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Maternal care and selection for low mortality affect post-stress corticosterone and peripheral serotonin in laying hens

T. Bas Rodenburg [a,*], J. Elizabeth Bolhuis [b], Rudie E. Koopmanschap [b], Esther D. Ellen [a], Eddy Decuypere [c]

[a] Animal Breeding and Genomics Centre, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[b] Adaptation Physiology Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[c] Laboratory of Livestock Physiology and Immunology, Department of Biosystems, Catholic University Leuven, Kasteelpark Arenberg 30, 3001 Leuven, Belgium

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ABSTRACT

The aim of the present study was to investigate the effect of brooding and group selection for low mortality on post-stress corticosterone and peripheral serotonin in laying hens. Birds in the experiment originated from the same population and were either group-selected for low mortality (low mortality line) or randomly selected (control line) for two generations. Twelve groups of seven birds from each line were used. Within each line, six groups were brooded by a foster mother and six groups were non-brooded. At 33 weeks of age, birds ($n = 42$/treatment) were manually restrained for 5 min, during which their behavioral response (number of struggles) was studied. Fifteen minutes after the start of the manual restraint, blood samples were drawn for assessment of plasma corticosterone and whole blood serotonin (5-HT) concentration. In the low mortality line, 80% of the birds struggled and vocalized vs. 72% in the control line (non significant). Birds from the control line had a higher plasma corticosterone concentration after manual restraint than birds from the low mortality line (7.7 vs. 6.0 nmol ml^{-1}). Furthermore, birds from the control line that were reared without a mother had a lower whole-blood 5-HT concentration than birds from the other treatments (45 vs. 48 nmol ml^{-1}). These results indicate that both brooding and selection for low mortality affect post-stress corticosterone and peripheral serotonin concentration, which may result in a reduced propensity to develop feather pecking.

1. Introduction

Feather pecking and cannibalism are major welfare problems in laying hens [1]. Feather pecking is a maladaptive behaviour, developing from ground pecking behaviour [2,3]. The development of feather pecking can be triggered by adverse environmental conditions during rearing or laying, such as absence of litter [2], inadequate feeding [4] or lighting [5] or large group sizes and high stocking densities [6]. It seems, however, that individual animals differ in their propensity to develop feather pecking and cannibalism, dependent on their behavioral and physiological characteristics [7–9].

It has been found that the propensity to develop feather pecking is related to fearfulness [10,11], stress reactivity [9,12] and activity of the serotonergic system [13,14]. Birds that show a long duration of freezing in an open-field test as chicks are more likely to develop feather pecking as adults [11]. Similarly, chicks from a high feather pecking (HFP) line were less active in an open field than chicks from a low feather pecking (LFP) line [10]. It has been shown that freezing in an open-field indicates increased fearfulness in the domestic hen [15–17]. In line with this, birds from a Rhode Island Red background had less feather damage and were more active in an open-field test compared with birds from a White Leghorn background [18], confirming the relationship between fear and feather pecking. Birds from a Rhode Island Red background also struggled more than birds from a White Leghorn background in a manual restraint test, in which a bird is restrained on its side for 5 min [18].

In the HFP and LFP lines, differences have been found in the corticosterone response to manual restraint. Birds from the LFP line had higher basal levels of corticosterone and also a higher corticosterone response to manual restraint [9,12]. These lines also differed in activity of the serotonergic system. Chicks from the HFP line were found to display lower 5-HT turnover levels in the brain than chicks from the LFP line [9]. Moreover, it was shown that feather pecking can be controlled by manipulating the serotonergic system, indicating that the performance of feather pecking is triggered by low 5-HT neurotransmission in the brain [13].

Behavioral and physiological characteristics related to feather pecking can also be influenced by breeding and rearing conditions, which in turn may lead to animals that are more or less prone to develop feather pecking and cannibalism. In animal breeding, novel breeding methods have been developed that allow us to select not only on individual performance but also on group performance, i.e.

* Corresponding author. Tel.: +31 317 483 936; fax: +31 317 483 929.
E-mail address: bas.rodenburg@wur.nl (T.B. Rodenburg).

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the influence of individuals on their group members [19–22]. One of the novel methods consists of selection of an individual which is partly based on performance of its relatives kept in family groups [21]. This method was used to select against mortality in laying hens. After one generation of selection this resulted in a considerable difference in mortality between the low mortality line and the unselected control line (20 vs. 30%, respectively). It has been shown in the second generation of selection that behavioral and neurobiological characteristics of selected birds had changed as well: birds from the low mortality line were found to be less fearful than birds from the control line [14,23]. Birds from the low mortality line were more active in an open-field test as chicks [23], and struggled and vocalized sooner and more frequently than birds from the control line in a manual restraint test as adults [14]. Furthermore, birds from the low mortality line displayed higher whole blood 5-HT concentrations and a lower platelet 5-HT uptake, indicating changes in peripheral 5-HT activity [14]. Similar results were found comparing birds from a (high feather pecking) White Leghorn line and birds from a (low feather pecking) Rhode Island Red line [24]. There are indications that peripheral (platelet) 5-HT activity may mirror brain 5-HT activity [24–26], which provides us with a less invasive measure of 5-HT activity.

In rodents and primates, long-term maternal separation or complete loss of maternal care have been shown to have a major and long-lasting impact on the development of behavior and monoamine neurotransmitter systems [27]. For instance, low maternal care is associated with high-anxiety related behaviour and exaggerated stress response in adulthood [28]. Also in domestic hens, it has been shown that rearing without or with a mother hen (brooding) has major effects on the chicks' behavioural development [23,29,30]. Brooded birds have been found to less fearful, already at a young age [23,31,32]. Furthermore, brooded chicks have been shown to display less feather pecking and cannibalism, resulting in lower mortality rates in brooded birds compared with non-brooded birds [29]. Effects of brooding on stress reactivity and on serotonergic activity in chickens are unknown.

Therefore, the aim of the present study was to investigate the effect of brooding and of selection on low mortality on behavioral and physiological responses to manual restraint, and on peripheral 5-HT concentration and uptake. It was hypothesised that brooded birds from a low mortality line would struggle and vocalise more in the manual restraint test and would have a lower corticosterone response after manual restraint and a higher peripheral 5-HT concentration and a lower uptake, compared with birds from the control line or non-brooded birds.

2. Materials and methods

The experiment was set up as a 2×2 arrangement, with genetic line (low mortality or control) and brooding (mother vs. no mother) as factors. For each treatment combination, six pens, each containing ten laying hens, were selected ($n = 24$ pens in total). The Dutch law on animal experiments was followed, which complies with the ETS123 (Council of Europe 1985) and the 86/609/EEC Directive. The Institutional Animal Care and Use Committee approved the experiment.

2.1. Animals and housing

A low mortality and a control line, originating from the same purebred White Leghorn layer line from ISA B.V., the layer breeding division of Hendrix Genetics, were used. The low mortality line was selected on low mortality due to cannibalism (in hens with intact beaks) for two generations, using the selection method previously described [14,21]. In brief, the selection criterion was based on the individual performance of the selection candidate (housed individually) and on the mortality levels of its sisters, housed in a family group of four birds. The control line originated from the same genetic population, but in this line no selection on low mortality was conducted.

After hatching, chicks were allocated to one of 24 floor pens, each containing 13 individuals from either the low mortality line or the control line. Twelve groups were reared with a foster mother from 1 to 7 wk of age (Silky hen or Wyandotte), 12 groups were reared with a conventional heating lamp with a 100 W ceramic bulb. The fostering procedure has been described previously [23]. In brief, foster hens were stimulated to become broody by increasing day length and by offering nests with non-fertilized eggs. Foster hens that were sitting on eggs were introduced to a group of 13 chicks. Only foster hens that showed positive interactions with the chicks were used in the experiment. There were no observable differences in maternal care between the Silky hens and the Wyandottes. The four treatments were equally distributed over 24 pens in two different houses (16 groups in one house, 8 groups in the other). Each group was housed in a floor pen measuring 1.9 * 1.2 m with wood shavings (2/3 of the surface) and sand (1/3 of the surface) on the floor. The areas were separated by a 10 cm high perch. A nest box was provided as well. Food and water were available ad libitum. The food supplied was a commercial mash diet, supplying a starter 1 diet (week 1–5), a starter 2 diet (6–16 weeks) and a laying diet (from 17 weeks onwards). Loose grains were supplied once a day around 8:00 in the sand area. From week 7 onwards, birds were housed with 10 individuals per pen and foster mothers and heating lamps were removed. A high perch (50 cm) was added as well. Birds were treated with routine vaccinations.

2.2. Manual restraint test

At 33 weeks of age, seven hens per pen ($n = 168$ birds) were individually subjected to a 5-min manual restraint test. These seven hens were pseudo-randomly chosen according to a planned schedule. Birds were tested by two experimenters on two consecutive days in four half-day blocks. Pens within different treatment combinations were evenly distributed over the experimenters and experimental days, and also testing order was balanced for genetic line and brooding. The test was carried out on a table placed in the corridor near the pens.

The experimental procedure was described previously [14]. In brief, the experimenter removed a hen from her home pen and placed the bird on her right side. The bird was then restrained with the right hand for 5 min, exerting mild pressure. The left hand was placed loosely on the stretched legs. After each struggle, hens were gently brought back in the original test position. Each bout of struggling was counted as an escape attempt. In addition, the number of vocalizations and latencies to the first struggle and vocalization were recorded. After the manual test, birds were housed individually in a plastic crate for 10 min, to allow the corticosterone concentration to reach its peak [33]. Fifteen minutes after the start of the test, blood samples of 6 ml were drawn from the wing vein for assessment of plasma corticosterone and whole blood 5-HT concentration. For three or four birds per pen, i.e. 21 chickens per genetic line × brooding combination, also platelet 5-HT uptake velocity was determined (see below). As this had to be done in fresh blood immediately after the test, only half of the birds were included in this analysis.

2.3. Physiological measurements after manual restraint

2.3.1. Corticosterone

Plasma samples for corticosterone analysis were stored at −20 °C until analysis. Corticosterone concentrations were determined using a radioimmunoassay kit (IDS Inc., Bolton, UK) as described earlier [34].

2.3.2. 5-HT in whole blood

Whole blood samples (1 ml), collected in EDTA-containing tubes, were placed on ice and stored at −70 °C until analysis. The 5-HT concentration in whole blood was determined by a fluorescence assay as previously described [14].