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The relation between fearfulness in young and stress-response in adult laying hens, on individual and group level

Elske N. de Haas [a,*], Marjolein S. Kops [b], J. Elizabeth Bolhuis [a], Ton G.G. Groothuis [c], Esther D. Ellen [d], T. Bas Rodenburg [d,e]

[a] Adaptation Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH, The Netherlands
[b] Department of Pharmaceutical science, Division Psychopharmacology, Utrecht University, 3584 CA, The Netherlands
[c] Behavioral Biology, Centre for Behavior and Neuroscience, University of Groningen, NL Nijenborgh 7, 9747 AG Groningen, The Netherlands
[d] Animal Breeding and Genomics Centre, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH, The Netherlands
[e] Behavioural Ecology Group, Wageningen Institute of Animal Sciences, P.O. Box 338, 6700AH, The Netherlands

HIGHLIGHTS

► Relation between fear as young and corticosterone as adult in laying hens.
► There are differences between groups in corticosterone response to stress.
► Fearful response as young was related with high corticosterone levels as adult.
► Presence of a fearful bird in a group led to high corticosterone of the group mates.
► High level of comb lesions was related to high corticosterone of all birds in a group.

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ABSTRACT

Fearfulness of an individual can affect its sensitivity to stress, while at the same time the social situation in which an animal lives can affect its fear level. It is however unknown what the long-term effects of high fearfulness on sensitivity to stress are, on individual or group level in laying hens. We hypothesize that increased fearfulness at a young age results in increased sensitivity to stress at an adult age, and that this relation can differ between groups, due to differences in group composition. Therefore, we studied the relation between fearfulness in an Open Field (OF) test at six weeks of age and plasma-corticosterone (CORT) levels after a 5-min Manual Restraint test (MR) at 33 weeks of age, and assessed behavior in the home pen. We used birds from a low mortality line, selected for four generations on low mortality due to feather pecking and cannibalism and a control line (n = 153 in total, eight pens/line). These lines are known to differ in fearfulness and stress physiology. Chicks from the low mortality line were more active in the OF compared to chicks from the control line. Chicks that showed a fearful response (no walking, no vocalizing) in the OF test had higher CORT at 33 weeks of age than chicks that walked and/or vocalized in the OF test and had higher activity in the home pen as adults. On group level, a passive response in the OF was related to high CORT levels after MR. Presence of at least one fearful bird in a group led to higher CORT in the other group mates compared to birds from groups with no fearful birds present. Birds from groups in which more than 50% of birds had severe comb lesions had higher CORT levels compared to birds from groups with less than 50% of birds affected. High fearfulness of laying hen chicks can on individual level have a long-term effect on stress sensitivity. The presence of fearful birds in a group as well as signs of social instability in a group, indicated by comb lesions, can affect sensitivity to stress of birds from the same group. The mechanism by which this occurs can lie in social transmission of (fear related) behavior, but this suggestion needs further investigation.

1. Introduction

Fear is an animal's state of apprehension to an imminent existing threat [1]. An animal's fearfulness is therefore the likelihood of responding fearfully to numerous potentially dangerous stimuli [2]. In response to a threatening situation, a physiological stress response is initiated, consisting of activation of the hypothalamic–pituitary–adrenal axis with release of catecholamines ((nor)adrenaline) and corticosteroids (cortisol and/or corticosterone) [3]. Behaviorally, animals become more alert, and they can either respond actively or passively in order to increase their survival [4]. An animal's fearfulness and its physiological stress-response are therefore, within a short time frame, related. In

* Corresponding author at: P.O. Box 338, 6700 AH, Wageningen, The Netherlands. Tel.: +31 317 48 29 20; fax: +31 317 48 50 06.
E-mail address: elske.dehaas@wur.nl (E.N. de Haas).

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some species it has been found that a high sustained fear level, causes an increased vulnerability to stress in later life [5–7].

Factors which can influence an animal's fear level can be its genetic make-up, unpredictable and uncontrollable early life experiences and associative learning experiences [8]. Furthermore, the social environment may also affect an animal's fear level [9]. Exposure to a stressed adult cage-mate can induce anxiety (perceiving a situation as threatening [10]) in young mice [11]. When this occurs in early life, it can influence stress sensitivity [12] and possibly also social behavior (suggested by [13]). In laying hens, fearful individuals may cause other hens to become more fearful [14]. For example, it has been shown that housing birds of a fearful line together with birds from a non-fearful line, led to increased fearfulness in the birds of the non-fearful line [15]. Others [16] also found an effect of mixing birds from a fearful line with birds from a less fearful line on basal corticosterone levels. Irrespective of mixing laying hens in groups which can be stressful as such [15], differences in fear level between stable groups of laying hens are known to exist [17]. Social interactions between animals with different behavioral characteristics could influence the development of fearfulness. As such, group composition may affect fear level within a group, and, potentially stress sensitivity.

Fearfulness in a group can also be affected by the method of breeding. Traditional breeding of laying hens consists of choosing the individuals with the highest egg-production. Individual egg-production can only be measured in individually housed hens and therefore no information on social behavior is available from these individually housed hens. A recently developed breeding method enables one to select birds on the basis of a combination of individual and group performance [18]. Selection is still based on individual egg-production, but with additional information on levels of mortality due to cannibalism and feather pecking of relatives living in a four-hen family cage [18]. This group selection method has been shown to reduce fear in the second generation of selection on low mortality (from now on labeled low mortality line) [19]. Chicks from the second generation for low mortality were more active in the Open Field test than chicks from a control line (selected on individual egg-production only) [20]. As adults, hens from the low mortality line had lower corticosterone levels after a restraint test [21] and higher whole-blood serotonin and lower serotonin-uptake [19] than control hens. Serotonin is known to be involved in fear, anxiety and stress [6,22–26]. Thus, selection on group performance seemed to have reduced fearfulness and response to stress in comparison to selection on individual performance only.

The aim of this study was to assess the long-term effects of fearfulness. Therefore, we used lines which were known to differ in fearfulness and stress physiology (low mortality line versus control line). Our hypothesis was that increased fearfulness at a young age results in increased stress sensitivity at an adult age, and that this relation can differ between groups. Additionally, we wanted to determine if differences between groups in home pen behavior could be affected by fear level of individuals within a group. As selection of the low mortality line was based on reduced levels of mortality due to cannibalism and feather pecking, we also assessed differences in body damage due to pecking.

2. Material and methods

2.1. Animals and housing

Two White leghorn lines from ISA BV, the layer breeding division of Hendrix Genetics, were used: a low mortality line and a control line. The low mortality line was selected for four generations on low mortality in group housing [18]. Selection candidates were housed individually, to enable recording of individual egg-production. Siblings of these selection candidates were housed in family groups and in those (non-beak trimmed) groups mortality was recorded. Only selection candidates with low mortality levels in the group of siblings and sufficient individual egg-production were selected as parents for the next generation of the low mortality line. In the control line, the standard commercial breeding program was implemented (focusing on individual egg-production). Eggs from both lines were brooded at the experimental farm 'De Haar' (Wageningen University, the Netherlands). After hatching, chicks received a neck tag with a color/number combination. Per line, 104 chicks were randomly assigned to one of eight 13-chick floor-pens measuring $1.9 * 1.2$ m, where they had access to sand (1/3 of the surface), wood-shavings (2/3 of the surface), a 10 cm high perch (dividing both areas) and had ad libitum water and food. The pens were situated in two different rooms, with an equal number of pens per line within both rooms. A commercial mash diet was provided; a starter diet (weeks 1–5), a starter 2 diet (weeks 6–16) and a layer diet (from week 17 onwards). Throughout the experiment whole grains were scattered once a day around 8 a.m. in the sand area (particle size adjusted to bird age). At seven weeks of age a 50 cm high perch was added in the wood-shavings area and group size was reduced to ten birds per pen. At 17 weeks of age, a group nest was added in each of the pens. One hundred and fifty three hens were retained in the experiment (11 pens with 10 hens/pen, 3 pens with 9 hens/pen, and 2 pens with 8 hens/pen). This experiment was approved by the Institutional Care and Use Committee and in accordance with Dutch legislation on the treatment of experimental animals, in conformation with the ETS123 (Council of Europe 1985) and the 86/609/EEC Directive.

2.2. Behavioral observations

Birds were individually subjected to two tests: an Open Field (OF) test at six-weeks of age and a Manual Restraint (MR) test at 33 weeks of age. Body damage was assessed at 30 weeks of age, and home pen observations took place between one and four weeks of age, and 30 and 33 weeks of age. Order of testing/observations was balanced for lines, birds and pens. The experimenter was blind to the allocation of lines and designation of pens and birds within pens.

2.2.1. Open Field test

Each bird ($n = 153$) was individually subjected to an Open Field (OF) test for a duration of 5 min (see [20] for a detailed description of test set-up). Birds were tested between 8 a.m. and 4 p.m. over a 10-day period. A square barren observation pen measuring $1.25 * 1.25$ m ($4.1 * 4.1$ ft) operated as OF, with wire walls through which camera recordings were obtained. A chick was placed in the middle of the OF, which was kept dark until the start of the test. Behavior was scored from a video-screen in an adjacent room by a single person using The Observer software package (Noldus Information Technology B.V., Wageningen, The Netherlands). Durations and latencies to walk, stand, sit and vocalize (distress calls [27]) were recorded, as well as the number of vocalizations. Chicks were transported to and from the OF in a cardboard box.

2.2.2. Body damage

Body damage was scored on each bird at 30 weeks of age. Damage to neck, back and belly were used as indicators for severe feather pecking [28]. All regions were scored on a three point scale: intact/slight wear (a), moderate wear (b) and featherless areas (c), and summed to give a whole body index. The total score was either; zero (all regions had "a"), one (only one region with "b" led to a total score of one) or two (only one region with "c" led to a total score of two) (Welfare Quality®, 2009). Comb lesions were scored on a three-point scale; zero (no lesions), one (less than three lesions), two (more than three lesions) modified from [29], based on Welfare Quality (2009).

2.2.3. Home pen observations

Home pen observations took place between one and four weeks of age, and 30–33 weeks of age, once a week between 9.30 a.m. and 4 p.m. alternating morning and afternoon in a balanced design (each pen was observed twice in the morning and twice in the afternoon) by the use of scan sampling (Martin and Bateson, 2007) with a duration of 20 min with four-minute interval at young age, while at adult