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Immunocompetence Profile of Indian Native *vs* Exotic Chicken Breeds

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ABSTRACT

Background: The native chicken breeds were evolved over natural selection since time immemorial. The immunocompetence status of chicken breed tells us about its general response to diseases. It was assumed that the native birds survive in harsh climatic conditions and has a higher degree of resistance to diseases as compared with other exotic breeds.

Methods: Indian native chicken breeds *viz.* Black Nicobari, Ghagus and exotic breed, Dahlem Red were studied for their growth and immunocompetence traits under deep litter management system. The native and exotic breeds were evaluated for Cutaneous Basophil Hypersensitivity (CBH) response against Phytohaemagglutinin-P (PHA-P), humoral immune response against Newcastle disease virus (NDV) antigen and Flow Cytometry analysis of CD8+ Cytotoxic T lymphocytes (CTLs).

Result: The Cytotoxic T lymphocyte (CTL/µl) population significantly differed (P<0.05) between native Ghagus (2132.34±69.526) and Black Nicobari (2023.53±75.14) vs exotic breeds Dahlem Red 1649.49±64.54) of chicken.CBH response to PHA-P significantly differed (P<0.05) between Ghagus (375.836±15.04) and Dahlem Red (267.603±13.99). Sex wise CBH response (based on pooled samples across breeds) showed that females had significantly higher response as compared to males. Serum haemagglutination inhibition titre (log_2) in response to ND vaccine was significantly (P<0.05) higher in Dahlem Red chicken (2.44±0.12) as compared to the two native breeds. Females were having significantly higher HI titre compared to males. In conclusion, the NDV response was considerably higher in exotic breeds as compared to native breeds; however, the trend was just the reverse in case of CBH response.

Key words: Chicken, Cytometry, Immunocompetence, NDV.

INTRODUCTION

The characteristic features of native chicken are constantly being used to develop location specific varieties which suit the Indian agro climatic milieu. In poultry industry, health aspect is an important factor which greatly influences the overall performance of the birds. Disease resistance trait is outcome of interaction of birds' immune system with the physiological and environmental factors (Zekarias *et al.,* 2002). Different strategies have been developed for genetic selection for different traits like immunocompetence and body weight in native and exotic chickens. For this, statistical models have been applied for estimating the body weight (Yadav *et al.,* 2019) which can be incorporated in the breeding programme as an alternative approach to develop the flock with better genetic resistance towards disease and weight gain. Nicobari fowl is an indigenous and endemic breed of Andaman and Nicobar Islands. This original bird is short legged with brownish matty coloured and having single comb. These birds are eggtype breed with shorter shanks and a compact body reported to be comparatively more adapted to tropical environment and resistant to common poultry diseases (Chatterjee and Yadav, 2008; De *et al.,* 2013), out of which, two strains of Nicobari fowl were developed *viz.* Black Nicobari and White Nicobari. Another native breed taken for the study is Ghagus which is one of the important chicken breeds of India. These birds are small in size and have single comb. Its native tract is Kolar district of Karnataka and adjoining areas of Andhra Pradesh and Karnataka state (Vij *et al.,* 2006). Dahlem Red is an exotic layer type breed originated from Germany, extensively used to improve local birds in developing country. The birds were maintained at ICAR-Directorate of Poultry Research, Hyderabad, India. The immune competence, an indicator of the bird's health can be assessed by several parameters like HI, CBH response and the circulating T lymphocyte population (Yadav *et al.,* 2018). Researcher evaluated peripheral blood lymphocyte population with respect to a specific disease (Yun *et al.,* 2000). Yadav *et al.* (2002) reported that haematological profile is vital for assessing the responses of animals to various physiological conditions. Therefore, understanding immunocompetence traits by evaluating HI, CBH and peripheral cytotoxic T lymphocyte population in

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chickens is of direct importance for integrating the information in the poultry breeding programmes and thereby selecting birds with better resistance or tolerance against the diseases.

MATERIALS AND METHODS

Birds and experimental setup

The experiment was conducted in the year 2017-18. A total of 399 chicks (simultaneously hatched) from three breeds *viz.* Black Nicobari (118; F: 82, M: 36), Ghagus (144; F: 101, M: 43) and Dahlem Red (137; F: 74 M: 63) were produced through random mating using pooled semen by taking 80 dams from each breed. The chicks were reared in deep litter system under similar milieu with *ad libitum* access to water and feed. The chicks from 0-8 week were provided standard commercial layer chick feed having 2817 Kcal metabolizable energy (ME) with 18.02% crude protein (CP). The temperature, during the birds reared for seven week period, ranged from 17.5°C to 24°C. The experiment was carried out with prior approval of Institute's Animal Ethics committee of Indian Council of Agricultural Research-Directorate of Poultry Research, Hyderabad (IAEC/DPR/16/1). The chicks of all the three breeds were immunized against Newcastle disease (ND) with lentogenic LaSota vaccine through eye drops at 7th day and again at 28th day of age. The blood samples of all the chicks were collected at seven week of age from wing vein under aseptic conditions. Blood samples were incubated at 37°C for 3 h and centrifuged at 1500 g for 20 min for Serum separation. Separated serum samples were stored at -20°C until used for HI titre estimation. Another set of blood samples were collected in EDTA-coated vials for flow cytometric analysis of Cytotoxic T lymphocytes.

Humoral immune response to newcastle disease vaccine

The haemagglutination (HA) titre of the NDV antigen (LaSota stock virus) was adjusted by dilution to contain 4 units of HA activity. HI titre was determined as the highest dilution of serum samples that inhibited NDV agglutination of chicken RBCs.

CBH response to PHA-P

Seven weeks old chicks were injected subcutaneously on the left wattle with 0.1 mg PHA-P in 0.1 ml of PBS (1 mg PHA-P/ml of PBS). The wattle thickness was measured using thickness gauge (Mitutoyo) before injecting mitogen and 24 h after injection. The cell mediated immune response was observed by calculating the difference in wattle swelling before and after injecting with PHA-P. (Wattle thickness before PHA-P injection - wattle thickness after PHA-P injection*100/ Wattle thickness before PHA-P injection).

Flow cytometric analysis of cytotoxic T lymphocytes

Mouse anti-Chicken CD8α-PE antibody (Southern Biotech, AL, USA) at a concentration of 0.2 ug for approximately 1 million cells was used to stain the chicken peripheral blood samples. The samples were incubated for 30 minutes at room temperature. BD-FACSLyse solution (BD Biosciences) was used to lysis of the RBCs as per the manufacturer's instructions. For absolute counting of CD8 positive cells, BD liquid counting Beads (BD Biosciences) were added and samples were analyzed within half an hour after lysis. BD LSR II flow cytometer fitted with 488 nm solid state laser and collection optics for PE (band pass filter 575/26 nm) using FACS Diva software was used for data acquisition and analysis.

Statistical analysis

The data of cutaneous basophil hypersensitivity, HI tests and T-Lymphocyte counts were analyzed separately using general linear mixed model:

$$X = \mu + Bj + Yk + Zjk$$

Where

μ = Population mean.

Bj = Breed.

Yk = Sex.

Zjk = Random effects in the model.

Duncan's multiple comparison test was used to compare the level of significance among factors. Statistical analysis was used using SAS software (version 9.3).

RESULTS AND DISCUSSION

Humoral immune response to ND vaccine

The vaccination against NDV fails to prevent the outbreak because of the genetic diversity and continuous evolution of ND virus. Different breeds and even individual birds of the same breed show different response to NDV. Therefore, the genetic selection and breeding strategies are important to enhance the genetic resistance of birds. Humoral immune response to ND vaccine as determined by $\log_2$ HI antibody titre was found to be highest in Dahlem Red (2.44±0.12) which significantly differed from the other two native breeds BlackNicobari (1.38±0.14) and Ghagus (2.00±0.13) as shown in Table 1. Sex wise analysis also revealed that females were having significantly higher antibody titre (2.12±.09) compared to males (1.75±0.12) as depicted in Table 2. The difference in the titre may be due to the genetic factor as the gene is located on the sex chromosome and the variation may be attributed to the fact that the breeds studied were not subjected to artificial selection for immune competence traits. The present study contradict the earlier report by Saxena *et al.,* 2012 in which they reported higher immune responses (HA) against SRBC in male (7.925 ± 0.1463) compared to female (7.57±0.161) native chicken. Santosh (1999) evaluated the response to SRBC in White Leghorn, Aseel, Kadaknath, Dahlem Red and their crosses and reported significant genotype differences for SRBC response and Dahlem Red birds showed the maximum titre.

Many workers have reported that native chicken varieties are more tolerance compared to exotic breeds against infectious diseases (Dessie *et al.,* 2011; Han *et al.,* 2013). Taha *et al.* (2012) reported significant differences in antibody titer responses to Newcastle disease virus between different breeds of chicken. Wondmeneh *et al.,* 2015 also reported that native chickens have significantly (*P*<0.05) higher natural antibody (Nab) levels compared to exotic and crossbred at different ages. In the present study, low HI antibody titre was observed as birds were given only two doses of lentogenic vaccine, at 7th and 28th day and the titres generally rise only after mesogenic booster ND vaccine. In the present trial, blood was collected at 7 weeks of age after which the experimental birds were administered mesogenic booster ND vaccine when the birds were 8 weeks of age. The present finding is in coherent with earlier studies as reported by Kokate *et al.* (2017) in which the exotic chicken and White Leghorn showed higher HI titre (2.60±0.12) against NDV compared to native breeds Aseel (2.43±0.16) and Kadaknath (2.38±0.06) at 7 weeks of age.

Cytotoxic T cell (CD8+)

It is well established that MHC class I restriction has a role of cytotoxic T cell (CD8+ T cells) in recognition and killing of the target cell in poultry (Weinstock *et al.,* 1989). To observe the immunocompetence traits in different breeds of chicken (*viz.* Ghagus, Dahlem Red and Nicoabri), total count of T lymphocyte bearing CD8 molecule in the peripheral blood of chicken was carried out by flow cytometry assay. The assay revealed that cytotoxic T cell population is highly inconsistent even within the breed (Fig 1). The study revealed a significant difference (P<0.05) between the three breeds. The T cell number bearing CD8+ was found to be highest in Ghagus (2132.34±69.526) followed by Nicobari (2023.53±75.14) and least in Dahlem Red (1649.49± 64.54). Sex wise results showed that males (1989.41± 65.47) were having high Tc cell compared to females (1880.83±47.14). In the present experiment, significant higher CD8+ lymphocytes proliferation was observed in Black Nicobari and Ghagus (native breeds) compared to Dahlem Red (exotic) breed, which indicate the existence of higher cell mediated immunity in native birds as compared to exotic birds. The proportion of peripheral blood T cell population is known to influence immunocompetence which is responsible for disease susceptibility or resistance in chicken (Yun *et al.,* 2000). Yadav *et al.* (2018) reported the higher cytotoxic T cell (CD8+) number in native Brown

Table 1: Breed wise variation of T-lymphocytes carrying CD8 molecules (cells/µl) CMI (response %) and HI antibody titre(log_2) in whole blood.

Breed	Cytotoxic T cell mean±SE	CMI response % mean±SE	Haemagglutination inhibition titre (log_2) to ND vaccine (LaSota) Mean±SE
Dahlem Red	1649.49± 64.54^{c} (n=137)	267.60±13.99^{b} (n=136)	2.44 ±0.12^{a} (n=136)
Ghagus	2132.34±69.53^{a} (n=144)	375.84±15.04^{a} (n=142)	2.00 ±0.13^{b} (n=141)
Nicobari	2023.53±75.14a^{b} (n=118)	281.41±16.22^{b} (n=117)	1.38±0.14^{c} (n=95)

Within column, the different superscripts indicate significant difference (P<0.05).

Table 2: Sex wise variation of T-lymphocytes carrying CD8 molecules (cells/µl) CMI (response %) and HI antibody titre(log_2) in whole blood.

Breed/ Sex	Cytotoxic T cell mean±SE	CMI response % mean±SE	Haemagglutination inhibition titre (log_2) to ND vaccine (LaSota) mean±SE
Female	1880.83±47.14 (n=257)	346.35±10.27^{a} (n=254)	2.12±.09^{a} (n=239)
Male	1989.41±65.47 (n=142)	270.22±14.11^{b} (n=141)	1.75±0.12^{b} (n=133)

Within column, the different superscripts indicate significant difference (P<0.05).

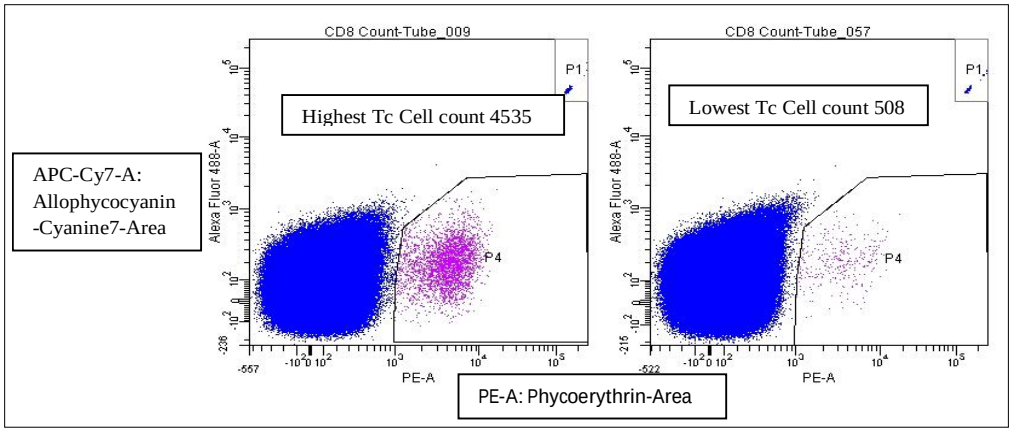

Fig 1: Dot plot with a region distinguishing the cytotoxic T cell (CD8+ve) population in ghagus chicken peripheral blood.

native breeds. The number of CD8+ T cells may act as one of the parameters to know the level of disease resistance. A higher number of CD8+ T cells in the joints and peripheral blood have been noted in WLH chickens which are resistant to *Enterococcus faecalis,* in contrast to the brown layer breed, which is susceptible to the same infection (Zekarias *et al.,* 2000). The study shows that the peripheral blood circulating cytotoxic T cell profile in native vs. exotic breed of chicken and may prove to be instrumental in developing and determining the health status of the birds especially against intracellular pathogens.

CBH response to PHA-P

The PHA-P boosts the immune cells to go through blast transformation and proliferation. It is one of the measures to quantify the proliferative capacity of certain cell types specifically T-lymphocytes which in turn indicate the general cellular immune response of the bird. A CBH response to phytohemagglutinin-P (100 µg/ per bird) in seven week old birds showed significant difference ($P<0.05$) in the wattle thickness (% increase after 24 hours) across the breeds and sexes. Highest response was observed in Ghagus (375.836±15.04) and least in Dahlem Red (267.603±13.99). Sex wise results showed that females (346.35±10.27) were having higher percentage of increase in wattle thickness compared to males (270.22±14.11). In contrary with present study, earlier workers reported that this T- cell mediated in vivo response (web index) was higher in males than in females (Cheng and Lamont, 1988). Yadav *et al.,* (2018) also reported the higher CMI response in females than in males. On the other hand, non-significant difference was reported between sexes for CMI response by Kundu *et al.* (2015) and Saxena (1993) which were not consistent with the findings of the present investigation. Pathak *et al.* (2018) reported that *in vivo* cell mediated response to PHA-P at seven weeks of age was highest in Aseel followed by synthetic broiler (IBL-80) and lowest in Kadaknath. Oladele *et al.* (2010) reported higher levels of natural antibodies and more intense delayed type hypersensitivity reaction in Nigerian indigenous chickens than exotic Nera breed. Higher CMI response in native breeds as compared to exotic breed probably is an attributed to their unique genetic structure or alleles evolved over years for being reared in a competitive open environment. The study revealed that the cytotoxic T cell number has direct relationship with PHA response *i.e.* the response of CTL and PHA have same pattern which is highest in Ghagus followed by Black Nicobari and least in Dahlem Red breed. The result indicates an important association between the activation of T-cell mediated immune system and local tissue swelling. Tella *et al.* (2008) also reported the highly significant correlation between individual tissue swelling and circulating CD5+ and CD8+ lymphocyte subsets.

CONCLUSION

In the present study, it was observed that HI antibody titre (log_2) to ND vaccine was found to be highest in exotic breed (Dahlem Red) which is significantly different from the other two native breeds (Ghagus and Black Nicobari). Sex wise analysis also revealed that females were having significantly higher response against ND vaccine. While the measure of cytotoxic T lymphocyte is perhaps the most relevant functional measure that reflects cell-mediated acquired immunity against viral infections, which is significantly higher in native breeds compared to exotic in the present study.CBH response, is the measure to quantify T-cell mediated immune response which was observed to be significantly higher in native breeds compared to exotic. Based on the observations, it can be concluded that there is significant higher humoral immunity against NDV in Dahlem Red compared to native breeds while the trend of cell mediated immunity is just the opposite *i.e.* significantly higher in Ghagus and Black Nicobari compared to Dahlem Red. This observation will help in selection of birds having higher immunocompetence at an early stage for bird selection programs.

Conflict of interest

All authors declare that they have no conflict of interest.

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