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Correlated Responses to Bi-Directional Selection for Sheep Red Blood Cell Titer in White Leghorns

[1, 2]Yao-Dong Hu, [2]Jie Wen, [2]Mai-Qing Zheng, [2]Ran-Ran Liu,
[2]Ji-Lan Chen, [1]Qing Zhu and [2]Gui-Ping Zhao
[1]Laboratories of Animal Genetics and Breeding, College of Animal Science and Technology,
Sichuan Agricultural University, Ya'an, 625014 Sichuan, China
[2]Institute of Animal Science, Chinese Academy of Agricultural Sciences, 100193 Beijing, China

Abstract: Four generations of bi-directional selection for antibody response to challenge with SRBC on days 56 were completed in White Leghorn chickens. The anti-SRBC titer and other immune traits were measured in up-selected (H), down-selected (L) and Control (C) lines in each Generation (G0-G4) and reproductive traits were measured in G4. In the 4th generation, the difference in SRBC titers (measured on days 61) between the selected lines was 7.18 versus 6.10, a 17% increase in the H compared to the L lines. The heritability of SRBC titer was 0.26. The genetic correlations between anti-SRBC and IgG (0.87) and IgM (0.81) were high and those between SRBC titers and those against AI (0.56), ND (0.48) and the H/L ratio (0.58) were moderate. Immune and reproductive traits compared among the H, L and C lines in the final Generation (G4) showed that AI, ND and IgM were improved significantly ($p<0.05$) by selection for SRBC but the age at first lay was slightly delayed (0.48 days) in the H line. This study demonstrates that selecting for antibody response to SRBC improves other immune traits consistent with broad disease resistance of chickens.

Key words: Immunology chicken, SRBC, selection, disease resistance, reproductive traits

INTRODUCTION

There is an increasing demand for poultry meat and eggs that are nutritious, wholesome and free of drug residues. This goal can be easily achieved in chickens by combining optimal management with genetic improvement of disease resistance (Lillehoj *et al*., 2004; Siwek *et al*., 2006). The incidence of disease is minimized by use of chicken strains with established disease resistance and appropriate manipulation of the immune system by vaccination. Inadequate understanding of the resistance mechanisms for the array of specific disease vectors (Gavora and Spencer, 1983) is constraining the rational development of broad spectrum disease resistance.

Antibody titer against Sheep Red Blood Cells (SRBC), a non-pathogenic multivalent antigen (Saxena *et al*., 1997; Baelmans *et al*., 2005) has been extensively used to measure immunocompetence. The immune response to challenge with SRBC reflects antigen handling, antigen presentation of macrophages and the proliferation of B lymphocytes (Sarker *et al*., 1999). It has been suggested that the immune response to SRBC is an indicator of disease resistance (Warner *et al*., 1987) as it is correlated with resistance to a spectrum of specific pathogens (Gross *et al*., 1980; Mashaly *et al*., 2000) and the ability of birds to respond to specific antigenic challenges can be indirectly improved by selection based on the response to non-infectious complex antigens such as SRBC (Siwek *et al*., 2006). However, there maybe negative relationships between SRBC antibody responses and the time of pullet maturing (Laan *et al*., 1998), production traits (Martin *et al*., 1990) such as juvenile Bodyweight at 28 days (BW_{28}) with L line was heavier than H line (Gross *et al*., 1980) but no differences between lines for BW at sexual maturity (Dunnington and Siegel, 1984). Kuehn *et al*. (2006) even thought SRBC selection may have a negative effect on fitness relative to other traits because of over-production of antibodies (Kuehn *et al*., 2006).

Several research groups such as Siegel, Gross, Van der Zijpp, Parmentier, Bovenhuis and Nieuwland, set up the bi-directional selection for response to SRBC in chicken whcih have detected some relation between anti-SRBC titer and anti-disease, production traits and animal welfare (Gross *et al*., 1980; Siegel and Gross, 1980; Van der Zijpp and Nieuwland, 1986; Pinard *et al*., 1992; Kreukniet *et al*., 1994; Parmentier *et al*., 1996; Adriaansen-Tennekes *et al*., 2009; Wijga *et al*., 2009) and

Corresponding Author: Zhu Qing, Laboratory of Animal Genetic and Breeding, College of Animal Science and Technology, Sichuan Agriculture University, 46# Xinkang Road, Ya'an, 625014 Sichuan, China

in this research, researchers perform four generations of selection for SRBC to gain insight into the underlying genetic control of immune responses and the correlation between SRBC selection and some important immune traits.

MATERIALS AND METHODS

Selection and maintenance of the chicken lines: Founders of White Leghorns, from the population maintained at the Institute of Animal Science, CAAS, Beijing, China were randomly chosen to establish two divergent selection lines based on the hemagglutination titer at 61 and 5 days after challenge with SRBC (High, H and Low, L) along with randomly selected Controls (C).

Selected individuals, within each Line (H, L) were mated (25 males, each with 3 females) to produce the next generation. Matings for future generations were within lines and sibling matings were avoided. If birds were infertile, they were eliminated and replaced with others having higher or lower SRBC titers in each line as appropriate. All progeny were retained for evaluating the response to SRBC; others were eliminated from the study. In each generation for the C line, about 40 sires and 120 dams were randomly chosen and hens were inseminated artificially using the pooled semen of all sires on the same day during the egg collection period. Generations were not overlapping.

All birds were wing-banded at 1 day of age and reared under the same conditions in stair-step caging under using standard conditions of temperature, humidity and ventilation: A temperature of 20-22°C with a relative humidity of 55-60% was maintained. The lighting program was: 0-1 week, 23 h and 2-9 weeks, 14 h. Three sizes of cages were used during the rearing period: 0-6 weeks, 10-12 birds/m^2 (cage dimension: 200×200×150 cm); 7-17 week, 2-4 birds/cage (cage dimension: 32.5×45×50 cm); 18-68 weeks, one bird/cage (cage dimension: 32.5×37×34 cm). The chickens were fed the same diet by the professional breeders and had free access to feed and water during the entire rearing period.

The present study was carried out in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China).

Schedule of immunization, sampling and H/L ratio: All birds vaccinated for NDV (Newcastle disease attenuated vaccine, Beijing Veterinary Biological Products Co. Beijing 100193, China) at 7, 15 and 28 days, AIV (H5N1, Re-4+Re-5, Qingdao Yebio Biological Products Co., QingDao, China) at 15 and 28 days, challenged with SRBC at 56 days (each leg was injected with 0.5 mL of a 25% (v/v) suspension of washed SRBC in PBS (Ghaffari Laleh *et al*., 2008).

After 5 days later, at 61 days, Live Weight was measured (LW_{61}) and approximately 4 mL of blood was collected from the brachial vein of each individual and was used to obtain serum after clotting at 37°C and centrifugation. In the meantime, freshly obtained blood (10 μL) was smeared on each of two glass slides, air-dried and dyed with May-Grunwald-Giemsa stain. The heterophil to lymphocyte ratio was calculated as Campo *et al*. (2008).

Antibody titers and ELISA assays (IgG and IgM): Antibody titers against NDV, AIV and SRBC were tested by hemagglutination or hemagglutination inhibition tests and expressed as $\log_2$ of the reciprocal of the highest serial dilution in which there was hemagglutination. Immunoglobulin concentrations (IgG and IgM) were determined by enzyme-linked immunosorbent assays using commercial chicken ELISA kits, according to the manufacturer's instructions (Sun Biomedical Technology Co., Beijing, 10039).

Reproductive performance and immune organ index: In the 4th generation, the data were recorded from age at first lay to the 55th week. About 30 hens randomly chosen from each line were weighed at 86 days (LW_{86}) and slaughtered to measure carcass weights (de-feathered but including feet and head) and weights of the major immune organs (bursa, thymus, spleen). The latter were also expressed as indices (organ weight in g/LW_{86} in kg). At the peak of egg production (30-31 weeks), 75 hens selected from each line were bred and fertilization, hatching and hatchling vigor were assessed.

Analyses of traits: Descriptive statistics including the test of the normality of the distribution for each trait and ANOVA were analyzed with SAS Software 8.0. Heritabilities (h^2) and genetic correlations were estimated using a Multivariate Additive Genetic Mixed Model; multivariate analyses were used to estimate genetic and phenotypic correlations between any pair of traits. These estimates were based on data from G_0-G_4 in the S line. The following mixed linear model was used:

$$y_i = X_i b_i + Z_i a_i + e_i \quad (1)$$

Where:

y_i = The an N×1 vector of observations for the ith trait

b_i = The ap×1 vector of fixed sex and generation effects for the ith trait

a_i = The N×1 vector of additive genetic effects for the ith trait
e_i = The N×1 vector of residuals for the ith trait
X_i and Z_i = The incidence matrices for the ith trait

Solutions for a_i and b_i can be obtained from the Henderson's Mixed Model equations (Campo *et al*., 2007). Parameter estimates were obtained using the MTDFREML Software.

RESULTS AND DISCUSSION

Response to divergent selection using anti-SRBC titers: All data collected from G_1-G_4 were used to compare changes in anti-SRBC titers between the H and L lines. Generational changes from divergent selection for antibody response to SRBC are shown in Fig. 1. As shown in Fig. 1, upward selection (H) resulted in about 4 fold greater change than downward selection (L) did. Differences between the selected lines were significant ($p<0.05$) in G_3 and in G_4 where the H line was 13.8% higher than the controls and 17% higher than the L line. The target trait clearly responded to the selection imposed here.

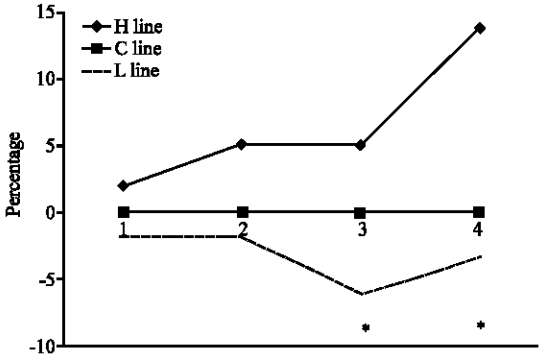

Fig. 1: Generational changes from divergent selection for antibody response to SRBC. The mean titers are plotted relative to the control line (%) and the X-axis is generation number. Differences between the H and L lines are significant ($p<0.05$)

Correlated responses to selection for SRBC response: After four generations of divergent selection, differences in some indices of immune function among the H, L and C lines by two-way ANOVA were shown in Table 1. As shown in Table 1, there were significant ($p<0.05$) overall increases in antibody titers against SRBC, AI, ND and IgM in the H line compared to the L and C lines, especially for IgM in the H line which increased 16.18% over the C line. Others traits responded slightly ($p>0.05$) when selecting for SRBC. In the case of the H/L ratio, the differences among the lines were significant (by ANOVA) and the H line had the lowest mean value (0.425). Males were heavier (LW_{61}) than females. There were no significant difference ($p>0.05$) between the L line and C line and no interactions were found between lines and sexes.

Effects of four generations of selection for SRBC response on the major immune organs and reproductive performances: The weights of the major immune organs including thymus, bursa and spleen, did not differ among the three lines (Table 2) with or without adjustment for LW_{86} but there was high variability of these measurements (CVs of 16 - 36%, n = 30 hens), especially for the bursa of Fabricius.

There were no significant differences, resulting from up or down-selection for SRBC titers for four generations, on the reproductive performance of the hens as measured in this study. At age of first lay, eggs produced to week 55 and the laying rate were determined for 196 (H), 172 (L) and 74 (C) birds and the 75 selected hens from each line were used to assess fertilization (H: 91.31, L: 92.39 and C: 91.82%), hatching (H: 79.80, L: 81.33 and C: 80.45%) and hatchling vigor (H: 79.13, L: 80.77 and C: 79.75%).

Genetic and phenotypic parameters: Genetic correlations (r_g), phenotypic correlations (r_p) and heritabilities (h^2) are shown in Table 3. Based on all measurements made in this study, the mean anti-SRBC titer was 7.27 and the calculated genetic standard deviation was 1.30 indicating considerable variation in the population.

Table 1: Differences in immune variables between the selected lines in G_4

Items	SRBC	AI	ND	H/L	LW_{61}	IgG	IgM
H line (n)	7.18 (353)^A	5.87 (341)^A	8.87 (350)^A	0.42 (360)	0.54 (360)	0.50 (116)	83.3 (162)^A
L line (n)	6.10 (347)^B	5.17 (336)^B	8.29 (346)^B	0.47 (346)	0.53 (358)	0.44 (104)	73.5 (155)^B
C line (n)	6.31 (115)^B	5.45 (114)^B	8.21 (117)^B	0.46 (120)	0.53 (120)	0.46 (120)	71.7 (120)^B
P-line	<0.001	<0.001	<0.001	0.04	0.51	0.11	<0.001
P-sex	0.70	0.13	0.06	0.51	0.03	0.44	0.34
P-line x sex	0.27	1.00	0.87	0.88	0.63	0.70	0.30
SD	2.29	1.55	1.68	0.22	0.10	0.20	14.95

Data are means with the number of observations. Probabilities and standard deviations are from the respective two-way ANOVA. Means within traits with common superscripts are not significantly different ($p>0.05$). H/L ratio: the number of heterophils divided by the number of lymphocytes; SRBC, AI and ND are serum antibody titers against sheep red blood cells, avian influenza and Newcastle disease, respectively; IgG (mg mL^{-1}) and IgM (ng mL^{-1}) are concentrations in serum; LW_{61} Is Live Weight measured on days 61 (kg)

Table 2: Changes in major immune organs after four generations of selection for SRBC titer in hens

Items	LW_{86}	CW_{86}	Thymus	Bursa of Fabricius	Spleen	Thymus index	Bursa of Fabricius index	Spleen index
H line	0.80[a]	0.67	4.58	2.91	2.37	5.73	3.64	2.96
L line	0.77[b]	0.65	4.36	2.79	2.16	5.66	3.63	2.81
C line	0.80[a]	0.66	4.49	2.90	2.36	5.61	3.63	2.95
p-value	0.05	0.15	0.72	0.87	0.11	0.96	0.99	0.46
SD	0.05	0.05	1.05	0.97	0.43	1.32	1.23	0.57

Data are means (n = 30 in all cases). Probabilities and standard deviations are from the respective ANOVA. LW_{86} = Live Weight at days 86 (kg), CW_{86} = Carcass Weight at 86 days (kg), weights of bursa of Fabricius, thymus and spleen are shown in grams while the indices are % of LW_{86}. Significantly different means within traits have different superscripts ($p<0.05$)

Table 3: Genetic and phenotypic correlations and heritabilities

	SRBC	AI	ND	H/L	IgG	IgM
SRBC	0.26	**0.56**	**0.48**	**0.58**	**0.87**	**0.81**
AI	-0.01	0.05	**0.56**	**-0.02**	**0.06**	**-0.32**
ND	0.05	0.12	0.16	**0.89**	**0.66**	**-0.19**
H/L	-0.04	-0.03	0.02	0.09	**-0.48**	**0.25**
IgG	0.15	0.10	0.07	0.11	0.20	**0.82**
IgM	0.12	-0.04	-0.01	0.00	0.05	0.73

Genetic correlations are shown in boldface (upper right), phenotypic correlations in the lower left, heritabilities are underline on the diagonal

The estimated h^2 of anti-SRBC titer at 61 days was 0.26 and other heritabilities were low or very low except those for IgM (0.73) and IgG (0.20). Phenotypic correlations between anti-SRBC and other measured variables were low (r_p = 0.15 with IgG and 0.12 with IgM) or non-existent. Anti-ND and anti-AI titers were weakly correlated (0.12) as were concentrations of IgG and the H/L ratio (0.11). Genetic correlations among most of these traits were usually moderate to high. Genetic correlations between anti-SRBC titer and the H/L ratio and titers against ND and AI were moderate (0.48-0.58) and were higher between anti-SRBC titer and IgG and IgM (0.87, 0.81). A strong negative genetic correlation existed between the H/L ratio and concentrations of IgG (-0.48) but not between H/L ratio and anti-AI titer (-0.02). The strong genetic correlation between H/L ratio and anti-ND was positive (0.89).

Direct selection response of SRBC: In the present study, the response to divergent selection for anti-SRBC titers was evaluated. During the four generations of selection, differences between the H and L lines gradually increased and were statistically significant by G_3. In the fourth generation, the difference in titers between the selected lines was 1.08 (7.18 versus 6.10, a 17% increase in the H lines over the L lines). Similar results have been obtained by others (Pinard *et al*., 1992; Bovenhuis *et al*., 2002). The C and L line did not differ for SRBC because when the data were analysis, the part of data which did not detectable antibody titer in L line has been precluded. The heritability of this SRBC-response trait can be influenced by the age when the SRBC were injected (Dunnington *et al*., 1992) because SRBC titer may be affected by maternally-derived antibody (Boa-Amponsem *et al*., 1997). Previous estimates of the heritability of the SRBC-response are inconsistent. Pinard *et al*. (1992) using 9 generations, derived h^2 of 0.31 (Pinard *et al*., 1992) estimated h^2 to be 0.18 based on 18 generations (Bovenhuis *et al*., 2002). Other estimates have been made, ranging from 0.17-0.52 (Van der Zijpp *et al*., 1983; Saxena *et al*., 1997).

The heritability, estimated in the present study (0.26), obtained when the SRBC were injected at 56 days and titers were measured at 61 days and the estimates of others, cited above indicate that response to SRBC has moderate heritability. Although, there are differences in environments and breeds used, the response to selection obtained here within four generations was similar to those described in the cited studies. Clearly, the relatively large σ_g of 1.30 and moderate heritability of the response to SRBC permits tangible genetic progress by selection on this trait.

SRBC and immune traits: In the current study, changes in the H/L ratio, antibody titers against ND and AI and concentrations of immunoglobulin (IgG and IgM) were observed as correlated responses to selection based on anti-SRBC titer. There were moderate to high genetic correlations between response to SRBC with anti-AI and anti-ND titers, the H/L ratio and concentrations of IgG and IgM whcih indicate that selection for response to SRBC can also improve these additional immune traits.

Anti-AI and anti-ND titers, examples of specific immunity involving humoral responses (Kleinbeck and McGlone, 1999) are generally used by veterinary inspection in poultry production. Gross *et al*. (1980) have previously shown that chickens selected for higher anti-SRBC titers had higher resistance to ND (Gross *et al*., 1980) and other similar reports exist (Laan *et al*., 1998). The results obtained in the present study are quite consistent with these earlier findings.

Of the serum immunoglobulins, IgM provides initial protection against various types of pathogens while IgG is produced as part of the humoral defence upon activation of the acquired immune system (Lee and Klasing, 2004). Concentrations of IgG and IgM were higher in the H line in the present study, similar to that reported by Okada and Yamamoto (1987) but the reverse of that reported by Sarkeret *et al*. (1999); both studies using selection based on IgG.

Some additional immune traits such as the weights of major immune organs with or without normalizing to live weight were also higher, though not significantly so in the H line. This trend was consistent with the findings of others who found larger spleens and bursas in their H lines compared to L lines (Boa-Amponsem *et al*., 2001; Renaville and Burny, 2001). Increased size of the major immune organs (bursa of Fabricius, thymus and spleen)

might indicate earlier maturation and enhanced cellular and humoral immune function (Xu *et al*., 2009). The very small differences found here and the sizeable CVs of these measurements indicate the need for larger samples when assessing potential differences in future comparisons, after additional generations of selection for the response to SRBC.

SRBC and reproductive performance: In the present study, the L lines had a slightly higher egg production at G_4 than H line but not reach the significant level. Furthermore, Siegel *et al*. (1982) found the L line had significantly higher egg production than H line at G_8 (Siegel *et al*., 1982). These results indicated that divergent selection for SRBC had effects on the egg production. In addition, there are few published papers on correlated responses in age at first lay and subsequent egg production in chickens selected for their response to SRBC. Chao and Lee (2001) found that phenotypic correlations between anti-SRBC titer and age at first lay, egg production in 52 weeks and average egg weight (at 25 weeks) were negative (-0.046, -0.038 and -0.05) (Chao and Lee, 2001).

CONCLUSION

The present study with White Leghorn chickens demonstrated improvement of several indicators of immune function as a result of four generations of up-directional selection for SRBC. The heritability of SRBC was estimated to be 0.26, sufficient to achieve tangible genetic progress, especially when large genetic variance exists and high selection intensity is possible. Baelmans *et al*. (2005) suggest that selecting for antibody and complement responses to SRBC can improve the genetic basis for broad disease resistance of chickens (Baelmans *et al*., 2005). This premise is further supported by significant increases in the H line in specific titers after vacination with AI and ND, serum concentrations of IgG and IgM and the decreased H/L ratio. The present findings and those of others are consistent with chickens having enhanced disease resistance after four generations of selection using SRBC.

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