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Comparison of various immune responsiveness traits in divergent stocks of chicken: A review

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Abstract
The poultry industry during the past two decades has been one of the most dynamic and ever expanding sectors in the world. Worldwide poultry production is being undertaken under a wide variety of climate, production and management conditions and the losses occurring due to diseases are large. Development of stocks resistant to wide variety of diseases i.e. general disease resistance, offers a good prospect of reducing the expenditure on prophylactic and vaccination programmes. The comparative evaluation of immunological responses of genetically diverse chicken stock of layer and broiler chicken will help in the development of stocks with higher production potential and with increased resistance to pathogen, parasites and non-specific stresses. The information presented in this review paper shows that it would be worth comparing these traits in divergent stocks of chicken.

Keywords: Poultry, immune responsiveness, non-specific stress

Introduction
Worldwide poultry production is being undertaken under a wide variety of climate, production system and management conditions and the losses occurring due to diseases are large and widespread. Development of stocks resistant to wide variety of diseases i.e. general disease resistance, offers a good prospect of reducing the expenditure on prophylactic and vaccination programmes. Incorporation of genetic resistance has several advantages including the enhancement of the immune response to vaccines (Gavora and Spencer, 1979) [13]. Immune responsiveness in poultry can be evaluated by challenging the birds with antigens. These antigens may be causative agent of disease like Marek's disease virus, New castle disease virus, but they are expensive, time consuming and will cause morbidity and mortality in flock since they are pathogenic in nature. So the appropriate way to study immune-competence in a species is to challenge it with non-pathogenic, non-specific antigen like Sheep Red Blood Cell (SRBC), Mollusk Haemocyanin, Chicken Egg White Lysosome and Bacterial Lipo-polysaccharids or to measure the level of antibody production against the vaccines. SRBC is the most commonly used antigen to study immune-competence in poultry (Vanderzipp, 1983) [4]. The comparative evaluation of immunological responses of genetically diverse chicken stock of layer and broiler chicken will help in the development of stocks with higher production potential and with increased resistance to pathogen, parasites and non-specific stresses.

Immune Response to Sheep Red Blood cells (SRBC)
Genetic enhancement of immune competence without challenging birds with disease agents may be a potential approach for improving health and production parameters as well as disease resistance. Indirect selection for immune response traits can improve genetic resistance to diseases (Vanderzipp, 1983) [41].

Among various immune competence traits, response to antigen, serum lysozyme concentration and IgG level are some important measures. Various antigens have been used to monitor immune responsiveness in poultry. Non-pathogenic antigen included synthetic glutamic acid alanine tyrosine (Cheng and Lamont, 1988) [10], Bovine Serum Albumin (Parmentier *et al* 1994, 1998b) [27, 28] and Sheep Red Blood Cells (Siegel and Gross 1980, Li *et al* 2000) [33, 24]. Genetic variation in immune response is well established in chicken. SRBC antibody response is one of the model systems used for genetic analysis of humoral immune response. SRBC are complex, non pathogenic, T-cell dependent antigen and most selection experiments in chicken have been conducted using antibody response to SRBC (Siegel and Gross 1980, Vanderzipp *et al* 1983) [33, 40]. SRBC antigen is thymus dependent antigen, which needs the help of lymphocytes to produce antibodies (Cheng *et al.* 1991) [11]. Parmentier *et al.* (1998b) [28] reported that birds having higher antibody response against non- specific and multi-determinant antigen

SRBC also produce more antibodies to a variety of other antigens. Indian native breeds of chicken are considered to have better general disease resistance for the tropical diseases than imported breeds because they have evolved through natural selection over a long period in the prevailing tropical environment of the country. Many workers had compared the immune response among the various breeds and strains (Vanderzipp and Leenstra, 1980; Hampl *et al.,* 1989; Martin *et al.,* 1989) [39, 15, 25].

Response to Mitogen Phytohaemagglutinin (PHA-P)

The cellular mechanism involved in immune responsiveness may be considered at two levels

i. Cell mediated immune response
ii. Phagocytosis

Cell mediated immunity is primarily mediated by T cells, macrophages and natural killer (NK) cells while phagocytosis is basic, nonspecific body response to microbial infection.

Mitogens are substances that stimulate a large proportion of immune cells to undergo blast transformation and proliferate. Mitogens, are therefore used to measure the proliferative capacity of certain cell types which are indicator of general cellular immune responsiveness. Phytohaemagglutinin (PHA-P) and Concanavalin (Con-A) are mitogens known to stimulate T cells in avian species. Cell mediated immunity is measured by localized *in vivo* inflammatory response of chicken to phytohaemagglutinin since long times.

Ubosi *et al* (1985) [38] studied the age dependency of antibody response to sheep red blood cells (SRBC) antigen in lines of chicken divergently selected for this trait and in reciprocal crosses between them. At 7 days of age there were differences among the populations for the frequency of responders and all populations reached serological maturity at 14 days of age. Chickens of high line had significantly higher antibody titre than those from the low line and cross populations.

Genetic analysis of immune competence measures in a White Leghorn chicken line of the Iowa State University S1 White Leghorn chicken line was studied. This line was divided into eight sublines based upon erythrocyte antigen B (Ea-B) allele (B1B1 or B19B19), antibody response to glutamic acid-alanine-tyrosine (GAT) (high or low), and response to Rous sarcoma virus-induced tumors (progression or regression). Antibody responses to Pasteurellamultocida (PM), Mycoplasma gallisepticum (MG), and infectious bursal disease virus vaccines were evaluated by enzyme-linked immunosorbent assay. Phagocytic activity and T cell-mediated response were measured by carbon clearance and phytohemagglutinin (PHA) injection assays, respectively. Significant haplotype (subline) differences and sire family differences were observed in all three measurements. Significant sex differences were observed in phagocytic activity and T cell-mediated responses. Haplotypes with high antibody responses to GAT had significantly higher antibody titers to PM and MG vaccines than haplotypes with low antibody responses. Significant positive correlations were observed between antibody levels to the two vaccines. A significant negative correlation was seen between phagocytic activity and T cell-mediated response of females. The data suggest that the total immunocompetence profile of an individual must be considered to select for optimum immune responsiveness (Cheng and Lamont, 1988) [10].

Parmentier *et al* (1998b) [28] studied the mitogen induced cutaneous hypersensitivity in chickens selected for High (H) and low (L) antibody response to Sheep Red Blood Cells (SRBC). Mitogens Phytohaemagglutinin, Con-A, pokeweed mitogen and *E. coli* lipo-polysaccharides induced significant acute 4 hours web swelling response followed by a significant late 24 hours wing web swelling response 4 and 24 hour responses were lower in L line.Evaluation of general immunocompetence in four pure breeds and their selective crosses by *in vivo* cell mediated immune response to Con-A revealed that the breeds did not differ significantly, however crosses showed significant differences among themselves (Haunshi and Sharma, 2002) [17].

Study on cell-mediated immune response in selected line (IWH) and control line at 40 weeks of age, conducted on high, medium and low feed efficiency groups showed that foot index did not follow any definite pattern in selected line, while in control line foot index value was highest in high feed efficiency group followed by medium, and low feed efficiency group (Reddy *et al*., 2005) [29].

Singh *et al* (2009) [35] conducted comparative study of immune response to sheep red blood cells and PHA-P inoculation in pure and crossbred chicken. The HA titre on day 10, was significantly higher in WLh (3.83±0.13) compared to the RIR (3.24±0.15). The crosses between breeds or between strains of the same breed did not exhibit any superiority as indicated by HA titre post primary inoculation. The *in vivo* cell mediated response to mitogen phytohaemaglutinin response (PHA-P) was highest in the unselected control line (PL3) (0.82 mm) followed by selected strains of WLH and RIR (PL1, PL2, Naked) (RIR-C and RIR-B). The PHA response of WLH was significantly higher than RIR. The crosses did not yield high PHA response than their corresponding pures.

Specific Immune Responses to Infectious Bursal Disease Virus

Assessment of passively acquired protection against infectious bursal disease virus in chickens by ELISA made it possible to estimate the ELISA titre necessary in vaccinated hens to provide the desired duration of passive protection; protection being assessed by an ELISA which measures IBD viral antigen in the bursa of Fabricius following challenge (Fahey *et al*., 1987) [12].

Study was conducted on a double antibody sandwich ELISA for rapid diagnosis of virus infection and to measure the humoral response against infectious bursal disease on clinical material. Serum titres obtained in indirect ELISA and serum neutralization test were compared with (double antibody sandwich) DAS-ELISA. The agreement was 80% between DAS-ELISA, and the conventional techniques, with high sensitivity (87%) and specificity (90%) (Cardoso *et al*., 1998) [9]. Correlation of Enzyme-Linked Immunosorbent Assay titers with protection against Infectious Bursal Disease Virus resulted that predicting the percentage of protection against classic or variant IBDV strains in broilers from vaccinated breeder flocks can be improved when VP2 (polyprotein antigen) is used as the only antigen in the ELISA (Jackwood *et al*., 1999) [20].

Study on validation of five commercially available ELISA for the detection of antibodies against Infectious Bursal Disease Virus (serotype 1) revealed that all ELISAs reached a sensitivity of 100% on sera between 14 and 21 days post-vaccination (d.p.v.) with two classical vaccines and a Delaware variant-E virus. Overall, most birds became positive between 8 and 11 d.p.v. As expected, the ELISA with the lowest specificity showed the highest sensitivity at 5 d.p.v. When the decrease in maternally derived antibodies against IBDV was measured, a highly significant correlation ($P <$

0.001) was found for all ELISAs and the virus neutralization test (VNT) (Wit *et al*., 2001) [42].

Hassan *et al* (2002) [16] conducted study on susceptibility of vaccinated and unvaccinated Egyptian chickens to very virulent infectious bursal disease virus. Neither pathological nor immunological measures correlated closely with susceptibility or resistance of the different breeds. The findings suggest that innate non-immunogenic factor(s) may play a critical role in resistance. Study on detection of serum antibody levels against Infectious Bursal Disease (IBD) Virus using indirect hemagglutination (IHA) test in commercial broilers pointed out that maximum positive samples belonged to 6-8 weeks of age (78.57%) followed by 3-6 weeks (60.29%) and 0-3 weeks (43.02%), respectively. Calculated geometric mean titers for groups 0-3, 3-6 and 6-8 wks of age, were found to be 6.37, 10.84 and 16.14, respectively (Hussain *et al*., 2003) [19].

Study on effect of different levels of maternally derived antibodies on protection against Infectious Bursal Disease Virus was conducted and Chicks with no maternal antibodies were not protected at any age. Chicks with medium levels of maternal antibodies were protected when challenged at 1 and 2 wk of age. Chicks with high levels of maternally derived antibodies were protected when challenged at all the ages tested (Al-Natour *et al*., 2004) [1]. Use of multiple antigenic peptides related to antigenic determinants of infectious bursal disease virus (IBDV) for detection of anti-IBDV-specific antibody in ELISA focused on the antigenicity of two eight-branched Multiple antigenic peptides (MAPs) to detect anti-IBDV antibodies in ELISA, would serve as safe, chemically defined, noninfectious alternative antigens to whole virus in sero-diagnosis (Saravanan *et al*., 2004) [31].

Herdt *et al* (2005) [18] conducted study on the enzyme-linked immunosorbent assay for the detection of antibodies against infectious bursal disease virus (IBDV) and the estimation of the optimal age for IBDV vaccination in broilers. Results demonstrated a linear relationship between mean ELISA titres and virus neutralization (VN) titres. It was concluded that the ELISA could be a useful tool in IBDV vaccination timing, but that interpretation criteria should be adjusted to avoid vaccination at too early an age. Study on detection of antibodies against serotypes 1 and 2 Infectious Bursal Disease Virus by commercial ELISA kit resulted that commercial ELISA kits detect antibodies elicited by the two serotypes of IBDV. Hence, the prevalence of serotype 2 antibodies in a flock should be considered while determining antibody profiles of the flock against serotype 1 viruses (Ashraf *et al*., 2006) [3].

Immune responsiveness to IBDV vaccine in broiler chicken lines divergently selected for immune competence index differ significantly ($P \leq 0.01$) in antibody response to IBDV vaccine at 14 dpi. Peak response was observed earlier in high index line (at 14 dpi) as compared to low line (21 dpi) (Sivaraman and Kumar, 2006) [36]. The line x sex interaction was significant at 7 and 28 dpi indicating that differences between sexes across the lines were not similar.

Significance of vaccination against infectious bursal disease virus (IBDV) at the optimal time point in broiler flocks with maternally derived IBDV antibodies showed that flocks IBD-vaccinated between 1 day before, at, or up to 3 days after the estimated optimal time point developed detectable humoral immunity up to 14 days post vaccination. If birds had been vaccinated more than 1 day before the calculated optimal vaccination date, the humoral immune response was delayed or non-detectable until slaughter (Block *et al*., 2007) [6]. Effect of arginine and vitamin E on improvement of antibody responses to infectious bursal disease virus (IBDV) and sheep red blood cells in broiler chickens showed that diets with high arginine (ARG) and high vitamin E (80 mg/kg) improved the humoral-mediated immune response of broilers to IBDV and SRBC, suggesting it could be a strategy to improve vaccination protection and resistance to diseases (Ruiz-Feria and Abdukalykova, 2009) [30].

Genetic background also influences the induction of innate and acquired immune responses in chickens depending on the virulence of the infecting infectious bursal disease virus (IBDV) strain. No significant differences were seen between genetic backgrounds in induction of the IBDV-specific humoral response and timing of IBDV-antibody induction and antibody levels were comparable between Broiler-Type and Layer-Type birds. It demonstrated a significant influence of chickens' genetic background on disease outcome and difference between backgrounds in IBDV susceptibility is influenced by the virulence of the infecting virus strain (Aricibasi *et al*., 2010) [2].

Comparison of Strains

Phenotype of an animal is function of its genotype and present and past environment. Environmental factors like mutation, feed, management and temperature influence growth and modify the sensitivity to infectious agents and immune responsiveness of birds (Glick *et al* 1983, Katanbaf *et al* 1988, Boa *et al* 1991, Zulkifli *et al* 1993, 1994) [14, 21, 8, 43, 44].

Immuno competence traits being under genetic control are influenced by selection and breeding. Marked breed, strain and line differences have been reported for various immunological traits (Cheng and Lamont 1988, Baelmans *et al* 2005) [10, 4]. For the formulation of a rational breeding policy, the breed and strain difference among various immune competence traits are of utmost value.

Total agglutinin antibody titre, 2-Merceptoethanol sensitive (MES) and resistant (MER) titre in 598 White Leghorn chickens at 0, 3, 7, 10 and 13 days post-injection was estimated by Vanderzipp and Leenstra (1980). Total antibody titre on day 0 and 5 represented completely 2-Merceptoethanol sensitive antibodies and natural antibodies were present in some chicks at day 0. Mean total titre was highest (5.2) on day 7 post immunization. Investigation done on the intensity of antibody production and wattle reaction to natural antigens with different thymus dependency in adult cocks of two layer breeds (White Leghorn and Rhode Island Red) and it showed that both humoral and cellular immune reactivity to sheep red blood cells were breed dependent than those to *Brucella abortus* crude antigen (Hampl *et al*., 1989) [5].

IgM and IgS response in high and low antibody selected lines of chicken revealed primary response pattern of total, MER and MES antibodies differed according to origin and Total antibodies increased rapidly, peaked and persisted at moderate level in high antibody selected line (HA). MES level peaked then declined in line HA but it persisted at low level throughout in line LA. Titre of MER antibodies was considerably higher in line HA than in line LA. Secondary total titre was greater at five days after injection than at three day and greater for HA chicks than for LA chicks (Martin *et al*., 1989) [25]. Immune responsiveness of White Leghorn and Rhode Island Red cocks immunized with sheep red blood cells and *Brucella abortus* crude antigen were evaluated by Benda *et al* (1990) [5]. Significant breed differences were

found for humoral and cellular immune responses. The wattle response was much higher in WLh than the RIR birds.

Genetic study on primary antibody response to sheep erythrocytes in guinea fowl, using haemagglutination test was conducted and Immune response to sheep erythrocytes was normally distributed in guinea fowl with mean titre 1.53 ± 0.01. The effects of sire and variety (feather colour) were significant on titre value while the sex and sex × variety interaction for titre values was not significant. The estimate h^2 for the immune response to SRBC was 0.35 ± 0.17(Saxena *et al.*, 1997) [32].

Antibody response to sheep erythrocytes in Indian native breeds *viz*; Aseel, Kadaknath, Naked Neck and Frizzle with imported breeds *viz*; Dahlem Red, White Leghorn, Synthetic Dam Line Broiler and Naked Neck and test on primary antibody response to sheep erythrocytes and revealed presence of natural antibodies in all groups and highest antibody titre was recorded on day 5 post immunization except in Dahlem Red which showed highest response throughout and among Indian native breeds (Kundu *et al*., 1999a) [23]. Naked neck has highest titre. There was significant variation in HA response among various genetic groups at different days post immunization.

However the immune competence status of Indian native and imported breeds of chicken by 2 Mercaptoethanol resistant (Ig G) and sensitive (Ig M) antibody responses to sheep erythrocyte showd that natural Ig G and lg M antibodies were present in all breeds under study. High Ig C response was obtained in Dahlem Red followed by Naked Neck, Frizzle, White Leghorn, Kadaknath, SDL broiler, Aseel, NN broiler at day 5 post immunizations while Ig M has highest peak in Aseel followed by Kadaknath, Dahlem Red, NN, WL, Frizzle, NNB and SDL broiler. There was significant variation to MER and MES response among various genetic groups on different day post SRBC immunization (Kundu *et al*., 1999b) [23]. Apparent differences between breeds and between sexes were not significant but the interaction between sex and breed was significant at 5 days post injection.

Boa *et al* (2000) [7] studied temporal pattern of SRBC antibody response after primary and secondary inoculation in White Leghorn lines selected for High (HA) and low (LA) 5 day antibody titres at 50 days of age. Antibody titre against SRBC was measured at 3, 5, 7, 9, 11, 13 and 20 days after inoculation. At 70 days of age half of birds were given a booster inoculation and antibody titre was measured 3, 6, 9 and 13 days after secondary injection. Pattern of antibody response to SRBC differed according to line, resulting in interaction of line by dosage by day. Antibody response to booster inoculation differed between lines with old age effect present for LA but not for HA chicks.

Msoffe *et al* (2001) [26] studied the variation in physical characters and immune competence among 84 adult scavenging local chickens from four ecoclimatic regions in Tanzania. They studied traits like adult body weight, body length, shank length, egg weight, antibody response to sheep red blood cells, serum hemolytic complement level and cutaneous response to Phytohaemagglutinin-P and reported significant differences between ecotypes for high and low response to sheep red blood cells.

Evaluation of general immunocompetence traits at 10-12 weeks of age in four pure chicken breeds *viz*. Dahlem Red, Aseel, Kadaknath and White Leghorn along with their selective crosses showed significant breed differences for antibody response to SRBC. Dahlem Red has higher HA titre (9.21±0.46) while cross-breds showed significant differences among themselves for HA titre to SRBC (Haunshi and Sharma, 2002) [17]. Singh *et al* (2004) [34] studied immune competence status in two turkey varieties in India. They measured the humoral immunity as response to Sheep Red Blood Cells by Haemagglutination Test 5 days post immunization with SRBC, Mercaptoethanol Resistant (MER) and Mercaptoethanol sensitive titre. They revealed that sexual dimorphism is apparent for body weights and males were heavier than females in both varieties. The economic and immunological traits in a synthetic dam line (SDL) of broiler chicken and also estimated by genetic and non-genetic parameters. No effect of sire on HA titre and body weights at 4, 5 and 6 weeks of age was found. Heritability estimates were high for body weight but low for immunological traits. Phenotypic correlations were high and positive among body weights, but were low between body weight and immunological traits (Sivaraman *et al*., 2005) [37].

Conclusion

Genetic selection that has been carried out for numerous years has led to considerable development in poultry production by improving productive, immune responsiveness traits and increasing the profitability of broiler breeder and layer hen production. There are indications that the defense mechanisms vary between layer- and broiler-type chickens. Genetic enhancement of immune competence without challenging birds with disease agents may be a potential approach for improving health and production parameters as well as disease resistance. Indirect selection for immune response traits can improve genetic resistance to diseases. The general and specific immune response traits are most important traits in both layer and broiler and this knowledge would find direct application in breed selection programs, which have to include selection for early immunocompetence, high disease resistance and efficient reproductive performance to meet the demands of modern intensive poultry production systems.

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