

Aseel, Kadaknath and White Leghorn Chicken Immune Response to Variation in Sheep Red Blood Cell

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ABSTRACT

A study has been directed towards a better understanding how the Aseel, Kadaknath and White Leghorn immune system responds to multi-determinant antigen, sheep red blood cells. In contemporary commercial poultry production myriad of Viral and bacterial diseases being lingering menace to poultry industry and retort to prevent and control them are needed due to enormous production losses. Our research has addressed the immune status of three native chicken innate immune mechanisms and components to develop disease resistant breeds by breeder and the characterization and production of native instinct chicken breeds as potential immune modulators in poultry industry. Indigenous breeds show a significant immune response and have considerable potential to reduce susceptibility to infectious diseases. With this premise, this paper asks and answers a series of pertinent questions on the utilization of native avian immunity for increasing resistance to a variety of potential pathogens problematic in today's commercial poultry industry. By immunizing chickens with multi-determinant sheep blood RBC, it demonstrated to affect relative antibody responses against each of the proteins found in that mixture. The paper determines and presents the differences of antibody titres between groups of three native chickens and was assessed by using the Statistical Package for Social Sciences (SPSS) software package for windows20. The effects of three breeds (Aseel, Kadaknath and White Leghorn) were determined by analyzing the data using one-way ANOVA followed by Duncan's multiple comparison tests.

Key words: Aseel, Kadaknath, White Leghorn, SRBC, Titre

INTRODUCTION

Chicken meat is consumed worldwide and provides nutritious, unadulterated healthy food to the human being in contemporary world. As per 19th livestock census, the total poultry population in India is 729.2 million as against

207.74 million in 13th Livestock Census¹³.

This rapid development in the modern poultry industry witnessed the evolution of newer promising poultry strains with higher production potentiality.

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The higher productivity by neophyte poultry strains also experienced the flurry of emerging diseases, even though stringent and comprehensive disease control programmes. Hence enhancement of immunity has permanent and cumulative effects in a breeding population, and has therefore been an environment friendly approach necessary required to maintaining the health of poultry.

Disease resistance in chicken is a polygenetic trait, controlled by immune system that plays an important role in maintaining the normal health and protects the individual from wide range of multiple pathogens. Analysis of variation in immune status which is involved in disease controls approach that commensurates with permanent enhancement of health. Genetic variation in disease resistance ability among different breeds/strains has been evaluated and found to be valuable in the genetic selection programme. Genetic improvement is cumulative over the generations and it reduces the recurring expenditure on vaccines/medicines and overcomes the problem of drug residues in poultry products. Apart from genetic factors, nutritional and environmental factors also play a role in immunity in chicken.

Improvement in disease resistance of poultry is achieved by selection of birds based on resistance against specific pathogens and by molecular analysis of general status of host's immune system i.e., immunocompetence traits. The foreign antigen especially sheep red blood cell challenge is an important part of complement test used to assess immunocompetence assay in chicken because it could assess an individual humoral response without impacting its health. Inoculation of sheep red blood cells generates specific antibody titres (anti-SRBC) which is used as an investigative tool to define the molecular mechanism that trigger vertebrate immune response.

In case of chicken, their heritability of antibody response to inoculation of SRBC is known to be moderate and this trait responds to divergent selection¹⁴. The antibody

responses to SRBC have been correlated to responses in production and disease-related traits¹². After divergent selection for SRBC response, the high antibody line displayed higher antibody to Newcastle disease virus and greater resistance to Marek's disease virus, *Eimeria tenella*, *Mycoplasma gallisepticum*, and *Eimeria necatrix* than the low antibody selected line^{2,3,4}.

This analyses of variation that involved in disease resistance or immunocompetence traits help to identify the differences in individual's resistance or susceptibility against the diseases. Therefore, genetic improvement of the host immune response can increase vaccine efficacy, disease resistance and overall health status of the flock. Under this background, the experiment was carried out.

MATERIALS AND METHODS

A biological trial using three breeds of chicken was conducted at Poultry Research Station, Madhavaram Milk Colony, Tamil Nadu Veterinary and Animal Sciences University, Chennai located between 13° and 9' and 13° and 15'N and longitudes 80° and 14' and 80° and 24'E with an altitude of 22 meters above mean sea level.

A total of 60 day old chicks each from Aseel, Kadaknath and White Leghorn were received from Poultry Research Station (PRS), TANUVAS, Madhavaram milk colony, Chennai for the experiment

The design of experiment consisted of three breeds as three treatments with three replicate of 20 birds each. All the experimental birds were wing banded and maintained under identical management conditions in cages. Birds were fed *ad libitum* with known quantity of standard feed. Clean potable water was provided *ad libitum*.

Immune response status of the experimental birds was assessed indirectly by haemagglutination (HA) titres against sheep red blood cell (SRBC) as a specific antigen. A total quantity of 20 ml blood was collected directly from jugular vein of healthy Madras Red sheep which was maintained in University

Research Farm, TANUVAS in a sterile heparinized vacutainer. The collected blood was centrifuged at 3000 rpm for 15 minutes at 4°C and then plasma and buffy coat were removed. RBC pellet was washed thrice with sterile 0.9 per cent sodium chloride at 2000 rpm for 10 minutes at 4°C for complete removal of serum and plasma protein components in the blood. After washing, 1ml of packed red blood cell (RBC) was mixed with 99 ml of 0.9 per cent sodium chloride for immunization. The prepared SRBC antigen suspension was stored at 4°C till further usage.

All the experimental birds in all the treatment groups were sensitized with one per cent SRBC suspension through intravenous route in jugular vein by using tuberculin syringe at six weeks of age for eliciting the immunity. One ml of blood was collected on 5th, 14th and 21st day of post immunization. Later, serum was separated from the blood and then stored at -20°C till further assay. This sera sample was subjected to haemagglutination assay. Haemagglutination assay for SRBC was carried out as per standard procedure²³.

The differences of antibody titres between groups were assessed by using the Statistical Package for Social Sciences (SPSS) software package for windows²⁰. The effects of three breeds (Aseel, Kadaknath and White Leghorn) were determined by analysing the data using one-way ANOVA followed by Duncan's multiple comparison tests.

RESULT AND DISCUSSION

In the present study wide variability in HA titre ranged from 1-12 against Sheep RBC was observed in Aseel, Kadaknath and White Leghorn breeds. The similar variability was observed in antibody production to sheep erythrocytes in chicken earlier studies²². In concurrence with this, previous report on 5th day antibody titre to sheep erythrocytes between lines of White Leghorn chicken¹⁶. The variability in HA titre against Sheep RBC might be attributed to the genetic basis of chicken breeds which in turn considered as well proven concept for multi trait selection

programme for various immune responses in chicken⁹.

Effect of breeds on HA titre against SRBC in chicken

Significantly higher HA titre was observed in Aseel followed by Kadaknath and White Leghorn during 5, 14 and 21 days of post immunization. HA titre on day 5 of post immunization was highest in Aseel (8.71 ± 0.35) followed by Kadaknath (8.59 ± 0.37) and White Leghorn (7.11 ± 0.34) which was presented in Table 1. There was no significant difference between Aseel and Kadaknath, whereas White Leghorn birds recorded significantly lowest HA titre than other two breeds.

Similarly, previous reported HA titre of 7.0 on 5th day of post immunization in White Leghorn against SRBC⁴. Whereas, few researchers recorded lower values of HA titre than observed in the present study in various strains of White Leghorn^{7,21}. Significantly higher antibody response to SRBC in Aseel breed observed in this study is in accordance with previous reports^{1,11,18}. Comparatively higher antibody titre against SRBC was recorded in Kadaknath breed in the present study than the earlier reports^{8,15,17}. The variations in the HA titre among the breeds observed in this study is coincided with the earlier reports in Chinese chicken breeds⁶.

The variations in the HA titre among the breeds might be due to the genetic makeup, immune status and adaptability to the environment of the breeds. Higher antibody titre in native breeds than exotic breed might be attributed to the general biological response of innate immunity in native breeds of chicken. This inferred that divergent selection based on SRBC response alone or combination of host immune system along with economic traits may improve the production and protection in chicken⁵.

Effect of different post immunization days on HA titre against SRBC in chicken

Reduction in HA titre was observed as days advanced in all three breeds of chicken and the data presented in Table 2. This is in accordance with the earlier reports in

indigenous breeds of chicken in White Leghorn birds^{11,10}. The higher HA titre on 5th day of post immunization might be attributed to the additive genetic variation. Absence of continuous stimuli and the weaning on antibodies in the blood stream resulted in the reduction in HA titre as the day of post immunization advanced in all the three breeds of chicken.

Effect of sex on HA titre against SRBC in chicken

This result found that no significant influence of sex of the chicken on HA titre was observed in all the three breeds of chicken (refer Table 3). However, the male birds had higher HA titre than the female birds of the corresponding breed. This finding is in concurrence with the earlier reports that studied the antibody response to sheep erythrocytes between Indian

and Native breeds of chicken including Aseel, Kadaknath and White Leghorn and reported that non-significant difference observed between sexes and numerically male had higher titre values than female¹¹. Similarly, another study observed non-significant difference in HA titre between male and female broiler chicken¹⁹. Further non-significant effect of sex on HA titre was reported in Kadaknath chicken⁸.

The numerically higher HA titre in male birds than female birds of all breeds might be due to the sexual dimorphism of chicken, which is attributed to the effect of sex hormone on the thymus and the immune cells as seen in mammals. The tendency of males to reveal higher titer might be due to influence of sex hormone which however needs further investigation.

Table 1: Effect of breeds on HA titre against SRBC in chicken

Breed	No. of observation	Post immunization days		
		5	14	21
Aseel	50	8.71 ^a ±0.35	6.64 ^a ±0.29	5.52 ^a ±0.29
Kadaknath	50	8.59 ^a ±0.37	6.56 ^a ±0.30	5.46 ^a ±0.29
White Leghorn	50	7.11 ^b ±0.34	4.46 ^b ±0.16	3.60 ^b ±0.12
F value		6.33**	23.39**	19.67**

Means within column bearing different superscripts differ significantly

** – Highly significant (P<0.01)

Table 2: Effect of different post immunization days on HA titre against SRBC in chicken

Post immunization days	No. of observation	Breed			Overall mean
		Aseel	Kadaknath	White Leghorn	
5 th day	50	8.71 ^a ±0.35	8.59 ^a ±0.37	7.11 ^a ±0.34	8.14 ^a ±0.21
14 th day	50	6.64 ^b ±0.29	6.56 ^b ±0.30	4.46 ^b ±0.16	5.89 ^b ±0.17
21 st day	50	5.52 ^c ±0.29	5.46 ^c ±0.29	3.60 ^c ±0.12	4.86 ^c ±0.16
F value		27.05**	24.51**	64.03**	100.84**

Means within column bearing different superscripts differ significantly

** – Highly significant (P<0.01)

Table 3: Effect of sex on HA titre against SRBC in chicken

Sex	Breed											
	Aseel				Kadaknath				White Leghorn			
	n	5	14	21	n	5	14	21	n	5	14	21
Male	28	9.18± 0.40	6.50± 0.36	5.54± 0.39	24	7.58± 0.34	5.87± 0.35	5.41± 0.36	26	7.57± 0.44	4.80± 0.22	3.90± 0.17
Female	22	8.75± 0.46	6.18± 0.41	5.04± 0.44	26	7.39± 0.34	5.79± 0.35	5.18± 0.36	24	6.62± 0.45	4.35± 0.23	3.68± 0.17
F value		0.78 ^{NS}	0.52 ^{NS}	1.07 ^{NS}		0.15 ^{NS}	0.02 ^{NS}	0.19 ^{NS}		2.71 ^{NS}	2.45 ^{NS}	0.99 ^{NS}

NS – Non significant

CONCLUSION

In general, any parameter or trait is influenced by genetic and non-genetic factor. The results may be due to variation in influences of genetic and non-genetic factors on antibody response against sheep RBC. The goal of poultry geneticists is to effect genetic enhancement of chicken health and productivity. The candidate approach is a powerful method to improve immune response as well as production traits. Discovery of the significant effect of antibody production response in chicken illustrates its potential value for use in marker assisted selection to improve immune response. Future studies needed to correlate the antibody titres against SRBC with immune related gene expression and its variation for validation SRBC as a multi-determinant antigen.

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