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Immunocompetency of two chicken backyard variety with 25% Aseel inheritance

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Abstract
The experimental study was carried out to evaluate the humoral and cell mediated immune response in Type I and Type II backyard chicken varieties with 25% Aseel inheritance. The humoral response was computed against sheep red blood cells (SRBCs) for haemagglutinin (HA) antibodytitre on 5 days post-immunization. It was expressed as log2 of the highest dilution which shows complete haemagglutination. The HA titers of Type I and Type II genetic groups were 8.17 ± 0.50 and 7.60 ± 0.48, respectively with males having higher titre over their counterpart females. The *in vivo* cell response to Phytohaemagglutinin-P (PHA-P) was quantified as Foot Index (FI) in mm where the respective Type I and Type II genetic group's1.61 ± 0.09 and 1.60 ± 0.09 with significant sex effect i.e males exhibited significantly higher response than females. The result of present study suggests that the two genetic groups, Type I and Type II are equally competent and had better immune response to fight against infections.

Keywords: Chicken, Aseel, inheritance, backyard, immune response, PHA-P, SRBC

Introduction
Indigenous chicken breeds are famous for their natural disease resistance over decades. Early immunocompetence and high disease resistance are considered as the two important entities that to be incorporated in breeding program in the near future (Vander ZIJPP, 1983a) [1]. The immunocompetence parameters such as humoral immune response to SRBCs (Sheep red blood cells) and cell mediated immune response to PHA-P(Phytoheamoglutinin-P) are considered as important factor in chicken in order to categorize birds as disease-susceptible and disease-resistant (Siegel and Gross, 1980; Pandey *et al*., 1992; Singh *et al*., 2010; Kumar and Kumar, 2011) [2, 3, 4, 5].
These parameters reveal the immune status of birds and act as a key players in understanding the various aspects of immune responses in the field of avian immunology.
The former parameter, SRBC is a non-pathogenic and T-cell-dependent multideterminant antigen, while the latter, PHA acts as a mitogen and induces skin swelling (Martin *et al*., 2006) [6] besides stimulating T-cells in avian species. Birds exhibiting higher antibody response against RBCs indicate the production of more antibodies over a variety of antigens (Parmentier *et al*., 1998) [7] and that of PHA-P show higher general level of cellular immunity influencing T-cell mechanisms (Weber, 1975) [8].
Two backyard chicken varieties (Type I and Type II) with the genetic inheritance of Black Australorp, WLH and Aseel (50:25:25) in Type I and Black Australorp, Synthetic population and Aseel (50:25:25) were evaluated for immunocompetence (humoral – SRBCs; cell mediated immune response – PHA-P). There were no literature available in the aspects of immunocompetence of birds. Thus, an experiment was carried out with the objective to evaluate humoral and cell mediated immune response in these two chicken varieties.

Materials and Methods
The birds of both the genetic groups are random-bred population that were maintained at AICRP on Poultry breeding, Rajendranagar, Hyderabad. They belong to third generation and were obtained in 8 hatches. The chicks (96 in total) were hatched and vaccinated immediately against Mareck's disease at day-old stage prior to rearing in brooder house. Standard floor spacing and brooding temperature with *ad lib* watering and feeding were provided. Good managemental practices were followed with standard vaccination protocol for Ranikhet, Mareck's, IBD and fowl pox at different stages of chicken growth. The birds were then transferred to cage system with a motive to record individual production potentials.

The general immune status of these backyard chicken varieties were studied at 25 – 30 weeks of age where the antibody response against SRBC was used to estimate humoral immune response using haemagglutination test (Van der ZIJPP and Leenstra, 1980) [9] and the response to a mitogen PHA – P was used for assaying *in – vivo* T – cell mediated immune response. SRBCs suspension was prepared by collecting approximately 5 ml of venous blood from Deccani ram, reared at ILFC, College of Veterinary Science, Rajendranagar, Hyderabad in to a sterile vial containing anticoagulant (5% EDTA). The vial was centrifuged (3000 rpm/min for 10 min's) and the supernatant was discarded. The suspension of SRBC was then washed thrice in Phosphate buffer saline (PBS) and resuspended in PBS to obtain 0.5% (v/v) SRBC antigen. The resulted 0.5% SRBC suspension were injected to the bird's wing vein. After 5 days post-injection, about 1ml blood was collected and incubated (2-3 hrs @ room temperature) and serum was separated and stored at – 20 °C for subsequent analysis. Sequentially, two fold serial dilutions were done using PBS in U – shaped micro - well plates where an equal volume of 0.75% SRBC was added and incubated (37 °C @ 4 hrs). Finally, the antibody titers were visually scored as the reciprocal of the highest two fold dilution of serum showing complete haemagglutination reaction and expressed in $\log_2$ values (Wegmann and Simithies, 1966) [10].

Also the birds were of both the genetic groups were immunized with 0.1mg of PHA – P in 0.1ml of normal saline (5 mg PHA–P dissolved in 5 ml of 0.9% normal saline solution) intradermally into one of the wattles. The pre and 24 hours post-injection wattle thickness was measured in millimeters using a constant tension gauge (Mitutoyo, Japan) (Corrier and De LOACH, 1990) [11] and the wattle thickness was calculated using the formula given below.

Thickness Index = (Post-injection wattle thickness – Pre-injection wattle thickness) × 100

The statistical analysis was performed by least squares ANOVA (Harvey 1990) [12] considering sex as a fixed effect in the model:

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

Where Y_{jk} - observation on k^{th} individual of j^{th} sex.

μ - population mean

W_j - fixed effect of j^{th} sex

e_{jk} - N (0,) σ^2 i.e random error associated with mean zero and variance $\sigma 2$.

Results and Discussions

Humoral immune response to SRBC immunization

Heamagglutination test was used to evaluate the humoral immune response in Type I and II chicken varieties (Fig. 1) that can be used for backyard farming. The SRBC immunization study in indigenous chicken expose various aspects of immune response besides their genetic basis. SRBC is a T-cell-dependent multideterminant antigen used to measure the complement activity as innate immune response to SRBC (Haunshi *et al*., 2002) [13]. The heredity of antibody responsiveness to SRBC was estimated at 0.18 (Bovenhuis *et al*., 2002) [14]. Significant hatch, line and sex on response to SRBCs have also been reported (Gross *et al*., 1980; Vander ZIJPP and Leenstra, 1980; Pinard *et al*., 1992) [15, 9, 16]. Analysis of variance for effect of sex (Table 1) and genetic group (Table 2) on response to SRBC were presented together with the mean values in respective genetic groups in Table 3.

The immunocompetence profile of the birds of both the genetic groups displayed a non-significant effect of genetic group with the mean values of 8.17 ± 0.50 and 7.60 ± 0.48 (in $\log_2$ values) in Type I and Type II varieties, respectively.This non-significant difference with respect to SRBCs signify that both the genetic groups are equally competent. This are in agreement with the findings of other study Haunshi *et al*. (2002) and Reddy *et al*. (2002) [13, 17]. However, the present estimates were higher than reported titrevalues [(Sivaraman *et al*. (2005) and Devi *et al*.(2012)] [18, 19] and lower than the titre values [(Haunshi and Sharma, 2002; Haunshi *et al*., 2002; Patra *et al*., 2004; Singh *et al*., 2010)] [13, 20, 21, 22] of various chicken breeds in their study. Further, a differential HA titre values observed in synthetic dam line and Aseel chickens [(Sivaraman *et al*. 2005; Chatterjee *et al*. 2007)] [18, 23]. In contrast, a significant difference among the breeds was found by other studied [Haunshi and Sharma (2002) and Reddy *et al*. (2002)] [17, 20]. This differential titres may be due to the difference in genetic background of chicken populations (Singh *et al*., 2010) [22]. The males mean SRBCtitres (in $\log_2$ values) were 8.62 ± 0.69 and 8.08 ± 0.63, while that of females were 7.68 ± 074 and 7.09 ± 0.72 in respective Type I and Type II genetic groups. In other words, the mean titres ranged from 7.09 to 8.62 (in $\log_2$ values) and were analogous to the various chicken breeds [(Reddy *et al*., 2002; Nath *et al*., 2005; Chatterjee *et al*., 2007; Singh *et al*., 2009;Tomar *et al*., 2012)] [17, 23, 24, 25, 26]. A non-significant ($P \leq 0.05$) sex effect was seen in Type I genetic group unlike in Type II chicken variety with the males being higher over females in terms of magnitude of HA titres. This nonsignificant sex effect was in concordance with the values reported by other researchers [Kundu *et al*. (1999); Nath *et al*. (2005); Sivaraman *et al*. (2005) and Kumar and Kumar, (2011)] [5, 18, 24, 27]. The wide variation observed in the published literature may be due to the dose and route of administration, term of age and type of estimation utilized in study (Van der ZIJPP, 1983b; Kundu *et al*., 1999) [27, 28] and the difference in genetic background of groups and sensitiveness (Gross and Siegel, 1988; 1993) [29, 30].

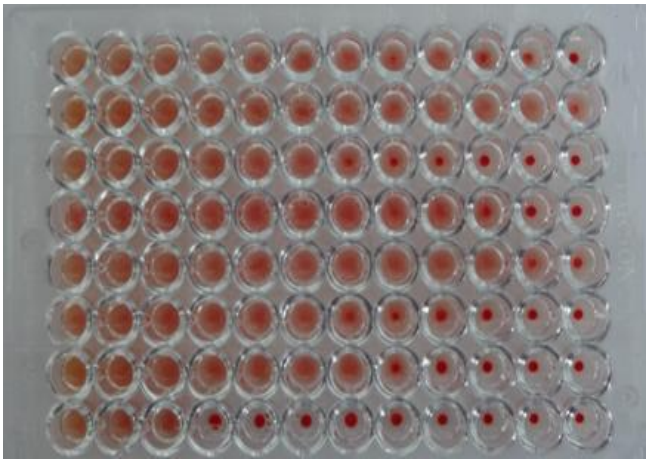

Fig 1: Haemagglutination test in response to SRBC

Table 1: ANOVA for effect of sex on response to SRBC

Source of variation	SRBC Titres			
	Type I		Type II	
	df	MSS	df	MSS
Sex	1	10.21	1	11.90**
Error	44	11.78	46	0.23

** Significant ($P \leq 0.01$)

Table 2: ANOVA for effect of genetic group on response to SRBC

Source of variation	SRBC Titres	
	df	MSS
Genetic group	1	7.49
Error	89	11.07

** Significant ($P \leq 0.01$)

Table 3: Mean response to SRBC (in log_2 values)

Genetic groups	SRB Ctitres		
	Males	Females	Pooled
Type I	8.62 ± 0.69	7.68 ± 0.74	8.17 ± 0.50
Type II	8.08 ± 0.63[a]	7.09 ± 0.72[b]	7.60 ± 0.48

Means among sexes within each genetic group with different superscripts (a,b) differ significantly ($P \leq 0.05$)

Cell mediated immune response (CMI) to PHA-P

Phytohaemagglutinin – P test was conducted to study *in–vivo* T–cell mediated immune response in the birds of 25 – 30 weeks of age that can acts as an indicator of general cellular immune – responsiveness in both the genetic groups. PHA-P, a mitogen, used to intensify the immune cells to undergo blast transformation and proliferation in larger amounts. It is one of the measure employed to assess the proliferative capacity of certain cell types specifically T-lymphocytes which later symbolize the general cellular immune response. It is used in its mucopolysaccharide form obtained from the red kidney bean (Phaseolus vulgaris) that have the potential to provoke responses. This response in turn altered by the sub-populations of T- helper and T- suppressor cells. PHA-P were used to assess the intensity of *in vivo* inflammatory response in chicken over decades (Stadecker *et al*., 1977; Goto *et al*., 1978) [31, 32]. This T- cell immune response generated *in vivo* was utilized as web index in chicken with sex difference i.e males exerting higher immune response in terms of magnitude than females (Cheng and Lamont, 1988) [33]. This *in-vivo* immunocompetence parameter was analyzed in various previous studies particularly in indigenous chicken (Kundu, 1997; Rajkumar *et al*., 2012) [34, 35]. The higher immune response in indigenous breeds of chicken indicate higher resistant power over infectious diseases. Thus, the present investigation was designed and conducted to evaluate the status of these flocks of Type I and Type II chicken varieties with respect to cell-mediated immune response by PHA–P. The results of ANOVA for genetic group (Table 4) indicate that Type I and Type II genetic groups did not differ significantly reflecting they are equally competent over infections. The CMI response ranged from 1.12 to 2.11 mm and is in close agreement with the values reported by Haunshi and Sharma (2002) and Haunshi *et al.* (2002) [13, 20] where a non-significant effect of genetic groups observed implicating equal competency in mounting the CMI response to PHA–P mitogen. Contrary, a significant influence of genetic group was found byotherstudy [Reddy *et al.* (2002) and Patra *et al.* (2004)] [17, 21]. However, in both the genetic groups, a significant effect of sex ($P \leq 0.05$) observed (Table 5).The mean PHA–P thickness index values (Table 6) were 2.11 ± 0.11 and 1.97 ± 0.11 mm in Type I and Type II male samples, while, the values were 1.12 ± 0.09 and 1.23 ± 1.04 mm in Type I and Type II female samples. Male birds of both the genetic groups showed significantly higher response against their counter parts (females) in contrast to others [Sivaraman *et al.* (2005)] [18]. In brief, the published literature revealed both higher and lower values than the present findings (Reddy *et al*., 2002; Nath *et al*., 2005; Sivaraman *et al*.,2005; Tomar *et al*., 2012) [17, 18, 24, 26] reported higher values, while, lower values were reported [Patra *et al*., 2004; Chatterjee *et al*., 2007; Singh *et al*., 2009; Singh *et al*., 2010; Devi *et al*., 2012)] [19, 21, 22, 23, 25] which could be due to the differences in genetic background, dose and site of injection, differences in usage of mitogen and sensitiveness of birds. Finally, it is concluded that both the genetic groups are equally competent to cope up against a wide variety of diseases. They can be utilized in chalking out programmes for simultaneous genetic improvement and as a backyard chicken variety in the farming community.

Table 4: ANOVA for effect of genetic group on response to PHA–P

Source of variation	PHA–P values	
	df	MSS
Genetic group	1	0.00
Error	93	0.44

**Significant ($P \leq 0.01$)

Table 5: ANOVA for effect of sex on response to PHA–P

Source of variation	PHA–P values			
	TYPE I		TYPE II	
	df	MSS	df	MSS
Sex	1	11.90**	1	6.51**
Error	46	0.23	45	0.27

**Significant ($P \leq 0.01$)

Table 6: Mean response to PHA–P values (in mm)

Genetic groups	PHA–P values		
	Males	Females	Pooled
Type I	2.11 ± 0.11[a]	1.12 ± 0.09[b]	1.61 ± 0.09
Type II	1.97 ± 0.11[a]	1.23 ± 1.04[b]	1.60 ± 0.09

Means among sexes within each genetic group with different Superscripts (a, b) differ significantly

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