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DIVERGENT SELECTION FOR HIGH AND LOW ANTIBODY TITER AGAINST SHEEP RED BLOOD CELLS IN FAYOUMI CHICKENS

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SUMMARY

To improve general disease resistance, Fayoumi chickens were divergently selected for high (H) and low (L) antibody titer against sheep red blood cells (SRBC) at 7 days post primary immunization for two generations. Control line was also maintaind. Primary antibody level against SRBC changed significantly over the course of selection. The divergence of selected lines became larger after the two successive generations of selection. After two generations primary antibody titer against SRBC at 7 days post immunization were 6.27 for H line versus 4.56 and 5.49 for L and control lines, respectively. Heritability of the antibody titer, 7 days post primary immunization, was estimated using sire components which ranged from 0.17-0.34. No significant differences in the delayed type hypersensitivity response (DTH) were found between any of the lines.

Keywords : Chickens, sheep red blood cells, immune response ,selection, heritability

INTRODUCTION

One of the major problems that encounter the poultry industry is infectious diseases , which is responsible for major economic losses and can have devastating effects particularly on intensive production. Although vaccination programs have dramatically reduced the incidence of diseases; vaccination program alone can not cope adequately with infectious diseases. Therefore, special attention has been made to the ability of chickens to respond to pathogenic challenges by enhancing the production of antibodies against non-pathogenic antigens . The relationship between genetic make-up and immune responseviness have been studied extensively . The existence of such a relationship has been utilized efficiently in developing chicken lines that diverge in their immune responses . Siegel and Gross (1980) and Van der Zijpp and Leenstra (1980) developed ISA Warren chicken lines that have either high or low in antibody production against SRBC and Heller *et al.* (1991) have selected broiler lines for antibody production against *E. coli* antigen .The ultimate goal of developing such lines is to improve some immune parameters ,improve resistance to specific diseases ,and decrease mortality rate . Which finally would lead to increase the economic profit of poultry production. For example ,chicken selected for high antibody response to SRBC showed higher resistance to some infectious diseases , such as *Marek's* , *Newcastle* disease , *Mycoplasma gallisepticum*,and parasites (Gross *et al.*,1980 and Dunnington *et al.*,1986).

The aim of this study was to enhance the genetic resistance against several diseases over generations by continuous divergent selection against a nonpathogenic antigen (SRBC) with different genetic parameters study over two generations.

MATERIALS AND METHODS

Experimental design

Chickens were bidirectionally selected from Fayoumi base population (generation G0 n=780) for 2 generations. The individual selection criterion was total antibody titer against SRBC 7d. after primary i.m. immunization (P7) with 1 ml of 25% (SRBC) at 50 d. of age . After titration, the flock was divided into three groups high (H), low (L), and control groups (C). The highest 25%(P7) dams and 10%(P7) sires constituted the H line.

In contrast, The lowest 25%(P7) dams and 10% (P7) sires constituted the L line inG0 and G1.Whereas the random females and males represented the C line. Random mating of the parents was used; the only restriction was the exclusion of mating between full and half-sibs .All birds were reimmunized with 1 ml 25% SRBC at 80d. of age to measure secondary antibody response.

Hatching eggs were collected from all selected parents (high ,control, and low lines) and were set in a forced air incubator that provided 37.5°c and 60% R.H. in the setter and 36.9°c and 80% R.H. in the hatcher .

On the 18th day of incubation, all eggs were candled to discard infertile (clear) eggs and dead embryos . The remaining fertile eggs were transferred to hatching basket and returned to hatcher

At hatching, all chicks were wing- banded,and placed intermingled in floor pens with wood shaving litter . The chicks were exposed to 33°c during the first 3 days , and then the temperature was reduced gradually by 0.3°c per day till reached 20-22°c at 42 days of age . During the first five weeks of age all chicks were fed a commercial starter breeder ration (20% crude protein and 2800 kcal ME/kg). From 6 weeks to 17weeks of age they were fed commercial growing ration (14% crude protein and 2700 kcal ME/kg),and thereafter received a commercial breeder ration (17% crude protein 2800Kcal ME/kg). Both feed and water were provided with *ad libitum* consumption . During the first three days of age The chicks were exposed to continuous light, then received only natural day light until 17-weeks of age. At 18 weeks of age ,they received 14 hrs of light a day ,and then light period was increased 30 minutes every other week until fixed at 17 hrs daily.

Antibody titer

Blood samples were collected at 3,7 and 10 days post primary and secondary immunization with SRBC to detect antibody titer using microhemagglutination technique (Van der Zijpp and Leenstra, 1980). Antibody titer values were expressed as log2 of the highest serum dilution giving agglutination.

Delayed type hypersensitivity response to Bovine serum albumin(BSA)

Delayed hypersensitivity to the BSA was measured for the selected cocks in each generation of the three lines at 26 wk of age. All cocks were injected with 4 mg BSA in 1ml saline s.c. in the back of the neck , After 7 days ,cocks were reinjected with 1mg BSA in 0.1ml saline into the flat surface of the right wattles whereas the left wattles were injected with 1ml saline and served as a control. At 24h post injection the thickness of both wattles were measured by paper thickness micrometer to calculate the differences in the thickness between saline and BSA injected birds.The DHT response was calculated as a relative response as the following:

$$\text{Relative response} = \frac{\text{Thickness of right wattle (BSA response)}}{\text{Thickness of left wattle (saline response)}}$$

Statistical analysis

The data were analysed by general linear model using (SAS) software package (1996). One-way analysis of variance, using the line as main effect was used as follows :

$Y_{ij} = \mu + L_i + e_{ij}$

Whereas:

Y_{ij} is the observation on the j^{th} bird in the i^{th} line , μ is the overall mean , L_i is the effect due to the i^{th} line and e_{ij} is the random error .

The separation of means was carried using Duncan multiple range test.

Heritability were estimated using mixed model . The data set containing both sexes was analyzed by line and generation using the following model :

$Y_{ijk} = \mu + S_i + X_j + e_{ijk}$

Whereas:

Y_{ijk} is the observation on the k^{th} bird of the i^{th} sire and j^{th} sex.

μ is the overall mean

S_i is the random effect of the i^{th} sire

X_j is the fixed effect of the j^{th} sex.

e_{ijk} is the random error.

RESULTS

Antibody titer

Primary (Table 1) and secondary (Table 2) antibody level against SRBC changed significantly ($P \leq 0.05$) over the course of selection. The divergence of selected lines became gradually larger with each successive generation of selection. Significant differences ($P \leq 0.05$) in antibody titer between High (H) and Low (L) lines were observed at early as three days post primary immunization of both G1 and G2 generations, whereas the control line was intermediate and being significantly higher than low line in the first generation only. But in the second generation, the control (C) line produced a significant

Table 1. Primary antibody titer ±SE against SRBC In H,C, and L lines at different days postimmunization

	Days post immunization								
	3			7			10		
Generation Line	G0	G1	G2	G0	G1	G2	G0	G1	G2
H		2.16±0.07[a]	2.55±0.13[a]		4.14±0.13[a]	6.27±0.23[a]		2.36±0.07[a]	2.21±0.12[a]
C	0.82±0.05	2.08±0.07[a]	2.16±0.11[b]	4.11±0.10	3.63±0.12[b]	5.49±0.21[b]	2.09±0.12	2.25±0.07[a]	2.27±0.11[a]
L		1.68±0.07[b]	1.89±0.09[b]		3.16±0.12[c]	4.56±0.17[c]		1.86±0.07[b]	2.31±0.09[a]

[a,b,c] values with different superscript within days postimmunization and within generation are significantly differ ($p \leq 0.05$)
G0: Base line
G1: First generation after selection
G2:Second generation after selection

Table 2. Secondary antibody titer ±SE against SRBC In H,C, and L lines at different days postimmunization

	Days post immunization								
	3			7			10		
Generation Line	G0	G1	G2	G0	G1	G2	G0	G1	G2
H		2.53±0.07[a]	2.83±0.11[a]		5.09±0.11[a]	5.81±0.16[a]		2.63±0.07[a]	3.51±1.53[a]
C	1.58±0.11	2.34±0.07[ab]	2.49±0.10[a]	5.09±0.17	4.78±0.11[b]	4.96±0.14[b]	3.02±0.14	2.49±0.07[a]	2.46±1.32[a]
L		2.63±0.07[b]	2.21±0.08[b]		4.23±0.11[c]	4.14±0.12[c]		2.29±0.07[b]	3.25±1.12[a]

[a,b,c] values with different superscript within days postimmunization and within generation are significantly differ ($p \leq 0.05$)
G0: Base line
G1: First generation after selection
G2:Second generation after selection

lower antibody titer than H line (Table 1). At 7d. post immunization the gaps were increased between the lines in both generations ,i.e. the H line had significant($P \leq 0.05$) highest antibody levels, also C line had intermediate titer with significant differences($P \leq 0.05$) from both H and L lines. At 10 d. post immunization the differences between lines were only observed in the first generation ,whereas both H and C lines yielded the highest levels than L line but no significant differences were observed between H and C lines. On the other hand, the significant differences between lines disappeared in the second generation.

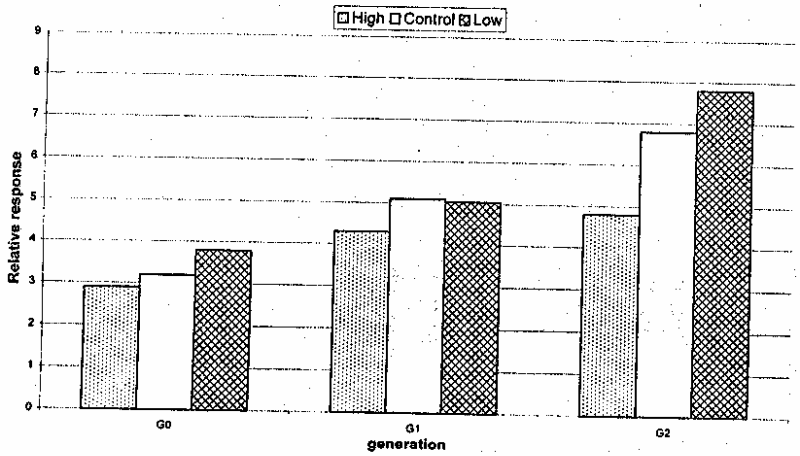

Figure 1. DHT-Test Differences between lines

*Scince no significant differences between lines,the superscript are excluded

The differences between lines in the secondary antibody response against SRBC had similar trend as in the primary titer (Table 2).

Delayed type hypersensitivity (DHT)

DHT is a simple and useful method for assessing *in vivo* cell mediated immunocompetence. The relative response to BSA as a wattle test in selected cocks is shown in Figure 1. There were no significant differences($P \leq 0.05$) between H,C,and L lines .But L line had higher numerical relative response than H line whereas , the control line was intermediate in all generations except in generation one where C line appeared slightly higher relative response than both H and L lines.

Estimated Heritability(h^2)

The heritability was estimated on the basis of half sib analysis for the selected trait (total antibody titer at 7 days post primary immunization with SRBCs).

The total antibody titer 7 days post primary immunization with SRBC was in general less heritable in all lines and the heritability estimates among lines ranged between 0.17 and 0.34. The heritability estimates from the second generation of selection were slightly lower than those from the first generation. (Table 3).

Table 3. Heritability estimates (h^2) for total antibody titer at 7 days post primary immunization of different lines

Generation / LINE	Generation	
	1	2
HIGH	0.22 ± 0.17	0.21 ± 0.19
CONTROL	0.34 ± 0.17	NC[1]
LOW	0.29± 0.09	0.17 ± 0.11

[1]NC= Not calculated due to negative additive genetic variance component

DISCUSSION

Antibody titer

The superiority of H line over L line in the present study is consistent with the results of Pinard *et al.* (1992) who divergently selected ISA Warren cross chickens for their primary antibody titer at 5 days after immunization against SRBC for nine generations. They found that the genetic trend was not linear and the response to selection tended to accelerate over generations, so, selected and control lines differed significantly for primary and secondary responses after three generations. Furthermore the antibody titer after nine generations were 10.6 , 1.94 for H and L line , respectively . In addition, they had yearly genetic gain of 0.48 and –0.44 antibody titer for the H and l line ,respectively.

Similar results were also found when selected lines were done for H and L antibody titer against SRBC (Ubosi *et al.*, 1985, Siegel and Gross 1980 and Kreukniet 1996). Significant differences were found ($P \leq 0.05$) at early generation.

The influence of divergent selection on the function of the immune competence had been documented. Kim *et al.* (1987) reported that immune responsiveness to various antigens like SRBC has been demonstrated to be influenced by non-MHC as well as MHC genes. Biozzi *et al.*, (1979,and 1984) reported that differences in antibody production withen lines selected for high (H) or low (L) antibody production to SRBC were associated with antigen handling and antigen presentation of macrophages and multiplication rate of B lymphocytes. Donker (1989) found that more immunocompetent cells were detected in H line spleen than in L line spleen.In addition, Siegel *et al.* (1992) found a higher B^+ cell percentage at the expense of T cells in the H line spleen and blood as well as a higher $CD4^+$ / $CD8^+$ ratio in the H line spleen than in L line spleen.

Whereas, Kreukniet (1996) reported that selection for low antibody response has favored the $CD8^+$ T cell populations , these cells might be able to suppress the antibody response . Although $CD8^+$ might also reflect a more active cytotoxic defense in the L line . In H line selection might be based on high number of the $CD4^+$ T_{helper} cell phenotype . However, the differences in antibody response might also be the result of differences in cytokine profiles , causing higher number of H line $CD4^+$ to differentiate into putative T_H2 cells and L line $CD4^+$ to differentiate into putative T_H1 cells . These combined effects of selection might have resulted in the high and low anti-SRBC response lines.

Regardless to the differences between lines in primary and secondary response, the antibody titer against SRBC increased gradually after immunization and reached its maximum level at 7 days post immunization and then declined again. This phenomenon is in agreement with that of Kuby (1992) who suggested that the primary response is characterized by lag phase ,during which the B cells undergo clonal selection in response to the antigen and differentiate into Plasma cells and memory cells. The lag phase is followed by a logarithmic increase in serum antibody level, which reaches a peak, plateaus for a variable time , and then declines. In the present study ,the lag phase lasts 3-4 days ; peak levels were attained within 4-5 days and peak serum antibody level are attained by 5-7 days . This time frame allows eight or nine successive cell division within the 4 to 5-day period generating plasma and memory cells. The memory B cells formed during a primary response stop dividing and enter the G_0 phase of the cell cycle. The capacity to develop a secondary response depends on the existence of a population of memory B cells and memory T cells. Antigen activation of these memory cells results in secondary antibody response that can be distinguished from the primary response in several ways: The response occurs more rapidly, reaches a greater magnitude, and lasts for a longer duration. The lag phase lasts only 1-3 days and the magnitude of peak secondary response attained by 3-5 days.

Delayed hypersensitivity type (DHT)

The current results indicating that the absence of a relationship between antibody titer and cell mediated immunity is in partial agreement with Parmentier *et al.* (1994) who reported that there were similarity in immune response to BSA between H and C line after 12 generation of divergent selection. They suggested that selection for an enhance immune response to one antigen (SRBC) may not necessary implicate improvement of immunity to another antigen (BSA). In spite of the fact that after 24 h, the L line differed significantly from the other lines (P? 0.05) and harvested the lowest value , there were no significant differences between H and C line. On other hand , Mashaly *et al.* (1994) showed that the high line had more active T and B cells than low line . This measure was in response to *in vitro* study to Con-A and PWM mitogen.

Estimated heritability(h^2)

The heritability is defined , in the narrow sense , as to the ratio of additive genetic variance to the total phenotypic variance. There are various methods to estimate the heritability such as sire, dam , sire plus dam component of variance and realized heritability. The most important function of the

heritability in the genetic study of metric characters is its predictive role , expressing the reliability of the phenotypic value as a guide to the breeding value of the next generation .

Our results agree with Pinard *et al.* (1992) who estimated the realized heritability of 0.21 and 0.25 in the high and low lines, selected for antibody production against SRBC , they also found that the genetic trend was not linear and the response to selection tended to accelerate over generations . Siegel and Gross (1980) estimated Realized heritabilities for 5-day antibody titers through the S3 generation and were 0.30 in the line H and 0.23 in the line L . Realized heritabilities of 5-day antibody titer for generation S10 through S14 were 0.25 and 0.23 for lines HA and LA, respectively.

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