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Research Article

Assessment of Immunocompetence Traits of Aseel, an Indian Native Chicken Breed

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Abstract

In the present study, Aseel chicken was studied to evaluate high and low immune responses by assessing immunocompetence traits like hemagglutination assay (HA), serum lysozyme activity, and immunoglobulin G (IgG) in blood. A total of 301 Aseel birds were selected to get all the important immunocompetence parameters. Antibody titers against sheep red blood corpuscles (SRBC) and serum IgG levels acted as the humoral immune response indicators. In contrast, the bacteriolytic activity of serum lysozyme acted as an indicator of a non-specific immune response. The mean HA titer, serum lysozyme and IgG levels were 8.14 ± 0.35, 4.85 ± 0.20, 10.82 ± 0.64 in males and 8.15 ± 0.31, 4.48 ± 0.22, and 12.64 ± 0.75 in females, respectively. Sex did not affect immunocompetence traits; however, the average serum IgG concentration was higher in females than in males. In conclusion, we accessed immunocompetence traits of male and female Aseel. A comparison of Aseel's immune competence status with broiler and other fowl chicken would be a piece of valuable information for poultry breeders.

Keywords: Aseel; Immunocompetence; Hemagglutination Assay; Lysozyme; IgG

Introduction

Aseel is one of India's twenty native chicken breeds well known for pugnacity, fighting ability, and great stamina [1]. The native breeds of chicken are the gold mines of genomes and possess many genes that have the scope for improving high-yielding exotic germplasm for tropical adaptability and disease resistance. The best specimen of the breed, although rare, are encountered in parts of Andhra Pradesh and Rajasthan. The most popular varieties are Peela (golden red), Yakub (Black and red), Nurie (White), and Kagar (black). The standard weight of cock, hen, cockerel, and pullet ranges from 4-5, 3-4, 3.5-4.5 kg, respectively. It is also known for good resistance to some poultry diseases [2] and delicious and flavored meat.

In selection experiments, several workers have studied different immunocompetence traits based on the immune response to a single antigen, such as sheep red blood cells (SRBC) in chicken [3-7]. Serum lysozyme, an index of macrophage functional status, demonstrated a positive correlation between the dam's serum lysozyme level and the natural resistance of progeny to diseases in chickens [8]. Lysozyme acts as an innate immune defense factor and protects birds against many bacterial, viral and fungal infections [9]. A study has shown that exogenous administration of dietary lysozyme to broiler chicken improved non-specific immunity mRNA [10], indicating enhancement of innate immunity. Likewise, immunoglobulin G (IgG; in chicken, called IgY, an evolutionary precursor of IgG) constitutes 75% of serum immunoglobulins and is responsible for providing humoral immunity. Avian IgY combines the functions of IgG and IgE and provides a defense against infection [11].

In the present study, the objective was to evaluate the immunocompetence status of Aseel chicks under the standard management system at Central Avian Research Institute, Bareilly, India.

Materials and Methods

Experimental birds

A total of 301 birds of Aseel chicks were evaluated for immunological responses to sheep RBC (SRBC), levels of serum lysozyme and IgG. Chicks of Aseel were maintained at the Desi Fowl unit at Central Avian Research Institute, Bareilly, under standard management conditions.

Immunological traits

Humoral immune response to SRBC: Response to SRBC was assessed through the HA test as per our published protocol [5,6]. Briefly, 50 µl of phosphate-buffered saline (PBS) was added to each well of 96 welled PCR plates. Then, 50 µl of serum was added in the first well of each row except the last row, which acted as a control where 50 µl of PBS was added. After thorough mixing, the sera were two-fold serially diluted by taking 50 µl from each of the wells and adding it to the subsequent wells, mixed thoroughly, and it was continued like this till the last column, from that column it (50µl) was discarded. An equal volume of 1% SRBC suspension was added in all the wells and was thoroughly mixed with sera samples by gentle rocking/rotation. Plates were then incubated at 37°C for 1 hour in a humid chamber. The highest dilution that gives complete agglutination (button-shaped clumping of RBCs that indicated hemagglutination reaction) was recorded as a titer and was expressed as $\log_2$.

Estimation of serum lysozyme level

The serum lysozyme activity was estimated using the Lysoplate assay method [12]. Briefly, standard lysozyme was prepared by dissolving 2 mg of standard lysozyme (SRL, India) in 1 ml of dibasic buffer. Several standards were prepared by serial dilution of above lysozyme stock (2 mg/ml) solution to obtain final concentrations as 50 µg/ml, 25µg/ml, 12.5µg/ml, 6.25 µg/ml, 3.125 µg/ml and 1.57 µg/ml. The agar lyso-plate was set up on a perfect horizontal surface. The glass plate was cleaned and sterilized with spirit and air-dried. The size of the gel was determined on the basis of a number of samples to be analyzed and was prepared by placing glass borders on the edges. All the four edges of the glass plate were sealed with 2% agar. About 50 ml of 1% Agarose in dibasic buffer was sufficient for a 15 X 15 cm^2 plate. After boiling the Agarose in dibasic buffer, it was cooled to 60°C and the pre-diluted *Micrococcus lysodieketicus* (Sigma, USA) at 50 µg/ml of dibasic buffer was poured into it and mixed well. Then the whole content was poured onto the sealed glass plate, which spread uniformly and was then left at room temperature for solidification. After polymerization of gel, the wells were cut at a distance of approximately 1.5 cm using a well cutter and 10 µl of serum sample was pipette into each well. Serial standards samples were also loaded in the wells at one side (4-5 dilutions). The plate was incubated at 37°C in a humidity-controlled chamber for 24 hours. It was stained with 0.2% Coomassie brilliant blue for 6 hours and the excess stain was removed with a de-staining solution. A digital Vernier caliper measured the diameters of the lysed zones. The concentrations (After $\log_2$ transformation) of known standards were regressed on the diameter of the lysed zones around these standards using the following equation, $Y = bx + c$. Where 'Y' represents the concentration of the unknown sample, 'b' represents the slope of the regression equation, 'c' represents the Intercept of the regression equation and 'x' is the diameter of the lysed zone around the unknown sample.

Estimation of serum IgG level

Agarose (2%) gel was used to assay IgG concentrations through Single Radial Immunodiffusion (SRID) assay as per the protocol described previously [13]. Briefly, the edges prepared with glass strips at the borders were sealed with 2% agar, and a 50 ml of 0.1 M Tris- HCl was divided equally into two halves. In the first half, 2% agarose was added and boiled and in the second half, 1.75 ml of anti-chicken IgG (Sigma, USA) was added. Contents were mixed thoroughly and kept at 50°C in a water bath. The temperature of first half (boiled and cooled) was brought down to about 50°C and then the second half was mixed. The whole content was poured onto the glass plate and allowed to solidify for 1-2 hours. Wells were punched on the solidified gel at a distance of 1 cm with the help of a well-cutter. The standards of IgG (Sigma, USA) namely, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, and 1.562 µg/ml, prepared by serial dilution of stock solution, were loaded in the wells to plot standard curve. A 5 µl of unknown sera was diluted 4 times with 0.1 M Tris and then loaded into the wells. The plate was incubated at 37°C for 24 hours in a humid chamber. The diameters of the ring around standard, as well as unknown samples, were measured with the help of a digital Vernier caliper. The serum IgG concentrations in unknown samples were determined with the help of a regression equation obtained by plotting $\log_2$ concentrations of IgG standards against the diameter of the precipitation ring as mentioned earlier for serum lysozyme.

Statistical analysis of immunological data

The data generated on immunological traits were analyzed by variance's least-squares analysis [14]. The following statistical model was employed.

$Yijk = \mu + Si + Sxj + eijk$

Where,

Yijk = value of a trait measured on ijkth individual

μ = Overall mean

Sxj = Effect of ith sire

Sxj = Effect of jth sex (j = 1, 2)

eijkl = random error associated with mean '0' and variance σ

Results and Discussion

Immunocompetence traits of Aseel chicken were assessed through antibody titers against SRBC, serum IgG level, and Lysozyme activity. Effects of sire and sex were also studied on these traits using LSML [14]. The results of least square ANOVA are presented in table 1, and their factor-wise means ± standard errors (SE) are shown in table 2.

Source of variation	df	Mean sum of squares		
		HA Titer	Serum Lysozyme level	Serum IgG level
Sire	24	17.29	7.106	48.84
Sex	1	0.0029	9.551	236.14*
Error	275	14.181	5.175	71.61(283)

Table 1: Least squares ANOVA of important immunocompetence traits in Aseel chicken.

*P ≤ 0.07, Figure in parenthesis is no. of observations.

Sex	No. of observations	HA Titer	Serum Lysozyme level (μg/ml)	No. of observation	Serum IgG level (mg/ml)
Overall	301	8.14 ± 0.25	4.66 ± 0.16	309	11.73 ± 0.49
Males	173	8.15 ± 0.31	4.85 ± 0.20	178	10.82 ± 0.64[b]
Female	128	8.14 ± 0.35	4.48 ± 0.22	131	12.64 ± 0.75[a]

Table 2: Least squares means+ SE of important immunocompetence traits in Aseel chicken.

Means in a column with different superscripts differ significantly (P < 0.07).

Antibody responses to SRBC

The antibody titers against 1% SRBC were measured through the hemagglutination (HA) test (Figure 1) on the 5th-day post-injection (dpi). The titer ranged from 1 to 17. The average HA titer was 8.14 ± 0.35 for females and 8.15 ± 0.31 for males, respectively. The overall average HA titer was 8.14 ± 0.25. Shivakumar reported a titer of 8.89 ± 0.29 in the base population of the IWG line of White Leghorn [15]. Similar results were shown in the mean HA titer of native chicken (7.74 ± 0.109), which was identical to the value that we observed in our study [16]. However, lower values of response to SRBC were reported in Kadaknath (5.31) and Aseel (5.10) on 5th dpi [2]. This difference could be due to the difference in genetic merits of birds associated with various poultry farms.

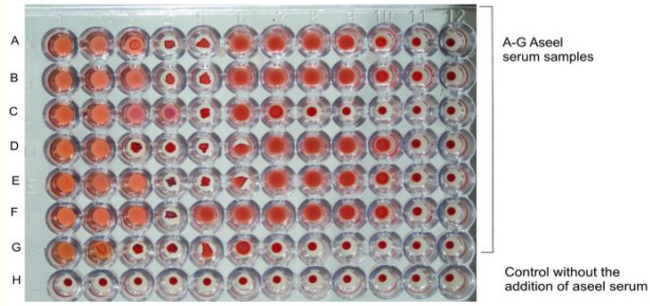

Figure 1: HA titer plate showing button formation in sera samples of Aseel native chicken along with control.

Sex had no effect on HA titer (Table 1). The finding is comparable to those reported in guinea fowl [16] and exotic birds [2]. The antibody response to SRBC antigen may vary between males and females depending on the site of injection (intramuscular vs. intravenous injection of antigen) and may or may not influence by the day post-injection [17].

Serum lysozyme concentration

The serum lysozyme concentration ranged from 1.90 to 12.82 μg/ml. Average lysozyme concentration in males and females was 4.85 ± 0.20 and 4.48 ± 0.22, respectively. The overall lysozyme concentration was 4.66 ± 0.16. The mean lysozyme levels observed in the present study were higher than earlier reports in various breeds of chickens [16]. Lysozyme concentrations in desi fowl and commercial broilers were 1.79 ± 0.07 and 1.33 ± 0.036 μg/ml, respectively [8].

The influence of sex on serum lysozyme was non-significant, consistent with the report of [3] in broiler chicken. However, a significant effect of sex was observed on serum lysozyme levels in IWG-WLH chicken [15]. The difference in the results could be due to different chicken types - broiler and layers.

Serum IgG concentration

The average serum IgG concentration in Aseel chicken was 11.73 ± 0.49. It was higher in females (12.64 ± 0.75) than males (10.82 ± 0.64). The serum IgG concentrations in broiler and indigenous birds were 8.01 ± 0.4 and 10.01 ± 0.4 mg/ml, respectively [16]. The sex of the birds had no significant ($P > 0.05$) effect on serum IgG levels. However, male birds revealed higher values ($P < 0.07$) than females. In high (HA) and low (LA) chicks of White Leghorn, five days after a single injection (0.1 mL) of 0.25% SRBC showed IgG levels at 7 and 13 days but not at 3- and 20-days PPI, and this temporal pattern of increased plasma IgG concentration was observed in both the HA and LA lines [18].

Conclusion

The present study demonstrates that Aseel has high immune competence. Increased knowledge of the immune status of these birds might be helpful in the selection of disease-resistant chickens.

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