INTRODUCTION

During the past decades, poultry breeders have successfully improved the production performance, either egg production in layers or meat production in broilers. However, considering the ever-increasing social concern, future animal husbandry is required to pay more attention to enhancing animal welfare. In laying hens, animal welfare is particularly focused on feather pecking behavior. Feather pecking is defined as pecking toward the plumage of other birds. Two major forms of feather pecking can be distinguished: gentle and severe feather pecking (Keeling, 1995). Severe feather pecking causes damage to the birds, results in bald patches, denuded area, hemorrhage, wounds, is painful for the birds, and can even lead to death (Gentle and Hunter, 1991). Feather pecking is not only a welfare but also a serious economic problem (Rodenburg et al., 2008). Decreased egg production caused by feather pecking was observed (Johnsen et al., 1998). Feather loss because of feather pecking can lead to heat loss, which results in higher maintenance energy requirements (Blokhuis and Wiepkema, 1998). Mortalities due to cannibalism, which is considered the ultimate phase of severe feather pecking, can be substantial. Hill (1986), for example, found up to 15% mortality in laying hens housed in aviaries, whereas Peeters et al. (2012) and Ellen et al. (2008) found around 32 and 48% mortality, respectively, due to cannibalism in cage-housed birds. Prohibition of both cage housing system and beak trimming because of animal welfare concern in many European Union member countries increases the risk of feather pecking and cannibalism.

Better understanding of the genetic and biological mechanisms of feather pecking is needed to find alter-
native ways of preventing this unfavorable behavior. Feather condition score (FCS) is a measure of feather damage, which has been shown to be closely related to feather pecking behavior in hens housed in groups (Bilčík and Keeling, 1999; Uitdehaag et al., 2008). Different from ordinary traits, FCS is a so-called interacting phenotype, a trait whose value is also affected by the behavior of an individual’s conspecifics (the cage mates that are kept with the focal individual in the same cage in case of laying hens; Moore et al., 1997). In contrast to the direct genetic effect of an individual on its own phenotype, the heritable effect of an individual on the phenotype of a conspecific is known as associative effect or indirect genetic effect (Griffing, 1967; Wolf, 2003; Bijma et al., 2007). Associative effects influence a trait’s inheritance and contribute to heritable variation (Moore et al., 1997; Bijma, 2011). For the genetic parameters estimation of survival days in non-beak-trimmed laying hens, the inclusion of associative effects in the model gave higher heritable variation than a traditional linear animal model (Muir, 2005; Ellen et al., 2008; Peeters et al., 2012). Consequences of feather pecking behavior in beak-trimmed and non-beak-trimmed laying hens are different, and therefore FCS can be a different trait in both types of birds. In the first part of the present study, we estimated genetic parameters for FCS in beak-trimmed and non-beak-trimmed laying hens, respectively, with 2 variance components models: a traditional linear model and a linear animal model combining direct and associative effects.

Besides modeling the FCS using the variance component approach, a trait-based approach can help to understand the biological mechanism of social interactions (Moore et al., 1997; Wolf et al., 1998). Knowledge of the traits that underlie the interacting phenotype is, however, needed for the trait-based approach (Kirkpatrick and Lande, 1989). Brain serotonergic levels (Chaouloff, 2000) and some neurotransmitters such as dopamine and hormones (Cheng et al., 2003) were correlated with feather pecking. El-Lethey et al. (2003) found that feather pecking was related with corticosterone levels, which also reduced immune responses. Recently, the effects of immunity on feather pecking behavior were suggested by several studies. Buitenhuis et al. (2004) reported a significant genetic and phenotypic correlation between feather pecking and primary antibody response to keyhole limpet hemocyanin (KLH). Parmentier et al. (2009) found that when chickens were challenged intratracheally and repeatedly at a young age with different doses of the endotoxin lipopolysaccharide and the protein human serum albumin, they showed different levels of feather damage at an older age. In addition, some SNP or QTL that were associated with FCS (Biscarini et al., 2010b) were also significantly associated with levels of natural antibody (NAb) isotypes IgM and IgG binding KLH (Sun et al., 2013a). These variations were mostly reported to be associated with the associative genetic effects on FCS, and few with the direct genetic effect (the genetic effect of the individual’s own genotype on its FCS). This suggests that the NAb isotypes titers may not only be related with the susceptibility to be pecked at, but also particularly with the propensity to perform feather pecking. In our previous study, NAb isotype titers were reported to be associated with survival of laying hens (Sun et al., 2011). As mentioned previously, severe feather pecking and cannibalism may also induce mortality. It is possible that the NAb isotype is associated with survival by regulating the feather pecking behavior. Therefore, in the second part of the present study, we model an individual’s FCS as a function of the NAb isotype titers of the individual and those of its cage mates to investigate the possible relationship between feather pecking behavior and levels of NAb isotype IgM and IgG in beak-trimmed and non-beak-trimmed laying hens.

**MATERIALS AND METHODS**

**Study Population**

Female crossbred offspring of 2 commercial purebred White Leghorn layer lines (male W1 and female WB) were provided by Institut de Sélection Animale (ISA) B.V., the layer breeding division of Hendrix Genetics (Boxmeer, the Netherlands). The 2 purebred lines and the crossbred show high mortality with intact beaks (Ellen et al., 2008; Peeters et al., 2012). The W1 and WB lines were verified as “high and low natural antibody isotype” lines, respectively (Star et al., 2007; Sun et al., 2011). Uitdehaag et al. (2008) showed that birds of the W1 lines have more severe feather pecking behavior and feather damage than birds of the WB line (Uitdehaag et al., 2008), whereas Ellen et al. (2008) found a higher mortality in the WB line. Fifty sires of line W1 were randomly chosen and mated with 908 dams of line WB, where dams were nested within sires. Sires and dams were housed individually in cages. Each sire was mated to approximately 18 dams. The eggs from the same dam were collected and incubated in a separate cell of the incubation plate, discriminated from the eggs of the other dams. After hatch, the female chicks from the same cell (dam) were assigned unique barcode wing bands, allowing for identification of individuals. Each dam contributed on average 3 female offspring, resulting in 2,724 offspring.

**Housing and Management**

All chickens were hatched, sexed, and wing-banded in the right wing for individual identification at the same time. Only female chicks were kept for this study. Offspring of 25 sires were beak trimmed, whereas offspring of another 25 sires were kept with intact beaks. Chicks were trimmed manually at 1 d old using a hot blade to remove and cauterize the tip of the beak. Chicks were allocated to rearing cages randomly with respect to beak trimming, 60 individuals per cage. From 5 wk
of age onward, the hens were housed with 20 individuals per cage. The cage number for each hen was not recorded. At 17 wk of age, all hens were transported to a high-light intensity laying house with conventional 5-bird cages (44 cm height \times 40 cm depth \times 55 cm width). Each pair of back-to-back cages shared 2 drinking nipples. A feeding trough was in front of the cages, with a length of 55 cm per cage. After placing the birds in the cages, hen were wing-banded in the left wing as well, to avoid loss of data. There were 6 rows (3 double rows) of cages in the laying house, with corridors in between to allow employees to have access to the cages (Figure 1). The outer 2 double rows consisted of 3 levels (top; middle, closest to the light; and bottom). The middle double rows consisted of 4 levels (super top; top; middle, closest to the light; and bottom). Hens were only placed in the top and middle levels. Five hens that were a mix of half sibs and full sibs were allocated to the same cage. The hens in each pair of back-to-back cages had received the same treatment regarding beak trimming and could contact their back neighbors through the wire mesh. Contact with hens in adjacent cages was impossible because of the closed wall in between. Water and standard commercial layer diet was provided ad libitum. Rearing started with a 9L:15D light scheme and increased 1 h per week until 16L:8D was reached when the hens were 26 wk of age. The hens received routine vaccinations for Marek’s disease (d 1), infectious bronchitis (d 1, wk 2, 10, 12, and 15), Newcastle disease (wk 2, 6, 12, and 15), infectious bursal disease (wk 3 and 15), turkey rhinotracheitis (wk 8 and 18), fowl pox (wk 15), chicken anemia virus (wk 15), and avian encephalomyelitis (wk 15).

Study Design

All hens were observed daily from 17 until 83 wk of age for survival. Hens that died were removed from the cages without replacement. Wing-band number and date of death were recorded. Cause of death was not determined. For each hen, information was collected on survival and survival days. Survival was defined as dead (0) or alive (1) at the end of the study. From these data, survival rate was calculated as the percentage of laying hens still alive at the end of the study. Survival days were defined as the number of days from the start of the observation until either death or termination of the present study, with a maximum of 457 d. At 24 wk of age, 2-mL blood samples of all birds were taken from the wing vein using the plastic vacuum blood collection tubes containing sodium heparin. The bleeding procedure for each bird was 15 to 30 s. The plasma samples were collected after the centrifugation of the blood and used to measure NAb isotype IgM and IgG titers binding KLH. At 53 wk of age, the individual feather condition of neck, back, rump, and belly areas was scored.

NAb Isotype IgM and IgG Titers Binding KLH

There are no antigens in the environment of laying hens that show immunological cross-reactivity with KLH based on the literature and pilot experiments. Thus, prior exposure or sensitization to this protein is considered unlikely. According to definitions, NAb are immunoglobulins present in animals in the absence of earlier (deliberate) immunization, vaccination, or infection (Avrameas, 1991). Therefore, the antibodies detected in the plasma binding KLH were regarded as NAb. Indirect ELISA as described earlier (Sun et al., 2011) was performed to measure levels of plasma NAb isotypes IgM and IgG binding KLH at 24 wk of age by the same person on different days. The NAb isotypes titers were only measured once for each sample. However, each plate was run with 2 duplicated positive plasma samples of 8 step-wise dilutions. The interassay CV and intraassay CV was calculated as 5.1 and 4.2%, respectively.

FCS

In the present study, feather damage of the laying hens was assessed by evaluating the individual’s feather condition at 53 wk of age of 4 body areas: neck, back, rump, and belly, which are the frequent targets of feather pecking. The scoring was performed by 4 persons, following the classification of Bilčik and Keeling (1999), as modified by Uitdehaag et al. (2008). In a pilot study, the average correlation between persons performing the scoring was estimated 0.82 for neck, back, and rump, and 0.72 for belly by E. D. Ellen (Wageningen University, Wageningen, the Netherlands, personal communication). There were 6 classes for FCS, ranging from 0 (intact feathers) to 5 (almost all feathers missing), with higher score indicating more damage. The sum of scores of 4 areas was used as an overall parameter of feather condition. Sum = individual neck score + individual back score + individual rump score + individual belly score (ranging from 0 to 20). Birds that died before 53 wk of age (252 out of 1,169 non-beak-trimmed,

Figure 1. The division of the stable, showing the light arrangement and numbers of the cages per row and level (Sun et al., 2013a).
and 31 out of 1,555 beak-trimmed laying hens) did not receive their FCS.

**Data Analysis**

Descriptive statistical analyses were performed using SAS 9.1.2 (SAS Institute Inc., Cary, NC). Effects were considered significant at the level of $P < 0.05$. A GLM was used to study the differences in FCS between beak-trimmed and non-beak-trimmed birds, and the differences in IgM and IgG titers binding KLH between both groups. The correlations between FCS of 4 different body areas were estimated by Pearson product-moment correlation. The average IgM and IgG titer of cage mates of every individual laying hen was also calculated.

**Variance Components Estimation of FCS.** A traditional linear animal model and a direct-associative effect model were used to estimate the variance component of FCS. The FCS of beak-trimmed and non-beak-trimmed laying hens were analyzed separately using the GLM procedure of the SAS program (SAS Institute Inc.). To correct for systematic nongenetic differences among observations, factors with $P < 0.10$ from the GLM were included as fixed effects in the model for estimating genetic parameters. Fixed effects for FCS in beak-trimmed laying hens were (1) row of cages, (2) level of the cages where the laying hens were located to account for infrastructural effects such as light intensity difference (Kjaer and Vestergaard, 1999), and (3) person who scored the feather condition. In non-beak-trimmed laying hens, only the person who scored the feather condition was included as a fixed effect.

To compare genetic parameters for FCS between beak-trimmed and non-beak-trimmed laying hens, a bivariate model was used by treating FCS as different traits for both populations.

(1) Traditional linear animal model: Genetic parameters of FCS were first estimated using a traditional linear animal model as implemented in the ASReml software package (Gilmour et al., 2006):

\[
\begin{bmatrix}
  y_1 \\
  y_2 \\
\end{bmatrix} = \begin{bmatrix}
  X_1 & 0 & b_1 \\
  0 & X_2 & b_2 \\
\end{bmatrix} \begin{bmatrix}
  Z_{1,D} & 0 & a_{1,D} \\
  0 & Z_{2,D} & a_{2,D} \\
\end{bmatrix} + \begin{bmatrix}
  V_1 & 0 & \text{cage}_1 \\
  0 & V_2 & \text{cage}_2 \\
\end{bmatrix} + \begin{bmatrix}
  a_{1,S} \\
  a_{2,S} \\
\end{bmatrix} + e,
\]

where subscript 1 indicates beak-trimmed laying hens and subscript 2 indicates non-beak-trimmed laying hens; $y$ is a vector of individual sum of FCS at 53 wk of age; $b$ is a vector of fixed effects, with incidence matrix $X$ linking FCS to fixed effects; $a$ is a vector of usual breeding values, with incidence matrix $Z_D$ linking FCS to the breeding value; $\text{cage}$ is a vector of independent random cage effects; $V$ is an incidence matrix linking observations to random cage effects; and $e$ is vector of random residuals. The direct genetic (co)variance structure was

\[
\text{var} \begin{bmatrix}
  a_{1,D} \\
  a_{2,D} \\
\end{bmatrix} = \begin{bmatrix}
  \sigma^2_{A_{1,D}} & \sigma_{A_{12,D}} \\
  \sigma_{A_{12,D}} & \sigma^2_{A_{2,D}} \\
\end{bmatrix} \otimes A,
\]

where $\sigma^2_{A_{1,D}}$ is the direct genetic variance for beak-trimmed laying hens, $\sigma^2_{A_{2,D}}$ is the direct genetic variance for non-beak-trimmed laying hens, $\sigma_{A_{12,D}}$ is the direct genetic covariance between beak-trimmed and non-beak-trimmed laying hens. The $\otimes$ indicates the Kronecker product of matrices. The $A$ is the additive genetic relationship matrix generated from a 5-generation pedigree. Phenotype variance was calculated as $\sigma^2_P = \sigma^2_A + \sigma^2_c + \sigma^2_e$. Heritabilities were calculated as $h^2 = \sigma^2_A / \sigma^2_P$.

(2) Direct-associative effect model: To estimate genetic parameters for both direct and associative effects, the following model extended from Muir (2005) was used:

\[
\begin{bmatrix}
  y_1 \\
  y_2 \\
\end{bmatrix} = \begin{bmatrix}
  X_1 & 0 & b_1 \\
  0 & X_2 & b_2 \\
\end{bmatrix} \begin{bmatrix}
  Z_{1,D} & 0 & a_{1,D} \\
  0 & Z_{2,D} & a_{2,D} \\
\end{bmatrix} + \begin{bmatrix}
  V_1 & 0 & \text{cage}_1 \\
  0 & V_2 & \text{cage}_2 \\
\end{bmatrix} + \begin{bmatrix}
  a_{1,S} \\
  a_{2,S} \\
\end{bmatrix} + \begin{bmatrix}
  e_1 \\
  e_2 \\
\end{bmatrix},
\]

where the vectors and incidence matrices correspond to those in the traditional linear animal model; $Z_S$ is an incidence matrix linking an individual’s FCS to its cage mates’ breeding value vector; and $a_S$ is a vector of social breeding values for all cage mates. The direct-associative genetic (co)variance structure was

\[
\text{var} \begin{bmatrix}
  a_{1,D} \\
  a_{2,D} \\
  a_{1,S} \\
  a_{2,S} \\
\end{bmatrix} = \begin{bmatrix}
  \sigma^2_{A_{1,D}} & \sigma_{A_{12,D}} & \sigma_{A_{1,D_S}} & \sigma_{A_{1,D_2_S}} \\
  \sigma_{A_{12,D}} & \sigma^2_{A_{2,D}} & \sigma_{A_{2,D_1_S}} & \sigma_{A_{2,D_2_S}} \\
  \sigma_{A_{1,D_S}} & \sigma_{A_{2,D_1_S}} & \sigma^2_{A_{1,S}} & \sigma_{A_{1_2_S}} \\
  \sigma_{A_{1,D_2_S}} & \sigma_{A_{2,D_2_S}} & \sigma_{A_{1_2_S}} & \sigma^2_{A_{2,S}} \\
\end{bmatrix} \otimes A,
\]

where $\sigma^2_{A_{1,D}}$, $\sigma^2_{A_{2,D}}$, and $\sigma_{A_{12,D}}$ are the same as in the traditional linear animal model. The $\sigma^2_{A_{1,S}}$ is the associative genetic variance for beak-trimmed laying hens; $\sigma^2_{A_{2,S}}$ is the associative genetic variance for non-beak-trimmed laying hens; $\sigma_{A_{1_2_S}}$ is the associative genetic covariance between beak-trimmed and non-beak-trimmed laying hens; $\sigma_{A_{1,D_2_S}}$ is the direct-associative ge-
netic covariance in beak-trimmed laying hens; \( \sigma_{A2_{DS}} \) is the direct-associative genetic covariance in non-beak-trimmed laying hens; \( \sigma_{A1_{D2_{SS}}} \) is the genetic covariance between the direct effect of beak-trimmed and the associative effect of non-beak-trimmed laying hens; \( \sigma_{A2_{D1_S}} \) is the genetic covariance between the associative effect of non-beak-trimmed laying hens and the direct effect of beak-trimmed laying hens. The total heritable variance for response to selection was \( \sigma^2_TBV = \sigma^2_{P0} + 2(n - 1)\sigma^2_{D0} + (n - 1)^2\sigma^2_A \) (Bijma et al., 2007). The \( \sigma^2_P \) is the phenotypic variance, \( \sigma^2_P = \sigma^2_{D0} + (n - 1)\sigma^2_A + \sigma^2_{cage} + \sigma^2_e \). The \( n \) is the number of laying hens kept in the same cage, and \( n = 5 \) in the present study. The \( T^2 \) expresses the total heritable variance relative to the phenotypic variance: \( T^2 = \sigma^2_TBV/\sigma^2_P \).

Likelihood ratio tests were used to test the significance of the random associative effect in a univariate model in beak-trimmed and non-beak-trimmed laying hens, respectively.

**Trait-Based Approach.** To investigate whether NAb can explain variation in FCS among individuals, a trait-based analysis for FCS was conducted by fitting a linear mixed model, following Moore et al. (1997). The fixed effects were the same as those detected in the variance components approach. Therefore, the model for beak-trimmed laying hens was

\[
y_{ijkl} = \mu + \text{row}_i + \text{level}_j + \text{person}_k + bx + \text{cage}_l, \quad [3]
\]

where \( y_{ijkl} \) is individual sum FCS at 53 wk of age; \( \mu \) is the overall mean; \( \text{row}_i \) is the fixed effect of row of the cage \( (i = 1, 2, 3, 4, 5, 6) \); \( \text{level}_j \) is the fixed effect of level of the cage \( (j = 1, 2) \); \( \text{person}_k \) is the effect of the \( k \)th \( (k = 1, 2, 3, 4) \) person who scored the feather condition; \( \text{cage}_l \) is the random effect of cage \( l \); \( x \) is the fixed effect of individual IgG or individual IgM or average IgM titers of the cage mates, \( b \) is the estimated parameter for the covariable \( x \). The model for non-beak-trimmed laying hens was

\[
y_{ijkl} = \mu + \text{row}_i + \text{person}_k + bx + \text{cage}_l, \quad [4]
\]

where all the terms are the same as those specified in model [3]. The sum FCS for beak-trimmed and non-beak-trimmed laying hens was tested for normality before model [3] and [4] were run with a MIXED procedure of the SAS program (SAS Institute Inc.).

**RESULTS**

**FCS of Beak-Trimmed and Non-Beak-Trimmed Crossbred Laying Hens**

The average individual FCS of the 4 body areas (neck, back, rump, and belly) at 53 wk of age for beak-trimmed and non-beak-trimmed laying hens is shown in Table 1. In both populations, the score for belly was the lowest among the 4 areas, indicating that the belly area was less pecked at. In contrast, the neck and rump areas were with the highest scores, indicating more damage in these areas. The CV ranged from 23 to 48% for non-beak-trimmed, and ranged from 31 to 70% for beak-trimmed laying hens. This indicated considerable variations for the FCS of both populations. The GLM analysis showed that the FCS for different body areas and the sum of the FCS in beak-trimmed laying hens was significantly lower than that in non-beak-trimmed laying hens, indicating that non-beak-trimmed laying hens had more feather damage (Table 1).

Both in beak-trimmed and non-beak-trimmed laying hens, as expected, the correlation coefficients between the scores of different body areas were positive (Table 2). The correlations between the areas that were close

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Neck***</th>
<th>Back***</th>
<th>Rump***</th>
<th>Belly***</th>
<th>Sum***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-beak-trimmed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td>813</td>
<td>3.78 ± 0.91</td>
<td>3.54 ± 1.04</td>
<td>3.76 ± 1.03</td>
<td>2.77 ± 1.31</td>
<td>13.77 ± 3.48</td>
</tr>
<tr>
<td>Nonsurvival</td>
<td>104</td>
<td>3.69 ± 0.85</td>
<td>3.70 ± 1.16</td>
<td>3.92 ± 1.12</td>
<td>2.92 ± 1.44</td>
<td>14.32 ± 4.10</td>
</tr>
<tr>
<td>Total</td>
<td>917</td>
<td>3.70 ± 0.85</td>
<td>3.56 ± 1.05</td>
<td>3.78 ± 1.04</td>
<td>2.79 ± 1.33</td>
<td>13.83 ± 3.55</td>
</tr>
<tr>
<td>CV (%)</td>
<td>23</td>
<td>29</td>
<td>28</td>
<td>48</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

| Beak-trimmed  |    |         |         |         |          |        |
| Survival      | 1,447| 2.97 ± 0.91| 2.64 ± 1.20| 2.84 ± 1.35| 1.79 ± 1.25| 10.23 ± 3.80 |
| Nonsurvival   | 77  | 2.94 ± 1.13| 2.78 ± 1.17| 3.13 ± 1.28| 1.86 ± 1.31| 10.70 ± 4.92 |
| Total         | 1,524| 2.96 ± 0.92| 2.65 ± 1.20| 2.85 ± 1.35| 1.79 ± 1.25| 10.25 ± 3.81 |
| CV (%)        | 31 | 45      | 47      | 70      | 37       |

1There are 6 classes for FCS, ranging from 0 (intact feathers) to 5 (almost all feathers missing), with higher score indicating more damage.

2Sum FCS = individual neck score + individual back score + individual rump score + individual belly score (ranging from 0 to 20).

3Survival indicated the birds survived until the end of the observation period (83 wk of age).

4Non-survival indicated the bird died between 53 wk of age (when feather condition scoring was performed) and the end of the observation period (83 wk of age) because the birds that died before were not scored.

5CV (%) = (SD/mean) × 100%.

***p < 0.0001, which indicates that FCS for beak-trimmed and non-beak-trimmed laying hens is significantly different.
to each other, such as back and rump, back and neck, were higher than those between the areas that were further away from each other, such as neck and belly. The sum of scores of these 4 body areas was used as the aggregated FCS.

Genetic Parameters of FCS

The estimated genetic parameters for FCS in beak-trimmed and non-beak-trimmed laying hens, using either a bivariate traditional linear animal model or a bivariate direct-associative effect model, are given in Table 3. Using a traditional linear animal model, similar and significant additive genetic variance (σ^2_A) were found in both populations. The proportion of phenotypic variance explained by direct genetic variance was denoted as h^2. The estimated h^2 of FCS was slightly higher in non-beak-trimmed laying hens (0.20, SE = 0.06) than in beak-trimmed laying hens (0.17, SE = 0.05).

Using a direct-associative effect model, direct (σ^2_Ad) and associative genetic variance (σ^2_As) for FCS were estimated in beak-trimmed and non-beak-trimmed laying hens, respectively. Total heritable variance relative to the phenotypic variance (T^2), and genetic correlations between the direct and associative effect (rDs) for FCS were also calculated based on those estimations.

Likelihood ratio tests were used to statistically compare the traditional linear animal model and the direct-associative effect model. In the beak-trimmed laying hens, final log-likelihoods as reported from traditional linear animal model and from the direct-associative effect model were −1,334.52 and −1,332.12, respectively. The test statistics were χ^2_{2df} = 2 × [−1,332.12 − (−1,334.52)] = 4.8, which corresponds to P = 0.09. In the non-beak-trimmed laying hens, final log-likelihoods as reported from the traditional linear model and direct-associative effect model were −2,258.99 and −2,261.48, respectively. The test statistics were χ^2_{2df} = 2 × [−2,258.99 − (−2,261.48)] = 4.98, which corresponds to P = 0.08. Therefore, using the common criterion of P < 0.05, the associative effect for FCS was not a significant random effect in both beak-trimmed or non-beak-trimmed laying hens. This observation agrees with the SE of the estimated associative genetic variance and the direct-associative genetic covariance, which were not significantly different from zero (Table 3).

NAb Isotype Titers in Beak-Trimmed and Non-Beak-Trimmed Laying Hens

Table 4 shows the average NAb isotypes IgM and IgG titers binding KLH in beak-trimmed and non-beak-trimmed laying hens. For IgG binding KLH, the titers in non-beak-trimmed hens were significantly higher than in beak-trimmed hens. Furthermore, in beak-trimmed laying hens, IgG titers in the nonsurviving birds were significantly higher than in the surviving birds. For IgM binding KLH, there was no significant difference between beak-trimmed and non-beak-trimmed laying hens. There was no significant difference for IgM between the surviving and nonsurviving birds within beak-trimmed nor non-beak-trimmed group. Overall, the IgM and IgG titers were higher in laying hens with higher FCS, although the difference was not significant.

Direct and Associative Effect of NAb Isotypes on FCS

In both populations, the direct effects of IgM and IgG on FCS were not significantly different from zero, which indicated that an individual’s FCS was not significantly affect by its own isotype titers (Table 5). In non-beak-trimmed laying hens, the estimated parameters for average titers of IgM and IgG titers of the focal individual’s cage mates were not significantly different from zero. In beak-trimmed laying hens, the average IgG titers of cage mates was a significant factor for the individual’s FCS (P = 0.03). The estimated parameter for average IgG was 0.36 (SE = 0.16), which indicated that when its cage mates had higher IgG titers, the individual may have worse feather condition. Average IgM titers of cage mates were not significant (P = 0.83).

DISCUSSION

In the present study, we compared FCS in beak-trimmed and non-beak-trimmed crossbred laying hens.
Table 3. Estimated parameters with SE from traditional and direct-associative animal model for beak-trimmed and non-beak-trimmed laying hens

<table>
<thead>
<tr>
<th>Item</th>
<th>Traditional linear animal model</th>
<th>Direct-associative effect model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-beak-trimmed</td>
<td>Beak-trimmed</td>
</tr>
<tr>
<td></td>
<td>Beak-trimmed</td>
<td>Non-beak-trimmed</td>
</tr>
<tr>
<td>Log-likelihood(^1)</td>
<td>—</td>
<td>2.40</td>
</tr>
<tr>
<td>(\sigma^2_{\text{cage}})</td>
<td>6.95 ± 0.78</td>
<td>7.18 ± 0.68</td>
</tr>
<tr>
<td>(\sigma^2_{\text{DP}})</td>
<td>2.31 ± 0.79</td>
<td>2.16 ± 0.60</td>
</tr>
<tr>
<td>(\sigma^2_{\text{P}})</td>
<td>10.76 ± 1.08</td>
<td>12.46 ± 0.71</td>
</tr>
<tr>
<td>(\sigma^2_{\text{A}_{12}})</td>
<td>2.40 ± 0.52</td>
<td>3.11 ± 0.39</td>
</tr>
<tr>
<td>(\sigma^2_{\text{A}_{12}})</td>
<td>10.76 ± 1.08</td>
<td>12.46 ± 0.71</td>
</tr>
<tr>
<td>(\sigma^2_{\text{TBV}})</td>
<td>4.84 ± 2.73</td>
<td>5.03 ± 2.15</td>
</tr>
<tr>
<td>(\sigma_{\text{DS}})</td>
<td>0.20 ± 0.06</td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td>(\sigma_{\text{DS}})</td>
<td>0.42 ± 0.24</td>
<td>0.41 ± 0.17</td>
</tr>
<tr>
<td>(r_{\text{DS}})</td>
<td>0.24 ± 0.89</td>
<td>0.63 ± 1.06</td>
</tr>
</tbody>
</table>

\(^1\)Log-likelihoods for the direct-associative model are expressed as a deviation from those of the traditional linear animal model. In the traditional linear animal model, \(\sigma^2_{\text{DP}}\) is the direct additive genetic variance, \(\sigma^2_{\text{P}}\) is the phenotypic variance, \(\sigma^2_{\text{A}_{12}}\) is the associative genetic variance, and \(\sigma^2_{\text{DRS}}\) is the direct-associative genetic covariance. In the traditional linear animal model, the total heritable variance is \(\sigma^2_{\text{DV}} = \sigma^2_{\text{DP}} + 2(n-1)\sigma_{\text{AR}} + (n-1)^2\sigma^2_{\text{A}_{12}}\) (Bijma et al., 2007). The \(\sigma^2_{\text{P}}\) is the phenotypic variance, \(\sigma^2_{\text{A}_{12}} = \sigma^2_{\text{DP}} + (n-1)\sigma_{\text{AR}} + \sigma^2_{\text{DRS}} + \sigma^2_{\text{A}_{12}}\); \(n\) is the number of laying hens kept in the same cage, \(n = 5\) in the present study. The \(T^2\) is the total heritable variance relative to the phenotypic variance: \(T^2 = \sigma^2_{\text{DV}} / \sigma^2_{\text{P}}\). The \(r_D\) is the genetic correlation between the direct and associative effect. The \(\sigma_{\text{A}_{12}}\) is the direct genetic covariance between beak-trimmed and non-beak-trimmed laying hens, and \(\sigma_{\text{A}_{12}}\) is the associative genetic covariance between beak-trimmed and non-beak-trimmed laying hens. The \(\sigma_{\text{A}_{12}}\) is the genetic covariance between the direct effect of beak-trimmed and the associative effect of non-beak-trimmed laying hens. The \(r_P\) is the genetic correlation between direct effect of beak-trimmed and non-beak-trimmed laying hens, \(r_P = \sigma_{\text{A}_{12}} / \sqrt{\sigma^2_{\text{A}_{12}} \sigma^2_{\text{A}_{12}}}\). The \(r_T\) is the genetic correlation between total heritable variance beak-trimmed and non-beak-trimmed laying hens, \(r_T = \sigma_{\text{A}_{12}} / \sqrt{\sigma^2_{\text{A}_{12}} + 2(n-1)\sigma_{\text{AR}} + (n-1)^2\sigma^2_{\text{A}_{12}}}\) (Peeters et al., 2012).

Variance component estimation indicated that there was relevant heritable variation for FCS in both populations using a traditional linear animal model. Using a linear animal model combining the direct and associative effects, there were no significant social genetic effects. A possible link between the NAb isotype titers and feather pecking behavior was also investigated.

**FCS in Beak-Trimmed and Non-Beak-Trimmed Laying Hens**

Beak trimming is the removal of the tip of the beak of a bird. This treatment is performed as part of an overall strategy to reduce feather pecking and cannibalism, especially in laying hens. In the present study, the feather condition in beak-trimmed birds was significantly better than that in non-beak-trimmed laying hens (Table 1). Thus, as expected, beak-trimming reduces feather damage. The variation of FCS in beak-trimmed laying hens was, however, larger than that in non-beak-trimmed laying hens. This indicated that beak-trimmed laying hens still have feather damage problems due to different extent of feather pecking behavior. Beak trimming only reduces the mortality instead of preventing the feather pecking propensity.

Among the 4 body areas, the belly received the least feather damage, whereas the neck and rump received the most. Similar patterns were also found in non-beak-trimmed purebred laying hens (Biscarini et al., 2010b). Bright et al. (2006) also found that the rump area of free-ranged laying hens was most damaged. This could be because the back and rump are the areas most exposed to other cage mates. However, in laying hens raised in floor pens, Bilčik and Keeling (1999) observed that the belly region became demuned first. The difference may be caused by the difference in housing systems. Both in beak-trimmed and non-beak-trimmed laying hens, the Pearson correlations between the scores of different body areas were positive and high, especially between
the areas that were close to each other, back and rump, for example (Table 2). Feather condition scoring as a behavioral measurement at the individual level is labor-intensive and time-consuming work. Given the extent of damage and correlations between the FCS for different body areas, it may be efficient and sufficient to only score one representative body area, such as the back or rump.

The survival of beak-trimmed laying hens was significantly higher than non-beak-trimmed laying hens (Sun et al., 2013b). This suggests that severe feather damage maybe a causative factor for the mortality afterward. However, neither in the beak-trimmed nor in the non-beak-trimmed laying hens, a significant difference was detected for the FCS between nonsurviving and surviving birds. This might rest not only on the limited number of nonsurviving hens after 53 wk of age (when the feather scoring was performed), but also on the fact that the laying hens that died before 53 wk did not receive a FCS [in non-beak-trimmed laying hens, a high death rate was observed from 18 to 26 wk of age, and around 35 wk of age (Sun et al., 2013b)]. Feather damage due to feather pecking cumulates over time. Feather pecking has been observed as early as 1 d after hatching (Roden and Wechsler, 1998) with sudden increases of cannibalism frequencies in the brooding period of 4 to 11 wk of age (Hughes and Duncan, 1972), and around the onset of egg laying at 20 wk of age (McKeegan and Savory, 1998). It could be speculated that the birds that died before the scoring had poor feather condition. Peeters et al. (2012) detected a substantial associative effect for survival days in non-beak-trimmed laying hens. This together with our analysis on FCS supports the argument that severe feather pecking contributed to the mortality in non-beak-trimmed laying hens.

### Direct and Associative Effect for FCS

Similar to other interaction phenotypes, such as survival days in laying hens (Ellen et al., 2008; Peeters et al., 2012), FCS of an individual hen is affected by both the individual and its conspecifics when kept in groups. Ignoring the social interaction among individuals that generate additional heritable variation may result in biased genetic parameter estimation. In the present study, we estimated the genetic parameters for FCS in beak-trimmed and non-beak-trimmed laying hens, using the traditional linear animal model, and direct-associative effect model, respectively. Using the traditional linear model, the heritability ($h^2$) for FCS in non-beak-

### Table 4. Number (n), and average titers (±SD) of natural antibody isotypes IgM and IgG binding keyhole limpet hemocyanin of beak-trimmed and non-beak-trimmed laying hens

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>IgM titers</th>
<th>IgG titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-beak-trimmed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,169</td>
<td>8.17 ± 1.07</td>
<td>6.71 ± 1.18</td>
</tr>
<tr>
<td>Survival¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surviving</td>
<td>813</td>
<td>8.17 ± 1.06</td>
<td>6.69 ± 1.16</td>
</tr>
<tr>
<td>Nonsurviving</td>
<td>356</td>
<td>8.18 ± 1.10</td>
<td>6.77 ± 1.24</td>
</tr>
<tr>
<td>Sum FCS²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>6</td>
<td>8.38 ± 1.30</td>
<td>7.92 ± 1.60</td>
</tr>
<tr>
<td>5–8</td>
<td>60</td>
<td>7.93 ± 0.99</td>
<td>6.46 ± 1.13</td>
</tr>
<tr>
<td>9–12</td>
<td>251</td>
<td>8.06 ± 1.10</td>
<td>6.53 ± 1.19</td>
</tr>
<tr>
<td>13–16</td>
<td>377</td>
<td>8.18 ± 1.04</td>
<td>6.81 ± 1.16</td>
</tr>
<tr>
<td>17–20</td>
<td>223</td>
<td>8.40 ± 1.05</td>
<td>6.76 ± 1.07</td>
</tr>
<tr>
<td>NA</td>
<td>272</td>
<td>8.18 ± 1.10</td>
<td>6.77 ± 1.27</td>
</tr>
<tr>
<td>Beak-trimmed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,555</td>
<td>8.24 ± 1.05</td>
<td>6.61 ± 1.22</td>
</tr>
<tr>
<td>Survival¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surviving</td>
<td>1,447</td>
<td>8.24 ± 1.05</td>
<td>6.59 ± 1.22</td>
</tr>
<tr>
<td>Nonsurviving</td>
<td>108</td>
<td>8.30 ± 1.03</td>
<td>6.86 ± 1.16</td>
</tr>
<tr>
<td>Sum FCS²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>89</td>
<td>8.30 ± 1.06</td>
<td>6.35 ± 1.42</td>
</tr>
<tr>
<td>5–8</td>
<td>436</td>
<td>8.23 ± 0.97</td>
<td>6.58 ± 1.24</td>
</tr>
<tr>
<td>9–12</td>
<td>594</td>
<td>8.20 ± 1.09</td>
<td>6.62 ± 1.19</td>
</tr>
<tr>
<td>13–16</td>
<td>308</td>
<td>8.30 ± 1.10</td>
<td>6.64 ± 1.22</td>
</tr>
<tr>
<td>17–20</td>
<td>97</td>
<td>8.32 ± 0.98</td>
<td>6.80 ± 1.19</td>
</tr>
<tr>
<td>NA</td>
<td>31</td>
<td>8.19 ± 1.00</td>
<td>6.59 ± 1.13</td>
</tr>
</tbody>
</table>

¹Surviving indicated the birds survived until the end of the observation period (83 wk of age), and nonsurviving indicated the birds died between 24 wk of age and the end of the observation period.

²Sum FCS = the sum of feather condition score of 4 body areas.

### Table 5. Parameter estimates with SE and the significance level (P-value) of the fixed effects of individual IgG titers, individual IgM titers, average IgG titers of cage mates, and average IgM titers cage mates, on individual feather condition score

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>Non-beak-trimmed</th>
<th>Beak-trimmed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate (SE)</td>
<td>P-value</td>
</tr>
<tr>
<td>IgG</td>
<td>0.09 (0.07)</td>
<td>0.20</td>
</tr>
<tr>
<td>IgM</td>
<td>0.09 (0.10)</td>
<td>0.38</td>
</tr>
<tr>
<td>IgG of cage mates</td>
<td>−0.15 (0.19)</td>
<td>0.43</td>
</tr>
<tr>
<td>IgM of cage mates</td>
<td>0.05 (0.19)</td>
<td>0.80</td>
</tr>
</tbody>
</table>
trimmed laying hens was estimated to be 0.20 (SE = 0.06), and in beak-trimmed laying hens was estimated to be 0.17 (SE = 0.05). Using a direct-associative effect model, the estimated associative genetic variance was substantial, causing the total heritable variation ($\sigma^2$) to be 2-fold greater than ordinary heritability (Table 3). Nevertheless, the estimated associative genetic variance was not significantly different from zero. This also agrees with the results from the likelihood ratio test for the significance of random associative genetic effect ($P = 0.09$ in beak-trimmed birds, and $P = 0.08$ in non-beak-trimmed birds). Hence, our results suggest that associative genetic effects may be important in those populations, but lacked the statistical power to accurately estimate those effects, probably due to a limited number of records (Table 1). T. Brinker (Wageningen University, Wageningen, the Netherlands, personal communication) showed that social effects had a substantial effect on the total heritable variation of FCS in purebred non-beak-trimmed laying hens ($n = 6,276$ and 6,916 for 2 purebred layer lines). Using large data sets, Ellen et al. (2008; $n = 3,988$ to 6,916 for different purebred layer lines) and Peeters et al. (2012; $n = 15,012$ for crossbred laying hens) also found large and strongly significant social effects on survival time in non-beak-trimmed laying hens. However, a suggestive heritable variation from the associative effect for FCS was still indicated from the comparison of analysis with 2 models in the present study. Ignoring the associative effect and the genetic correlation between direct and associative effect may induce underestimation of heritable variation for FCS and inappropriate breeding strategies for less feather damage in laying hens. Behavioral measurement on an individual level is a real effort. Hence, our results also illustrate the difficulty of collecting sufficient data to accurately estimate genetic parameters for behavioral traits. As we discussed before, to get proper estimation for genetic parameters for FCS, enlarging the numbers of birds involved maybe more valuable than scoring for multiple body areas, because of the high and positive correlation between the FCS of closely-located body areas. Further improvement of statistical power may come from optimizing the cage composition, as results in Bijma (2010) indicate that the SE of the estimated associative genetic variance is minimized when each cage consists of members of 2 families, each family contributing half.

Direct and Associative Effect of NAb Isotype IgM and IgG Titers Binding KLH on FCS

Several studies showed links between feather pecking and the immune system (Buitenhuys et al., 2004; Biscarini et al., 2010b). The NAb is claimed to be an important parameter of the immune system (Star et al., 2007). To investigate the possible relationship between receiving feather pecking and NAb isotype titers (direct effect of individual NAb on individual FCS), and the relationship between performing feather pecking and NAb isotype titers (associative effect of individual NAb on cage mates’ FCS), a mixed model with either the focal individual’s or cage mates’ average isotype titers as fixed effects was fitted for FCS of the individual in beak-trimmed (model 3) and non-beak-trimmed laying hens (model 4), respectively.

In both populations, the direct effects of IgM and IgG for individual FCS were not significant (Table 5). This indicated that the individual’s own NAb isotype titers may not affect its FCS, although Biscarini et al. (2010a) detected a link between receiving feather pecking and the individual’s innate and adaptive immune parameters.

A link between performing feather pecking and the immune parameters was detected (Buitenhuys et al., 2004; Parmentier et al., 2009; Biscarini et al., 2010b; Hughes and Buitenhuys, 2010; Brunberg et al., 2011). In non-beak-trimmed laying hens, a significant associative effect of NAb titers on feather damage was not detected. However, in beak-trimmed laying hens, the parameter estimate for average titers of IgG of the cage mates was 0.34 (SE = 0.16, $P = 0.03$). This indicated that when the cage mates have higher IgG titers, the individual may have higher suffer from more feather damage. However, multiple hypothesis testing will increase the false-positive results. As a statistical method used to correct for multiple comparisons, the false discovery rate adjusted $P$-value was 0.43. This suggested that the relationship between the individual FCS and the average IgG titers of its cage mate may need to be replicated for further confirmation. The relationships were also fitted for the 2 populations together, by adding beak treatment as an extra fixed effect. Still, the relationships were not significant (data not shown).

In our previous study about the relationship between NAb isotype titers and survival in laying hens, NAb isotypes especially IgM was shown to be a protective factor for health-related survival (Sun et al., 2011), and therefore a promising trait to be bred for higher survival of the population. The nonsignificant relationship between NAb isotype titers (both IgG and IgM) and individual FCS and nonsignificant relationship between isotype IgM titers of the cage mates’ and the individual FCS as shown in the present study suggest that the improvement of individual IgM levels does not result in more feather damage. However, the suggested relationship between individual FCS and the cage mates’ average IgG isotype titers needs further confirmation to determine the potential greater feather damage caused by the improvement of IgG levels.

Conclusions

To the best of our knowledge, this is the first time that FCS of the laying hens was modeled by a direct and associative effect model, and the first time that the
direct and associative effects of NAb isotype titers on individual FCS were investigated. The estimated associative genetic variance for FCS was substantial, but not significantly different from zero, probably due to the limited number of records. Results suggested, however, that including associative effects in the model, both in the beak-trimmed and non-beak-trimmed laying hens, is important to estimate genetic parameters for FCS. Although the effects of immunity on feather pecking behavior were suggested by several studies. The NAb isotypes titers did not show significant direct effects or an associative effect for individual FCS in the present study. However, further studies are needed to confirm the suggestive relationship between IgG titers and feather pecking.

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REFERENCES


