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Genetic analysis of immunocompetence and growth performance in a selected strain of Rhode Island Red chicken#

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ABSTRACT

This investigation was aimed to assess genetics of immunocompetence (IC) and growth performances in a selected strain of Rhode Island Red (RIR) chicken maintained at Central Avian Research Institute, Izatnagar. Five weeks aged pedigreed RIR chicks (265) were immunized against 1% (v/v) sheep red blood cells (SRBCs) suspension and 5th day post immunization (dpi) haemagglutination (HA) titre, serum lysozyme and immunoglobulin G (IgG) concentrations were estimated. Data on these IC traits and body weights of different age groups were analyzed by least squares ANOVA taking sire as random, sex and IC levels as fixed effect in the linear model. Analysis revealed that sire had significant effect on serum IgG concentration, and both the sire and sex had also significant effect on body weights. The heritability estimates were 0.302±0.188, 0.516±0.22, 0.468±0.219, 0.302±0.188 and 0.344±0.21 for serum IgG, body weights at 16th, 20th, 40th and 64th week of age, respectively. The serum IgG concentration had moderate to high genetic correlations with the body weights of different age groups in positive trend. HA titre showed negative low phenotypic correlations with serum lysozyme and IgG concentrations. Birds with low serum IgG level had significantly the highest body weights at 40th and 64th week of age than those having medium or high levels. The results indicated that RIR birds' IC is sire-influenced and may also impact on birds' body weights.

Key words: Body weights, Correlation, Heritability, Immunocompetence, RIR Chicken, Sire effect

A growing global population combined with factors such as changing socio-demographics causes increased meat consumption worldwide, where demand for chicken is increasing day by day due to its wide acceptance compared to red meat as driven by increased health and dietary awareness (Henchion *et al.* 2017). Modern commercial strains of layers and broilers are the results of long-term selection programs based on phenotypes (Saxena and Kolluri 2018). Poultry breeders have consistently being applying selection pressure on economic traits in high yielding populations which led to severe decline in immune response capabilities and increased susceptibility to numerous diseases (Tomar *et al.* 2012). Indirect genetic selection for better IC traits provides an effective alternative approach because the progress per generation is cumulative, heritable, conserves natural resources and avoids repeated expenditure on medicines and vaccines (Gupta *et al.* 2010). Therefore, the present investigation was carried out aiming at genetic analysis of RIR birds' IC and body weights after long term selection to generate information for chalking out future breeding programs for simultaneous improvement of production as well as protection status.

MATERIALS AND METHODS

Two hundred and sixty five single hatched out pedigreed chicks of 25 sires of a selected strain of RIR maintained at the Experimental Layer Farm, ICAR-Central Avian Research Institute, Izatnagar were investigated. The population undergone long-term selection program based on part-period egg production covering 30 generations of selection. The chicks were raised on deep litter, thereafter only pullets in cages under standard management and nutritional practices (Rahim et al. 2016).

The chicks at 5th week of age were immunized by injecting intravenously 1 ml of 1% (v/v) Muzaffarnagari sheep red blood cells (SRBCs) suspension in phosphate buffer saline (pH 7.2) (Das *et al* 2016a). The immune sera were harvested after 5th day post-immunization (dpi) and stored at –20°C for further analysis. The *in vivo* antibody

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response against the inoculated SRBCs antigenic suspension was assessed by Haemagglutination (HA) test (Van der Zijpp and Leenstra 1980). The highest dilution (n) of sera that yielded complete agglutination was recorded as HA titre and expressed as $\log_2 n$ (Siegel and Gross 1980). The serum lysozyme concentrations were determined by Lysoplate assay (Lie *et al.* 1986) using *Micrococcus lysodieketicus* (Sigma, USA) as substrate on 1% agarose in dibasic buffer. The standard curve was prepared by plotting $\log_2$ values of known lysozyme (SRL, India) concentrations (2 µg/µl in dibasic buffer and two-fold serially diluted) against the diameter of the lysed zones. The serum immunoglobulin G (IgG) concentrations were determined by Single Radial Immunodiffusion (SRID) assay (Mancini *et al.* 1965). The standard curve was prepared by plotting $\log_2$ values of known chicken IgY (Rabbit anti-chicken IgG) (GenScript, USA) concentrations (4 mg/ml in 0.1M tris-HCl and two-fold serially diluted) against the diameter of the precipitation ring. The serum lysozyme and IgG concentrations in the unknown sera samples were determined using regression equation:

$$Y = bx + c$$

where, Y, concentration of serum lysozyme/IgG in unknown sera sample; b, slope of regression equation; c, intercept of regression equation and x, diameter of the lysed/precipitation zone around the unknown samples.

The body weights of both male and female birds were recorded at 16^{th}, 20^{th}, 40^{th} and 64^{th} week of age using digital weighing balance and measures were made during morning hour before feeding.

Statistical treatment: The data were analyzed by least squares analysis of variance (Harvey 1990) incorporating sire as random and sex as fixed effects in the linear model:

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

where, Y_{ijk}, observation on k^{th} individual belonging to i^{th} sire and j^{th} sex; µ, population mean; S_i, random effect of i^{th} sire; W_j, fixed effect of j^{th} sex; and e_{ijk}, random error associated with mean zero and variance σ^2. Genetic parameters of IC and growth traits were estimated using parental half-sib correlation methods (Becker 1975). Again, the IC traits were classified as high group which had estimates on and above mean±standard deviation, low group included those on and below mean ± standard deviation and medium group which had estimates between high and low groups. Data on body weights were further analyzed taking IC levels as independent variable in the statistical model. Critical difference (CD) test at 5% level of probability of significance was performed for assessing critical differences among the least squares means under each IC trait.

RESULTS AND DISCUSSION

The estimated least squares means of different traits of IC, i.e. HA antibody titre, serum lysozyme and serum IgG concentrations, body weights at 16^{th}, 20^{th}, 40^{th} and 64^{th} week of age are presented in Table 1. The higher HA titre indicates presence of more total serum antibody, i.e. immunoglobulins IgG, IgM, IgE, IgD and IgA produced till 5 dpi when the chicks were challenged with SRBCs antigens; and the greater the titre value better the antibody response (Das *et al.* 2016a). More the concentration of lysozyme in the body fluids, stronger the first line defense to protect the birds being infected by bacteria (Gram negative) (Das *et al.* 2016a). More serum IgG concentration also indicates birds' better general immune response against any antigen (Das *et al.* 2016a). The present estimated least squares means of 5 dpi HA titre, serum lysozyme and serum IgG concentrations might be compared to the earlier reports in RIR chicken (Das *et al.* 2016a, Das *et al.* 2015, Rahim *et al.* 2015), and divergently selected strains of White Leghorn for humoral response to SRBCs suspension (Gupta *et al.* 2010). However, the present estimates were relatively lower than the reports in Aseel (Kokate *et al.* 2017), Kadaknath (Kokate *et al.* 2017, Jaiswal *et al.* 2014) and White Leghorn chicken (Kokate *et al.* 2017), but higher than the reports in different RIR crosses namely CARI-Debendra (Das *et al.* 2014a) and CARI-Sonali (Das *et al.* 2014b). Whatever discrepancy is present in various reports might be due to different genetic make-up of different stocks, age at immunization, day post immunization for sera collection and environmental factors.

Analysis revealed that sire component of variance (Table 1) was mostly highly significant (P≤0.01) for the estimates of serum IgG concentration, and body weights at 16^{th}, 20^{th},

Table 1. Estimated least squares means along with different genetic factors of different traits of immunocompetence and body weights at different age groups in a selected strain of Rhode Island Red chicken

Factor	Obs.	Least squares means±standard error						
		HA titre	Lysozyme (µg/ml)	Serum IgG (µg/µl)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)
Means	253	9.35±0.29	4.64±0.11	8.61±0.34	1364.41±21.96	1800.48±25.51	2185.21±25.58	2440.19±34.68 (219)
Sex	253	ns	ns	ns	***	***	***	***
Male	143	9.39±0.22	4.77±0.15	8.56±0.42	1504.56±25.08[a]	2069.07±29.37[a]	2626.70±31.19[a]	2804.00±42.52[a] (117)
Female	110	9.33±0.34	4.51±0.16	8.67±0.45	1224.26±26.83[b]	1531.89±31.53[b]	1743.73±34.09[b]	2076.38±44.42[b] (102)
Sire	25	ns	ns	**	***	***	*	**

Obs., number of observations; ns, non-significant (P>0.05); *P≤0.05, **P≤0.01, ***P≤0.001; Means within a factor having different superscripts in a column differ significantly (P≤0.05); Figures within parentheses denote number of observations.

40th (P≤0.05) and 64th week of age indicating sire-inheritance of these traits which would be utilized in selection program. In the similar line of these present findings, Das *et al.* (2016a) reported significant sire effect on the estimates of serum lysozyme and serum IgG concentrations in RIR chicken, and Sivaraman *et al.* (2005) reported for HA titre in synthetic dam line of broiler chicken. Earlier authors also reported significant sire effects on different body weight estimates in different chicken genotypes (Das *et al.* 2017, Das *et al.* 2016b, Kumar *et al.* 2016, Debnath *et al.* 2015) rather than dam effect (Das *et al.* 2017). Analysis revealed that sex component of variance was non-significant for all the IC traits in consistence with the earlier reports in different chicken genotypes (Kokate *et al.* 2017, Das *et al.* 2014a&b, Sivaraman *et al.* 2005). It could be infered that the IC traits might be sex-independent, although, males had higher (P>0.05) estimates of antibody titre and serum lysozyme concentration than the females, while reverse trend was followed for serum IgG concentration in the present study. Similar findings were also reported in RIR (Das *et al.* 2016a, Das *et al.* 2015), Aseel (Kokate *et al.* 2017), Kadaknath (Kokate *et al.* 2017, Jaiswal *et al.* 2014), and WLH chicken ((Kokate *et al.* 2017, Gupta *et al.* 2010). The sex-component of variance was highly significant (P≤0.001) for the body weight estimates at different age groups with males being heavier than the females throughout the ages in the line of earlier reports (Das *et al.* 2017, Rahim *et al.* 2016, Eid *et al.* 2010) indicating significant sex-differences and counter role of sex, a genetic factor to control the growth of body mass.

Results of the association study (Table 2) envisaged significant (P≤0.05) effect of serum IgG levels on body weight estimates at 40th and 64th week of age. It was observed that birds with low level of serum IgG concentration had the highest body weights followed by the birds with high and medium levels of IgG concentrations. HA titre and serum lysozyme levels could not demonstrate any significant (P>0.05) effect on any body weight estimate. Although, Parmentier *et al.* (1998) reported that chicken lines with high immune response to SRBCs had significantly lower body weight at 38 weeks of age than the control and low lines. Eid *et al.* (2010) also reported that birds with high antibody titre had lower body weight as compared to the birds with low antibody titre levels in ISA Hubbard and Ross 308 broiler chicken. Das *et al.* (2014a) reported that CARI-Debendra chicken having low or medium serum lysozyme level achieved comparatively heavier (P≤0.05) body weight at 40 weeks of age than the birds having high lysozyme level. Das *et al.* (2015) also reported that birds with high serum IgG levels had significantly (P≤0.05) more body weight at 20th week of age than those having medium and low IgG level in control line of RIR chicken.

The heritability estimates of IC and growth traits and correlations among different traits are presented in Table 3. The heritability estimates for body weights at different age groups and serum IgG concentration were moderate to high in magnitude, h^2 for HA titre and serum lysozyme could not be estimated. Similar magnitude of heritability estimates for body weights were also reported earlier (Das *et al.* 2017, Kumar *et al.* 2016, Rahim *et al.* 2016, Qadri *et al.* 2013). Moderate to high level of heritability estimates for different IC traits were earlier reported in RIR chicken (Das *et al.* 2016a), Kadaknath (Jaiswal *et al.* 2014) and low heritability in HSRBC line of White Leghorn (Gupta *et al.* 2010). The present heritability estimates for serum IgG and body weights could indicate that effective improvement would be achieved using combined selection based on individual's own records and family performance for IC as well as growth parameters.

Results of correlation coefficients (Table 3) revealed that the serum IgG concentration demonstrated moderate to high genetic correlations (r_G) with the body weights of different age groups in positive trend. HA titre showed negative low

Table 2. Estimated least squares means of body weights at different age groups under different immunocompetence levels in a selected strain of Rhode Island Red chicken

Factor	N	Least squares means±standard error			
		BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)
HA titre levels					
Low	49	1348.55±39.15	1774.06±46.27	2217.76±50.60	2465.74±67.68 (40)
Medium	180	1364.90±30.13	1807.14±35.17	2180.77±35.01	2432.24±46.26 (159)
High	24	1393.37±50.15	1802.76±59.69	2152.71±68.27	2453.16±89.965 (20)
Serum lysozyme levels					
Low	35	1372.22±43.11	1812.39±50.88	2125.51±57.25	2435.44±72.30 (34)
Medium	182	1364.01±28.48	1795.51±32.80	2185.81±32.59	2436.53±45.36 (155)
High	36	1358.57±43.20	1815.09±50.99	2242.98±57.40	2465.10±76.93 (30)
Serum IgG levels					
Low	35	1379.52±43.29	1832.50±51.13	2303.89±57.78^{a}	2588.41±74.36 (30)^{a}
Medium	184	1359.77±28.29	1785.72±32.78	2161.85±33.13^{b}	2400.99±42.85 (156)^{b}
High	34	1373.88±43.82	1847.03±51.78	2189.71±58.62^{b}	2492.95±72.33 (32)^{ab}

N, number of observations; Means within a factor having different superscripts in a column differ significantly (P≤0.05); Figures within parentheses denote number of observations.

Table 3. Estimated heritability (values marked with aestriks), genetic (above diagonal) and phenotypic (below diagonal) correlations among different traits of immunocompetence and body weights at different age groups in a selected strain of Rhode Island Red chicken

Trait	HA titre	Serum lysozyme	Serum IgG	BW16	BW20	BW40	BW64
Serum IgG	–0.088	0.005	0.302±0.188*	0.162±0.395	0.302±0.392	0.928±0.459	0.796±0.507
BW16	0.033	0.049	0.015	0.516±0.22*	1.050±0.049	1.086±0.179	0.972±0.168
BW20	0.029	0.049	0.064	0.786	0.468±0.219*	1.047±0.131	0.829±0.193
BW40	–0.033	0.108	–0.005	0.552	0.691	0.302±0.188*	1.011±0.117
BW64	–0.037	0.026	–0.002	0.521	0.581	0.752	0.344±0.21*

Number of observations was 219 for each estimate.

phenotypic correlations (r_P) with serum lysozyme (–0.097) and IgG concentrations (–0.088) while serum lysozyme had very low positive phenotypic correlation with serum IgG concentration (0.005). Sivaraman *et al.* (2005) had also reported low to high level of negative genetic correlations of serum IgG concentration with body weights at four, five and six weeks of age in synthetic dam line of broiler chickens associated with high standard errors. Saini *et al.* (2007) reported positive genetic correlations between HA titre and body weights at 12, 24, 28 and 40 weeks of age in a selected strain of White Leghorn. Jaiswal *et al.* (2014) reported negative r_G among IC traits associated with high standard errors. Various researchers also reported lower phenotypic correlations (r_P) among IC traits in various chicken breeds (Das *et al.* 2016a, Das *et al.* 2014a&b, Jaiswal *et al.* 2014, Singh *et al.* 2010). HA titre had low and negative phenotypic correlation with BW40 and BW64, and positive r_P with BW16 and BW20. Serum lysozyme concentration had low positive phenotypic correlation with body weights at all age groups. HA titre and Serum IgG concentration had positive and low phenotypic correlation with early body weights (BW16 and BW20) and very low and negative correlation with older body weights (BW40 and BW64). Kundu (1997) reported inconsistent trend either in direction or magnitude of r_P for different IC traits with growth traits. The genetic and phenotypic correlations among body weights at all age groups were positive and higher in magnitude. Similar trends were also reported by various researchers (Kumar *et al.* 2016, Rahim *et al.* 2016, Rajkumar *et al.* 2011, Barot *et al.* 2008).

From the present investigation it can be concluded that RIR chicken after long term selection based on part period egg production demonstrated better IC status. Serum IgG concentration and body weights at different age groups had sire-inheritance, and moderate to high magnitude of heritability and genetic correlations. Again birds with low serum IgG level were heavier followed by the birds with high and medium IgG levels. Thus this study generated useful information for chalking out the inheritance pattern of IC and growth traits and incorporation of such traits into the breeding program for improvement of both production as well as protection status.

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