Influences of environment and personality on cognitive judgment bias in chickens

Emelie Jansson

LiTH-IFM- Ex--15/3002--SE

Supervisors: Hanne Løvlie and Josefina Zidar,
Linköping University

Examiner: Jordi Altimiras, Linköping University
Influences of environment and personality on cognitive judgment bias in chickens

Emelie Jansson

Cognitive processes include biases, such as cognitive judgment bias. Cognitive judgment bias influences how the surrounding is interpreted, and this can differ between individuals. However, thus far no formal framework exists to understand how cognitive judgment bias works. Here I investigated how environmental factors and personality influence cognitive judgment bias in Gallus gallus chicks. First I investigated how two environmental factors affected the cognitive judgment bias of laying hen chicks. Chicks were exposed to stress and/or environmental enrichment, and tested in a cognitive judgment bias test before and after collective unpredictable stressors were presented. The results showed that chicks living in enriched environments were faster to reach all cues after the collective stressors than chicks living in non-enriched environments. Individual differences are often observed in animals, even when raised under identical conditions; therefore I also investigated if variation in personality influence cognitive judgment bias. Red junglefowl chicks were thereby raised in equal environments and exposed to personality assays in addition to a cognitive judgment bias test. I demonstrated that less nervous chicks were more optimistic towards ambiguous and negative cues than more nervous chicks. Also previous studies have found indications of connections between cognitive bias and environment or personality. I conclude that environmental enrichment can buffer the influence of stress on cognitive judgment bias and that personality has a small influence on interpretations of stimuli. In the future, experiments in this field should focus on exploring more aspects on how cognitive biases occurs to improve our understanding of cognitive processes.

Cognitive judgment bias, personality, welfare, environmental enrichment
## Content

1 Abstract ........................................................................................................................................................................ 2
2 Introduction ......................................................................................................................................................................... 2
3 Methods ............................................................................................................................................................................... 6
   3.1 Influence of environmental factors on cognitive judgment bias ................................................................. 6
      3.1.1 Animals and housing ................................................................................................................................. 6
      3.1.2 Test room conditions ................................................................................................................................. 8
      3.1.3 Associative learning ................................................................................................................................. 9
      3.1.4 Cognitive judgment bias test ..................................................................................................................... 11
   3.2 Linking variation in personality to cognitive judgment bias ............................................................................ 12
      3.2.1 Animals and housing ................................................................................................................................. 12
      3.2.2 Associative learning ................................................................................................................................. 12
      3.2.3 Cognitive judgment bias test ..................................................................................................................... 13
      3.2.4 Personality assays ........................................................................................................................................ 13
   3.3 Statistical analysis ..................................................................................................................................................... 17
      3.3.1 Influence of environmental factors on cognitive judgment bias ................................................................. 17
      3.3.2 Linking variation in personality to cognitive judgment bias .................................................................... 18
4 Results ............................................................................................................................................................................. 18
   4.1 Influence of environmental factors on cognitive judgment bias .......................................................................... 18
   4.2 Linking variation in personality to cognitive judgment bias ............................................................................... 21
5 Discussion ....................................................................................................................................................................... 24
   5.1 Influence of environmental factors on cognitive judgment bias ........................................................................... 25
   5.2 Linking variation in personality to cognitive judgment bias ............................................................................. 26
   5.3 Societal and ethical considerations ......................................................................................................................... 27
6 Conclusions ...................................................................................................................................................................... 28
7 Acknowledgements ........................................................................................................................................................ 28
8 References ........................................................................................................................................................................ 29
1 Abstract

Cognitive processes include biases, such as cognitive judgment bias. Cognitive judgment bias influences how the surrounding is interpreted, and this can differ between individuals. However, thus far no formal framework exists to understand how cognitive judgment bias works. Here I investigated how environmental factors and personality influence cognitive judgment bias in Gallus gallus chicks. First I investigated how two environmental factors affected the cognitive judgment bias of laying hen chicks. Chicks were exposed to stress and/or environmental enrichment, and tested in a cognitive judgment bias test before and after collective unpredictable stressors were presented. The results showed that chicks living in enriched environments were faster to reach all cues after the collective stressors than chicks living in non-enriched environments. Individual differences are often observed in animals, even when raised under identical conditions; therefore I also investigated if variation in personality influence cognitive judgment bias. Red junglefowl chicks were thereby raised in equal environments and exposed to personality assays in addition to a cognitive judgment bias test. I demonstrated that less nervous chicks were more optimistic towards ambiguous and negative cues than more nervous chicks. Also previous studies have found indications of connections between cognitive bias and environment or personality. I conclude that environmental enrichment can buffer the influence of stress on cognitive judgment bias and that personality has a small influence on interpretations of stimuli. In the future, experiments in this field should focus on exploring more aspects on how cognitive biases occurs to improve our understanding of cognitive processes.

2 Introduction

Cognition is the process of gathering, processing, storing and acting upon information from the surrounding environment (Shettleworth 2010). Animals rely on cognitive processes to be able to find food, shelter and mates as well as to avoid predators. Individual variation in cognitive abilities can therefore have great impact on animal lives (Shettleworth 2010). Some known individual difference in the cognitive process is cognitive biases. One of these biases is cognitive judgment bias, defined as a bias in the cognitive process where the interpretation of stimuli differs dependent on the mood (i.e. a prolonged affective state, affective state being subjective experiences of different valence that do not occur as a response to a stimuli, Russell 2003, Mendl et al. 2010) of the individual (Mathews & MacLeod 1985, Eysenck et al. 1991).
Work on cognitive judgment bias in humans show that individuals experiencing a negative mood (e.g. depression or anxiety) interpret stimuli more negatively than individuals experiencing a positive mood (e.g. happy or excited, e.g. Mathews et al. 1989, Eysenck et al. 1991). A well-known example of cognitive judgment bias is when humans are asked if a glass half-filled with liquid, is half full or half empty. Within animal science, cognitive judgment bias was first investigated by Harding and colleagues (2004). They observed that different environmental conditions changed the response of male rats to sound cues indicating reward or punishment (Harding et al. 2004). Rats that were housed in an unpredictable environment showed more pessimistic responses to cues predicting reward and ambiguous cues resembling the rewarded cue, than animals living in predictable housing systems (Harding et al. 2004). Thus, by observing animals’ reactions to ambiguous stimuli that are intermediate to cues associated with punishment and reward, we can interpret the individuals’ affective states.

Cognitive judgment bias tests are generally performed by training the animal to associate one cue (e.g. tone, colour or placement) with a reward (mostly a treat) and its opposite (e.g. white vs. black or right vs. left), with a lack of reward or a punishment (e.g. electric shock or white noise). How the animal behaves towards intermediate, ambiguous, cues is then investigated as indicative of the affective state of the animal (i.e. the response to the ambiguous cues are interpreted with regards to the previously rewarded and unrewarded or punished cues). Reactions to ambiguous stimuli that resemble the response to unrewarded or punished cues are interpreted as reactions of a more pessimistic individual (Harding et al. 2004, Matheson et al. 2008). On the other hand, reactions to ambiguous stimuli resembling the response to rewarded cues are interpreted as the animal being more optimistic (Harding et al. 2004, Matheson et al. 2008).

The knowledge that human cognitive judgment bias is influenced by their mood has been used to further the research of animal welfare. Animal welfare being defined as an individual's attempts and level of success in coping with its environment (Broom 1986, 1991). Studies of animal welfare highlight the importance of evaluating quality of life of animals that we keep in captivity (Fraser et al. 1997). An important component of animal welfare is the importance of subjective states of animals (Fraser et al. 1997, Hewson 2003, Mendl et al. 2009). According to Duncan (1996) it is even the most important part of assessing animal welfare. Previously, focus has been on preventing poor welfare such as feather pecking in chickens (e.g. Dixona 2008), tail biting in pigs (e.g. Sutherland et al. 2008).
focus has been directed towards measures of positive welfare. One way of estimating both positive and negative animal welfare is by observing the subjective states of animals. Animal welfare can consequently be measured by investigating the mood of animals (Russell 2003, Mendl et al. 2010). To investigate the mood of animals cognitive judgment bias tests have been used. Cognitive judgement bias tests are ideal to use as they do not only measure the arousal of an individual’s mood (which gives no distinction between positive and negative affective states), but also the valence of it (which can be positive or negative, Mendl et al. 2009).

Variants of the method to measure cognitive judgment bias in animals have been used to assess welfare in starlings (Sturnus vulgaris, Bateson and Matheson 2007, Brilot et al. 2010, Matheson et al. 2008), chickens (Gallus gallus, Salmero et al. 2011, Seehuus et al. 2013), rats (Rattus norvegicus, Enkel et al. 2010) and calves (Bos taurus, Neave et al. 2013). These studies have found that enriched and/or predictable housing conditions result in more optimistic animals, interpreted as animals with better welfare. However, Wichman and colleagues (2012) did not find a relationship between welfare and enrichment in a study on welfare in laying hens. Moreover, the studies mentioned above have typically focused on one single factor (e.g. stress or environmental enrichment) at the time that could influence the emotional state of the animals and are therefore potentially missing important factors that might influence the individuals’ biases. To understand what influences variation in cognitive judgment bias, it is vital to investigate how several different factors, such as enrichment and stress (the non-specific response of the body to any demand made upon it, Selye 1973) affect the response of an individual. However, it is also important to investigate how interactions of factors affects individual interpretation of stimuli as different stimuli might influence each other.

While trying to understand animal emotions and welfare through cognitive judgment bias tests, it has become apparent that there are large individual differences in how individuals’ responses are affected. For example individual differences have been observed when observing the effects of stress on animal welfare (Wichman et al. 2012). Intraspecific individual variation in behavioural responses over time and / or across context (Gosling 2001, Dall et al. 2004, Réale et al. 2007) is used to describe animal personality. Thereby personality could be the reason behind the individual differences observed in cognitive judgment bias
tests. Animal personality has been demonstrated in a broad range of species (Gosling 2001), including the fowl (Favati et al. 2014a,b). Personality gradients that animals are described along are typically shyness-boldness, exploration-avoidance, activity, sociability, aggressiveness (Réale et al. 2007), and nervousness (Gosling & John, 1999). Personality is known to interlink with variation in physiology, commonly investigated in rodents and under the term ‘coping styles’ (Koolhaas et al. 1999, 2010). Further, connections between cognitive processes and personality have been seen as success in associative learning (a process in which a behavioural response becomes altered or acquired as a consequence of sensory information from the environment, Lachman 1997) have been connected to boldness (Dugatkin & Alfieri 2003). With this cognitive process shown to be related to variation in personality it is possible that also biases in cognitive processes are related to personality. In fact, attention bias has been shown to correlate with personality in parrots (*Amazona amazonica*, Cussen & Mench, 2014). However, no formal framework has, been developed for how personality and cognitive biases may be related. Although one may predict that individuals that are more nervous or stress-sensitive would be prone to have more negative biases.

In order to thoroughly understand how cognitive judgment bias occurs and what influences it, I here investigate why differences in cognitive judgment bias occur among individuals by (1) investigating how environmental enrichments and stress influence cognitive judgment bias in chicks, and (2) investigating how variation in personality relates to variation in cognitive judgment bias among chicks.

When exploring the influence of environmental complexity, stress and personality on variation in cognitive judgment bias, I used two different breeds of fowl (*Gallus gallus*). Fowl can easily be breed and are easy to handle. Further, fowl are precocial, meaning that they can take care of themselves already after they have hatched. As a consequence, parental influences and other accumulated experiences can be minimized if using young chicks. Chickens in the egg- and meat industries are exposed to a lot of stressors, therefore I chose to use a common breed of laying hen, the Bovans Robust for the first experiment (influence of stress and environment on cognitive judgment bias). When testing the influences of personality on cognitive judgment bias I found it more suitable to use a breed that had not undergone strong artificial selection in the same way as domesticated breeds have done. Strong selection typically reduce individual differences and genetic variation. For this reason the wild ancestor of our domesticated chickens, the Red Junglefowl, was used for
the second experiment (influence of personality on cognitive judgment bias).

3 Methods

3.1 Influence of environmental factors on cognitive judgment bias

3.1.1 Animals and housing

During the summer of 2014, female Bovans Robust chicks from Swefarm AB were used to investigate potential differences in cognitive judgment bias in chicks raised under different environmental conditions. At the day of hatching, chicks (n = 96) were collected and transported to Funbo research facility (SLU) at Lövsta, Uppsala, where they were housed in eight equally sized pens with twelve chicks per pen (1.2 x 1.2 m, figure 2). The walls of the pens prevented visual, but not vocal, contact with neighbouring chicks. Chicks were exposed to cognitive judgment bias tests on two separate occasions, first 14 - 16 days post-hatching (n = 96), and again 35 - 36 days post-hatching (n = 65, figure 1). The second cognitive judgment bias test was performed after a unpredictable, collective stressors had been introduced (figure 1). During the first test the temperature was kept to around 28 °C with light maintained at a 16:8 hour light:dark ratio. During the second cognitive judgment bias test the temperature was kept around 26 °C and lights maintained a 9:15 hour light:dark ratio. The conditions of light and heat was used to simulate the conditions in laying hen farms in Sweden at the different ages.
Figure 1. Timeline overview of the different experimental phases that female Bovans Robust chicks were exposed to. See main text for details about each phase and test.

Chicks were randomly assigned to four different treatment groups (n = 24 / treatment): stressed non-enriched, unstressed non-enriched, stressed enriched and unstressed enriched (figure 1, 2). The non-enriched pens had wood shavings on the floor, a round feeder with a commercial chick feed and a water bell. The enriched pens had access to cover where the chicks could hide, four perches at different heights, a small roof over a part of the top perch and wooden blocks on the floor to provide different types of structures, as well as wood shavings on the floor, a round feeder.
with commercial chick feed and a water bell (figure 3). To simulate a stressful event that could occur during transport of commercial chicks, the chicks in the stressed treatment groups were exposed to a cold stress at two days of age (hereafter called ‘cold stress’). This was done by keeping pairs of chicks in boxes (20W x 15L x 15H cm) for six hours while the room temperature was lowered to 18 - 20 °C. Pilot studies were performed by Irene Campderrich Lecumberri prior to the test on another set of chicks of the same breed showing that a temperature of 18 - 20 °C lowered the chicks’ body temperature, whereby it was regarded to be stressful for the chicks. Conversely, the chicks not subjected to the cold stress had a temperature of 33 °C during the same time period. At 22 - 27 days of age, between the first and second cognitive bias test all chicks from all four treatment groups were exposed to a battery of stressors using unpredictable lighting and sound at 90 dB (animal and mechanical noises as well as music) in their home pens (hereafter called ‘collective stressors’, figure 1).

Figure 3. Drawing (not to scale) of an enriched pen for the female Bovans Robust chicks exposed to an enriched treatments. The pen is seen from a) above and b) the left side, where the slatted box is a cover, grey lines are perches, the black filled box is the roof over the top perch, the black boxes with grey filling are wooden blocks and the circles indicate water bell (black) and feeder (grey).

3.1.2 Test room conditions

During training and testing, four to twelve chicks from the same home pen were collected using a small, dark box and transported to the testing room, located in an adjacent building. Birds were left in the dark box a maximum of 30 minutes while waiting to be tested. The test room had covered windows and was lit by standard fluorescent lamps to prevent UV-light from altering the colours of the cues used. The temperature in the test room was 24 - 25 °C during the first test and reached 27 - 28 °C
in the second test. The mean temperature was higher during the second test because of the extreme temperature in Sweden during this period of testing. This was not expected to affect the behaviour of the birds in any biased manner, and all birds were exposed to the same temperature within each test round.

3.1.3 Associative learning

In order to investigate variation in cognitive judgment bias among treatment groups, we trained chicks to associate white (n = 47) or black (n = 49) visual cues with a reward (a small piece of mealworm) after which we investigated their reaction to three intermediate (grey) visual cues. Arenas were constructed out of plastic boxes covered with single faced corrugated cardboard. Prior to testing the chicks were trained to be alone in the arena by placing groups into the arenas and successively decreasing the group size until each chick was calm even when alone in the arena. A small partition was put at the far short end of the test arena to separate the cues and thereby make sure the chick had to make an active choice (figure 4a). On either side of the partition, signs (9 x 9 cm) of black and white colour were placed. The signs were squares of laminated photo paper, 9 x 9 cm in size, placed on the ground, leaning against the wall. In front of the signs were bowls (5Ø x 3H cm) made of transparent plastic, inside of which laminated paper of black or white was lined to show both from the inside and the outside to maximize the cue stimuli for the chicks. During training the rewarded cue was always placed on the left side of the partition for the first choice, after which right and left placement of the cues were alternated randomly according to a pre-determined schedule to prevent chicks from associating placement with a reward.
Figure 4. Drawing of the arena used for training (a) and testing (b) Bovans Robusta and Red Junglefowl chicks in a cognitive judgment bias test. Chicks were first trained to associate a colour cue with a reward before their reactions to also intermediate cues were scored. Cues are at the top of the figure and the chick at the bottom (closest to the handler). The size of the arena differed between the breeds; for Bovans Robust chicks the arena had the size of 46W x 68L x 42H cm and for the Red Junglefowl chicks it was 28W x 37L x 18H cm.

At the start of a training session the chick was placed in the opposite short side of the arena from the cues (figure 4a), closest to the handler, with a hand initially held in front of the chick to prevent jumps or rushes to the cues. In the beginning of a training session the chicks received help finding the mealworm in the bowl by the handler tapping in the correct direction with the fingers or lifting the chick to the correct cue. Once the chick started walking towards the cues on its own upon being placed in the arena, no further help was offered.

A training session lasted for 15 minutes, and chicks were allowed to make an unlimited amount of trials during each session. Each choice the chick made was noted as a correct choice (walking to the rewarded colour), an incorrect choice (walking to the unrewarded colour) or as help, if the chick had received help as explained above. If the chick made an incorrect choice it was immediately picked up, meaning that it did not receive a reward, and the cues were switched accorded to a pre-determined pseudo-random order. The association between cue and reward was considered to have been learned when the chick had made six correct choices in a row. To learn the association chicks were allowed three training sessions, after which 87 chicks had learned the association. Upon reaching the criteria of six correct choices in a row the chick was placed back into the cage and given at least one hour of rest before it was exposed to a cognitive judgment bias test.
3.1.4 Cognitive judgment bias test

We tested cognitive judgment bias by presenting different cues one by one to the chicks. The cues consisted of the previously rewarded and unrewarded cues together with three intermediate cues (25%, 50% and 75% in between black and white), creating a ‘near rewarded’ cue, a ‘middle’ cue and a ‘near unrewarded’ cue, regardless of which cue chicks had initially been trained to associate with a reward. The responses of chicks towards each cue were scored separately.

Before the cognitive judgment bias test, we made sure that each chick had learned the association between cue and reward by exposing them to a ‘refresh’ trial, where chicks again had to make six correct choices in a row in the same fashion as described above. Once this association was made, chicks were exposed to one cue at a time in the same arena as described above. However, this time the partition was taken away and the cue placed in the middle of the short end side (figure 4b), thereby eliminating possible problems of chicks favouring one side.

The chick was placed in the arena with its back towards the cue (figure 4b) to make the chick see the cue first once it turned around, thereby preventing the chick from making a decision before starting to move. A stopwatch was started as soon as the chick turned its head towards the cue, and latency until the chick had its head over the bowl was measured. A maximum latency of 30 seconds to reach the cue was set to prevent chicks from getting tired or weary of the test. If the chick jumped out of the arena, it was given a score of the maximum latency (30 seconds). The first and the second cue presented to chicks during this test were always the rewarded cue followed by the unrewarded cue. These first two cues were not included in the analysis since they were considered to be acclimatisation trials to the new test set-up.

After the first two cues, the order of cues was presented according to a pre-determined, pseudo-randomized schedule. Each intermediate cue was presented to the chicks two times and the rewarded and unrewarded cues were presented eight and seven times respectively, for a total of 21 cue presentations. The rewarded cue was rewarded throughout the cognitive judgment bias test to keep the chicks motivated to investigate the cues, but this was the only cue where chicks received a reward as this was the only cue they were pre-trained to interpret as positive.

For the second cognitive bias test the arenas were larger to adjust to the larger size of the older chicks. But from his, everything else was performed as described as above, with the chicks being through a
‘refresh’ trial before proceeding to the cognitive bias test. At the time of
the second cognitive judgment bias test 22 chicks had passed away,
whereby the remaining 65 chicks were tested.

3.2 Linking variation in personality to cognitive judgment bias

3.2.1 Animals and housing

Red Junglefowl chicks (males and females) from 29 different families (n
= 1 - 4 from each family) were used to investigate the relationship
between cognitive judgment bias and personality. The study was carried
out in the spring of 2014 at Linköping University. Sixty eight chicks were
hatched, out of which 50 were randomly selected to take part in the
cognitive judgment bias test. The remaining 18 chicks lived with the test
chicks, however they were not used in this study. Chicks were housed in
pens (70W x 72L x 60H cm) in the laboratory where the experiments
were to take place. Initially, chicks were all housed together, but as they
grew larger they were randomly parted into mixed-sex groups in five
pens.

In their home pens, chicks had access to a perch and a heating plate for
warmth, as well as commercial chick feed in a feed bell and water in a
water bell. Room temperature in the laboratory was kept around 25 °C
throughout the five weeks of testing.

3.2.2 Associative learning

From the day of hatching, the chicks were accustomed to getting handled
and being alone in the test arena. The test arena was a mesh arena
covered with single faced corrugated cardboard. Thereafter, at three days
of age, learning began by teaching the chicks to associate a randomly
assigned colour cue, white (n = 25) or black (n = 25), with a reward (a
piece of mealworm). As in the test above, a partition was put up in one
short end of the arena to clearly separate the cues placed on either side
(figure 4a). The same cues as above, consisting of a sign with a matching
bowl in front of it, were used. As the test room had no windows UV-light
was not an issue, and the room was also here lit up by standard
fluorescent lighting.

As in the previous test the assigned rewarded colour was placed on the
left side for the first choice in a trial however it was then altered every
time the chick had chosen a cue. Each choice was marked in a protocol in
the same way as described above and this continued for 15 minutes or
until the chick had made a correct choice five times in a row, whereby the
association was considered learned. In total chicks were allowed seven
trials of fifteen minutes each to learn the association between cue and reward, after which all chicks had learned the association. During each session the chick could choose between the colours for as many times as it had time for, as mentioned above. When the association was learned the session was ended and the chick put back in the home pen and given at least an hour of rest before it was tested in the cognitive judgment bias test. For more details about the associative learning, see above.

3.2.3 Cognitive judgment bias test

A cognitive judgment bias test was performed to investigate differences in how chicks with known personality interpret their surroundings. At this time the same five colour cues as in the test described above (the previously rewarded and unrewarded cues as well as three intermediate cues) were presented to the chicks one at a time. Chick responses were recorded by measuring latency to reach each cue in the same fashion as described above.

Directly following a successful ‘refresh’ trial of associative learning, where they had to choose the correct bowl six times in a row. Afterwards the chicks (at the time six to nine days old) were exposed to a cognitive judgment bias test, as described above.

Each intermediate cue was presented three times and the rewarded and unrewarded cues were presented eleven to thirteen times each in a pseudo-randomized pre-determined order, for a total of 33 cue presentations. In this test the previously rewarded cue kept being rewarded to keep the chicks motivation up throughout the test. The rewarded cue was always presented first followed by the unrewarded cue. However, these two initial cues were not included in the analysis, as mentioned above. The maximum latency to reach the cue was set to 60 seconds. A stopwatch was started as soon as the chick turned its head towards the cue, from the position of having the back towards the cue, and latency until the chick had its head over the bowl was measured. Jumping out of the arena was recorded as the maximum latency of 60 seconds.

3.2.4 Personality assays

Upon finishing the cognitive judgment bias test the chicks were exposed to a range of personality assays (n = 49, one chick died previous to personality assaying).
3.2.4.1 Novel arena and novel object

Novel arena (NA) and novel objet (NO) tests are commonly used and validated tests for investigating personality in fowl (Forkman et al. 2007; Favati et al. 2014a,b). Chicks were tested in NA and NO tests when 27-29 days old, with a NA test directly followed by a NO test. Two arenas, made out of solid wood walls with a mesh roof, were set up next to each other on the floor in the lab. In these arenas the floors were covered with rubber mats, upon which some wood shavings were sprinkled. Two empty food bells and one empty large water bell were set up at the same place in each arena so that the chick could not see the entire arena from the start position, thereby encouraging exploration. Cameras from two different angles were used to record chick behaviour during the tests. Two chicks were tested simultaneously in two identical arenas. The handlers placed the birds in the upper left corner of each arena (figure 5) and then hid behind a screen during the test.

![Figure 5. Drawing of arenas used to test responses of Red Junglefowl chicks to novel arenas and novel objects. Large, unfilled, circles indicate placement of large water bell, small, grey filled, circles indicate placements of food bells, black triangles indicates placements of cameras, the dotted lines divides the imaginary squares used to record activity and the chick placement at the beginning of the novel arena test is in the top left corner. Each arena measures (114L x 76W x 40H cm).](image)

After the chicks had been exposed to the NA for ten minutes lights were turned off and a NO, being a yellow, round stuffed animal with big eyes and a tail (figure 6), were placed into each arena as far away from the chick as possible. Lights were then turned back on after the handlers had hidden behind the screen. This procedure was used in order to influence the chicks as little as possible as the NO was put into the arena. Chicks were exposed to the NO test for ten minutes, after which they were put
back into the home cage. In between each trial the arenas were cleaned to remove any faeces left from the previously tested bird.

![Stuffed animal](image)

*Figure 6. Stuffed animal used as novel object to investigate personality in chicks (ca. 10 cm in diameter and 20 cm tall).*

I later decoded the videos, recording latency to move, latency to explore all six imaginary areas the arenas were divided into, number of square changes, number of escape attempts, and the chick's behaviour every ten seconds using instantaneous sampling (table 1). The behaviours observed were chosen in order to measure fear response and activity in the chicks.
Table 1. Observed behaviours of Red Junglefowl chicks during instantaneous sampling of behavioural responses in novel arena and novel object test

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigilance</td>
<td>The chick has its neck stretched out, while either walking around or standing still with legs straight.</td>
</tr>
<tr>
<td>Eat</td>
<td>The chick holds its head in a position lower than in a relaxed, neutral position, or it pecks on the ground or at an object (not the novel object).</td>
</tr>
<tr>
<td>Activity</td>
<td>The chick is moving around in the arena, body position or pace not important.</td>
</tr>
<tr>
<td>Stand</td>
<td>The chick is in a still position, legs extended so that the stomach does not touch the ground.</td>
</tr>
<tr>
<td>Other</td>
<td>Behaviours not listed above (e.g. lying down).</td>
</tr>
</tbody>
</table>

3.2.4.2 Tonic immobility

A validated test of fear responses in chicks is the tonic immobility test (TI, Forkman et al. 2007). In the TI test, latency until the chick starts to move is used to measure fear. A long duration in TI is considered to show higher levels of fear. The TI test was performed on all chicks in this study at 30 days of age by the same observer, in order to make sure that it was done in the same way for all chicks. During this test a chick was placed on its back in a v-shaped construction (figure 7). After being placed on the back, chicks were held down for fifteen seconds with one of the handlers’ hands gently holding down its chest while the other hand covered the head. As the fifteen seconds ended the pressure of the hand on the chick was released and a stopwatch was started. During the time when the chick was on its back the handler avoided eye contact to minimize human influence of the duration of the TI. Latency to first head movement and latency until the chick jumped up on its feet were recorded in seconds. Each chick was given three trials to go into tonic
immobility, which was regarded as achieved when the chick stayed on its back for three seconds or longer. The number of trials it took to induce tonic immobility was also noted and if the attempts to reach tonic immobility failed the latency was noted as zero seconds and the chick was released back into the home cage. A maximum latency of ten minutes was set for the TI, after which the chick was helped up from the position by a gentle nudge to its side and maximum latency (600 s) was noted.

![Figure 7. V-shaped construction used to induce Red Junglefowl chicks in tonic immobility to investigate fear response as a part of a personality assessment.](image)

3.3 Statistical analysis

Statistical analyses were performed using R Studio Version 0.98.1062.

3.3.1 Influence of environmental factors on cognitive judgment bias

When investigating environmental impact on cognitive judgment bias, linear mixed-effect models (LMM) were used for the first and second cognitive judgment bias test. Mean latencies per chick and cue were calculated per test (creating a total of ten mean latencies per chick) and log transformed before used as the response variable. The variables ‘cold stress’ (i.e. whether the chick was exposed to a cold stress or not), ‘environmental enrichment’ (i.e. whether the chick was raised in an enriched environment or non-enriched environment) were included in the LMMs as fixed effects. The interaction between cold stress and environmental enrichment was initially included but was later dropped from the final model because it was not significant. ‘Pen’ (i.e. pen 1 - 8) and ‘ID’ (i.e. the individual id number of the chicks) were added as random effects. An Analysis of variance (ANOVA) was performed on the LMMs from the first and second cognitive judgment bias test separately to search for significant results. Post-hoc unpaired t-tests were performed when there were a significant effect in the LMMs to investigate if there was a specific factor, such as one cue, that influenced the results, this was
not the case. Furthermore a Mann-Whitney U test was performed to investigate if the differences detected could be because of differences in learning.

### 3.3.2 Linking variation in personality to cognitive judgment bias

To reduce the behaviour variables obtained from the personality assays a principal component analyses (PCA) was performed. The behaviours added to the PCA were latency to move and latency to visit all areas from the novel arena test, activity, vigilance, number of escape attempts and number of square changes from both the novel arena and the novel object test, and latency to move head and latency to jump up from the tonic immobility test (for more information about these behaviours and other behavioural responses, see above under Personality assays). Four principal components (PCs) had eigen values larger than 1 and were consider from the PCA. These PCs contained data describing mainly variation in activity (PC 1), fearfulness (PC 2), escape proneness (PC 3) and nervousness (PC 4, table 2). Individual scores were obtained for each PC and analysed using a LMM to investigate if personality could predict individual scores in the cognitive judgment bias test. Mean latencies per chick and cue from the cognitive judgment bias test (for a total of five mean latencies per chick) were used as response variable and the four PCs were added as fixed effects. As random effects ‘ID’ was used. Due to lack of significant effects, the variables ‘Sex’ (i.e. male, female), ‘Family’ (i.e. family 1 - 29) and PC*Sex interactions, were dropped from the model. An ANOVA was used to search for significant results from the final model of LMM. The interactions between PC and Cue (the cues used in the test, i.e. rewarded, near rewarded, middle, near unrewarded and unrewarded cues) were further investigated in a linear model (LM) carried out for each ambiguous cue (i.e. one for each of the three grey cues). These LMs were searched for significant results using ANOVAs.

### 4 Results

#### 4.1 Influence of environmental factors on cognitive judgment bias

Chicks required 9 - 89 choices to learn the association between cue and reward, with no effect of cold stress ($w = 995, p = 0.67$) or enrichment ($w = 918, p = 0.82$). Neither stress nor enrichment had an influence on how the chicks performed in the first cognitive judgment bias test, prior to the collective stressors (table 2, figure 8a). During the second cognitive judgment bias test, stress once again had no effect on latency to reach the cues (table 2). However, chicks that were raised in enriched environments had significantly shorter latencies to reach the cues in comparison to
chicks that had lived in non-enriched environments (table 2, figure 8b). The difference was explained by chicks in non-enriched pens being slower to reach the cues in the second cognitive judgment bias test (df = 1, $\chi^2 = 44.03$, $p = 0.00000000003$), not by chicks from enriched pens being faster (df = 1, $\chi^2 = 0.01$, $p = 0.91$). This indicates that enrichment buffered stress and that chicks in enriched environments were able to have a more positive cognitive judgment bias in comparison to chicks living in non-enriched environments, after the collective stressors.

Table 2. Results from ANOVA when performing an linear mixed effects model. It was investigated if stress and / or enrichment have an effect of response to the cognitive judgment bias tests in female Bovans Robust chicks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\chi^2$</th>
<th>Df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First cognitive judgment bias test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrichment</td>
<td>0.10</td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td>Stress</td>
<td>0.007</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Second cognitive judgment bias test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrichment</td>
<td>5.07</td>
<td>1</td>
<td>0.02*</td>
</tr>
<tr>
<td>Stress</td>
<td>1.13</td>
<td>1</td>
<td>0.29</td>
</tr>
</tbody>
</table>

* $p < 0.05$
Figure 8. Responses of female Bovans Robust chicks to two cognitive judgment bias tests. Chicks were tested before (a) and after (b) being subjected to unpredictable stressors. Chicks displayed more optimistic responses (lower latency to reach the cues) in the second test when living in enriched environments in comparison to chicks living in non-enriched environments. Mean latencies ± standard error are presented. * p<0.05, ** p<0.01
4.2 Linking variation in personality to cognitive judgment bias

Red Junglefowl chicks needed 13 - 54 choices to learn the association between colour (black or white) and reward. Results from the cognitive judgment bias test show that chicks displayed a shorter latency to reach the rewarded cue in comparison to all other cues, and that latencies to reach the cues increased with its resemblance to the unrewarded cue (figure 9). The PCA clustered behaviours into 4 components describing variation in activity, fearfulness, escape proneness and nervousness (table 3). Performing a LMM and ANOVA showed a significant interaction between individual nervousness score and latency to reach the cues, depending on cue (table 4). Analysing each cue on its own showed no effect of the PCs on latency to reach the rewarded cue, the cue close to rewarded or middle cue (table 5). However, the cues near unrewarded and unrewarded were positively correlated with nervousness, meaning that more nervous individuals were slower to reach the near unrewarded (figure 10a) and unrewarded (figure 10b) cues (table 5).

![Figure 9](image_url)

*Figure 9. Responses of red junglefowl chicks in a cognitive bias test. Chicks were faster (latencies in seconds) towards rewarded cues, and slowest towards unrewarded cues. Mean ± standard error are presented.*
Table 3. Personality scores describing variation among red junglefowl chicks. The personality gradients are obtained from a principal component analysis (PCA) based on data from Novel arena (NA)-, Novel object (NO)- and Tonic immobility (TI) tests. Bold numbers indicate factors loading over 0.3, which are considered having a strong loading in the PC.

<table>
<thead>
<tr>
<th></th>
<th>Personality gradients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active</td>
</tr>
<tr>
<td>NA activity</td>
<td>0.37</td>
</tr>
<tr>
<td>NA vigilance</td>
<td>0.15</td>
</tr>
<tr>
<td>NA latency to move</td>
<td>-0.21</td>
</tr>
<tr>
<td>NA latency to visit all areas</td>
<td>-0.29</td>
</tr>
<tr>
<td>NA number of escape attempts</td>
<td>0.22</td>
</tr>
<tr>
<td>NA number of square changes</td>
<td>0.38</td>
</tr>
<tr>
<td>NO activity</td>
<td>0.39</td>
</tr>
<tr>
<td>NO vigilance</td>
<td>0.26</td>
</tr>
<tr>
<td>NO number of escape attempts</td>
<td>0.29</td>
</tr>
<tr>
<td>NO number of square changes</td>
<td>0.39</td>
</tr>
<tr>
<td>TI latency to move head</td>
<td>-0.21</td>
</tr>
<tr>
<td>TI latency to jump up</td>
<td>-0.07</td>
</tr>
<tr>
<td>Eigen value</td>
<td>2.17</td>
</tr>
<tr>
<td>Proportion of variance</td>
<td>39.1 %</td>
</tr>
<tr>
<td>Accumulative proportion of variance</td>
<td>39.1 %</td>
</tr>
</tbody>
</table>
Table 4. ANOVA output from comparison between personality principle component analysis (PCA) and data from a cognitive judgment bias test in Red Junglefowl chicks using a linear mixed effects model (LMM). The components compared are the four PCs (active, fearful, escape prone and nervous, see table 3)

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\chi^2$</th>
<th>Df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.49</td>
<td>1</td>
<td>0.48</td>
</tr>
<tr>
<td>Active</td>
<td>0.0007</td>
<td>1</td>
<td>0.98</td>
</tr>
<tr>
<td>Fearful</td>
<td>1.10</td>
<td>1</td>
<td>0.30</td>
</tr>
<tr>
<td>Escape prone</td>
<td>0.10</td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td>Nervous</td>
<td>2.01</td>
<td>1</td>
<td>0.16</td>
</tr>
<tr>
<td>Active*Cue</td>
<td>2.04</td>
<td>4</td>
<td>0.73</td>
</tr>
<tr>
<td>Fearful*Cue</td>
<td>1.13</td>
<td>4</td>
<td>0.89</td>
</tr>
<tr>
<td>Escape prone*Cue</td>
<td>7.58</td>
<td>4</td>
<td>0.11</td>
</tr>
<tr>
<td>Nervous*Cue</td>
<td>14.50</td>
<td>4</td>
<td>0.006**</td>
</tr>
<tr>
<td>Active*Sex</td>
<td>0.62</td>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>Fearful*sex</td>
<td>0.69</td>
<td>1</td>
<td>0.41</td>
</tr>
<tr>
<td>Escape prone*sex</td>
<td>1.10</td>
<td>1</td>
<td>0.29</td>
</tr>
<tr>
<td>Nervous*sex</td>
<td>0.79</td>
<td>1</td>
<td>0.37</td>
</tr>
</tbody>
</table>

** $p < 0.01$
Table 5. Results from linear models (LM) per each cue used in a cognitive judgment bias test compared to ‘Nervousness’ (see table 3) observed in Red Junglefowl chicks.

<table>
<thead>
<tr>
<th>Cue</th>
<th>F-value</th>
<th>Df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rewarded</td>
<td>0.01</td>
<td>1</td>
<td>0.90</td>
</tr>
<tr>
<td>Near rewarded</td>
<td>0.71</td>
<td>1</td>
<td>0.41</td>
</tr>
<tr>
<td>Middle</td>
<td>0.73</td>
<td>1</td>
<td>0.40</td>
</tr>
<tr>
<td>Near unrewarded</td>
<td>4.95</td>
<td>1</td>
<td>0.03*</td>
</tr>
<tr>
<td>Unrewarded</td>
<td>7.95</td>
<td>1</td>
<td>0.007**</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01

Figure 10. Relations between ‘nervousness’ (see table 3) and latency to reach two different cues in a cognitive judgment bias test in Red Junglefowl chicks. ‘Nervousness’ correlated with latency to reach a) the near unrewarded cue \((r = 0.34, p = 0.03)\) and b) the unrewarded cue \((r = 0.21, p = 0.007)\).

5 Discussion

I have here demonstrated that chicks housed in enriched environments were faster to reach ambiguous cues in a cognitive judgment bias test performed after a battery of collective stressors, in comparison to chicks.
housed in non-enriched environments. This suggests that living in enriched environments buffers the influences of unpredictable stressors and keeps chicks more optimistic. When exploring effects of personality on cognitive judgment bias, I demonstrated a negative correlation between nervousness and optimism. This indicates that the more nervous a chick is, the more negatively it interprets its surroundings. However, the influence of personality on optimism was limited.

5.1 Influence of environmental factors on cognitive judgment bias

Results from the cognitive judgment bias tests showed that chicks housed in enriched environments are more optimistic in comparison with chicks living in non-enriched environments after being subjected to unpredictable stressors. Thus it is possible that enrichment buffers stress, indicating that providing enrichment to chicks supports better welfare and helps them to cope with unpredictable stressors, should they happen to occur.

The finding that unpredictable environments, such as the one created with the collective stressors, induce a more pessimistic judgment bias, supports previous studies which have found a similar pattern (Harding et al. 2004, Reefmann et al. 2012). However, here I also showed that environmental enrichment can buffer the negative influence on cognitive judgment bias since chicks living in enriched environments showed no altered response from the first cognitive judgment bias tests to the second. When physiological and behavioural responses to stress previously have been investigated, environmental enrichment has been found to reverse the effects of stress (e.g. Francis et al. 2002, Laviola et al. 2004, Del Arco et al. 2007). Nevertheless, it has previously not been investigated how environmental enrichment affects stress responses by investigating the animals’ subjective experience, as delineated in this thesis.

The collective stressors, which consisted of unpredictable sound and light schedules, led to more pessimistic responses in the cognitive judgment bias test from the chicks that lived in non-enriched environments. Latencies to reach the cues differed between chicks living in enriched and non-enriched environments in a way that resembled previous findings, which linked pessimistic responses towards both unrewarded and rewarded cues to depression in rats (Enkel et al. 2010, Richter et al. 2012). What these studies found were that rats bred to model human depression exhibited reduced anticipation of positive events in addition to an increased anticipation of negative events (Enkel et al. 2010, Richter et al. 2012). As chicks living in non-enriched environments increased their
latencies to reach both the rewarded, unrewarded and ambiguous cues, they could have been induced into a depressed state. This would be bad for their welfare, as the cognitive judgment bias test indicates.

No effects of initial stress or environmental enrichment were seen in the first cognitive judgment bias test. It is possible that the initial stressor was less stressful in comparison to the collective, unpredictable stressors. Further it is possible that the initial stressor did not alter the behaviours of the chicks, whereas the other stressors did because of their unpredictability, which have been seen to have an effect as stated above. However, this does not explain the lack of effect of environmental enrichment in the cognitive judgment bias test before the collective stressors. It is possible that the environment does not have a large effect on cognitive judgment bias until other factors, such as the unpredictable stress in this case, are applied. This is a possibility that should be investigated in future studies.

My results demonstrated that providing enrichment to chicks made them more optimistic when unpredictable stressors occurred. This in turn provides support that environment influences cognitive judgment bias, and thus optimism, for chicks.

5.2 Linking variation in personality to cognitive judgment bias

When investigating the link between variation in personality and optimism, I showed that personality has a significant effect on interpretation of stimuli in a cognitive judgment bias test. However, this effect was rather small. Other studies have also found personality and cognition to be related, for example in the form of learning (e.g. Dugatkin & Alfieri 2003). In psittacines, more neurotic animals had a more negative attention bias (Cussen & Mench, 2014), showing similarities with my findings of nervousness being positively correlated with pessimism.

There is a possibility that the tests used to investigate personalities in the chicks did not represent the full range of their personalities, and that some other aspect of personality would better explain variation in optimism. However, the novel arena-, novel object-, and tonic immobility tests performed here were chosen in order to cover a variety of situations in which personality differences have previously been found. Novel arena- and novel object tests are methods of assessing behavioural differences in a variety of species (e.g. mice (Hood & Quigley 2008), pigs (Janczak et al. 2003), rabbits (e.g. Andersson et al. 2014) and cattle (MacKay et al. 2014). Also the tonic immobility test is a test that is used in different types of animals, including chickens (Jones 1984), muroid rodents
(Webster et al. 1981) and pigs (e.g. Janczak et al. 2003). The three personality assays used have also been reviewed and deemed fit for testing fear response (Forkman et al. 2007).

However, because personality only had a limited influence of performance in a cognitive judgment bias test after environmental influences have been minimised, it is still unclear what causes the observed individual differences in cognitive judgment bias tests. It is possible that observed individual differences arise from variations in other cognitive biases such as attention, and memory bias. Further, the observed variation can be dependent on variation in genetics, physical status, social status or other individual differences. Future studies should investigate the contributions to variation in responses to cognitive judgement bias tests further.

5.3 Societal and ethical considerations

Investigations of welfare in the common laying hen, which was done in the first experiment in this thesis, is an important part of egg production since it is important to be able to house the laying hens in a suitable way. Although it is mostly important from an ethical point of view to have animals with good welfare producing the food we eat it has also been seen that animals with better welfare produce higher quality and quantity of animal products (e.g. Castellini & A Dal Bosco 2002, Pascual-Alonso et al. 2015). Consumers have, in addition, become more and more concerned about the welfare of the animals that produce their food (Blokhuis et al. 2003) in particular in pork and poultry production (Verbeke & Viaene 2000). This means that products produced using practices with better welfare aspects will probably sell better than products where the welfare aspect has not been taken into consideration.

Research regarding personality influence on cognitive judgment bias is important in trying to understand how the human mind works. Although bird brains are different to mammalian brains, homologues of several areas exist, such as the mammalian neocortex being homologous to the dorsal ventricular ridge in birds (Dugas-Ford et al. 2013). Furthermore, it is of interest to explore species resembling those of humans to further our understanding of how our minds work more in depth without having to perform tests in humans. This is especially prevalent since more invasive studies might be of interest to perform and since animal environments are more possible to control and affect. Invasive studies and studies done when the study subjects are housed in a controlled environment are not possible o perform in humans, whereby an animal model is of great use in
future studies. By using animals to understand our mind we can get deeper into functions that are thus far unexplored.

6 Conclusions

In this study, I first showed that environmental enrichments could buffer the negative influences unpredictable stressors can have on the cognitive judgment bias in female Bovans Robust chicks. In turn, this supports that environmental enrichments can buffer negative moods and thereby poor welfare in these animals. No effects of initial environmental enrichment nor early cold stress were detected in the animals. Perhaps the lack of effect indicates that environmental enrichments are most important when other factors, such as stressors, are prevalent. Furthermore the lack of effect from the initial stressor might indicate that cold stress was easier to recover from than the large stressor of unpredictable stimuli.

In the second test, I showed that personality has a limited influence on individual cognitive judgment bias in Red Junglefowl chicks. This influence consists of individuals scoring higher in nervousness also portraying more pessimistic interpretations of unrewarded stimuli. With this influence being small and individual differences being observed in cognitive judgment bias in previous studies, there question remains on where these differences originate from. This question is interesting to investigate in future studies, along with questions of the underlying physiological mechanism explaining variation in cognitive judgment biases.

7 Acknowledgements

I would like to thank my supervisors Hanne Løvlie and Josefina Zidar for all feedback and support. I would also like to thank Alexandra Balogh, Anna Favati and Johan Almberg for help collecting data in Linköping. A special thank you goes to Linda Keeling and Irene Campderrich Lecumberri for inviting ‘the Linköping chick crew’ to take part in their study. Furthermore I would like to thank Anette Wichman, Helena Jones, Rebecca Katajamaaa and Sonja Broberg for their help with data collection in Uppsala. Lastly I thank the people helping me finishing the report, consisting of Charlotte Rosher, Mikaela Hanson and Johan Jönsson, as well as my examiner Jordi Altimiras.
8 References


Gosling SD. 2001. From mice to men: what can we learn about personality from animal research? *Psychological Bulletin*. 1, 45-86.


