



Performance of laying hens in a cognitive bias task; the effect of time since change of environment

*Kognitiv förskjutning hos värphönan;
effekten av förfluten tid sedan miljöbyte*

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Skara 2010

Studentarbete 189

*Swedish University of Agricultural Sciences
Department of Animal Environment and Health
Ethology and Animal Welfare programme*

Student report 189

ISSN 1652-280X



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**Grund C, 15 hp, Etologi och djurskyddsprogrammet, självständigt arbete i biologi,
kurskod EX0293**

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Nyckelord: cognitive bias; laying hen; gallus gallus; animal behaviour; animal welfare

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Abstract

In the debate on laying hen welfare, and specifically housing conditions, the main focus has been on physiological and behavioural measures. What is lacking is knowledge of how the hen experiences the situation – her state of mind.

This study is an attempt to gain insight into the private mental states of former battery hens. It uses a cognitive bias method based on spatial judgement, i.e. judgement of an ambiguous stimulus placed spatially in between a reinforced and an unreinforced stimulus. This method has previously shown differences in judgement by animals in enriched versus poor housing. The aim of the current study was to measure such biases expressed by hens two months compared to four months after moving from battery cages to littered pens. The animals were also tested in a novel object task for a measure on general anxiety, and their plumage condition was scored. Their social rank was established by testing penmates in pairwise competitions over a limited food resource.

The hens showed longer latencies to reach the intermediate position four months after leaving the cages, compared to two months after. Possible reasons for this could be that the positive effects of the improved environment were largest when the hens had recently left the battery cages, with the effect of the improvement gradually being diminished or even reversed. It is likely that novelty in itself is positive to hens, and a static environment becomes boring in time even though it is far more complex than a battery cage.

No difference was found in the reactions to ambiguous cues by hens of different social status. A strong correlation was found between feather score and social dominance.

Sammanfattning

I djurskyddsdebatten runt värphönor och hur de hålls har fokus huvudsakligen legat på fysiologiska faktorer och mätbara beteenden. Däremot saknas kunskap om hur hönan själv upplever sin situation, hennes inre tillstånd.

Den här studien är ett försök att få tillträde till de privata mentala upplevelserna hos före detta burhönor. Metoden som används går ut på att se hur en höna tolkar ett tvetydigt stimulus, som ligger spatiellt mellan två inlärda stimuli; en belönad och en obelönad. Metoden har tidigare visat att djur som lever i berikade jämfört med oberikade miljöer bedömer sådana stimuli olika. Den här studien jämför bedömningarna hos hönor som flyttat från konventionella, oinredda burar till ströade boxar, två och fyra månader efter flytten. Höornas reaktion på ett främmande föremål testades för att mäta deras allmänna nervositet, och deras fjäderdräkt bedömdes. Deras dominantstatus fastställdes genom parvis konkurrens över en begränsad födoresurs.

Hönorna tog längre tid på sig att nå den mittersta positionen fyra månader jämfört med två månader efter flytten. Detta kan bero på att den positiva effekten av miljöbytet var störst kort efter flytten, och därefter gradvis jämnades ut eller till och med omvändes. Det verkar troligt att förändring i sig är positivt för hönor, och att en statisk miljö blir tråkig med tiden, även om den är mycket mer komplex än en konventionell bur.

Ingen skillnad hittades i reaktionerna på tvetydiga signaler hos hönor med olika social status. Det fanns en stark korrelation mellan befjädring och rang.

Introduction

In large parts of the Western world there has been an intense discussion on laying hen welfare during the last two to three decades. In Europe as well as in California and Michigan the barren battery cage is being phased out. It is being replaced by floor rearing, multi-level aviaries or free range systems, but also by enriched cages containing perches and nests. Cage-free systems receive criticism from scientists and animal welfare organisations for their unnaturally large group sizes, leading to social stress, while all forms of cages are criticized for lack of space to perform natural behaviours such as foraging and stretching of limbs (Appelby & Hughes, 1991; Rodenburg et al., 2005; CIWF, 2007).

The scientific basis underlying these discussions is voluminous (see AHAW, 2005, for a review). Research in this area has focused on physiological measures, such as egg quality, body weight, prevalence of disease and mortality (e.g. Fossum et al., 2009; Shimmura et al., 2007) and behavioural measures such as use of enrichment (e.g. Bubier, 1996a; Wall, 2003). Other areas of focus have been the occurrence of natural behaviours (e.g. Mollenhorst et al., 2005; Albentosa et al., 2004) and abnormal or agonistic behaviours (e.g. Nicol et al., 1999; Pöttsch et al., 2001). Experimental assessments of what enrichments or facilities a hen is willing to work for, based on consumer demand theory, have also been useful (Dawkins, 1983; Bubier, 1996b; Cooper & Appelby, 2003). But what is largely lacking is knowledge of the mental experiences of the hen that do not manifest themselves in spontaneous behaviour or in physiology.

The subjective emotional experiences of nonhuman animals have long been, and are still by some, seen as inaccessible to science (Nagel, 1974; counter-argued by Allen & Bekoff, 1997). Still, this is one of the most important aspects to consider when it comes to animal welfare (Duncan & Petherick 1991; Broom & Johnson, 1993). To gain knowledge of some of these "private" experiences, a method has been developed to assess the state of mind in animals as they interpret an ambiguous signal. In humans, this is commonly known through the rhetorical expression of the glass that is either half full or half empty, depending on the state of mind of the observer. An optimistic individual would tend to see the glass as half full, while a pessimist would refer to the same glass half empty – they are both cognitively biased and this affects their judgement. Psychological studies in humans have confirmed that there is an effect of mood on cognition. Paul et al. (2005) review several studies where persons in a state of depression or anxiety make more pessimistic judgements of ambiguous stimuli or future events. By assuming that the same is true for nonhuman animals, scientists have created situations where animals can show by their behaviour whether they judge a certain stimulus optimistically or pessimistically. By manipulation or knowledge of the subjects' living conditions, it is possible to see how this affects cognitive function and specifically, judgement.

The first study of cognitive bias in animals was published by Harding et al. in 2004. Here, rats were trained to press a lever in response to a tone of a specific frequency, to receive a food reward. If they pressed the lever in response to a tone of a different frequency, they instead received an unpleasant burst of white noise. During the test, the rats were presented with tones of intermediate frequencies, and their responses (pressing/not pressing the lever) were recorded. The experiment showed that rats housed in a presumed more negative environment made significantly slower and fewer lever presses in response to an ambiguous signal. This indicates a more pessimistic view on life (Harding et al., 2004).

Since then, a number of studies have confirmed this method as reliable in reflecting differences in living conditions believed to affect welfare (reviewed by Mendl et al., 2009). Another advantage of this method compared to other measures of the welfare of animals is the possibility to measure an emotion's *valence*, i.e. if it is experienced as pleasant or unpleasant, not only the *level of arousal* which is what most physiological measures detects (Paul et al., 2005). For example, one can have high levels of cortisol or adrenaline when in distress, but also when playing or mating.

The aim of the present study was to investigate whether the time passed since leaving a conventional battery cage in exchange for a littered pen would lead to a change in judgement of ambiguous cues in domestic hens. In this study a special kind of cognitive bias task was used, based on spatial judgement bias which was developed by Burman et al. (2008a), and further tested by Doyle (2008) and Burman et al. (2009).

The animals were also tested in a novel object task for a measure on general anxiety, and their social rank was established by assessing the winners in a pairwise competition over a limited resource. These parameters were tested for correlations with the results in the cognitive bias test, to investigate other factors that might influence the birds' behaviour in the test.

Animals and methods

Animals and housing

The hens were acquired from a conventional battery farm at slaughter age, 67 weeks. They were transported to the research facility Rørrendegård, belonging to Copenhagen University, Faculty of Life Sciences. Here they were housed with unfamiliar hens in groups of two to five individuals, and kept in pens of 1.5×1.7 m², all fitted with a perch, litter and three nests. They were fed a commercial laying hen feed *ad lib*. Five of the ten pens were additionally supplied with a large amount of litter, elevated perches, a peat bin for dustbathing and given extra treats once per day. This difference between enriched and less enriched pens will not be considered here, but was the focus of another project which this study was part of. However, hens from the two housing conditions are used in a balanced way throughout the study.

Thirty-three hens were housed in the facilities during the experiment. Of these, 13 individuals were tested twice for their cognitive bias, and are the main subjects of this study. The first test period was carried out two months (days 65-70) and the second test four months (days 129-134) after leaving the cages and arriving to the research facility. Another 13 individuals were tested only once, days 113-117. The remaining seven hens did not succeed in learning the cognitive bias task. One hen did not like corn, so she had to be excluded from all elements of this study. Her two pen mates had to be excluded from social status testing, as this was based on competition over corn.

Cognitive bias training

For the cognitive bias training and testing, an arena of 2.2×2.35 m was used (Figure 1). The hens were taught to expect a reward, consisting of a few pieces of corn, if the bowl was placed in either the opposite left or right corner of the arena, and an empty bowl if it was placed in the other corner (Figure 2). Half of the birds were only rewarded at the left position and the other half at the right position.

The hens were taken from their home pens and put in a start box, separated from the arena with a manually handled guillotine door (Figure 3). At first, they were presented to the arena in pairs, and with corn scattered on the floor. When the birds were feeding of the

corn they were on the next training session presented to the arena singly and with corn in the bowl. The bowl was placed at the rewarded position and always contained corn.

When a hen walked from the start box to the bowl and ate the corn in less than two minutes, three times in a row, she was considered ready for training sessions including the unrewarded position. At this point, the bowl was also fitted with a lid made of a half circular piece of cardboard, to prevent the birds from seeing whether it contained corn or not from a distance. The time was measured from when the door was opened until the bird either pecked at or looked closely into the food bowl. After the bird had done so, alternatively after 60 seconds, the bird was picked up and returned to the start box for the next run. Each training session consisted of eight runs, pseudo-randomly altered between the rewarded and unrewarded position with a maximum of two runs to the same positions in a row and an equal number of both. Thus, a training session could look like this: R-R-U-R-U-U-R-U. The birds were considered ready for testing when they had reached the learning criteria to take *at least five seconds longer* to reach the unrewarded compared to the rewarded position on *at least three occasions* (out of four possible) in a session.

To reach these criteria before the first test period, two months after arriving at the research facility, most hens needed 12 training sessions or more. Two months later, most birds needed some additional training to once again reach the learning criteria before the second test period could begin. All birds were given at least four training sessions this time, though one bird fulfilled the criteria at the first training session and one at the second. All hens had reached the criteria after seven training sessions.

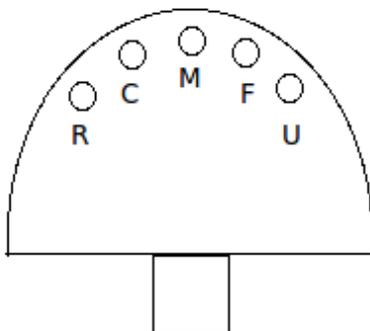


Figure 1: Sketch of the cognitive bias arena with the five possible bowl positions marked out: Rewarded (R), Closest to rewarded (C), Middle position (M), Furthest from rewarded (F) and Unrewarded (U). For the hens that were rewarded at the right position the letters are reversed.



Figure 2: The bowl placed at the right position



Figure 3: The start box door opening into the arena

Cognitive bias testing

During the test sessions, three runs with probe positions (Figure 1) were mixed with ten runs with left or right positions, making a total of 13 runs during each test session. The three probes were labelled 'C' (closest to the rewarded position), 'M' (middle position) and 'F' (furthest from the rewarded position; see Figure 1). A test session could look like this: U-R-U-R-M-U-R-U-C-R-U-R-F. Each individual was tested three times over a period of five days, with one day of no testing between each test day. The time taken from the door opened until the hen pecked at or looked closely into the bowl was registered. Maximum time was 60 seconds.



Figure 4: Seven Brown finds the bowl in the middle position to be empty.

Novel object test

The reaction of each individual to a novel object was established as follows: first all hens were removed from their home pen and then put back one at a time, this time presented with a tin can with some pieces of corn. The hens were considered ready to be tested when they fed from the can in less than ten seconds from being released in the home pen. Most hens did so at the first or second try.

The procedure was then repeated, but this time a red and yellow plastic ball, about 12 cm in diameter, with two eye-like holes in it was placed about 20 cm behind the can (Figure 5). The time for the hen to feed from the can in the presence of the ball was measured. Maximum time was 60 seconds. The tests were carried out on days 130-135 after leaving the batteries.



Figure 5: The ball used as novel object and the can with corn as presented to the hens.

Social status test

To determine the dominance relationships within the ten social groups all hens were removed from the home pen, and then put back in pairs. The pair was served corn from a can, with the rather narrow opening making it possible for only one hen to feed at a time. All successful feeding attempts for both individuals were recorded by one-zero interval sampling (Lehner, 1998) in five second intervals during 180 seconds. The dominant individual in each pair was defined as any individual who fed during at least twice as many intervals as the other during the first 90 seconds of sampling (eighteen 5-second intervals). The winning part was assigned one point. If the set was ending in a draw, so to speak, both individuals received one point. The ratio of won or undecided sets to total number of sets 'played' was calculated for each individual, and resulted in a number between 0 and 1. All hens were subsequently assigned a rank of 0, 0.5 or 1, where hens winning all their sets receiving status 1, hens losing all their sets receiving status 0, and all intermediates put in the 0.5 category. The tests were carried out on days 135-136 after leaving the batteries.

Feather scoring

All hens at the facility were examined and scored with regard to feather plucking. They were given a score between 0-3, defined as follows: 0 – No obvious naked parts. 1 – Naked part <5x5 cm. 2 – Naked part >5x5 cm. 3 – More than $\frac{3}{4}$ of the body is naked. The scoring was made days 113-130 after leaving the batteries.

Statistical analyses

The effect of time since change of environment on latencies to reach the three probe positions was analysed with a Wilcoxon signed-rank test. The effect of social status (3 levels) on the average latencies to reach probes during the hens most recent test period was analysed with a Kruskal-Wallis analysis of variance. Correlations between feather score and social dominance, as well as between reaction to novel object and latencies to reach probe positions, were tested with Spearman rank-order correlation. Differences in reaction to novel object by hens of different social status was tested for with a Mann-Whitney U-test. All calculations were made in Minitab.

Ethical considerations

As the experiment was non-invasive, the national (Danish) legislation did not require an ethical approval. The cognitive bias task can be seen as a type of environmental enrichment in itself by posing a cognitive challenge to the hens (Meehan & Mench, 2007). There were no negative reinforcers or stimuli used but the absence of reward. After the study was completed, all 33 hens were rehomed to a private home in the country with outdoor access and rooster company, where they will be able to lead the rest of their lives regardless of their utility for humans (Figure 6-7).



Figure 6-7: The life of the hens after the study.

Results

The hens did perform differently in the second test period compared to the first, by making a more pessimistic judgement of the middle probe. No difference was found in the reactions to ambiguous cues by hens of different social status. The hens who reacted strongly to the novel object were more optimistic in their judgements of all probes, though this was not statistically significant. A strong correlation was found between feather score and social dominance.

Effect of time since change of environment

The average times to reach the five positions for all 13 hens during the three test sessions of each of the two test periods is depicted in Figure 8. The graph shows that the hens, on average, judge the value of the probes – expressed as their keenness to go and check them – according to their proximity to the reinforced cue.

The results of the Wilcoxon signed-rank test were for position C $W_+ = 67$, $p = 0.14$, for position M $W_+ = 81$, $p = 0.014$, and for position F $W_+ = 44$, $p = 0.94$. Thus, the hens showed significantly longer latencies to reach the middle probe four months compared to two months after leaving the batteries.

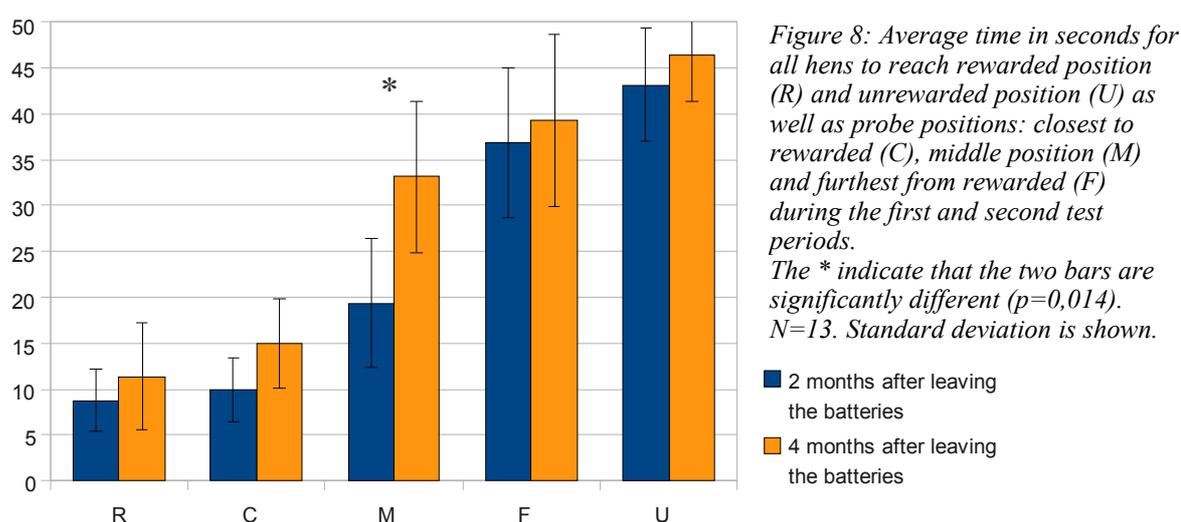


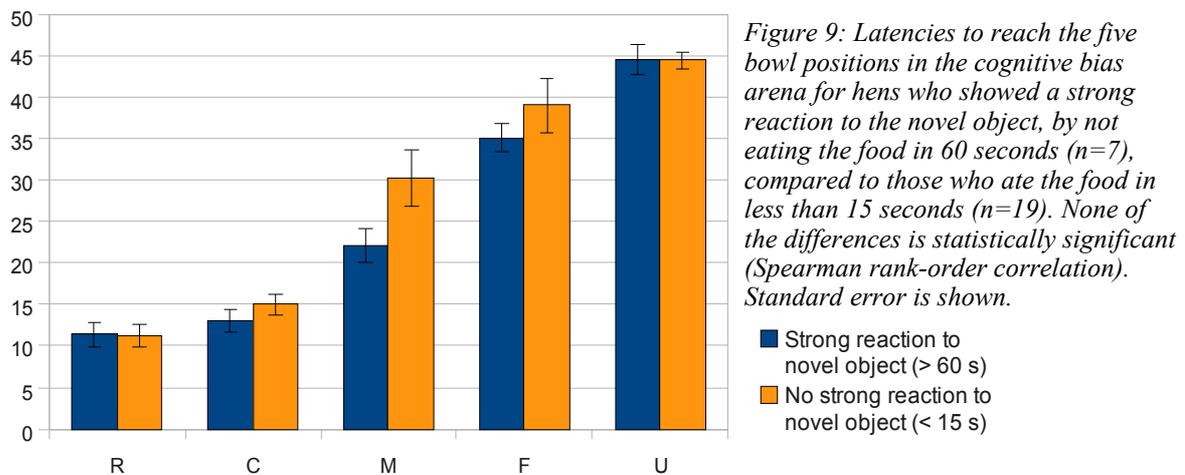
Figure 8: Average time in seconds for all hens to reach rewarded position (R) and unrewarded position (U) as well as probe positions: closest to rewarded (C), middle position (M) and furthest from rewarded (F) during the first and second test periods. The * indicate that the two bars are significantly different ($p=0,014$). $N=13$. Standard deviation is shown.

Novel object test

There was a large individual variation in the reaction to the novel object, where the hens could be divided into one of two distinct groups. Most hens (20 out of 32) ate from the can within 15 seconds in presence of the novel object. The remaining twelve did not eat within 60 seconds. In these cases, the ball was removed, and all hens subsequently ate the corn within one minute. This indicates that it was indeed the novel object that had prevented them from eating.

Of all 32, six hens had not been susceptible to training in the cognitive bias task; five of the twelve who showed a strong reaction to the novel object, and one who did not show a strong reaction. Complete data on both reaction to novel object and cognitive bias testing was thus available for 26 hens. A Spearman rank-order correlation test revealed no significant relationships between the reaction of the hens to the novel object, and their interpretation of the ambiguous cues in the cognitive bias arena. However, the hens who showed a strong reaction to the novel object had shorter average latencies to reach all three probe positions, with the most pronounced difference at the middle position (Figure 9).

There were no significant differences in the reactions to the novel object by hens of different social status.



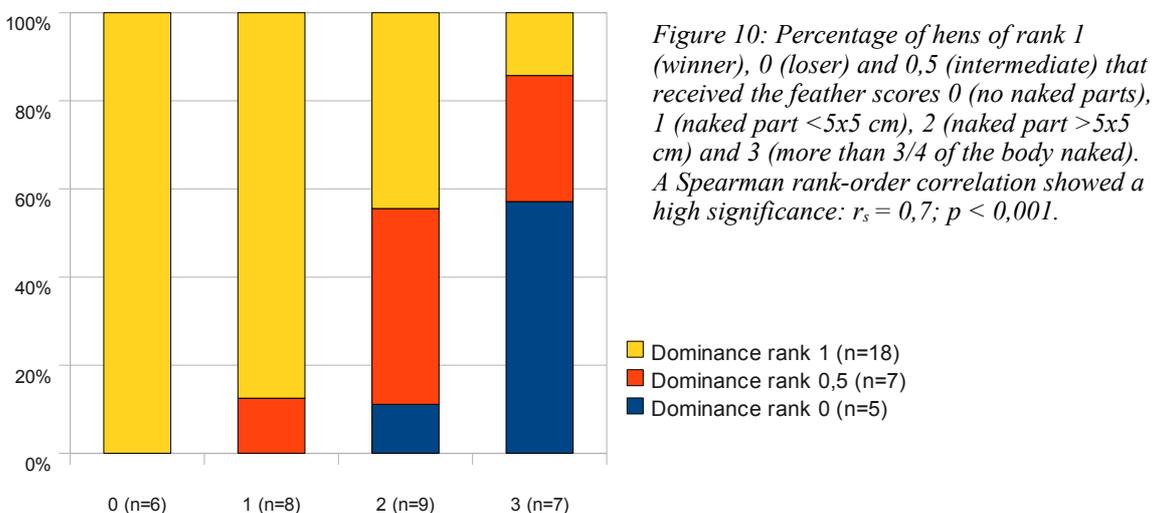
Social status test

The hen who did not eat corn and her two pen mates had to be excluded from this test, but the remaining 30 hens were tested. Of these, 18 was assigned rank 1 as all of their sets were won or undecided. Five individuals lost all of their sets and received rank 0, while the remaining seven won some and lost some of their sets and were assigned rank 0,5.

A Kruskal-Wallis analysis of variance was then performed to see if social status had any effect on average time to reach probe positions during the hens most recent test period (i.e., tests performed days 113-134). Only 25 birds could be included in the analysis, as five birds had not completed any cognitive bias test period. No significant difference was found.

Correlation between feather score and social status

The relationship between feather score (0-3) and social status (0-0,5-1) for all 30 hens was tested with a Spearman correlation. A strong correlation between these measures was shown: $r_s = 0,7$; $p < 0,001$ (Figure 10).



Discussion

The hens showed longer latencies to reach the middle probe after a longer stay in the pens. According to cognitive bias theory (Paul et al., 2005; Mendl et al., 2009), this means that they made a more pessimistic judgement of the probe, indicating that they were more negatively biased during the second test period. This could be because the assumed positive effects of the improved environment are largest when the hens have recently left the battery cages, with the effect of the improvement leveling out in time. It is likely that novelty in itself is positive to the hens, and a static environment becomes boring with time even when it is far more complex than a battery cage. This would be in line with the opponent-process theory of motivation, proposed by Solomon & Corbit (1978). According to this theory, any emotional reaction to a stimulus is followed by an opposing reaction before returning to normal. If it is assumed that the theory applies here – and that the move to a more enriched environment is a positive event for the hens which induces an increase in positive emotions – the opposing, negative emotions gradually follow before the hens return to a normal state. The first test results would thus have been gathered while the hens were still positively affected by the change, and the second test results during either the negative or the normal phase, occurring four months after the sudden improvement.

A possible source of error is that the hens remembered from the previous test period that the probe positions were unrewarded, and that this made them less eager to approach. This seems unlikely, as most hens needed several training sessions to re-learn to differentiate between the left and right position correctly, and they had only encountered each probe position three times, two months earlier, interspersed between the usual left and right positions.

Previous studies (Bateson & Matheson 2007; Burman et al., 2008a; Burman et al., 2008b) have found increased pessimism in animals deprived of previously experienced environmental enrichment. At least one previous attempt to find the opposite – increased optimism in animals obtaining improvements in their environment – failed to find any such effect (Bateson & Matheson, 2007). It might be that the cognitive bias test method is more prone to measuring effects of lowered life quality or expectancies, rather than different levels of good quality of life. This is another possible source of error in the present study, as the life of the hens is presumed to be at a comparable, and rather positive, level during the first and second test periods.

Burman et al. (2009) documented the effect of *relative* rather than *absolute* properties of the environment, by examining the effect on judgement by different light intensities in rats. The contrasting effect of lowered light intensity (perceived as positive to a nocturnal animal) induced more positive judgements of ambiguous cues, while there was no difference in judgement between rats subjected to unchanged – either high or low – light intensities. This supports the possibility that the contrasting effect of an improved environment was stronger in the hens shortly after leaving the cages, and was leveled out with time.

This, however, does not explain the consistent shorter latencies to reach all intermediate positions by the hens that exhibited a fear response towards the novel object. These hens show a negative interpretation of the ball ('dangerous') but a positive interpretation of all three intermediate probes ('likely to contain food'). How these apparently nonconcordant reactions can coexist in the same individuals is hard to say and, albeit interesting, beyond the scope of this study. However, as the results are non-significant, the reaction to a novel

object can not be used generally as a predictor of judgement biases, and is therefore of minor relevance.

There were no differences in average latencies to reach any of the bowl positions for winners or losers in the pairwise competitions. This indicates that the social status of a bird in her group does not have a significant influence on her state of mind when judging the ambiguous probes. It could also be that such an effect exists, but is compensated for by the lower-ranking birds taking the opportunity to claim a resource when they are, for once, without competition.

As such, the concepts of social dominance and rank orders in hens are complicated. The two main methods to assess dominance relationships is by recording agonistic versus submissive encounters in the group, or by staged pairwise competitions between either familiar or unfamiliar individuals. It is well known that results from the two methods are not always congruent (Rushen 1983; Craig & Ramos, 1986; Cloutier & Newberry, 2000). Neither is plumage condition always a good predictor of social status (Cloutier & Newberry, 2000). This is because the objectives underlying feather pecking in hens are multifaceted, and far from only the result of agonistic pecking (Rodenburg et al., 2004; Jensen et al., 2005). Nevertheless, feather pecking is more frequently performed by dominant individuals (Vestergaard et al., 1993) and the strong correlation between feather pecking and competitive rank order in the present study confirms this.

The results of this study show that it is possible to find pronounced differences in the cognitive bias of hens over time, though the difference in living conditions between tests is rather small. It is therefore a promising approach for future studies. It would be interesting to test hens in certain living conditions, for example while living in cages, and compare the results with subsequent testing of the same individuals after moving to another environment. It would also be interesting to test cognitive bias in hens immediately after moving from cages to an enriched environment, but here the extensive training procedure is an impediment. Though it is clearly possible to teach hens to differentiate between two separate locations and to judge intermediate probes according to their proximity to the rewarded location, there is a need for a less time-consuming way to access their state of mind. The current cognitive bias method nevertheless comprises a promising approach to further studies and new insights in animal cognition and welfare.

Acknowledgements

My warmest thanks to Johan and Livia who gave room in their home and hearts for the 33 hens after the study. Two months after arriving there, they are reported to all have feather score 0 and “look like little angels”. Thanks also to my supervisors Jenny Loberg and Anette Wichman for continuous ideas and great support with practical and statistical issues, and to Björn Forkman, Irene Bontell, Cecilia Mille, Ingvill Riise and Sara Asteborg for comments on a draft.

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