



Association study of some immunological traits with layer performances in Rhode Island Red chicken lines*

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ABSTRACT

This investigation aimed to study association of some immunological traits with layer performances in Rhode Island Red (RIR) chicken lines at experimental layer farm of this institute. Five to six weeks aged pedigreed RIR chicks of selected and control lines were immunized against 1% (v/v) sterile sheep erythrocytes suspension (SRBC) to assess humoral immune response through haemagglutination (HA) test. Serum lysozyme and immunoglobulin-G (IgG) concentrations were estimated through agarose lysoplate and single radial immunodiffusion assays. HA titre, serum lysozyme and IgG concentrations were classified in high, medium and low levels based on their means and standard deviations. Layer performance data under various immunocompetence levels were analyzed by least squares analysis of variance. Pullets having high HA titre and serum IgG levels laid heavier eggs at 40th week of age than those with medium or low levels of HA titre and serum IgG in the selected line. Again, hens of the selected line containing high and medium serum IgG levels also laid heavier eggs at 28th week than those with low IgG level. Birds of the control line containing high serum IgG level had heavier body weights at 20th week of age than those with medium IgG level. This generated some valuable information that might be used in the genetic improvement programme of the chicken production and protection status.

Key words: Association, Egg and body weight, HA titre, IgG levels, RIR chicken lines, Serum lysozyme

Understanding of the genetics and association of various immunological traits and layer performances provides significant information to be used in programmes for genetic improvement of birds with higher production and protection status. Birds with higher antibody response to sheep erythrocytes antigen suspension produce more antibodies to a variety of antigens (Parmentier *et al.* 1998). The serum lysozyme plays an important role in body's defense against infection and serum IgG is regarded as an indicator of general immune response. The present investigation was undertaken to study association of these immunological traits with some layer performances in the selected and control lines of Rhode Island Red (RIR) chicken maintained at this Institute.

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MATERIALS AND METHODS

Experimental birds and sampling procedures: Single hatched out pedigreed chicks (153) of each RIR selected (79) and control (74) lines, 5- to 6-week-old, maintained at Experimental Layer Farm of this institute were immunized with 1 ml of 1% (v/v) sterile sheep erythrocytes (SRBC) suspension in PBS (pH 7.4). The antigen suspension was *de novo* prepared from venous blood collected from Muzaffarnagari sheep breed maintained at Sheep and Goat Farm, Indian Veterinary Research Institute, Izatnagar (India).

Poultry husbandry adopted: The institute has been rearing exotic Rhode Island Red chicken since her inception in 1979 and segregated as RIR selected and control lines. The institute itself maintains the chicken lines by mating the parental female lines in individual laying cages artificially inseminating with semen collected from the individual sires of respective male line taking records for dam and sire numbers and selected for egg production up to 40 weeks of age along with some independent culling for egg weights at 28th and 40th weeks of age. The day-old chicks were wing banded and pedigreed by sire and dam in the hatchery itself. Standard litter brooding, housing and feeding *ad lib.* on the institute formulated feed were provided with optimum management as elaborated in an

earlier literature (Das *et al.* 2014a). Chicks were vaccinated following standard vaccination schedule being followed at this institute as described in an earlier literature (Das *et al.* 2014b).

Harvesting of immune sera: The hyper immune sera were harvested in 0.5 ml sterile tubes from approximate 1 ml of anticoagulant-free blood collected in 1.5 ml sized sterile tubes on day 5 post immunization (dpi) from jugular/wing vein of the immunized chicks. For this the blood was allowed to clot keeping the tubes slanting way in sun ray. Sera samples were stored at -20°C till further analysis.

Estimation of immunological traits: The humoral immune response of chicks was assessed by estimating *in vivo* antibody response to SRBC through haemagglutination (HA) test (Van der Zijpp and Leenstra 1980). The highest dilution (n) of sera that yielded complete agglutination was recorded as titre and then expressed as $\log_2 n$ (Siegel and Gross 1980). The serum lysozyme concentration was estimated through lysoplate assay (Lie *et al.* 1986) using 1% agarose as solidifying base, in which *Micrococcus lysodieteticus* @ 50 mg / ml of dibasic buffer was added. Lysozyme stock solution was prepared diluting 2 μg standard lysozyme into 1 μl dibasic buffer (0.066 M, pH 6.3). Two-fold serial dilutions of the stock solution were made to get the final concentrations of lysozyme as 40 $\mu\text{g}/\text{ml}$, 20 $\mu\text{g}/\text{ml}$, 10 $\mu\text{g}/\text{ml}$, 5.0 $\mu\text{g}/\text{ml}$, 2.5 $\mu\text{g}/\text{ml}$ and 1.25 $\mu\text{g}/\text{ml}$ as working solutions. These working lysozyme standards were loaded in the wells (5.4 mm diameter) in a row on to the lysoplate in order to plot standard curve. Then 10 mg of each unknown sera sample was loaded in the rest wells on to the lysoplate. A 3% (w/v) agarose gel in 0.1M Tris-HCl was used as solidifying base to assess serum IgG concentrations through single radial immunodiffusion (SRID) assay (Mancini *et al.* 1965) and IgY stock solution was prepared diluting 25 mg chicken IgG (IgY) into 1 ml of 0.1M Tris-HCl. Working standards of 25 $\mu\text{g}/\text{ml}$, 12.5 $\mu\text{g}/\text{ml}$, 6.25 $\mu\text{g}/\text{ml}$, 3.125 $\mu\text{g}/\text{ml}$ and 1.562 $\mu\text{g}/\text{ml}$ concentrations were prepared by serial dilution of the IgY stock solution and loaded in the wells (2.5 mm diameter) in a row to plot standard curve. Five μl of unknown sera was diluted to 4 times with 0.1M Tris-HCl and then 10 μl of each sera sample was loaded in the rest wells. Lysoplate and IgG plates were incubated at 37°C in humidity-controlled chamber for 24 h, thereafter stained with 0.2% coomassie brilliant blue staining solution for 6 hours and excess stain was removed with destaining solution. The diameters of the lysed zones around standards as well as unknown samples were measured with the help of digital vernier calipers. The concentrations (after $\log_2 n$ transformation) of standards were regressed on diameter of the lysed zones around these standards. The slope of the curve and intercept were determined. The serum lysozyme and IgG concentrations in the unknown sera samples were estimated using the regression equation:

$$Y = bx + c$$

where, Y, concentration of serum lysozyme or diluted serum

IgG in unknown sera sample; b, slope of regression equation; c, intercept of regression equation; and x, diameter of the lysed zone around the unknown sera sample.

Layer performances assessed: Layer performances were assessed undertaking the traits of body weights at 20th (BW20) and 40th (BW40) weeks of age, age at first egg (AFE), egg weights at 28th (EW28) and 40th (EW40) weeks and part period egg production up to 40 weeks of age (EP40). Live body weights were measured in grams using digital weighing balance (least count 100 mg) during morning at birds' empty stomach. Egg weights were taken in grams using the digital weighing balance (least count 5 mg) for successive 3 days' records being averaged to a single record. AFE was recorded in days as of laying her first egg and EP40 in numbers was calculated from the production data sheet.

Statistical treatment and analysis: Association between three immunological traits and some layer performances was assessed by least squares analysis of variance (Harvey 1990), for which, HA titre, serum lysozyme and serum IgG concentrations were classified in high, medium and low levels based on their means and standard deviations (SD) and wherein levels of HA titre, serum lysozyme or serum IgG was taken as independent variable in the linear model:

$$Y_{jk} = \mu + I_j + e_{jk}$$

where, Y_{jk} , observation on layer performance on kth individual under jth immunocompetence level; μ , population mean; I_j , fixed effect of jth immunocompetence level; and e_{jk} , random error associated with mean zero and variance σ^2 . Critical Difference (CD) test at the 5% level of probability of significance was performed for assessing critical differences between the least squares means under individual immunocompetence level.

RESULTS AND DISCUSSION

Overall means $\pm$ SD of HA titre, serum lysozyme and serum IgG concentrations were estimated as 9.55 ± 4.06 , 5.69 ± 2.93 $\mu\text{g}/\text{ml}$ and 6.98 ± 2.95 $\mu\text{g}/\text{ml}$, respectively. Analysis revealed significant ($P < 0.05$) influence of two immunological traits on the estimates of some layer performance traits. HA titre and serum IgG levels demonstrated its significant ($P < 0.05$) association with egg weights in the selected line; whereas body weights at 20th week of age of the birds of the control line had association ($P < 0.05$) with the birds' serum IgG levels. Least squares means estimates of egg weights at 28th and 40th weeks of age had some influences ($P < 0.05$) of the birds' serum IgG levels, whereas the estimate of egg weight at only 40th week of age only was found to be influenced ($P < 0.05$) by the birds' HA titre level. Hens of the selected line containing high HA titre and serum IgG levels laid heavier ($P < 0.05$) eggs at 40th week of age than those with medium or low levels of HA titre and serum IgG. Again pullets having high and medium serum IgG levels also laid heavier ($P < 0.05$) eggs at 28th week of age than those with low IgG level in the selected line (Table 1). But birds of the control line

Table 1. Least squares means of various layer performance traits under various immunocompetence levels in RIR selected and control line chickens

Factors	Obs.	Least squares means $\pm$ standard errors					
		BW20 (g)	AFE(days)	EW28 (g)	BW40 (g)	EW40 (g)	EP40(nos.)
RIR selected line							
<i>Haemagglutination (HA) titre levels</i>							
Low	8	1660.0 $\pm$ 84.0	157.3 $\pm$ 6.8	43.01 $\pm$ 1.30	1775.0 $\pm$ 60.2	50.66 $\pm$ 1.22 ^b	88.9 $\pm$ 6.5
Medium	27	1627.0 $\pm$ 45.7	155.8 $\pm$ 3.7	44.56 $\pm$ 0.71	1743.0 $\pm$ 32.8	52.10 $\pm$ 0.66 ^b	97.1 $\pm$ 3.5
High	5	1690.0 $\pm$ 106.2	156.2 $\pm$ 8.6	47.40 $\pm$ 1.65	1810.0 $\pm$ 76.2	55.66 $\pm$ 1.54 ^a	93.4 $\pm$ 8.2
<i>Serum lysozyme concentration levels</i>							
Low	4	1692.5 $\pm$ 115.5	152.3 $\pm$ 9.6	47.68 $\pm$ 1.86	1875.0 $\pm$ 80.3	54.58 $\pm$ 1.82	97.8 $\pm$ 9.3
Medium	32	1656.6 $\pm$ 40.8	156.9 $\pm$ 3.4	44.39 $\pm$ 0.66	1761.3 $\pm$ 28.4	52.10 $\pm$ 0.64	94.4 $\pm$ 3.3
High	4	1470.0 $\pm$ 115.5	153.5 $\pm$ 9.6	43.23 $\pm$ 1.86	1612.5 $\pm$ 80.3	51.25 $\pm$ 1.82	97.0 $\pm$ 9.3
<i>Serum IgG concentration levels</i>							
Low	6	1530.0 $\pm$ 95.4	154.7 $\pm$ 7.9	40.98 $\pm$ 1.43 ^b	1683.3 $\pm$ 68.8	49.67 $\pm$ 1.39 ^b	93.0 $\pm$ 7.6
Medium	28	1663.6 $\pm$ 44.2	156.6 $\pm$ 3.7	44.95 $\pm$ 0.66 ^a	1764.6 $\pm$ 31.8	52.21 $\pm$ 0.64 ^b	94.6 $\pm$ 3.5
High	6	1650.0 $\pm$ 95.4	155.3 $\pm$ 7.9	46.60 $\pm$ 1.43 ^a	1800.0 $\pm$ 68.8	55.08 $\pm$ 1.39 ^a	98.8 $\pm$ 7.6
IR control line							
<i>Haemagglutination (HA) titre levels</i>							
Low	12	1175.8 $\pm$ 44.2	191.4 $\pm$ 3.8	41.28 $\pm$ 1.22	1435.0 $\pm$ 43.5	48.84 $\pm$ 1.35	52.2 $\pm$ 5.8
Medium	23	1234.8 $\pm$ 31.9	191.7 $\pm$ 2.7	41.72 $\pm$ 0.88	1486.1 $\pm$ 31.4	49.38 $\pm$ 0.97	62.7 $\pm$ 4.2
High	7	1147.1 $\pm$ 57.9	195.6 $\pm$ 4.9	40.83 $\pm$ 1.59	1447.1 $\pm$ 56.9	48.21 $\pm$ 1.76	55.9 $\pm$ 7.6
<i>Serum lysozyme concentration levels</i>							
Low	5	1208.0 $\pm$ 70.4	188.2 $\pm$ 5.8	39.64 $\pm$ 1.86	1426.0 $\pm$ 67.8	47.46 $\pm$ 2.06	62.8 $\pm$ 9.1
Medium	32	1204.7 $\pm$ 27.8	193.5 $\pm$ 2.3	41.85 $\pm$ 0.73	1473.8 $\pm$ 26.8	49.02 $\pm$ 0.82	57.0 $\pm$ 3.6
High	5	1190.0 $\pm$ 70.4	188.2 $\pm$ 5.8	40.66 $\pm$ 1.86	1448.0 $\pm$ 67.8	50.68 $\pm$ 2.06	64.4 $\pm$ 9.1
<i>Serum IgG concentration levels</i>							
Low	4	1205.0 $\pm$ 72.3 ^{ab}	188.8 $\pm$ 6.5	43.65 $\pm$ 2.06	1472.5 $\pm$ 75.8	50.18 $\pm$ 2.31	64.5 $\pm$ 10.1
Medium	32	1175.6 $\pm$ 25.6 ^b	193.0 $\pm$ 2.3	40.97 $\pm$ 0.73	1457.2 $\pm$ 26.8	48.61 $\pm$ 0.82	59.7 $\pm$ 3.6
High	6	1350.0 $\pm$ 59.0 ^a	190.7 $\pm$ 5.3	42.53 $\pm$ 1.69	1501.7 $\pm$ 61.9	50.55 $\pm$ 1.88	48.7 $\pm$ 8.2

Means within an immunological trait having different superscripts differ significantly ($P < 0.05$). BW20, body weight in grams at 20th week of age; AFE, age at first egg in days; EW28, egg weight in grams at 28th week; BW40, body weight in grams at 40th week; EW40, egg weight in grams at 40th week; and EP40, part period egg production in numbers up to 40 weeks of age.

containing high serum IgG level had higher ($P < 0.05$) body weights at 20th week of age than those having medium IgG level (Table 1).

Significant influence of the immunological traits on some layer performances observed in the present study was of interest and reports in this regard were limited. Van der Zijpp and Nieuwland (1986) reported higher egg number and egg weight for those birds containing high HA titre than low HA titre in ISA Warren chicken line. Moreover, the birds with lower body weight at 38th week of age contained high HA response to SRBC than the control and low HA response (Parmentier *et al.* 1998) and this was on contrary to the another report of non-significant influence of HA titre on any performance traits in CARI-Debandra crossbred chicken (Das *et al.* 2014b). In the present study serum lysozyme levels had no significant effect on any performance traits also on contrary to the earlier report of Das *et al.* (2014b) who obtained comparatively heavier body weights of the birds containing low or medium serum lysozyme level at 40th week of age than the birds having high lysozyme level ($P < 0.05$). But the present findings in respect to serum IgG levels were in the line of earlier report of egg weight heavier ($P < 0.05$) for those hens containing

high serum IgG level than low or medium IgG level (Das *et al.* 2014b). The present investigation thrive a research with a large number of samples to accurately associate immunocompetence levels with layer performance traits in more accuracy.

The present investigation concluded that if improved the immunocompetence levels of the layer birds, then concurrently its housing body weights and egg size would also improve. Thus layer production and protection status would be nourished.

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