

Assessment of Immunocompetence Status of Native Breed of Chickens

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ABSTRACT

Immunocompetence is known to be controlled by genetics depending on the B-haplotype Major Histocompatibility complex (MHC), for instance and the selection for production traits. Genetic resistance to disease involves many facets of the body's defense system and interactions, and it is extremely complex. Production traits had negative correlation to disease resistant traits. The aim of the current study was to estimate the immune responses against SRBC in selected lines, estimating the genetic trend and genetic correlation between male and female immune responses against SRBC. Humoral response was estimated in the form of titer against sheep RBC, each birds was received an intravenous injection of 0.1 ml of 0.5% suspension of packed SRBC in normal saline at 6 week of age Five day later the blood was collected from Jugular vein of each birds in the individual test tube and sera were collected. In the present investigation, chicks belonging to the selected lines of native chickens were used for the study of immunological traits viz., response to sheep RBCs, IgG concentrations. Finally, 1% sheep RBC suspension was prepared by mixing 1 ml of packed sheep RBCs and 99 ml of PBS which was then used for injection in the birds. The Average means for HA was observed (7.74±0.109) and IgG (1.94±0.72) in native breed. Sex wise HA results showed that male have higher mean (7.925 ± 0.1463) than females (7.57± 0.161) for HA while the same was also estimated for IgG by having mean (2.1194±8.9) and female (1.77±8.18). Test of Homogeneity of Variances was recorded as (0.39) for HA which was significant and IgG level (0.937).

Key words: immune competence, native breed. HA & HI titer, IgG.

INTRODUCTION

Increasing population of India with increased demand of nutritious food and shrinkage of land has drawn the attention of people towards the livestock production and poultry farming. Due to high nutritive value and less input cost, the poultry farming has grown up nicely. Hence, economics of poultry production can be effectively improved by decreasing the input costs. It has been amply proved that immunocompetence or resistance to infections/diseases is under the control of gene or group of genes called gene complexes includes MHC, Interleukin, Interferon.

Immuno-competence is known to be controlled by genetics depending on the B-haplotype (major Histocompatibility complex [MHC]), for instance and the selection for production traits. It has been shown that the proportion of immune cell subsets can be correlated to susceptibility and resistance to disease^{1,2}.

Genetic enhancement of immunocompetence without challenging animals with antigens may be a potential approach for improving health and production parameters as well as general disease resistance. Selection of chickens for serum IgG isotypes, which represents the

response to a wide variety of unknown antigens, may be a new breeding strategy to improve resistance to infectious diseases. In this regard, genetic control of humoral and cell-mediated immunities and phagocytic ability of chicken lines selected for serum IgM and IgG has not been well investigated.

Avian immune system is a highly evolved and complex system involving different cell types and soluble factors that act in concert to elicit immune response against invading pathogens and thus play a central role in determining survivability. Immune response and disease resistance in chicken have been found to be affected by multiple genetic and environmental factors³. The latest discoveries in the area of molecular and immunogenetics have tremendously added to the vast knowledge of genome organizations, number of genes, their structures, expression and regulation and nucleotide polymorphism.

The major part of the immunological defense may be grouped in to three different and interdependent functional facets viz. cellular system, which provides cell mediated immunity or direct cellular intervention against pathogens, humoral system, which acts by producing humoral immunity or by antibody production, the non-specific system (e.g. complement system, phagocytosis etc.).

It has been demonstrated that different immune challenges produce diverse responses and so require different cells to be activated^{4,5,6,7}. For this reason, an assay intended to understand the current status of commercial poultry and the response capability of its immune system must comprise parameters that refer to these different pathways.

The majority of B-cells express major histocompatibility (MHC) II molecules, and they are classified as antigen presenting cells. Early events in B-cell activation include antigen contact with specific B-cells, that leads to transmembrane signaling, increased expression of Class-II MHC molecules as secreted molecules in plasma and other bodily fluids, they are able to bind foreign antigens, either neutralizing them directly or indirectly through activation of the complement

system or antibody dependent cell cytolysis by monocytic phagocytes⁸.

A meta-analysis of experiments increasing/decreasing clutch/brood size to alter parental effort found a significant negative relationship between parental effort and immune responses⁹.

Genetic resistance to disease involves many facets of the body's defense system and interactions, and it is extremely complex. Production traits had negative correlation to disease resistant traits. In addition, disease resistant traits had low heritability, which made it difficult to use conventional breeding to improve the disease resistant traits. New opportunities to improve our understanding of the genetic nature of disease resistance have been produced through recent advances in molecular biology, gene mapping, and immunology which make selection for disease resistance possible in the future. However, testing and selection for disease resistance and improved immune responsiveness require knowledge of the genetic correlations.

This implies a cost of rather short duration, at least in physiologically non exhausted individuals; that is, when the strenuous activities cease, immune responses should be restored within days or weeks¹⁰.

Many factors regulating the immune responses in birds have been identified and characterized in last few decades. Macrophages are one of the most important immune-competent cells of the birds immune system, which are involved in initiating and directing the innate specific immune response. Selection based on these immune effectors may helps in enhancing disease resistance immunocompetence of the poultry flock. Cytokines play an important role in immune response. These are secreted by a variety of cells and two principal producers are the dendritic cells and macrophages. Cytokines are involved in development of cellular and humoral immune responses, induction of the inflammatory response, regulation of haematopoiesis, control of cellular proliferation and differentiation and healing of wounds. The present experiment studied the effect

that selecting chickens divergently for antibody response to a single i.e. inoculation of SRBCs.

The genetics of a bird or flock has a profound impact on its ability to resist disease, because genetics define the maximum achievable performance level. Careful attention should be paid to genetics as an important component of a comprehensive disease management program including high-level biosecurity, sanitation, and appropriate vaccination programs. Some specific genes (e.g. MHC) are known to play a role in disease resistance, but resistance is generally a polygenic phenomenon. Future research directions will expand knowledge of the impact of genetics on disease resistance by identifying non-MHC genetic control of resistance and by further elucidating mechanisms regulating expression of genes related to immune response.

The immune system of an individual consists of three major facets antibody response (humoral immunity), T cell-mediated immunity and phagocytosis. Coordination of these systems enables an individual to resist infection and disease. Genetic control of these facets of the immune system may be independent of each other¹¹⁻¹⁴. The kinetics of antibody responses depends on the Ig isotype. The immune system of an individual consists of three major facets antibody response (humoral immunity), T cell-mediated immunity and phagocytosis. Coordination of these systems enables an individual to resist infection and disease. The challenging of these lines and the crosses between them with a range of challenging agents. The defense mechanisms were not only resource expensive, but depending on the genetic mechanisms involved, the populations were ranked depending on the challenging agent. Concomitant with the direct response of SRBC antibody titers to selection are correlated responses in production and disease-related traits¹⁵.

The level of maternal antibodies increases during the first 2 to 4 d of age¹⁶. Where after, it decreases until 2 to 4 wk of age^{17,18}.

Genetic control of antibody response and cell mediated immunity is polygenic. The three major

parts or systems of the immunological defense are under independent genetic control¹⁹. They may be distinguished as three sets of host response genes such as:

- (a) Genes which control the susceptibility or resistance of an innate immunity.
- (b) Genes which govern the specificity of acquired or adaptive immune response.
- (c) Genes which affect the quality of specific immune response.

The control of immunosuppressive diseases is of prime importance for the nascent poultry industry in developing countries.

The aim of the current study was to estimate the immune responses against SRBC in selected lines, estimating the genetic trend and genetic correlation between male and female immune responses against SRBC.

Estimation of Humoral Immune Responses

Humoral response was estimated in the form of titer against sheep RBC, each birds was received an intravenous injection of 0.1 ml of 0.5% suspension of packed SRBC in normal saline at 6 week of age Five day later the blood was collected from Jugular vein of each birds in the individual test tube and sera were collected. The total antibody titer was determined by haemagglutination test perfumed in micro titer plate from individual serum sample two fold serial dilutions was carried out in normal saline solution and equal amount of 1% SRBC suspension was added in each well. The serum was not added in control well the plates were incubated at 37°C for about one hrs. The reciprocal of highest dilution showing 50% agglutination was expressed as titer (n) the titer was transformed into \log_2 for further analyses.

MATERIAL AND METHODS

In the present investigation, chicks belonging to the selected lines of native chickens were used for the study of immunological traits viz., response to sheep RBCs, IgG concentrations. The statistical analysis was performed by using SPSS10 software for evaluating various parameters.

Experimental Animals -Birds and their genetic background

Approximate 100 chickens of native randomly selected from a flock of 300 birds being maintained at experimental farm of CARI, Izatnagar, India as closed flock. Screenings of population for SRBC response in each of the above was used for current study.

Sheep

Healthy Muzaffarnagari breed of sheep maintained at Sheep and Goat farm of Livestock Production Research Centre, Indian Veterinary Research Institute, Izatnagar were used for collection of blood from the Jugular vein to prepare SRBC suspension.

Assaying of Immunological traits

The immunocompetence status of the birds can be assessed by analyzing various components of immune system. A few important facets of immune responses were evaluated in this investigation. Total IgG antibody titre and humoral immune response manifested by its components like antibody titer against an antigen were estimated.

Humoral Response to Sheep RBC

Preparation of Sheep RBC antigen

Approximately 10 ml of heparinized (20 IU/ml) blood was collected from jugular vein of healthy sheep. It was centrifuged at 4°C 1200 rpm on 3-5 thousand rpm. For 10 minutes to settle down the RBCs. The RBCs were then washed thrice with PBS/NSS by mixing and centrifuging it to remove other serum components. Finally, 1% sheep RBC suspension was prepared by mixing 1 ml of packed sheep RBCs and 99 ml of PBS which was then used for injection in the birds.

Administration of antigen

0.1 ml of 1% sheep RBC suspension was injected into the jugular vein of each bird with tuberculin syringe. Jugular vein was the choice of injection as it led to minimum bleeding in comparison to other veins like brachial vein etc.

Harvesting of immune sera from SRBC sensitized birds

Two ml of blood was collected in sterilized

glass tubes at 5 days post immunization (5 dpi) and allowed to clot for 2-3 hours at 37°C. The hyper immune sera oozed out of the clot or the clot was broken. Sera were collected by centrifuging the tube at 1000 rpm for 3-4 min. and stored at -20°C till further analysis.

Estimation of antibody titer against sheep RBCs

The antibody response to SRBC was assessed using haemagglutination test as mentioned below-

The test was performed in round bottom (U shaped) micro titer plates. 50 µl of phosphate buffered saline (PBS) was added in each well. Then, 50 µl of serum was added in first well of each row except the last row where 50 ml of PBS was added, that acted as control. After thorough mixing thoroughly, the sera were two fold serially diluted by taking 50 µl from each of the well and adding it to the subsequent wells, mixing thoroughly and it was continued like this till last column, which was discarded. Equal volume (50 µl) of 1% SRBC suspension was added in all the wells and was thoroughly mixed with sera samples. The plates were then incubated at 37 °C for 1 hour. The highest dilution that gives complete agglutination (button shaped clumping of RBCs indicated haemagglutination reaction) and it was recorded as titer and was expressed as log₂n.

Estimation of serum IgG by Single Radial Immuno-diffusion (SRID) Assay

Chicken serum IgG neutralizes the anti chicken IgG Agarose gel was used as a solidifying base to assay IgG concentrations through Single Radial Immunodiffusion (SRID) assay. The procedure is briefed below.

Clean and sterilized glass plate was placed on leveled horizontal surface. The edges prepared with glass strips at the borders were sealed with agar. About 50 ml of 0.1 M Tris - HCl was divided equally into two halves. In first half, of 3% Agarose concentration was added 3% (w/v) and boiled. In second half, 1.750 ml of anti-chicken IgG (Sigma, USA) was added and after thorough mixing, it was kept at 50 °C in a water bath. The temperature of first half (boiled and cooled) was brought down to about 50 °C and second half was

mixed. The whole content was poured on to the glass plate. The gel was allowed to solidify for 1-2 h. Then wells were punched at a distance of 1 cm with the help of well-cutter. The standards of IgG (Sigma, USA) were loaded in different concentration in the wells to plot standard curve. 5 ml of unknown sera were diluted to 4 times with 0.1 M Tris and then loaded the wells. The plate was incubated at 37 °C for 24 h in humid chamber. The diameters of the ring around standard as well as unknown samples were measured with the help of Digital Vernier Calipers. The serum IgG concentrations in unknown samples were determined with the help of regression equation obtained by plotting log₂ concentrations of IgG standards against diameter of the precipitation ring as mentioned in section 2 for serum lysozyme.

Statistical analysis

The data generated on immunological traits were analyzed by LS ANOVA using following Statistical model: -

$$Y = \mu + L_i + L_i S_{ij} + H_k + Sx_l + e_{ijklm}$$

Where,

Y	=	value of a trait measured on ijklm th individual
μ	=	Overall mean
L _i	=	Effect of line (i = 1, 2)
L _i S _j	=	Random effect of j th sire in i th line
H _k	=	effect of k th hatch
Sx _l	=	Effect of l th sex (l = 1, 2)
e _{ijklm}	=	random error associated with mean '0' and variance σ ²

RESULTS AND DISCUSSION

The observed results are listed in the following tables and graphs by using SPSS10 software of data Analysis for evaluating various statistical parameters of native Chickens.

The Average means for HA was observed (7.74±0.109) and IgG (1.94±0.72) in native breed. Sex wise HA results showed that male have higher mean (7.925 ± 0.1463) than females (7.57± 0.161) for HA while the same was also estimated for IgG by having mean (2.1194±8.9) and female (1.77±8.18). (Table 1&2).

One way Anova recorded significant between the groups of male and females having less than 0.05 values which is recommended for significant level. The square mean value was observed for HA (4.3) & IgG (4.145) between the groups, while with in the group was observed as (1.628) for HA and IgG (0.499). (Table 4)

Test of Homogeneity of Variances was recorded as (0.39) for HA which was significant and IgG level (0.937). (Table 3).

The native breeds have unique alleles in their genetic constitution being reared in agro-climatic conditions for their well adaptability in open environment. Result showed that native breed was estimated for HA was observed (7.74±0.109) and IgG (1.94±0.72) which was higher being a native breed and it was preserved in open environment under extreme climatic conditions by which their immunity levels has increased. A mean titer of 6.289±0.246 in SDL broiler chickens. Aseel breed of native chicken demonstrated higher HA titer than most of the other breeds²⁰. The wide variations observed in the present study might be due to the reason that no artificial selection has been applied on this breed of chicken for immune response or production traits.

Sex wise HA results showed that male have higher mean (7.925 ± 0.1463) than females (7.57± 0.161) for HA while the same was also estimated for IgG by having mean (2.1194±8.9) and female (1.77±8.18). This difference might be due to male and female antibody titers being genetically different traits, due to the genes being located on the sex chromosome. This possibility can be studied by estimating the genetic correlation between male and female antibody titers. The overall male has better immunity level then females. The least squares mean of HA titer as 12.38±0.60 in Aseel native chicken. In males, the average was 12.80±0.74 whereas in case of females, it was 12.35±0.62.

Besides, genetic factors other factors have also been reported to influence the response to sheep RBCs 20, 21. One way Anova recorded significant between the groups of male and females having less than 0.05 values which are

Table 1. Mean for HA and IgG Sex wise of Native Chicken

SEX	Mean	HA	IGG
Male		7.9254	2.1194
	Std. Error of Mean	.1463	8.902
	Std. Deviation	1.1974	.7287
Female	Mean	7.5714	1.7714
	Std. Error of Mean	.1610	8.183
	Std. Deviation	1.3467	.6846
Total	Mean	7.7445	1.9416
	Std. Error of Mean	.1097	6.196
	Std. Deviation	1.2835	.7252

Table 2: Descriptive One way Anova for HA and IgG Traits

Traits	Sex	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
HA	Male	67	7.9254	1.1974	.1463	7.6333	8.2174
	Female	70	7.5714	1.3467	.1610	7.2503	7.8925
	Total	137	7.7445	1.2835	.1097	7.5277	7.9614
IGG	Male	67	2.1194	.7287	8.902	1.9417	2.2971
	Female	70	1.7714	.6846	8.183	1.6082	1.9347
	Total	137	1.9416	.7252	6.196	1.8191	2.0641

Table 3: Test of Homogeneity of Variances for HA and IgG Traits

Traits	Levene Statistic	df1	df2	Sig.
HA	4.363	1	135	.039
IGG	.006	1	135	.937

Table 4: One Way ANOVA Analysis for HA and IgG Traits

Traits	Type	Sum of Squares	df	Mean Square	F	Sig.
HA	Between Groups	4.289	1	4.289	2.634	.107
	Within Groups	219.770	135	1.628		
	Total	224.058	136			
IGG	Between Groups	4.145	1	4.145	8.304	.005
	Within Groups	67.388	135	.499		
	Total	71.533	136			

recommended for significant level. The square mean value was observed for IgG (4.145) between the groups, while with in the group was observed as (0.499). Serum IgG is the most abundant antibody and constitutes approximately 80% of the total immunoglobulin. The bird's ability to mount antibody responses to other antigens is primarily revealed by serum IgG concentration. The average serum IgG concentration was 6.987 ± 6.57 it was higher in males (7.409 ± 8.13) than females (6.533 ± 10.20). The serum IgG concentrations in broiler & indigenous birds were reported to be 8.01 ± 0.4 and 10.01 ± 0.4 mg/ml, respectively²¹.

Factors Affecting Immunity

There are several factors which may effect immunity level of an host are as follows -

Age of the birds had no significant ($P > 0.05$) effect on serum IgG level. However, older birds revealed somewhat higher values. Sex of the birds had no significant ($P > 0.05$) effect on serum IgG level. However, male birds revealed higher values. Singh (2005) reported non-significant effect of sex on IgG concentrations. The least squares means for males and females were 59.09 ± 7.69 and 52.08 ± 7.74 mg/ml in WLH, respectively.

The influence of age was significant ($P \leq 0.05$) on HA titer (Table 4.1.1), older birds had higher mean antibody titer (13.0 ± 2.13) than younger birds (10.88 ± 0.54). This results has also reported effect of age on HA titer¹⁹. The influence of sex on HA titre was statistically non-significant although males had higher mean antibody titer (12.80 ± 0.74)

A Graph was plotted between Mean and Sex for determining the level of immunity as follows:

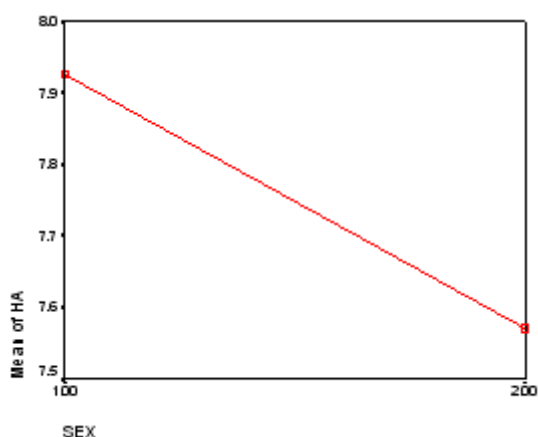
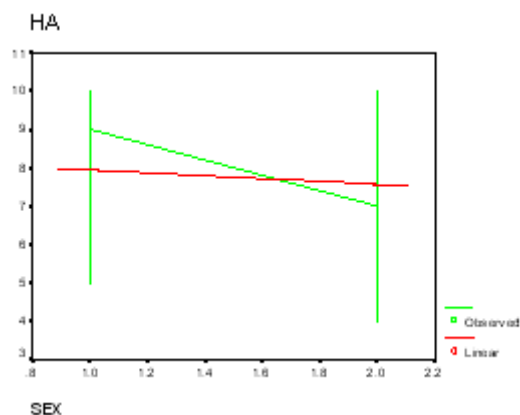


Fig. 1: Mean for HA sex wise



Linear Reg. for HA

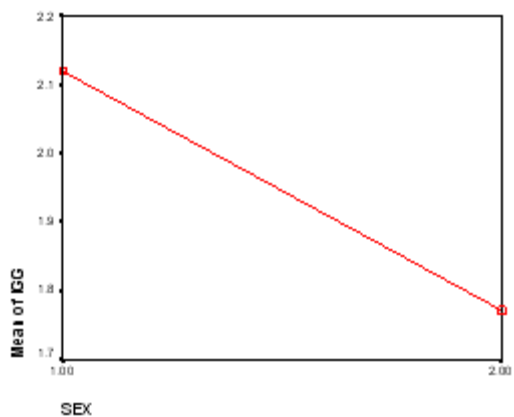
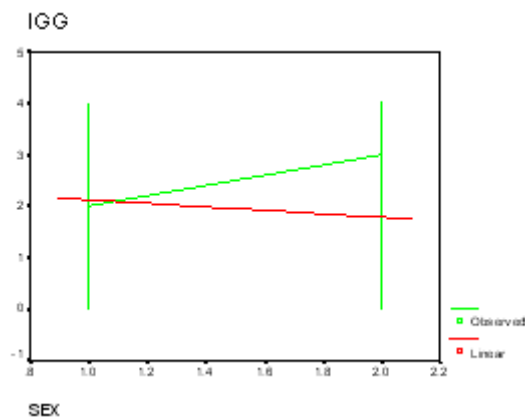


Fig. 2: Mean for IgG



Linear Reg. for IgG

than females (11.96±0.64). The finding is comparable to those reported by in different genetic stock^{20, 21, 22}.

Sex had non-significant effect on HA titre in guinea fowl too²¹. However significant sex differences (females having more titer than males) have been reported in 9th generation of divergent selection based on SRBC response. Sexual dimorphism was attributed to the effect of sex hormone on the thymus and the immune cells as

seen in mammals¹⁷. Some outcome we get, Native Breed of chicken demonstrated relatively higher humoral response to Sheep erythrocytes than exotic chicken breed, Serum IgG level was also relatively significant in native chicken, Male showed higher immune response than the females.

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