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Investigating immunocompetence in Aseel, Kadaknath and White Leghorn chicken

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ABSTRACT

The study aimed to investigate immunocompetence in Aseel, Kadaknath and IWH line of White Leghorn chicken maintained at this institute. Five to six weeks aged pedigreed 75, 55 and 100 chicks progenies of 8, 8 and 20 sires of these respective genotypes were immunized against 1% (v/v) SRBC suspension. Five dpi sera were analyzed through haemagglutination (HA) test to assess humoral immune response. Serum lysozyme and immunoglobulin-G (IgG) concentrations were assessed through agarose lysoplate and single radial immunodiffusion assay, respectively. The data were analyzed by least squares analysis of variance. The estimated corresponding means of HA titre, serum lysozyme and serum IgG concentrations were 10.84±0.18, 21.28±0.78 µg/ml and 24.23±2.05 µg/µl in Aseel, 11.62±0.21, 16.91±0.93 µg/ml and 15.70±2.44 µg/µl in Kadaknath, and 11.94±0.15, 9.42±0.68 µg/ml and 10.27±1.79 µg/µl in IWH line of White Leghorn chicken. The estimates significantly varied among the three genotypes. The HA titre mean was higher in IWH line of WLH followed by Kadaknath and Aseel chicken; whereas serum lysozyme and serum IgG concentration means were higher in Aseel followed by Kadaknath and IWH line. Sire within genotype or sire and sex of the birds had nonsignificant effect on the traits. This information could be important while selecting birds for improvement in general immune responsiveness and the high immunocompetence in Aseel and Kadaknath might be exploited by their selective introgression in high productive but less immunocompetent chicken germplasms.

Key words: Aseel, HA titre, Kadaknath, Serum IgG, Serum lysozyme, White Leghorn chicken

There are several traits of immunocompetence, which can be considered for improving genetic resistance to diseases in poultry. The immune system in the birds with higher antibody response to sheep erythrocytes antigenic suspension produces more antibodies to a variety of antigens (Parmentier *et al.* 1998). Birds' serum lysozyme also plays an important role in their body's defense against infection and serum IgG is regarded as an indicator of the birds' general immune response (Das *et al.* 2015). Genetic improvement in the immunocompetence is a target of poultry improvement programmes for the future and knowledge of its genetic and non-genetic kinetics is the prerequisite for any such programme. Hence, the present study was undertaken to investigate immunocompetence in Aseel, Kadaknath and IWH line of White Leghorn chicken.

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MATERIALS AND METHODS

Experimental birds and husbandry: Single hatched out 5 to 6 weeks aged 75, 55 and 100 pedigreed chicks progenies of 8, 8 and 20 sires of Aseel, Kadaknath and IWH line of White Leghorn chicken, respectively, maintained at experimental farms of this institute was used in this study. The day-old chicks were wing banded and pedigreed by sire and dam in the hatchery itself along with following a standard vaccination schedule being followed at this institute (Das *et al.* 2014a,b). Standard litter brooding, housing and *ad lib.* feeding were provided with optimum management and birds were fed on the institute-formulated chick mash at 0–8 weeks of age followed by grower mash at 9–20 weeks and layer mash at 20 weeks onwards (Das *et al.* 2014a).

Measurement of immunocompetent traits: The immunocompetent traits, viz. humoral immune response to sheep erythrocytes (SRBC), serum lysozyme concentration and serum IgG concentration were assayed at an age of 5–6 weeks. The *in vivo* antibody response to SRBC was determined by injecting intravenously 1 ml of 1% SRBC suspension (Das *et al.* 2014a, 2015, 2016) in phosphate buffer saline (pH 7.2) to each bird. The immune sera were collected on 5 days post-immunization (dpi). The

antibody titres against the inoculated SRBC antigenic suspension were determined by haemagglutination (HA) test (Van der Zijpp and Leenstra 1980) and expressed as $\log_2$ values for the reciprocal of the highest titre where complete agglutination was observed (Siegel and Gross 1980). The serum lysozyme concentrations were determined by the lysoplate assay (Lie *et al.* 1986) using 1% agarose as solidifying base. Lyophilized *Micrococcus lysodeikticus* was used @ 50 µg/ml of dibasic buffer (pH 6.3) required for the agarose gel. Two-fold serial dilutions of the standard lysozyme stock solution (2 µg standard lysozyme diluted into 1 µl dibasic buffer) were made to get the final concentrations of 40, 20, 10, 5.0, 2.5 and 1.25 mg/ml as working solutions. These working lysozyme standards were loaded in the wells in a row onto the lysoplate. The standard curve was prepared by plotting $\log_2$ values of known lysozyme concentrations against the diameter of the lysed zones and the serum lysozyme concentrations of unknown samples were estimated with the help of this standard curve (Das *et al.* 2015, 2016). The serum IgG concentrations were estimated by the single radial immunodiffusion (SRID) assay (Mancini *et al.* 1965) using 3% (w/v) agarose in 0.1M tris-HCl as solidifying base. Rabbit anti-chicken IgG was used @ 35 µl/ml of 0.1M tris-HCl required for the gel. The standard chicken IgG (IgY) stock solution was prepared diluting 25 mg IgY into 1 ml 0.1M Tris-HCl. Working standards of 25, 12.5, 6.25, 3.125and 1.562 mg/ml concentrations were prepared by serial dilution of the IgY stock solution and loaded in the wells in a row onto the gel. The standard curve was prepared by plotting $\log_2$ values of known chicken IgG concentrations against the diameter of the precipitation ring and the concentrations in unknown samples were determined (Das *et al.* 2015, 2016).

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 kth individual belonging to ith sire and jth sex; µ, population mean; S_i, random effect of ith sire; W_j, fixed effect of jth sex; and e_{ijk}, random error associated with mean zero and variance σ^2. The data after pooling over the chicken genotypes were also analyzed by least squares ANOVA taking sire within genotype as random, and genotype and sex as fixed effects, followed by Critical Difference test (Stevens 1999) to assess critical differences in the least squares means under different genotypes.

RESULTS AND DISCUSSION

The estimated least squares means of the immunocompetent traits i.e. haemagglutination (HA) antibody titre, serum lysozyme and serum immunoglobulin-G (IgG) concentrations are presented in Table 1.

The present estimated least squares means of HA antibody titre, serum lysozyme and serum IgG concentrations were relatively higher as evident when compared to the available reports in RIR chicken (Das *et al.* 2014a, 2015, 2016), CARI-Debendra (Das *et al.* 2014b), CARI-Sonali (Das *et al.* 2014c), White Leghorn chicken lines (Gupta *et al.* 2010), Dahlem Red chicken populations (Chatterjee *et al.* 2007) and synthetic dam line of broiler chicken (Sivaraman *et al.* 2005). The present HA titre estimates might be compared to the reports in RIR selected line (Das *et al.* 2015, 2016) and Aseel chicken (Kumar and Kumar 2011), whereas the estimates of serum lysozyme and serum IgG concentrations were in the higher line as reported in Aseel by Kumar and Kumar (2011). The differences in the estimates might be due to the different native and exotic breed, strain or line studied.

The least squares analysis of variance revealed that the present 3 chicken genotypes significantly (P<0.01) varied

Table 1. The estimated least squares means of various immunocompetent traits in Aseel, Kadaknath and IWH line of White Leghorn chicken

Factor		Least squares means ± standard errors		
		Haemagglutination (HA) antibody titre	Serum lysozyme concentration (µg/ml)	Serum IgG (µg/µl) concentration
Chicken genotypes		**	**	**
Aseel chicken		10.84±0.18^b (73)	21.28±0.78^a (73)	24.23±2.05^a (73)
Kadaknath chicken		11.62±0.21^a (52)	16.91±0.93^b (52)	15.70±2.44^b (52)
IWH line of WLH chicken		11.94±0.15^a (96)	9.42±0.68^c (96)	10.27±1.79^c (96)
Sex: Aseel birds		ns	ns	ns
	Male	10.68±0.27 (41)	22.08±1 .09 (41)	21.83±4.52 (41)
	Female	11.02±0.31 (32)	20.38±1.20 (32)	27.10±5.08 (32)
Sex: Kadaknath birds		ns	ns	ns
	Male	11.93±0.45 (20)	18.84±2.46 (20)	16.88±2.20 (20)
	Female	11.44±0.39 (32)	15.41±2.02 (32)	15.23±1.80 (32)
Sex: IWH line of WLH birds		ns	ns	ns
	Male	11.96±0.21 (41)	10.14±0.68 (41)	10.57±0.67 (41)
	Female	11.93±0.18 (55)	8.68±0.60 (55)	8.68±0.60 (55)

**P<0.01; ns, nonsignificant (P>0.05); figures within parenthesis denote number of observations; means within a factor and same column with different superscripts differ significantly (P<0.05).

in the estimated means of the 3 immunocompetent traits in accordance to the earlier reports (Das *et al.* 2014a, 2016). The estimated HA titre mean was higher (P<0.05) in IWH line of WLH followed by Kadaknath (P>0.05) and Aseel chicken (P<0.05); whereas the means of serum lysozyme and serum IgG concentrations were higher (P<0.05) in Aseel followed by Kadaknath (P<0.05) and IWH line (P<0.05) indicating better immunocompetence in indigenous chicken populations, though HA titres were lesser than in IWH line but more closer in figure. The HA titre demonstrates presence of total serum antibody (all types of immunoglobulins) produced when the chicks were challenged with SRBC antigens, and the greater titre value indicated better antibody response; the greater IgG level also indicated better general immune responsiveness (Das *et al.* 2016). It is notable that neither egg nor developing embryo produces immunoglobulins until about 7 days before hatching (Jaiswal 2009) and the hatchling is endowed with maternal IgG in the last few days before hatching (Kowalczyk 1985). Hence, egg has high lysozyme content to maintain vigilance until the embryo has developed the capacity to produce immunoglobulins. The lysozyme is present in various body fluids (Jaiswal 2009) and acts as a first line defense against infection. Aseel with higher serum lysozyme and serum IgG contents might be a more immunocompetent native chicken as demonstrated by this investigation.

The analysis also revealed that the sire within genotype or sire and sex of the birds could not significantly (P>0.05) influence the estimates of the traits. Das *et al.* (2016) also reported nonsignificant sire-effect when estimated means of these traits in RIR control line, though it was significant for serum lysozyme and serum IgG concentrations in RIR selected line. Previously, Sivaraman *et al.* (2005) reported that HA titre was significantly affected by sires when studied immunocompetence in synthetic dam line of broiler chicken.

The present findings of nonsignificant effect of sex in the estimates of the traits were in consistence with the earlier reports (Das *et al.* 2016, 2014b,c, Gupta *et al.* 2010, Sivaraman *et al.* 2005). Although statistically nonsignificant, the means of HA titre and serum IgG concentration were higher in females in Aseel, whereas all the estimated means were higher in males in Kadaknath and IWH line of WLH chicken; thus serum lysozyme estimates were higher in males uniformly in the 3 chicken genotypes. But females with higher (P>0.05) estimates of serum lysozyme concentration were reported earlier in RIR control and selected lines (Das *et al.* 2016). Females with higher (P>0.05) estimates of the 3 traits in RIR control line and males with higher (P>0.05) estimates of HA titre and serum IgG in RIR selected line were also reported (Das *et al.* 2016). Whereas, males with higher (P>0.05) estimates of the three traits were reported earlier in CARI-Debendra chicken (Das *et al.* 2014b). Males with higher (P>0.05) estimates of HA titre and serum lysozyme were also reported in CARI-Sonali chicken (Das *et al.* 2014c). These current results indicated that sex could not follow any specific trend for any immunocompetent trait and the genetic mechanisms responsible for mounting of antibody response to SRBC and regulation of the serum lysozyme and serum IgG levels might be sex-independent.

The present investigation concluded that the Aseel and Kadaknath native chicken might have better immunocompetence than IWH line of White Leghorn chicken and these indigenous chickens might be utilized in improving disease resistance of high productive but less immunocompetent chicken germplasms.

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