Short Communication

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In vivo cell – mediated immune response in various indigenous and exotic breeds of chicken

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Much of the variations in resistance to disease are genetically determined (Miller and Cook 1992). This knowledge of differences in disease resistance ability between different strains of birds may be valuable in genetic selection programs (Das *et al.* 2014). There is interest in identifying a genetic marker, which can be used for selection for general immune responsiveness and improving general immunocompetence profile of the birds (Singh *et al.* 2010). Mitogens stimulate large proportion of immune cells to undergo blast transformation and to proliferate, hence are used to measure proliferative capacity of certain cell types (such as T- lymphocytes) as an indicator of general cellular immune responsiveness. Mitogens, viz. phytohaemagglutinin-P (PHA-P) and concanavalin A (Con A) stimulate T- cells in avian species (Weber 1975). Phytohaemagglutinin type-P is used in its mucopolysaccharide from the red kidney bean, PHA-P provoke responses, influenced by sub- population of T-helper and T- suppressor cells. Good responder to PHA-P means higher general level of cellular immunity influencing T- cell mechanisms restricting or preventing lymphoma formation.

Localized *in vivo* inflammatory response of chickens to PHA-P has long been used as a measurement of cell-mediated immunity (Stadecker *et al.* 1977, Goto *et al.* 1978). T- cell immune response to PHA-P were used used *in- vivo* as web index in chicken and observed significant sire family and sex differences having higher value in male than female (Cheng and Lamont 1988). *In-vivo* immunocompetence measures in indigenous chickens were studied earlier also (Kundu 1997, Rajkumar *et al.* 2012). All observed significantly higher CMI response for Khadakanath compared to Aseel and values were also higher in males than females. Scientific information on cell-mediated immune response of indigenous breeds of chicken believed to be resistant to many infectious diseases is scarce. The present investigation was designed and conducted to evaluate the status of these flocks with respect to cell-mediated immune response by phytohaemagglutinin-P (PHA-P) vis-a-vis for the exotic breeds of chickens.

Birds,7-week-old, of both sexes belonging to different breeds were used. The innate immunocompetence trait in respect of cell-mediated immune response against phytohaemagglutinin-P (PHA-P) 100 microgram PHA-P/ 0.1 ml of phosphate buffer saline (PBS) of various indigenous and exotic breeds of chicken were measured at 7 weeks of age. Chickens (433) of both sexes and of 8 lines and native breeds Aseel (44), Kadakanath (69), Naked Neck (53), and Frizzle (49), Dahlem Red (49), White Leghorn (80), Synthetic dam line (SDL) broiler (43) and Nacked Neck (NN) broiler (46) were selected for the study.

In assay technique, *in-vivo* cell mediated immune response to PHA-P was evaluated as per Cheng and Lamont (1988). Phytohemagglutinin type P was used, which provokes responses, influenced by subpopulation of T-helper and T-suppressor cells. Good responder to PHA-P means a higher general level of cellular immunity influencing T-cell mechanisms restricting or preventing lymphoma formation. The procedure adopted was as follows:

(i) PHA-P, 0.1 ml (1 mg /ml PBS) was interdigitally administered between the third and fourth toe of the right foot of the chicken.

(ii) The left foot served as control and was injected with 0.1 ml PBS.

(iii) The skin index (foot index was calculated as the difference between the swelling, measured by a cutimetre type Harpenden skin fold caliper) in the right minus left foot, before and 24 h after injection and expressed as millimeter.

(iv) F1 (mm) = (post inj – pre inj) - (post PBS-pre PBS); where, post inj, thickness of test foot 24 h injection of PHA-P; pre-inj, thickness of test foot pre-injection; post-PBS, thickness of control foot 24 h post injection of PBS; pre-PBS, thickness of control foot before injection.

The statistical procedures (Snedecor and Cochran 1994) were used for analysis of data, which included computation of mean standard error and analysis of variance to evaluate the effect of genetic groups and sex. Differences in the

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means of various genetic groups were compared by use of Duncan's multiple range tests.

The response to PHA-P was evaluated by measuring interdigital foot skin thickness (in mm) at about 7 weeks of age. Change in skin swelling was used to determine CMI response and expressed as foot index (FI). The means along with standard errors for FI for separate and combined sexes of various genetic lines of chickens are presented in Table 1. The mean response to PHA-P (FI) averaged overall indigenous breeds of chicken was 0.34±0.01 with no difference between sexes. The mean response was highest in Dahlem Red followed by Kadakanath, Naked Neck, Frizzle-SDL broiler, White Leghorn and NN broilers. The overall mean for broilers was 0.27±0.02. Aseel, Naked Neck and Frizzle genetic groups did not differ significantly from each other. Kadakanath revealed significantly higher CMI response than all other indigenous chickens for this trait (Fig.1). Analysis of variance revealed significant line effect on response to PHA-P. Sex and interaction effects were not significant (Table 2). Response to an antigen is manifested

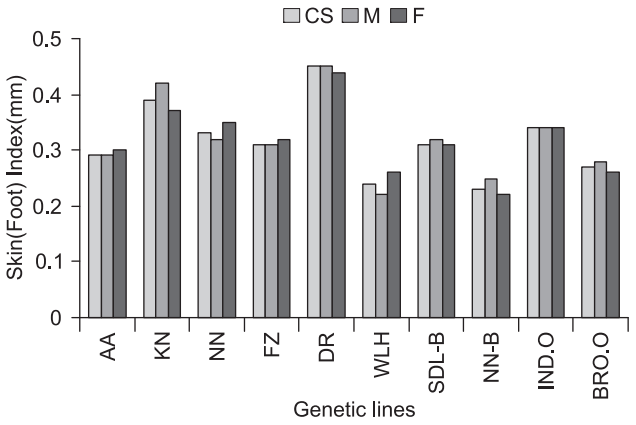

Fig. 1. CMI response (in mm) to PHA-P injection (FI) in various genotypes of chickens at 10 weeks of age. Aseel (AA), Kadakanath (KN),Naked Neck (NN),Frizzle (FZ); German Dahlem Red (DR), White Leghorn (WLH), SDL broiler (SDL-B), Naked Neck broilers (NN-B), averaged for indigenous chicken (IND.O), averaged for broilers (BRO.O).

by interaction of B- cells, T- helper cells and T- suppressor cells. The cellular interaction for generating immune response are rigorously regulated by class II MHC antigen on the surface of interacting cells but class II molecules do not regulate the ability of T- cells to recognize the specific epitopes. PHA-P is used in its mucopolysaccharide form from the red kidney bean, which provokes responses. This is also called delayed hypersensitivity occurring as a result of interaction between injected antigen and mitogen and T-lymphocytes (sub- population of T- helper and T- suppressor cells) and T- lymphocytes provide a measure of T-cell mediated response. Good responders to PHA-P means a higher general level of cellular immunity influencing T-cell mechanisms restricting or preventing lymphoma formation. T- cell mediated cytotoxicity is the major mechanism to destroy viral infection. However, the natural killer (NK) cells also take part in destruction of virus-infected cells. The cytotoxic T- cells recognize the foreign antigen in association with class I MHC antigens. The *in-vivo* proliferative response of T- lymphocyte to diversified phytomitogens was used as a tool by many workers for assessing the ability of an individual to mount the cell-mediated immune response. Several methods were suggested in the literature to measure cell-mediated immunity. Skin test, however, was widely used as a screening test for cellular immunodeficiency because of its simplicity and practicality (Bonforte *et al.* 1972). A cutaneous response to intradermal injection of skin agents has the characteristics of delayed type hypersensitivity (Lawlor *et al.* 1973). The data obtained in the present investigation clearly indicated that a cutaneous response by intradermal injection of PHA-P could be elucidated in normal chicken.

The CMI response observed for the various genotypes studied in this investigation are presented in Table 1. Most of these values were lower than Guinea fowls, Kadakanath and broiler chickens reported by Saxena (1993) and by Cheng and Lamont (1988) for White Leghorn chickens. Lasilla *et al*. (1979) observed *in vitro* responsiveness of lymphocyte to PHA-P ranged between 36,875±1,283 and

Table 1. Means and standard errors for CMI response (in mm) to PHA-P injection (FI) at 10 weeks of age

Geno types/ Sex	AA	KN	NN	FZ	DR	WLH	SDL-B	NN-B	IND.O	BRO.O
CS	0.29±	0.39±	0.33±	0.31±	0.45±	0.24±	0.31±	0.23±	0.34±	0.27±
(N)	0.03^{abc} (44)	0.02^{e}	0.02^{a}	0.02ab	0.04d	0.02c	0.03b	0.02c	0.01	0.02
		–69	–53	–49	–49	–80	–43	–46	–215	–89
M	0.29 ±	0.42 ± 0.02	0.32 ±	0.31 ±	0.45 ±	0.22 ± 0.03	0.32 ±	0.25 ±	0.34 ± 0.01	0.28 ± 0.02
(N)	0.03	–36	0.02	0.03	0.06	–44	0.04	0.03	–113	–43
	–29		–23	–25	–23		–20	–23		
F	0.30 ±	0.37 ± 0.02	0.35 ± 0.02	0.32 ±	0.44 ±	0.26 ± 0.03	0.31 ±	0.22 ±	0.34 ± 0.01	0.26 ± 0.02
(N)	0.05	–33	–30	0.03	0.05	–36	0.04	0.02	–102	–46
	–15			–24	–26		–23	–23		

CS, Combined sex; M, male; F, female. Means having the same superscript (column wise) do not differ significantly (P < 0.05). Aseel (AA), Kadakanath (KN), Naked Neck (NN), Frizzle (FZ), averaged for Indigenous chicken (IND.O), German Dahlem Red (DR), White Leghorn (WL), SDL broiler (SDL-B), Naked Neck broilers (NN-B), Averaged for broilers (BRO.O).

81,397±16,435 CPM in chickens. The low values observed in the present study may be due to variation in the injection site (footpad in the present study) vs. wing web used in other studies. Kean and Lamont (1994) indicated that caution must be exercised in comparing cell- mediated immune responses when different PHA-P injection sites were used to assess response. Significant differences among genotypes for CMI response was in agreement with findings of Miggiano *et al*. (1976) and Lasilla *et al.* (1979. Among the 3 breeds studied by Vanderzijpp (1983), Plymouth Rocks had the highest CMI response followed by warren and White Leghorns with differences between breeds being significant. Nonsignificant differences between sexes for CMI response as reported by Lasilla *et al.* (1979) and Saxena (1993) were consistent with the findings in the present investigation. Significant genetic group differences for FI as observed in this study was in agreement with the findings of Miggiano *et al.* (1976) and Lasilla *et al.* (1979).

From the results obtained, it is concluded that chicken populations utilized in this study differed widely with respect to CMI response to PHA-P. This trait was utilized to select the birds for disease resistance capability.

SUMMARY

Birds (433) of both sexes, 7-week-old, belonging to 4 Indian breeds, viz. Aseel, Kadakanath, Naked neck and frizzle and 4 exotic breeds, viz. Dahlem Red, White leghorn, Synthetic Dam Line (SDL) and Naked Neck Broiler (NNB) were evaluated for cell mediated immune response (CMI) by observing change in skin swelling expressed as foot index (FI). 0.1 ml of phytohaemagglutinin-P (PHA-P) was injected interdigitally (100 micrograms PHA-P /0.1 PBS) between third and fourth toe of the right foot of each chicken. The mean response to PHA-P averaged over all indigenous breeds of chicken was 0.34±0.1 with no difference between sexes. The mean response was highest in Dahlem Red followed by Kadakanath, Naked Neck, Frizzle, SDL Broiler, White Leghorn and NN Broilers. The Aseel, Kadakanath and Frizzle did not differ significantly from each other. Kadakanath revealed significantly higher CMI response than any other indigenous chicken breeds for this trait. Difference between lines showed a significant effect on response to PHA-P. Sex and interaction effects, however, were not significant.

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