to metabolic and energetic constraints. Interestingly, findings by Dudde et al. (2018) suggest that this reallocation does not impair cognition altogether; rather, it may drive specific enhancements, such as more efficient learning strategies that support energy acquisition under the constraints of high metabolic demand. This could be of adaptive value to cope with the energetic pressures imposed by artificial selection. However, in the present study, we did not find evidence that resource allocation shifts caused by strong selection for productivity in domesticated chicken breeds also impacts ordinal task performance.

Our results are also in contrast with previous studies investigating domestication effects on cognition in fowl. Lindqvist and Jensen (2009), for instance, compared the performance of Red Jungle Fowl chicks and White Leghorn chicks in a 2-arm

maze. According to the results, domestic chicks showed less contrafreeloading and an impaired spatial learning ability compared to the wild ancestor. Although Red Jungle fowl chicks demonstrated superior spatial learning, this advantage did not translate into significantly better performance in the ordinal task compared to the other breeds. Most importantly our results contrast with those reported by Rugani et al. (2023). Rugani and colleagues found that Hybro chicks, a less strongly selected broiler breed, outperformed Ross308 chicks in an ordinal task. In our study, the difference in selection between breeds was even greater, as both *Gallina Padovana* chicks and Red Jungle fowl chicks are much less strongly selected for productivity, or not selected and domesticated at all in the case of the Red Jungle fowl, than Hybro chicks. Consequently, these two breeds should perform better than Hybro chicks and even better than Ross308 chicks. Possible reasons for these contrasting results could lie in the differential methodologies applied. For instance, Rugani et al. (2023) trained chicks to select the 4<sup>th</sup> out of 10 containers, whereas in our study chicks had to select the 2<sup>nd</sup> out of 6. Possibly, the fact that our task was easier led to a ceiling effect in which all breeds perform so well that potential breed differences are masked. Hence, it would be worthwhile to train chicks of various breeds on a more challenging ordinal task including more than 6 possible food locations.

## **CONCLUSION**

In conclusion, our findings highlight that both domestic and wild-type chicks (*Gallus gallus*) are capable of successfully performing in an ordinal task. They were able to identify the second item in a sequence across the three experimental conditions, even when the position of the correct container was in conflict with spatial cues (increased spacing between bottle caps) and their arrangement was rotated by 90°. This indicates the use of ordinal-numerical processing alongside spatial information. Another important finding is that the side bias in the fronto-parallel test does not seem to be limited to domestic chicks: all breeds preferentially searched for the correct container from the left rather than from the right. This may suggest that both wild and domestic breeds use the Mental Number Line (MNL) to ordinally represent numbers.

When examining the effects of domestication, we found that domestic chicks' performance was as good as that of their wild relatives. Wild-type chicks might rely more on strategies suited to unpredictable environments—like the “working-up” strategy—whereas domestic chicks, raised in stable, controlled settings with consistent access to food and safety from predators, may adopt less cognitively demanding foraging strategies that are still effective in controlled task settings. Possibly, if ordinal abilities are not influenced by artificial selection or domestication, they may be fundamental skills that have been preserved throughout evolution. A more challenging ordinal task could provide deeper insight into possible differences.

Ultimately, research into ordinal cognition and broader cognitive abilities in chicks holds significant potential for improving their welfare, contributing to the growing recognition that these animals are capable of complex thought, learning, and problem-solving. Recognizing this complexity allows for the development of enriched environments that provide appropriate cognitive challenges and opportunities for agency, which are known to reduce stress-related behaviours such as feather pecking (Ferreira et al., 2021). Furthermore, artificial selection in intensive production systems, unlike natural selection, often pushes animals beyond ecological limits, leading to higher performance but potentially harming their welfare (Beilharz & Nitter, 1998).

The findings that emerged from this study raise important questions for future research: how would the different breeds and sexes behave in a more challenging task (for example when increasing the number of caps)? Would differences in ordinal-numerical and broader cognitive abilities emerge then? And ultimately, what are the real implications of artificial selection on animals/chickens' welfare?

## AUTHORS' CONTRIBUTIONS

This work was carried out as part of a larger research project led by Prof. Rosa Rugani<sup>1</sup> and Kimberly Brosche<sup>1</sup>, with statistical assistance by Filippo Gambarota<sup>3</sup>, Gianmarco Altoè<sup>3</sup>.

Chiara Pernechele contribution is restricted to a part of the project adapted to meet the requirement criteria for the bachelor mandatory internship.

Author contribution:

**Rosa Rugani**<sup>1</sup>: Project administration, Supervision, Methodology. **Kimberly Brosche**<sup>1</sup>: Conceptualization, Supervision, Investigation, Formal analysis, Writing – Review & Editing. **Filippo Gambarota**<sup>3</sup>: Formal analysis. **Gianmarco Altoè**<sup>3</sup>: Formal analysis. **Chiara Pernechele**<sup>1,2</sup>: Investigation, Data Curation, Formal analysis, Writing – Original Draft.

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## REFERENCES

- Abeyesinghe, S. M., Nicol, C. J., Hartnell, S. J., & Wathes, C. M. (2005). Can domestic fowl, *Gallus gallus domesticus*, show self-control? *Animal Behaviour*, *70*(1), 1–11.  
<https://doi.org/10.1016/j.anbehav.2004.10.011>
- Al-Nasser, A., Al-Khalaifa, H., Al-Saffar, A., Khalil, F., Albahouh, M., Ragheb, G., Al-Haddad, A., & Mashaly, M. (2007). Overview of chicken taxonomy and domestication. *World's Poultry Science Journal*, *63*(2), 285–300. <https://doi.org/10.1017/S004393390700147X>
- Andersson, M. (2000). Domestication effects on behaviour: foraging, parent-offspring interactions and antipredation in pigs and fowl. *Doctoral thesis, Swedish University of Agricultural Sciences*.  
<https://res.slu.se/id/publ/117478>
- Andersson, M., Nordin, E., & Jensen, P. (2001). Domestication effects on foraging strategies in fowl. *Applied Animal Behaviour Science*, *72*(1), 51–62. [https://doi.org/10.1016/S0168-1591\(00\)00195-7](https://doi.org/10.1016/S0168-1591(00)00195-7)
- Beilharz, R. G., Luxford, B. G., & Wilkinson, J. L. (1993). Quantitative genetics and evolution: Is our understanding of genetics sufficient to explain evolution? *Journal of Animal Breeding and Genetics*, *110*(1–6), 161–170. <https://doi.org/10.1111/j.1439-0388.1993.tb00728.x>

- Beilharz, R. G., & Nitter, G. (1998). The missing E: The role of the environment in evolution and animal breeding. *Journal of Animal Breeding and Genetics*, *115*(1–6), 439–453.  
<https://doi.org/10.1111/j.1439-0388.1998.tb00366.x>
- Belyaev, D. K. (1979). Destabilizing selection as a factor in domestication. *Journal of Heredity*, *70*(5), 301–308. <https://doi.org/10.1093/oxfordjournals.jhered.a109263>
- Brannon, E. M., & Terrace, H. S. (1998). Ordering of the Numerosities 1 to 9 by Monkeys. *Science*, *282*(5389), 746–749. <https://doi.org/10.1126/science.282.5389.746>
- Burt, H. E. (1916). A study of the behavior of the white rat by the multiple choice method. *Journal of Animal Behavior*, *6*(3), 222–246. <https://doi.org/10.1037/h0074872>
- Chen, S., Swartz, K. B., & Terrace, H. S. (1997). Knowledge of the Ordinal Position of List Items in Rhesus Monkeys. *Psychological Science*, *8*(2), 80–86. <https://doi.org/10.1111/j.1467-9280.1997.tb00687.x>
- Chittka, L., & Geiger, K. (1995). Can honey bees count landmarks? *Animal Behaviour*, *49*(1), 159–164. [https://doi.org/10.1016/0003-3472\(95\)80163-4](https://doi.org/10.1016/0003-3472(95)80163-4)
- Clutton-Brock, J. (1992). The process of domestication. *Mammal Review*, *22*(2), 79–85. <https://doi.org/10.1111/j.1365-2907.1992.tb00122.x>
- Coburn, C. A., & Yerkes, R. M. (1915). A study of the behavior of the crow *Corvus Americanus* Aud. By the multiple choice method. *Journal of Animal Behavior*, *5*(2), 75–114. <https://doi.org/10.1037/h0076090>
- Davis, H., & Bradford, S. A. (1986). Counting Behavior by Rats in a Simulated Natural Environment. *Ethology*, *73*(4), 265–280. <https://doi.org/10.1111/j.1439-0310.1986.tb00809.x>
- Dehaene, S., Bossini, S., & Giraux, P. (1993). The mental representation of parity and number magnitude. *Journal of Experimental Psychology: General*, *122*(3), 371–396. <https://doi.org/10.1037/0096-3445.122.3.371>
- Dudde, A., Krause, E. T., Matthews, L. R., & Schrader, L. (2018). More Than Eggs – Relationship Between Productivity and Learning in Laying Hens. *Frontiers in Psychology*, *9*. <https://doi.org/10.3389/fpsyg.2018.02000>
- Duncan, I. J., & Petherick, J. C. (1991). The implications of cognitive processes for animal welfare. *Journal of Animal Science*, *69*(12), 5017–5022. <https://doi.org/10.2527/1991.69125017x>

- Ferreira, V. H. B., Guesdon, V., & Calandreau, L. (2021). How can the research on chicken cognition improve chicken welfare: A perspective review. *World's Poultry Science Journal*, 77(3), 679–698. <https://doi.org/10.1080/00439339.2021.1924920>
- Ferreira, V. H. B., Lansade, L., Calandreau, L., Cunha, F., & Jensen, P. (2023). Are domesticated animals dumber than their wild relatives? A comprehensive review on the domestication effects on animal cognitive performance. *Neuroscience & Biobehavioral Reviews*, 154, 105407. <https://doi.org/10.1016/j.neubiorev.2023.105407>
- Höhne, A., Petow, S., Bessei, W., & Schrader, L. (2023). Contrafreeloading and foraging-related behavior in hens differing in laying performance and phylogenetic origin. *Poultry Science*, 102(4), 102489. <https://doi.org/10.1016/j.psj.2023.102489>
- Judge, P. G., Evans, T. A., & Vyas, D. K. (2005). Ordinal Representation of Numeric Quantities by Brown Capuchin Monkeys (*Cebus apella*). *Journal of Experimental Psychology: Animal Behavior Processes*, 31(1), 79–94. <https://doi.org/10.1037/0097-7403.31.1.79>
- Lemaire, B. S., Rugani, R., Regolin, L., & Vallortigara, G. (2021). Response of male and female domestic chicks to change in the number (quantity) of imprinting objects. *Learning & Behavior*, 49(1), 54–66. <https://doi.org/10.3758/s13420-020-00446-1>
- Lindqvist, C., & Jensen, P. (2009). Domestication and stress effects on contrafreeloading and spatial learning performance in red jungle fowl (*Gallus gallus*) and White Leghorn layers. *Behavioural Processes*, 81(1), 80–84. <https://doi.org/10.1016/j.beproc.2009.02.005>
- Meehan, C. L., & Mench, J. A. (2007). The challenge of challenge: Can problem solving opportunities enhance animal welfare? *Applied Animal Behaviour Science*, 102(3–4), 246–261. <https://doi.org/10.1016/j.applanim.2006.05.031>
- Nieder, A. (2020). The Adaptive Value of Numerical Competence. *Trends in Ecology & Evolution*, 35(7), 605–617. <https://doi.org/10.1016/j.tree.2020.02.009>
- Pastore, N. (1961). Number Sense and ‘Counting’ Ability in the Canary. *Zeitschrift Für Tierpsychologie*, 18(5), 561–573. <https://doi.org/10.1111/j.1439-0310.1961.tb00242.x>
- Pepperberg IM (2012). Further evidence for addition and numerical competence by a Grey parrot (*Psittacus erithacus*). *Animal Cognition*, 15(4):711-7. doi: 10.1007/s10071-012-0470-5
- Petrazzini, M. E. M., Lucon-Xiccato, T., Agrillo, C., & Bisazza, A. (2015). Use of ordinal information by

- fish. *Scientific Reports*, 5(1), 15497. <https://doi.org/10.1038/srep15497>
- Regolin, L., Garzotto, B., Rugani, R., Pagni, P., & Vallortigara, G. (2005). Working memory in the chick: Parallel and lateralized mechanisms for encoding of object- and position-specific information. *Behavioural Brain Research*, 157(1), 1–9. <https://doi.org/10.1016/j.bbr.2004.06.012>
- Rugani, R., Kelly, D. M., Szelest, I., Regolin, L., & Vallortigara, G. (2010). Is it only humans that count from left to right? *Biology Letters*, 6(3), 290–292. <https://doi.org/10.1098/rsbl.2009.0960>
- Rugani, R., & Regolin, L. (2021). Numerical Abilities in Nonhumans: The Perspective of Comparative Studies. In M. Danesi (A. c. Di), *Handbook of Cognitive Mathematics* (pp. 1–33). Springer International Publishing. [https://doi.org/10.1007/978-3-030-44982-7\\_39-1](https://doi.org/10.1007/978-3-030-44982-7_39-1)
- Rugani, R., Regolin, L., & Vallortigara, G. (2007). Rudimental numerical competence in 5-day-old domestic chicks (*Gallus gallus*): Identification of ordinal position. *Journal of Experimental Psychology: Animal Behavior Processes*, 33(1), 21–31. <https://doi.org/10.1037/0097-7403.33.1.21>
- Rugani, R., Vallortigara, G., Vallini, B., & Regolin, L. (2011). Asymmetrical number-space mapping in the avian brain. *Neurobiology of Learning and Memory*, 95(3), 231–238. <https://doi.org/10.1016/j.nlm.2010.11.012>
- Rugani, R., Zhang, Y., Scarsi, B., & Regolin, L. (2023). Hybro chicks outperform Ross308 in a numerical-ordinal task. Cognitive and behavioral comparisons between 2 broiler strains of newborn domestic chicks (*Gallus gallus*). *Poultry Science*, 102(12), 103148. <https://doi.org/10.1016/j.psj.2023.103148>
- Suzuki, K., & Kobayashi, T. (2000). Numerical competence in rats (*Rattus norvegicus*): Davis and Bradford (1986) extended. *Journal of Comparative Psychology*, 114(1), 73–85. <https://doi.org/10.1037/0735-7036.114.1.73>
- Vámos, T. I. F., Tello-Ramos, M. C., Hurly, T. A., & Healy, S. D. (2020). Numerical ordinality in a wild nectarivore. *Proceedings of the Royal Society B: Biological Sciences*, 287(1930), 20201269. <https://doi.org/10.1098/rspb.2020.1269>