Genetic parameters of natural antibody isotypes and survival analysis in beak-trimmed and non-beak-trimmed crossbred laying hens

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ABSTRACT Natural antibodies (NAb) are important humoral components of innate immunity. As the first line of defense, NAb provide protection against infection and support adaptive immunity. An earlier study indicated that serum levels of NAb isotypes IgM and IgG at a young age were predictive for survival in non-beak-trimmed purebred laying hens during the laying period. In the present study, genetic parameters of NAb isotypes were estimated and relationships between survival and NAb isotypes levels in crossbred laying hens were investigated. In total, 1,555 beak-trimmed and 1,169 non-beak-trimmed crossbred laying hens were used. Genetic parameters of IgM and IgG titers binding keyhole limpet hemocyanin at 24 wk of age were estimated with a linear animal model. The heritabilities of NAb isotypes IgG and IgM were 0.21 (SE = 0.04) and 0.26 (SE = 0.04), respectively. The genetic correlation between IgG and IgM isotypes was 0.43 (SE = 0.11). These results indicated that NAb isotype titers were heritable traits in the crossbred laying hens. Both NAb isotypes can be selected for simultaneously because the detected positive genetic correlation (0.43, SE = 0.11) between them is positive. Both row and level of the cage were indicated to be associated environmental factors for NAb isotype titers. Different from an earlier study with purebred hens, survival analysis showed no significant associations of survival with NAb isotype titers in beak-trimmed or non-beak-trimmed crossbred hens. Non-health-related causes of mortality, especially in birds with intact beaks, overruled the anticipated relationships between NAb isotype titers and survival.

Key words: immunoglobulin M, immunoglobulin G, heritability, beak treatment, mortality

INTRODUCTION

Antibodies are defined as immunoglobulins produced by plasma cells after naïve B lymphocytes recognize antigen during infection or immunization with antigens including vaccines. Therefore, conventionally, antibodies are antigen-triggered and characterized by their antigen specificity. In contrast, natural antibodies (NAb) are defined as antibodies being present in healthy individuals without any previous antigen exposure (Avrameas, 1991; Haury et al., 1997).

Natural antibodies are found in every species tested so far, including humans (Guilbert et al., 1982), mice (Ochsenbein et al., 1999), fish (Sinyakov et al., 2002), and chickens (Neu et al., 1984). This conservation during evolution suggests that NAb are not simple non-specific byproducts of exogenous immunization but may play a vital physiological role (Cohen, 2007). Alike specific antibodies (SpAb), NAb can be either of the IgM, IgG (IgY), or IgA isotypes in birds. The broad reactivity of IgM, the principal NAb isotype, provides a preexisting defense that enables animals to rapidly recognize and protect against infection by pathogens that have not been encountered previously (Baumgarth et al., 2000). This protection fills the gap between the onset of infection and the emergence of the adaptive immune response (Baumgarth et al., 2000). For example, the antigen-induced antibodies to influenza virus can be detected in the serum at 5 d after infection (Baumgarth, 2000), whereas the preexisting NAb can prevent major viral replication and consequent virus-induced tissue destruction (Ochsenbein et al., 1999). The NAb facilitate specific immunity by activating the classical complement pathway (Ochsenbein et al., 1999), and capture and present antigens to T helper cells (Elluru et al., 2008). It was proposed that screening for NAb against pathogens may predict the strength of an antigen-induced immune response and could be used as a tool for vaccine development (Kohler et al., 2003). Natural antibodies were reported to react with self or foreign novel molecules (Quintana and Cohen, 2004).
Keyhole limpet hemocyanin (KLH) is large metalloprotein and used as an example of a naïve antigen for detecting NAb in laying hens (Parmentier et al., 2004; Star et al., 2007).

In an earlier study with non-beak-trimmed laying hens from multiple purebred White Leghorn lines and Rhode Island Red lines, serum levels of NAb, especially IgM binding KLH at 20 wk of age, were found to be significantly associated with and predictive for survival during the laying period [i.e., the higher the titers of NAb at a young age, the higher the probability of layers to survive (Sun et al., 2011)]. The odds ratios estimated for the isotypes IgM and IgG as factors in the survival analysis also indicated that distinguishing isotype titers is less predictive for the survival in White Leghorns than in the Rhode Island Red layers. A hypothesis for different prediction power of NAb isotype titers in the 2 breeds was that NAb isotype titers are associated with the health-related survival, but in non-beak-trimmed White Leghorns, part of death may be caused by non-health-related reasons, such as severe feather pecking and cannibalism. Therefore, in the present study, we investigated the relationships between NAb isotype titers binding KLH and survival in beak-trimmed and non-beak-trimmed crossbred laying hens. Genetic parameters for the NAb isotype titers were also estimated. Crossbred laying hens are more common as commercial products than purebred ones in the poultry industry. Identifying the predictive parameters for survival and better understanding of the genetic parameters in crossbred hens will be valuable for designing a breeding strategy for improved survival.

MATERIALS AND METHODS

Study Population

Female crossbred offspring of 2 commercial purebred White Leghorn layer lines (W1 and WB) with pedigree information was provided by the Institut de Sélection Animale (ISA) B.V., the layer breeding division of Hendrix Genetics (Boxmeer, the Netherlands). Fifty sires of line W1 were randomly chosen and mated with 908 dams of line WB. Dams and sires were housed individually. Each sire was mated to approximately 18 dams, and each dam contributed on average 3 female offspring, resulting in 2,859 offspring.

Housing and Management

All chickens from the cross between W1 and WB lines were hatched, sexed, and wing-banded, respectively, at the same time. Only female chicks were kept for this study. The offspring of 25 sires were beak-trimmed, whereas the offspring of another 25 sires were kept with intact beaks. Chicks were trimmed manually at 1 d of age using a hot blade to remove and cauterize the tip of the beak. Hens 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. At 17 wk of age, all hens were transported to a high-light-intensity laying house with battery cages. There were 3 double rows of cages in the laying house, with rows in between to allow employees to have access to the cage. The outer 2 double rows consisted of 3 levels (top; middle, closest to the light; and bottom). The inner double rows consisted of 4 levels (very top; top; middle, closest to the light; and bottom). Hens were only placed in the top and middle levels (Figure 1). Five half-sibs or full-sibs with the same beak treatment were allocated to a single cage. Water and standard commercial layer diet was provided ad libitum. The chickens 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), infectious bronchitis (d 1, 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. Hens that died were removed from the cages and not replaced. Wing-band number, cage number, and date of death were recorded. Cause of death was not determined. Each hen, information was collected on survival and number of 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 study, with a maximum of 457 d (actual age of 581 d, since the date of hatch).

NAb Isotypes IgM and IgG Titers Binding KLH

At 24 wk of age, blood samples from all hens were taken from the wing vein for measurements of titers of NAb isotypes IgM and IgG binding KLH in the serum using ELISA as described earlier (Sun et al., 2011).

Data Analysis

Descriptive Statistics. Descriptive statistical analyses were performed using SAS 9.1.2 (SAS Institute Inc., Cary, NC). Effects were considered significant at $P < 0.05$. A multiple comparison test (Bonferroni) was conducted to study the differences in NAb isotype IgM and IgG titers binding KLH in contrast groups.

Genetic Parameters Estimation of NAb Isotype Titers Binding KLH. A linear animal model was used
to estimate variance components, heritability of NAb isotype IgM and IgG for the whole population including both beak-trimmed and non-beak-trimmed laying hens, using the ASReml program (Gilmour et al., 2006). Fixed effects were established using GLM with SAS 9.1.2 (SAS Institute Inc.). Fixed effects considered in this study were beak treatment, row, and level of the cage. The final model for the variance components estimation was

$$y_{ijk} = \mu + row_i + level_j + a_k + e_{ijk},$$

where $y_{ijk}$ was NAb isotype IgM or IgG titers binding KLH, $\mu$ was the overall mean, and $row_i$ was the fixed effect of row of the cage ($i = 1, 2, 3$). There were 6 rows as shown in Figure 1. Rows 1 and 6, rows 2 and 5, and rows 3 and 4 were treated as the same row, respectively; $level_j$ was the fixed effect of level of the cage ($j = 1, 2$); $a_k$ was the random additive genetic effect of the animal $k$ (direct genetic effect); and $e_{ijk}$ was the residual term. (Co)variance structures of model terms are $\text{var}(a) = A \sigma_a^2$, with $A$ being the additive genetic relationship matrix based on 4 generations of ancestors extracted from the pedigree file provided by ISA, and $\sigma_a^2$ is the additive genetic variance and $\text{var}(e) = I \sigma_e^2$, in which $I$ is an identity matrix and $\sigma_e^2$ is the residual variance. Genetic and phenotypic correlations between traits were estimated based on bivariate analyses using equation [1].

The heritability for NAb isotype titers was calculated as $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$, and phenotypic variance was $\sigma_P^2 = \sigma_a^2 + \sigma_e^2$.

Survival Analysis with NAb Isotype Titers as Explanatory Variables. Multivariable multilevel logistic regression analyses were used to assess the relationships between survival (binary variable taking the values 0 for survived and 1 for dead) from 24 to 83 wk of age) and NAb isotypes IgM or IgG titers binding KLH at 24 wk of age, $x_2$ was the effect of IgG titers binding KLH at 24 wk of age, $x_3$ = the effect of row of cage, $x_4$ = the effect of level of cage. $\beta_0$ is the intercept from the equation (the value of the criterion when the explanatory variables are equal to zero), $\beta_1, \ldots, \beta_4$ are the regression coefficients indicating the relative effect of a corresponding explanatory variable ($x_1, \ldots, x_4$) on the outcome. The continuous variables IgM and IgG titers were inspected for linearity in the log-odds by dividing them into classes. The likelihood ratio test was used for the significance of variables. The other covariates included row and level of the cage where the laying hens were located. Nonsignificant covariates ($P > 0.05$) were removed from the model one by one starting with the effect showing the highest $P$-value. If a removed covariate was deemed a confounder (i.e., one or more regression coefficients of the remaining variables relatively changed over 25%), it was forced back into the model. The fit of the logistic models was assessed by the Hosmer and Lemeshow goodness-of-fit test (Hosmer and Lemeshow, 1989). Outcomes of logistic regression analyses were presented as odds ratios, which indicate the ratio of risks to die dependent on the titers of IgM and IgG binding KLH (Sun et al., 2011).

RESULTS

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

Before the serum samples were collected at 24 wk of age, 135 laying hens died. In total, NAb isotype IgM and IgG titers were determined in 1,555 beak-trimmed and 1,169 non-beak-trimmed hens (Table 1). The NAb IgG titers in non-beak-trimmed hens were significant higher than those in beak-trimmed ones. There was no
significant difference for IgM titers between 2 populations. There were no significant differences for IgM titers between the surviving and nonsurviving hens within either population. However, in beak- trimmed hens, IgG titers in nonsurviving were significantly higher than in the surviving hens (Table 1).

Average IgG and IgM isotype titers of both beak- trimmed and non-beak- trimmed hens located at the top level were significantly higher than the hens located at middle level. A significant difference of the average IgG and IgM isotype titers of both beak- trimmed and non- beak- trimmed hens was also observed among the row categories where the cages were located: the hens located in the middle 2 rows (row category 3) of the house had higher NAb isotype titers (Table 1).

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

Table 2 shows the survival and average survival days of beak- trimmed and non-beak- trimmed hens. The beak- trimmed hens had high survival rate of 93.1% and average survival days of 447.8 d. The hens with intact beaks had low survival rate of 69.5% and average survival days of 383 d. The difference for survival and survival days in the 2 populations was significant. Kaplan- Meier survival function $S(t) = P(T \geq \tau)$ curves also show significant different survival experience versus time for beak- trimmed and non- beak- trimmed laying hens (Figure 2). In Figure 3, the probability density function curve for non- beak- trimmed laying hens increased sharply from 18 to 26 wk of age, and from 34 to 36 wk of age (at the peak of egg production). This indicates that the death rate increased during these time periods. The increase from 18 to 26 wk of age might be because the laying hens were moved to new cages, meeting unfamiliar cage mates and experiencing increased light intensity. The increase from 34 to 36 wk of age might be caused by the increasing production of eggs. In beak- trimmed laying hens, the probability of death was highest at 63 wk of age, which might be caused by aging.

### Table 1. Number (n) and average natural antibody (NAb) isotype IgM and IgG titers binding keyhole limpet hemocyanin (KLH) with SD1 in beak- trimmed and non- beak- trimmed laying hens

<table>
<thead>
<tr>
<th>Population</th>
<th>Variable</th>
<th>Class</th>
<th>n</th>
<th>IgM titer</th>
<th>IgG titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-beak trimmed</td>
<td>Survival</td>
<td>Survival</td>
<td>813</td>
<td>8.17 (1.06)</td>
<td>6.69 (1.16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonsurvival</td>
<td>356</td>
<td>8.18 (1.10)</td>
<td>6.77 (1.24)</td>
</tr>
<tr>
<td></td>
<td>Level</td>
<td>Middle</td>
<td>578</td>
<td>8.02 (1.01)b</td>
<td>6.47 (1.13)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Top</td>
<td>591</td>
<td>8.33 (1.11)a</td>
<td>6.95 (1.18)a</td>
</tr>
<tr>
<td></td>
<td>Row category</td>
<td>1</td>
<td>398</td>
<td>8.13 (1.14)b</td>
<td>6.55 (1.12)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>398</td>
<td>8.03 (1.10)b</td>
<td>6.42 (1.19)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>373</td>
<td>8.38 (0.94)a</td>
<td>7.29 (1.09)a</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>1,169</td>
<td>8.17 (1.07)</td>
<td>6.71 (1.18)x</td>
</tr>
<tr>
<td>Beak trimmed</td>
<td>Survival</td>
<td>Survival</td>
<td>1,447</td>
<td>8.24 (1.05)</td>
<td>6.59 (1.22)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonsurvival</td>
<td>108</td>
<td>8.30 (1.03)</td>
<td>6.86 (1.16)a</td>
</tr>
<tr>
<td></td>
<td>Level</td>
<td>Middle</td>
<td>802</td>
<td>8.13 (0.95)b</td>
<td>6.44 (1.22)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Top</td>
<td>753</td>
<td>8.36 (1.13)a</td>
<td>6.79 (1.19)a</td>
</tr>
<tr>
<td></td>
<td>Row category</td>
<td>1</td>
<td>525</td>
<td>8.18 (1.04)b</td>
<td>6.51 (1.16)a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>513</td>
<td>8.11 (1.11)b</td>
<td>6.39 (1.22)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>517</td>
<td>8.42 (0.95)a</td>
<td>6.93 (1.22)a</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>1,555</td>
<td>8.24 (1.05)</td>
<td>6.61 (1.22)y</td>
</tr>
</tbody>
</table>

a,b Different superscripts indicate there is significant difference between different classes within the same variable ($P < 0.05$).

x,y Indicate there is a significant difference for all beak- trimmed and non- beak- trimmed laying hens ($P < 0.05$).

1SD are in parentheses.

### Table 2. Number (n), survival, and average survival days with SD1 for beak- trimmed and non- beak- trimmed crossbred laying hens

<table>
<thead>
<tr>
<th>Population</th>
<th>Birds</th>
<th>n</th>
<th>Survival2 (%)***</th>
<th>Survival days3***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-beak trimmed</td>
<td>Total</td>
<td>1,169</td>
<td>69.5 (1.4)</td>
<td>383.0 (4.0)</td>
</tr>
<tr>
<td></td>
<td>Survival</td>
<td>813</td>
<td></td>
<td>457.0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Nonsurvival</td>
<td>356</td>
<td></td>
<td>214.2 (7.3)</td>
</tr>
<tr>
<td>Beak trimmed</td>
<td>Total</td>
<td>1,555</td>
<td>93.1 (0.6)</td>
<td>447.8 (1.2)</td>
</tr>
<tr>
<td></td>
<td>Survival</td>
<td>1,447</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nonsurvival</td>
<td>108</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1SD are in parentheses.

2Survival is the percentage of hens still alive at the end of the study (83 wk of age).

3Survival days are the average number of days from day of observation (17 wk of age) till death or the end of the study, with a maximum of 457 d.

***$P < 0.0001$. 

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Genetic Parameters Estimation for NAb Isotype Titers Binding KLH

In beak-trimmed and non-beak-trimmed laying hens, heritability of NAb isotypes IgG and IgM titers binding was 0.21 (SE = 0.04) and 0.26 (SE = 0.04), respectively (Table 3). Genetic and phenotypic correlations between IgG and IgM titers were estimated to be 0.43 (SE = 0.11) and 0.28 (SE = 0.02), respectively, based on bivariate analyses using equation [1].

Relationship Between NAb Isotype Titers and Survival in Beak-Trimmed and Non-Beak-Trimmed Laying Hens

The final variables for survival analysis in beak-trimmed and non-beak-trimmed laying hens are shown in Table 4. In beak-trimmed hens, row of the cage was not a significant factor ($P = 0.29$) or an important confounder and thus was removed from the logistic model. The fit of the ordinary logistic model was sufficient (chi-squared = 2.86, $P = 0.90$). Level was a significant factor ($P = 0.002$). Preliminary analysis indicated that IgM and IgG titers at 24 wk of age were not linearly related to the survival of laying period and were therefore

Table 3. Estimates of genetic parameters$^1$ with SE$^2$ for natural antibody (NAb) isotype IgM and IgG titers binding keyhole limpet hemocyanin (KLH) at 24 wk of age in crossbred laying hens

<table>
<thead>
<tr>
<th>Item</th>
<th>IgG titer</th>
<th>IgM titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_a^2$</td>
<td>0.29 (0.06)</td>
<td>0.28 (0.05)</td>
</tr>
<tr>
<td>$\sigma_e^2$</td>
<td>1.06 (0.05)</td>
<td>0.81 (0.04)</td>
</tr>
<tr>
<td>$\sigma_P^2$</td>
<td>1.35 (0.04)</td>
<td>1.10 (0.03)</td>
</tr>
<tr>
<td>$h^2$</td>
<td>0.21 (0.04)</td>
<td>0.26 (0.04)</td>
</tr>
<tr>
<td>$r_g$</td>
<td>0.43 (0.11)</td>
<td></td>
</tr>
<tr>
<td>$r_P$</td>
<td>0.28 (0.02)</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ $\sigma_a^2$ is the additive genetic variance, $\sigma_e^2$ is the random residual variance, $\sigma_P^2$ is the phenotypic variance: $\sigma_P^2 = \sigma_a^2 + \sigma_e^2$. $h^2$ is the heritability; $r_g$ is the genetic correlation, and $r_P$ is the phenotypic correlation.

$^2$ SE are in parentheses.
categorized into 5 classes, with IgG titers <5.0 and IgM titers <7.0 as reference class, respectively. The odds ratios for higher IgM or IgG titers groups (except for the IgM titers group 8.0–8.9) were smaller than 1.00, but not significant ($P > 0.05$; Table 4).

In non-beak-trimmed laying hens, row and level of the cage had no direct significant effect on survival ($P = 0.08$ for both variables). However, when removing them from the model, the relative change of the coefficient for IgM and IgG titers at 24 wk of age was larger than 25%. Therefore, row and level of the cage were both included in the final model to get proper estimation for NAb isotype titers. The fit of the ordinary logistic model was not sufficient (chi-squared = 6.31, $P = 0.61$). The IgM and IgG titers at 24 wk of age were not linearly related to the survival of laying period and were therefore categorized in 5 classes, with IgG titers <5.0 and IgM titers <7.0 as reference class, respectively. The odds ratios for higher IgM titers groups (except for the group 9.0–9.9) were smaller than 1.00, but not significant ($P > 0.05$).

The odds ratios for higher IgG titers groups were larger than 1.00, but not significant either ($P > 0.05$; Table 4).

**DISCUSSION**

**Genetic Analysis of NAb Isotype Titers Binding KLH in Crossbred Laying Hens**

In crossbred laying hens, moderate heritabilities were found for NAb isotypes: 0.21 (SE = 0.04) for IgG and 0.26 (SE = 0.04) for IgM. This indicated that NAb isotype titers in crossbred birds show genetic variation. In a previous study, the heritability for IgG and IgM titers binding KLH at 20 wk of age was estimated as 0.31 and 0.41, respectively, in purebred laying hens (Sun et al., 2012). The difference may rest on different populations, and slight differences of the traits studied (isotype titers at 24 wk in the present and 20 wk in the previous study). The heritability for IgM was higher than IgG in our study. This is in accordance with our previous study, and the possible explanations were discussed by Sun et al. (2012). The positive genetic and phenotypic correlation between IgM and IgG isotype was also observed in the previous study with purebred birds (Sun et al., 2012). The moderate genetic correlation suggests that IgM and IgG share partially the same genetic background but are relatively independently controlled. However, the positive genetic correlations indicated that 2 isotypes may be selected for simultaneously. Selection for improved NAb isotype titers is possible by selection in the purebred lines based on NAb measured in crossbreds, if the genetic correlation between the traits in purebred and crossbred lines is high. Our data set only contained NAb isotype titers measured on crossbred offspring; it was, therefore, not possible to estimate this genetic correlation. It is, however, important to estimate the genetic correlation before developing a strategy to breed for enhanced NAb titers in crossbred birds.

Direct heritability can be overestimated when maternal effects exist but are neglected in model analy-
NAb Isotype Titers Is Not Predictive for Survival in Beak-Trimmed or Non-Beak-Trimmed Crossbred Laying Hens

Total NAb titers and isotype IgM and IgG titers binding KLH at 20 wk of age were shown to be significantly associated with and predictive for survival in purebred hens in the laying period in previous studies (Star et al., 2007; Sun et al., 2011). In the egg production industry, commercial laying hens are usually crossbred. To investigate the predictive value of NAb isotype titers for survival in crossbred hens, a population of crossbred female offspring from purebred W1 (male) and WB (female) lines was used. The W1 line was typed to be a high NAb line, whereas WB line was a low NAb line (Star et al., 2007; Sun et al., 2011). The survival of W1 line was higher than that of the WB line (Ellen et al., 2008). These observations were in line with our previous study that high NAb isotype IgM and IgG titers were associated with higher survival of laying hens. In the present study, the survival of non-beak-trimmed laying hens was significantly higher than that of beak-trimmed laying hens (Table 2; Figures 2 and 3). There was no significant difference of IgM titers in the 2 populations, but the IgG titers in non-beak-trimmed laying hens were significantly higher (Table 1). We performed multivariable multilevel logistic analysis of IgM and IgG titers at 24 wk of age for survival of 24 to 83 wk of age. In beak-trimmed laying hens, odds ratios of smaller than 1.00 were found for all IgG and IgM isotype groups (except for the IgM titer group 8.9–9.9), which indicated that survival was higher in birds with higher antibody titers, but the associations were not significant, and titers and survival were not completely linearly related. In the non-beak-trimmed laying hens, odds ratios of lower than 1.00 were found for IgM titer groups (except for the titer group 9.9–10.0), which indicated that the survival was lower for the hens with the lowest IgM titers. Different from the finding in beak-trimmed laying hens, odds ratios larger than 1.00 were found for all IgG titer groups. This indicated that survival was higher for birds with lower IgG titers, although all the associations were not significant and there was no completely linear relationship between survival and isotype titers. In the present study, we also estimated the breeding value (EBV) of the 50 sires for NAb isotype titers and investigated the linear regression between sire EBV and the survival of their offspring. Beak-trimmed laying hens showed high survival irrespective of the sire EBV for NAb isotypes. Sires with higher EBV for non-beak-trimmed laying hens did not always predispose their non-beak-trimmed offspring for higher survival either. However, the regression indicated that survival of offspring was higher for the sires with higher EBV for IgM, whereas survival was lower for the sires with higher EBV for IgG (see Appendix). This was in agreement with the logistic regression analysis.

In general, the observations from logistic regression analysis were not consistent with our previous findings, where odds ratio of 0.56 ($P < 0.0001$) and 0.72 ($P = 0.02$) was found for IgM and IgG, respectively, in Brown purebred laying hens and odds ratio of 0.74 ($P = 0.01$) and 0.99 ($P = 0.99$) was found for IgM and IgG, respectively, in White Leghorn purebreds (Sun et al., 2011). As we hypothesized previously, different causes of mortality in 2 populations may account for this. In non-beak-trimmed laying hens, death rate increased from 18 to 26 wk of age, and from 34 to 36 wk of age. The increase from 18 to 26 wk of age might be because the laying hens were moved to new cages, meeting unfamiliar cage mates and experiencing increased light intensity. The increase from 34 to 36 wk of age might be caused by the increasing production of eggs. These reasons were causing factors of feather pecking. Therefore, mortalities in non-beak-trimmed laying hens are likely mainly due to cannibalism, which is considered the ul-
imate phase of severe feather pecking. In the study of Peeters et al. (2012), as high as 99.7% (6,683 out of 6,706 crossbred laying hens with intact beak) of dead birds was related with feather pecking behavior or cannibalism. Beak trimming is an effective way of preventing the severe feather pecking and thus improving the survival of laying hens (Table 2 and Figure 2). Based on our observation, the feather condition of beak-trimmed laying hens was also significantly better than those in non-beak-trimmed ones (data not shown). Analysis of survival days for the 2 populations in the present study using direct-associative effect animal models (Bijma et al., 2007) indicated that in non-beak-trimmed laying hens, the heritable variation for survival days was 6-fold larger than that in beak-trimmed laying hens, and almost all variation was contributed by the associative effect (data not shown). Therefore, although some of the deaths of non-beak-trimmed laying hens may be due to health-related causes, the majority of death in this population was caused by harmful social interactions, such as feather pecking and cannibalism.

In the earlier study, NAb isotype titers were also shown to be more sensitive and acute parameters for survival in the Brown laying hens, which show much less feather pecking behavior than White Leghorn laying hens (Uitdehaag et al., 2008). These results indicate more complex relationships between NAb isotype titers and survival of the layer population when the cause of mortality is also complex.

In beak-trimmed crossbred hens in the present study, mortality was less likely caused by severe feather pecking. The survival was significantly higher than that in non-beak-trimmed hens (Table 2 and Figure 2). However, the association between the NAb isotype titers and survival was not significant either (Table 4). This observation, which is different from that in purebred laying hens, may rest on crossing. In the present study, a survival of 93.1% was found for beak-trimmed crossbred offspring. In the study of Star et al. (2007), a survival of 89.6 and 87.1% was found for paternal W1 and maternal WB line, respectively. The higher survival of crossbred offspring indicated a heterosis for survival. It is possible that heterosis has a more prominent effect on survival than the NAb isotypes levels in the offspring.

In conclusion, NAb isotypes IgM and IgG titers binding KLH at 24 wk of age are heritable traits in crossbred laying hens. Environmental effects on isotype titers are also indicated. Environmental factors including row and level of the cage (light intensity) were associated with NAb isotype titers. Different from purebred laying hens, there was no significant associations between NAb isotype titers and survival in beak-trimmed or non-beak-trimmed crossbred laying hens. The present results confirmed our previous hypothesis that non-health-related causes of mortality (severe feather pecking) overruled the anticipated relationships between NAb isotype titers and survival in birds with intact beaks.

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**APPENDIX**

The EBV of the 50 sires for IgM and IgG titers, respectively, was estimated from the following sire model implemented in the ASReml software package (Gilmour et al., 2006):

$$y = Xb + Zs + e,$$

where $y$ is a vector of IgM or IgG titers; $b$ is a vector of fixed effects, with incidence matrix $X$ linking IgM or IgG titers to fixed effects; $s$ is a vector of the sire effect (half of breeding values), with incidence matrix $Z$ linking IgM or IgG titers to the sire effect; $\text{var}(s) = \mathbf{A}_s \sigma^2_s$, with $\mathbf{A}_s$ being the sire additive genetic relationship matrix and $\sigma^2_s$ being the sire genetic variance; $\text{var}(e) = \mathbf{I} \sigma^2_e$, with $\mathbf{I}$ being an identity matrix and $\sigma^2_e$ being the residual variance.

The survival of the offspring of the 50 sires was linearly regressed on the EBV of the sires for IgM (Figure 4A) and IgG (Figure 4B) titers. The linear regression models were also shown on the trend lines.
Figure 4. The regression of survival of the offspring on the estimated breeding value (EBV) for natural antibody isotype A) IgM and B) IgG titers binding keyhole limpet hemocyanin. The linear regression model is shown on the trend line.