Genetic and Phenotypic Correlations Between Feather Pecking Behavior, Stress Response, Immune Response, and Egg Quality Traits in Laying Hens

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ABSTRACT The objective of the current study was to estimate genetic and phenotypic correlations among feather pecking (FP) behavior and stress response, immune response, and egg quality parameters. These traits have been measured in an F2 cross, coming from a cross between a high and a low FP line of laying hens. Heritabilities (h²) of stress response (32 wk), primary immune response to keyhole limpet hemocyanin (KLH) (36 wk) and *Mycobacterium butyricum* (39 wk), and egg quality parameters (35, 44, and 50 wk of age) were calculated. The h² was 0.05 ± 0.05 (SE) for stress response, 0.15 ± 0.07 for antibody response to KLH, and 0.08 ± 0.06 for antibody response to *M. butyricum*. The h² for egg quality traits were in the range of 0.12 to 0.30. Significant phenotypic correlations were found between gentle FP in adult hens and stress response, egg weight at 44 and 50 wk, and egg deformation at 50 wk. Significant additive genetic correlations were found between severe FP in adult hens and antibody response to KLH (0.79 ± 0.35), and between ground pecking in adult hens and egg deformation at 50 wk (0.63 ± 0.26), and between ground pecking and eggshell strength at 35, 44, and 50 wk of age (−0.86 ± 0.29, −0.81 ± 0.20, −0.76 ± 0.24, respectively).

(Key words: behavior, chicken, egg quality trait, feather pecking, genetic correlation)

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INTRODUCTION

In Europe, increasing concern for animal welfare has resulted in stricter legislation concerning animal housing. In poultry husbandry there is a shift from battery cages to large group housing systems. Feather pecking (FP) is a problem in all current housing systems, but in large group housing systems, the problem is more difficult to control. Feather pecking is characterized as pecking at and pulling of feathers of another bird. There are different forms of FP. Severe FP causes damage to the bird (Savory, 1995), results in bald patches and is painful for the bird (Gentle and Hunter, 1990). Besides impaired animal welfare, feather loss because of FP can lead to heat loss resulting in higher energy requirements (Blokhuis and Wiepkema, 1998).

Feather pecking is influenced by many different environmental factors (Green et al., 2000). However, it has been shown that there is a genetic component in feather pecking behavior (Cuthbertson, 1980; Bessei, 1984; Kjaer and Sørensen, 1997). Selection for or against FP behavior using direct observations is feasible (Kjaer et al., 2001). Divergent selection for 3 generations on FP behavior resulted in a significant difference between the high and the low lines (Kjaer et al., 2001). Additionally, Muir (1996) was successful in selection against cannibalistic behavior using group selection.

Molecular genetics can be helpful in the identification of the underlying genes affecting FP behavior. Recently, QTL involved in gentle and severe FP have been identified on chicken chromosome 2 (GGA2) (Buitenhuis et al., 2003). Identification of QTL or genes may be helpful in the selection for behavioral traits in chickens. Before applying selection for behavioral traits in breeding programs, it is beneficial to know more about possible correlated responses. To date, the focus in the literature is mainly on FP only and not on the relation to production traits. Kjaer

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**Abbreviation Key:** CORT = corticosterone; FP = feather pecking; h² = heritability; HFP = high feather pecking line; KLH = keyhole limpet hemocyanin; LFP = low feather pecking line.
and Sørensen (1997) showed that there is a negative correlation between body weight and pecking behavior. This was also observed by Rodenburg et al. (2004). Korte et al. (1997) suggested that corticoids play a role in the appearance of FP behavior, because the animals of the high FP line showed a low corticosterone (CORT) response and animals from the low FP line showed a high CORT response to a stress test. This was shown to be consistent over age (Van Hierden et al., 2002). This observation fits in the coping style theory of Koolhaas et al. (1999) in which active animals (fight/flight response) show lower CORT levels, and reactive animals (conservatism/withdrawal response) show high CORT response under stressful situations. However, no phenotypic or genetic correlations between FP and stress response are known. In addition, correlations between FP and physiological or immune parameters may provide a better insight into the use of these traits as predictor traits for FP behavior.

The objective of the current study was to estimate the genetic and phenotypic correlations between FP behavior and stress response, immune response, and egg quality characteristics. Results would provide information on possible desirable or undesirable side effects when selecting for FP behavior.

**MATERIALS AND METHODS**

**Experimental Populations**

An F2 population of 630 hens was created from a cross between 2 commercial White Leghorn lines of laying hens as previously described (Buitenhuis et al., 2003). The high feather pecking (HFP) and low feather pecking (LFP) lines differ for behavioral traits such as FP and open-field behavior (Jones et al., 1995; Rodenburg and Koene, 2003), as well as physiological traits, i.e., CORT response to manual restraint (Korte et al., 1997; Van Hierden et al., 2002), and egg production traits (unpublished data).

**Housing Conditions**

The F2 hens arrived at the experimental farm as day-old chicks in 5 batches at 2-wk intervals. The birds were not beak-trimmed and each individual was marked with a wing-band. Each batch was divided over 2 pens, giving 200 birds per group. The floor area of the pen was 4.75 m×2 m and was covered with wood shavings. There were 2 light tubes (2×40W) in each pen, and during wk 0 to 4, a heating lamp was provided. From wk 0 to 4, continuous light was provided by the heating lamp, and in wk 5 to 6, the scheme was changed to 8 h of light per day from 0800 to 1600 h. From 16 wk of age onwards the light scheme was extended 1 h per week until the animals had a 16-h light day from 0300 to 1900 h. Feed (152 g/kg CP and 2,817 kcal/kg ME) and water was provided ad libitum. The Wageningen University Committee on Animal Care and Use approved the use of the birds in the current experiment.

**Social FP Test**

At 6 wk of age, 625 F2 birds and, at 30 wk of age, 550 F2 birds were tested using a social FP test, described by Rodenburg et al. (2003). The traits measured at 6 and 30 wk were: gentle FP (gentle pecks, ignored by recipient), severe FP (forceful peck, reaction of the receiver), and aggressive pecking (forceful peck aimed at the head or neck). The number of pecks and the number of bouts, a period of continuous pecking directed toward the same part of the body of the conspecific, were recorded for each trait. A detailed description concerning the distribution, averages, and standard deviation of the traits were presented in an earlier paper (Rodenburg et al., 2003).

**Manual Restraint Test**

After the FP test at 30 wk, the chickens were housed individually. At 32 wk of age, the hens (n = 524) were exposed to a manual restraint test. The test was performed between 0900 and 1200 h. For this test, the bird was placed manually on its side for 8 min. A blood sample (1 mL) was taken from the wing vein after 8 min of manual restraint. Blood samples were transferred to heparin-coated centrifuge tubes, chilled on ice (0°C), and centrifuged at 3,000 × g for 10 min at 4°C. The supernatant was stored at 4°C until analysis. The CORT concentration (ng/mL) was measured in duplicate (De Jong et al., 2001). Average values of the 2 CORT samples were used in the analysis.

**Immune Response Traits**

At 36 wk of age, total antibody responses to keyhole limpet hemocyanin (KLH) were measured in individual plasma samples obtained at 7 d after s.c. immunization with 1 mg of KLH in 1 mL of PBS (pH 7.2). At 39 wk of age, antibody responses to Mycobacterium butyricum were measured in individual plasma samples at 11 d after s.c. immunization with 1 mg of M. butyricum in 1 mL of PBS. Antibody titers were measured as an indirect ELISA as described by Sijben et al. (2000). Titers were expressed as the log2 values of the highest dilution giving a positive reaction.

**Egg Quality Traits**

The egg quality parameters were measured at 35, 44, and 50 wk of age. Eggs were collected during 5 subsequent days. Most of the hens laid 5 eggs. Hens with less than 5 eggs were excluded from the data set (n = 14). The measurements were: 1) egg weight (g); 2) eggshell strength (g), measured as compressive fraction force with
Tables and figures are not present, but the text is readable and comprehensible.
with data from commercial lines may be biased (Rodenburg et al., 2003). Therefore, it would be of interest to examine the egg production traits and pecking traits in the founder lines to validate the genetic and phenotypic correlations.

Ground pecking showed a negative correlation with breaking strength of the eggshell at the genotypic level, meaning that selection for stronger eggs would reduce ground pecking behavior. Ground pecking is considered a foraging behavior, therefore, the present results agree with the observation of Schütz and Jensen (2001). In the study of Schütz and Jensen (2001), 3 chicken lines were compared based on different types of behavior. From their study, it was clear that the commercial laying line showed less foraging behavior compared with the lines not selected for egg production.

It would be of interest to have a physiological parameter to predict FP behavior. Van Hierden et al. (2002) showed that there was a consistent difference in CORT response to manual restraint in the HFP and LFP lines in young and in adult chickens. In rodents, it was shown that CORT levels in the blood influence the behavioral status of an animal. Based on these observations, Koelhaas et al. (1999) proposed the coping style theory where 2 styles could be distinguished, the proactive (fight/flight response) and the reactive (conservation/withdrawal response) coping style. Korte et al. (1997) suggested that these coping styles could be applied to chickens as well.

### TABLE 1. Means, SD, and heritability ($h^2$) estimates with SE of the traits measured

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>SD</th>
<th>$h^2$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORT (ng/mL)</td>
<td>5,306</td>
<td>1,712</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>KLH (g)</td>
<td>6.53</td>
<td>1.18</td>
<td>0.15*</td>
<td>0.07</td>
</tr>
<tr>
<td>M. butyricum</td>
<td>7.66</td>
<td>1.11</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>Egg weight 35 wk (g)</td>
<td>61.6</td>
<td>4.0</td>
<td>0.28*</td>
<td>0.10</td>
</tr>
<tr>
<td>Egg weight 44 wk (g)</td>
<td>63.2</td>
<td>4.4</td>
<td>0.29*</td>
<td>0.10</td>
</tr>
<tr>
<td>Egg weight 50 wk (g)</td>
<td>62.9</td>
<td>5.1</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Egg deformation 35 wk (μm)</td>
<td>73.7</td>
<td>18.2</td>
<td>NE*</td>
<td>NE</td>
</tr>
<tr>
<td>Egg deformation 44 wk (μm)</td>
<td>78.1</td>
<td>16.3</td>
<td>0.30*</td>
<td>0.11</td>
</tr>
<tr>
<td>Egg deformation 50 wk (μm)</td>
<td>76.3</td>
<td>23.5</td>
<td>0.19*</td>
<td>0.08</td>
</tr>
<tr>
<td>Eggshell strength 35 wk (g)</td>
<td>4,464</td>
<td>539</td>
<td>0.23*</td>
<td>0.10</td>
</tr>
<tr>
<td>Eggshell strength 44 wk (g)</td>
<td>4,183</td>
<td>553</td>
<td>0.19*</td>
<td>0.08</td>
</tr>
<tr>
<td>Eggshell strength 50 wk (g)</td>
<td>3,904</td>
<td>589</td>
<td>0.19*</td>
<td>0.08</td>
</tr>
</tbody>
</table>

1CORT = corticosterone response (ng/mL) after a manual restraint test as measurement for acute stress response at 32 wk of age.
2KLH = total antibody responses to keyhole limpet hemocyanin at 36 wk of age. Expressed as the log2 values of the highest dilution giving a positive reaction.
3M. butyricum = total antibody responses to Mycobacterium butyricum at 39 wk of age. Expressed as the log2 values of the highest dilution giving a positive reaction.$\text{NE} = \text{nonestimable.}$

### TABLE 2. Phenotypic and additive genetic correlations between traits measured in the social feather pecking test at 30 wk of age and egg quality traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>CORT $^1$</th>
<th>KLH $^2$</th>
<th>Egg weight</th>
<th>Egg deformation</th>
<th>Eggshell strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 wk</td>
<td>44 wk</td>
<td>50 wk</td>
<td>35 wk</td>
<td>44 wk</td>
</tr>
<tr>
<td>Phenotypic correlation $^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentle FP (n bouts)</td>
<td>-0.11*</td>
<td>0.04</td>
<td>-0.14</td>
<td>-0.18*</td>
<td>-0.16*</td>
</tr>
<tr>
<td>Gentle FP (n pecks)</td>
<td>-0.11*</td>
<td>0.04</td>
<td>-0.16</td>
<td>-0.17*</td>
<td>-0.18*</td>
</tr>
<tr>
<td>Severe FP (n bouts)</td>
<td>0.05</td>
<td>0.07</td>
<td>0.02</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>Severe FP (n pecks)</td>
<td>0.06</td>
<td>0.07</td>
<td>0.05</td>
<td>0.11</td>
<td>0.05</td>
</tr>
<tr>
<td>Ground pecking</td>
<td>0.02</td>
<td>-0.03</td>
<td>-0.10</td>
<td>0.01</td>
<td>-0.05</td>
</tr>
<tr>
<td>Additive genetic correlation $^5$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentle FP (n bouts)</td>
<td>0.31</td>
<td>0.30</td>
<td>-0.09</td>
<td>-0.03</td>
<td>0.47</td>
</tr>
<tr>
<td>Gentle FP (n pecks)</td>
<td>0.29</td>
<td>0.29</td>
<td>-0.11</td>
<td>-0.06</td>
<td>0.40</td>
</tr>
<tr>
<td>Severe FP (n bouts)</td>
<td>-0.10</td>
<td>-0.79*</td>
<td>-0.30</td>
<td>-0.22</td>
<td>-0.11</td>
</tr>
<tr>
<td>Severe FP (n pecks)</td>
<td>-0.21</td>
<td>-0.72*</td>
<td>-0.38</td>
<td>-0.29</td>
<td>-0.16</td>
</tr>
<tr>
<td>Ground pecking</td>
<td>-0.07</td>
<td>0.33</td>
<td>-0.11</td>
<td>-0.17</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1CORT = corticosterone response (ng/mL) after a manual restraint test as measurement for acute stress response at 32 wk of age.
2KLH = total antibody responses to keyhole limpet hemocyanin at 36 wk of age. Expressed as the log2 values of the highest dilution giving a positive reaction.
3Standard errors were in the range of 0.03 to 0.08.
4FP = feather pecking.
5Standard errors were in the range of 0.20 to 0.40, except for egg deformation at 35 wk of age (SE = 0.54 to 0.77).
*Estimate significantly different from zero.
In the current study, the $h^2$ for CORT response to manual restraint was low (0.05), similar to that in a study in Japanese quail (Odeh et al., 2003). In quail, it was shown that selection for stress responsiveness was possible and that the $h^2$ for CORT response in a randombred line was low (0.05). With regard to a possible predictor for FP behavior, there was no relation between FP behavior in young hens and the stress response in adult laying hens. However, at the phenotypic level there was a low negative correlation between FP in adult hens and CORT response, indicating that pecking birds show a lower CORT response compared with nonpecking birds. This is in agreement with the observations of Van Hierden et al. (2002) for gentle FP behavior. The HFP birds show a consistently lower CORT response to manual restraint than the LFP birds. However, at the genetic level the relationship remains uncertain.

For severe FP, no correlation with CORT response was found. However, a high genetic correlation between severe FP and primary antibody response to KLH was found, indicating that there are genes simultaneously involved in both severe pecking and antibody response. The QTL detected for severe FP (Buitenhuis et al., 2003) and primary antibody response to KLH (Siwek et al., 2003), however, did not give any information on possible pleiotropic genes. For both traits the QTL detected were on different chromosomal regions.

There was a low positive phenotypic correlation between antibody response to KLH and antibody response to $M. butyricum$ (0.28 ± 0.05), but there was no genetic correlation between these traits. In mammals, the antibody response to KLH is mediated via the Th1 pathway, whereas the antibody response to $M. butyricum$ is mediated via the Th2 pathway (Bliss et al., 1996, Mossmann and Sad, 1996). The antibody responses generated via either pathway have different features in processing the antigens and are controlled by different genes, therefore, the lack of genetic correlation between these traits might be expected. The positive phenotypic correlation between KLH and $M. butyricum$, however, may point to a common environmental correlation.

In the search for a valid predictor trait for FP behavior in adult animals, one may use CORT response as a predictor for FP behavior, however, it is not practical in a breeding program (Buitenhuis et al., 2003). The primary antibody response to KLH might be an interesting predictor for severe FP. Whether this correlation would hold when KLH was measured before the onset of lay, however, remains unclear. The actual FP problem starts just after the onset of lay. Therefore, one would like to predict which animal will be more likely to become a pecker before the problem starts.

In conclusion, there was a relationship between gentle FP behavior and egg quality traits, and between severe FP and immune response. In addition, there was a strong genetic correlation between egg quality traits and ground pecking behavior, indicating that selection for egg quality may influence foraging behavior in the laying hen.

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