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In vivo phagocytic activity in various indigenous and exotic breeds of chicken for genetic selection

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The defensive functions of phagocytosis come into effect immediately upon the invasion by the foreign materials (El-safty 2012). Awadhiya *et al.* (1981) studied the role of thrombocytes in phagocytosis in chicken using colloidal carbon as foreign material. The rate of clearance of injected colloidal carbon is used as an index to evaluate the phagocytizing ability of an individual. Kundu *et al.* (1996) observed significantly higher PI for Kadakanath compared to Aseel. The mortality up to 5 weeks of age were also low for Kadakanath. Males had lower PI values than females. Nachimuthu *et al.* (1989) observed increased phagocytic activity in vaccinated chicks compared to unvaccinated chicks after 10 days of vaccination. Disease conditions and physical injuries reportedly affected the phagocytosis.

Indigenous breeds of chickens are relatively resistant to many infectious diseases. Variation in disease resistance capability is also a widespread phenomenon in all species and much of the variations are genetically determined. This knowledge of difference in disease resistance ability between different strains of birds may be valuable in genetic selection programme (Miller and Cook 1992). The highly evolved avian immune system plays a central role in the disease resistance capability. Efficient and effective antibody response depends upon contributions of the immunogen and the host system. The feasibility of improving genetic resistance to specific diseases and breeding for general disease resistance in poultry are indicated in literatures (Gavora and Spencer 1983, Bacon 1992). There is a great deal of interest in identifying a genetic marker, which can be used for selection for general immuno responsiveness (Gavora and Spencer 1983, Vanderzijpp 1983a). Phagocytosis of monocytes-macrophages is a marker for breeding excellent progeny with strong disease resistance (Ma *et al.* 2010). Scientific information on phagocytic activity of indigenous breeds of chicken believed to be resistant to diseases is scarce. The present investigation was designed and conducted to generate information for immunological traits in respect to *in- vivo* phagocytic activity.

Experimental stock: The chicks of Aseel, Kadakanath, Naked Neck, Frizzle, Dahlem Red, White Leghorn, synthetic broiler dam line, Naked Neck broiler line constituted the base material for this investigation. Management and feeding regimens were kept identical as far as possible for all the genetic groups under study. They were given a standard starter ration up to 8 weeks of age and a grower ration from 9 to 20 weeks of age.

General plan of experiment: Chickens (433) of both sexes from indigenous Aseel (44), Kadakanath (69), Naked Neck (53), and Frizzle (49), Dahlem Red (49), White Leghorn (80), synthetic dam line (SDL) broiler (43) and Naked Neck (NN) broiler (46) were chosen for studying *in vivo* phagocytic activity at the age of 10 weeks of age by means of reticuloendothelial carbon clearance, a measure of phagocytic activity as phagocytic index (PI) test.

Assay technique: Method of Cheng and Lamont (1988) was followed for the evaluation of phagocytic activity (PI). It was measured at 7 weeks of age using intravenous injection of colloidal carbon. The phagocytic activity was measured by injection of the Indian ink into the birds under study and comparing their ability to clear the injected carbon from blood circulation over a period. An increase in the percentage of optical density (OD) value would be indicative of more carbon present in the sample at the time of quantification, in turn less phagocytosis. The procedure was as follows:

(1) India ink was centrifuged at 4,000 rpm for 45 min and supernatant fraction was collected.
(2) This supernatant fraction (carbon) was injected in brachial vein @1ml/ kg body weight of experimental birds.
(3) Three blood samples of 200 ml were collected in 4 ml of 1% sodium citrate solution from opposite wing of each bird. First blood sample was collected before injection, then after 3 min and 15 min of carbon injection.
(4) These samples were centrifuged at 500 rpm for 5 min.

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Table 1. Means and standard errors for phagocytic index (PI) at 10 weeks of age of various indigenous and exotic breeds of poultry

Sex×genotype	AA	KN	NN	FZ	Ind.O	GDR	WL	SDL-B	NNB	BRO.O
CS	0.13± 0.01ab (44)	0.14± 0.01ab (69)	0.13± 0.01a (53)	0.27± 0.02c (49)	0.16± 0.01(215)	0.13± 0.01a (49)	0.16± 0.01b (80)	0.14 ±0.01ab (43)	0.13± 0.01a (46)	0.13± 0.01(89
M	0.12± 0.01(29)	0.13± 0.01(36)	0.12± 0.01(23)	0.23± 0.02(25)	0.15± 0.01(113)	0.13± 0.01(23)	0.14± 0.01(44)	0.13± 0.01(20)	0.13± 0.01(23)	0.13± 0.01(43)
F	0.14± 0.01(15)	0.16± 0.01(33)	0.13± 0.01(30)	0.30± 0.03(24)	0.18± 0.01(102)	0.14± 0.01(26)	0.18± 0.01(36)	0.15± 0.01(23)	0.13± 0.01(23)	0.1423± 0.01(46)

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

(5) The relative amount of carbon remained in supernatant was measured spectrophotometrically in red light (675nm). The optical density of sample collected before carbon injection was taken as zero values.
(6) The density readings were converted into logarithmic scale.
(7) The phagocytic indices were calculated as the negative of slope of line determined between time and optical density readings.
(8) The slope was determined by the following formula:

$$\text{Slope} = \frac{Y_2 - Y_1}{X_2 - X_1}$$

where Y_1 and Y_2 are the ordinates at Y-axis, (i.e. the optical density reading at 3 and 15 min, respectively, and X_1 and X_2 are the ordinates at X-axis (i.e. the time 3 and 15 min, respectively).

Means and standard errors for the various traits measured were calculated by use of standard statistical procedures. Analysis of variance was used to evaluate the effects of genetic groups, sexes and their interactions (genetic group×sex) for phagocytic index trait under study.

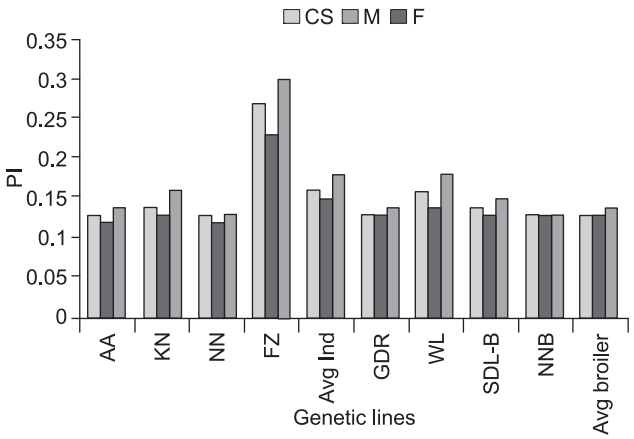

Fig. 1. Phagocytic index (PI) at 10 weeks of age of various genotypes of chicken. Aseel (AA), Kadakanath (KN), Naked Neck (NN),Frizzle (FZ), Averaged for Indigenous chicken (IND.O), German Dahlem Red (GDR), White Leghorn (WL), SDL broiler (SDL-B), Naked Neck broilers (NN-B), Averaged for broilers (BRO.O).

Differences in the means of various genetic groups were compared by use of Duncan's multiple range tests. The statistical procedures as described by Snedecor and Cochran (1994) were used for analysis of data.

The means and standard errors for phagocytic activity are presented in Table 1 according to sex and genetic groups. The phagocytic index (PI) for combined sex averaged over all indigenous breeds of fowl. The mean PI was highest in Frizzle followed by White Leghorn, SDL broiler and Kadakanath and rest of the genetic lines in that order (Fig. 1). Analysis of variance revealed significant differences between genetic groups and sex (P<0.01) although breed sex interaction was statistically non-significant. Phagocytosis is a basic defence mechanism of body. Among various methods used to assay this important immune phenomenon, carbon clearance assay is widely used. This procedure was therefore used in the present study for evaluation of phagocytic activity.

Significant genetic group's difference for phagocytic activity was in agreement with the findings of Cheng and Lamont (1988). Saxena (1993), however did not observe any difference between genetic groups for phagocytic activity.

Phagocytic activity values as obtained in this study although compared favorably well with those of Cheng and Lamont (1988) was different from those from Cheng and Hamilton (1979 a, b), Pachauri and Mukherjee (1972) and Nachimuthu *et al*. (1989), which may be due to different methods used by these workers for assaying of the phagocytic index in chickens.

Breed × sex interaction was nonsignificant as reported by Saxena (1993) in chicken and Guinea fowl. The nonsignificant differences among guinea fowl strains might be due to species specificity. The female showed higher response than male in the present study. In addition, the birds which showed comparatively better humoral antibody response (HA titre) are poorer in phagocytic index (Kundu 1997, Kundu *et al.* 1999a, Kundu *et al.* 1999b).

SUMMARY

Birds (433) of both the sexes developed from Aseel, Kadakanath, Naked Neck, Frizzle, Dahlem Red, White Leghorn, SDL and Naked Neck broilers, were evaluated

for phagocytic activity at the age of 10 weeks of age. The mean phagocytic activity in terms of phagocytic index (PI) determined using *in-vivo* carbon clearance assay averaged over both the sexes was highest for Frizzle (0.27) followed by White Leghorn (0.16), SDL broiler and Kadakanath (0.14) and PI value for rest of the genetic lines was 0.13. Sex and genetic group differences were significant but breed and sex interaction was not statistically significant. The combination of humoral and cell- mediated immunity and macrophages may be needed for providing optimum immune responses to chickens with a complete spectrum of resistance processes. Differences between genetic groups for phagocytic activity suggest, that selection for this trait will be effective to improve phagocytic activity. All immunocompetence traits however are to be taken into account for the development of disease resistant strain of poultry.

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REFERENCES

Awadhiya R P, Vegad J L and Kotle G N. 1981. A microscopic study of increased vascular permeability and leukocyte emigration in thermal injury in the chicken skin. *Avian Pathology* **10**: 313–20.

Bacon L D. 1992. Measurement of immunocompetence in chicken. *Poultry Science Reviews* **4**: 187–95.

Cheng C F and Hamilton P B. 1979a. Impaired phagocytosis by heterophils from chickens during aflatoxicosis. *Toxicology and Applied Pharmacology* **48**: 459–66.

Cheng C F and Hamilton P B. 1979b. Refractory phagocytosis by chicken thrombocytes during aflatoxicosis. *Poultry Science* **58**: 559–61.

Cheng S and Lamont S J. 1988. Genetic analysis of immunocompetence measures in a White Leghorn chicken line. *Poultry Science* **67**: 989–95.

El-safty Salah Abdel Rahman. 2012. Comparative study on some immunological traits in two different genetic groups of chicken. *Veterinary World* **5**: 645–50.

Gavora J S and Spencer J L. 1983. Breeding for immune responsiveness and disease resistance. *Animal Blood Groups and Biochemical Genetics* **14**: 159–80.

Kundu A. 1997. 'Comparison among genetically diverse lines of chicken for immunocompetence measures.' Ph.D. Thesis submitted to Deemed University, IVRI, Izatnagar.

Kundu A, Singh D P, Mohapatra S C, Moudgal R P, Dash B B and Bisht G S. 1999a. Immunocompetence status of Indian native vis-à-vis important breeds of chicken 2–Mercaptoethanol resistant (IgG) and sensitive (IgM) antibody response to sheep erythrocytes. *Indian Journal of Poultry Science* **34:** 295–302.

Kundu A, Singh D P, Mohapatra S C, Moudgal R P, Dash B B and Bisht G S. 1999b. Antibody response to sheep erythrocytes in Indian native vis-à-vis imported breeds of chickens. *British Poultry Science* **40**: 40–43.

Kundu A, Dash B B, Mohapatra S C, Singh D P and Singh H. 1996. *In vivo* immunocompetence measures in indigenous breeds of chickens. *Proceedings of World's Poultry Congress*, 2–5 Sep 1996, New Delhi, Vol.IV, pp.92.

Ma H, Ning Z H, Lu Y, Han H B, Wang S H, Mu J F, Li J Y, Lian Z X and Li N. 2010. Monocytes-macrophages Phagocytosis as a potential marker fr disease restance in generation 1 of dwarf chickens. *Poultry Science* **89**: 2022–29.

Miller C C and Cook M E. 1992. Immune response difference in different strains on ducks. *Poultry Science* **71**: Abstract Nos–96,166.

Nachimuttu K, Masiuamony R and Padmanaban V. 1989. Carbon clearance test to assess phagocytic activity. *Indian Veterinary Journal* **66**: 905–07.

Pachauri S P and Mukherjee S K. 1972. Phagocytic capacity of RES: Comparison in rabbit and poultry. *Indian Journal of Poultry Science* **7:** 5–7.

Saxena V K. 1993. 'Genetic studies on immunocompetence in Guinea fowl (*Numida meleagridis*).' Ph.D. thesis IVRI, Izatnagar, India.

Snedecor G W and Cochran W G. 1994. *Statistical methods*. 9th edn.Affliated East-West Press Pvt. Ltd Iowa State University Press, Iowa.

Vanderzijpp A J. 1983a. Breeding for immune responsiveness and disease resistance. *World's poultry Science Journal* **39**:118–31.