GENETIC ANALYSIS OF THE HUMORAL IMMUNE RESPONSE IN CHICKEN

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY)

PUSA, SAMASTIPUR, (BIHAR)

In partial fulfilment of the requirements

For the Degree of

Master of Veterinary Science

IN

ANIMAL BREEDING AND GENETICS

By

Dr. Rajiv Ranjan

Registration No. – M/ABG/93/2000-01

DEPARTMENT OF ANIMAL BREEDING AND GENETICS

BIHAR VETERINARY COLLEGE

PATNA – 800 014

2003

Dedicated

to

Respected

Late Dadi Maa

&

Late Dada Jee

Dr. S. R. Singh
Ph.D.
Associate Professor and Head,
Department of Animal Breeding and Genetics,
Bihar Veterinary College, Patna – 14
Rajendra Agricultural University,
Pusa, Samastipur, Bihar (India)

CERTIFICATE – I

This is to certify that the thesis entitled **"GENETIC ANALYSIS OF THE HUMORAL IMMUNE RESPONSE IN CHICKEN"** submitted in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Animal Breeding and Genetics) of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, Pusa is the record of bonafide research carried out by **Dr. Rajiv Ranjan** under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

11/12/03

(S. R. Singh)
Major Advisor

Endorsed :

11/12/03

(Chairman/Head of the Department)

CERTIFICATE – II

We, the undersigned members of the Advisory Committee of **Dr. Rajiv Ranjan**, a candidate for the degree of Master of Veterinary Science with Major in Animal Breeding and Genetics, have gone through the manuscript of the thesis and agree that the thesis entitled **"GENETIC ANALYSIS OF THE HUMORAL IMMUNE RESPONSE IN CHICKEN"** may be submitted by **Dr. Rajiv Ranjan** in partial fulfilment of the requirements for the degree.

11/12/03

(S. R. Singh)
Chairman
Advisory Committee

Members of Advisory Committee :

1. **Dr. K. G. Mandal** 11.12.03
   Assistant Professor-cum-Junior Scientist,
   Department of Animal Breeding and Genetics,
   Bihar Veterinary College, Patna – 14

2. **Dr. B. K. Sinha** 11/12/03
   Associate Professor and Head,
   Department of Veterinary Microbiology,
   Bihar Veterinary College, Patna – 14

3. **Dr. B. K. Sinha** 11/12/03
   Associate Professor and Head,
   Department of Veterinary Microbiology,
   Bihar Veterinary College, Patna – 14
   (Nominee of DRI-cum-Dean, P.G.)

CERTIFICATE – III

This is to certify that the thesis entitled **"GENETIC ANALYSIS OF THE HUMORAL IMMUNE RESPONSE IN CHICKEN"** submitted by **Dr. Rajiv Ranjan** in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Animal Breeding and Genetics) of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, Pusa was examined and approved on .....

(S. R. Singh)
Chairman
Advisory / Examination
Committee

Members of Advisory / Examination Committee :

1. **Dr. K. G. Mandal**

2. **Dr. B. K. Sinha**

3. **Dr. B. K. Sinha**
   (Nominee of DRI-cum-Dean, P.G.)

DRI-cum-Dean P.G. Studies.

ACKNOWLEDGEMENT

With stupendous ecstasy and profundity of complacency, I pronounce my utmost degree of gratitude to my major advisor Dr. S. R. Singh, Ph.D, Associate Professor and Head, Department of Animal Breeding and Genetics, Bihar Veterinary College, Patna, for his valuable guidance, critical appreciation, constant inspiration and constructive counselling during the course of my research work and preparation of this manuscript. I am indebted to him for all his efforts to help me to complete my master programme in time. Nonetheless a formal statement could hardly meet the end of justice in matter of expression of my deep sense of gratitude to him.

I am highly grateful to the esteemed members of my advisory committee – Dr. B. K. Sinha, M.Sc. (AIIMS), Assoc. Prof. and Head (Deptt. of Vety. Microbiology) and Dr. K. G. Mandal, Asstt. Prof. (Deptt. of Animal Breeding & Genetics), B. V. C. Panta for their valuable suggestions, prudent guidance and intense interest in going through the manuscript.

Neither words nor space will suffice to express my heartfelt gratitude to all my teachers especially Dr. S. B. Verma, Assoc. Prof., (Deptt. of Animal Breeding & Genetics), Dr. S. S. Singh, Assoc. Prof. and Head (Deptt. of Livestock Production and Management) and Dr. K. C. P. Singh, Ex-Assoc. Prof., (Deptt. of Veterinary Microbiology) for their guidance in gaining a better insight into the subject, sympathetic inspiration ad moral boosting during my scientific pursuits.

I gratefully acknowledge Dr. V. K. Saxena, Senior-Scientist, CARI, Izatnagar, for Statistical analysis of experimental data very meticulously.

I am highly obliged to Dr. R. P. Pandey, Assoc. Prof., Office-in-Charge and Dr. M. K. Choudhary, Assoc. Prof., Superintendent of APRI, Pusa, RAU and Dr. R. P. Mishra, G.M., CPF, Patna, who provided me experimental birds, valuable suggestions and generous help.

Cordial gratitude is expressed to Dr. A. Prasad, Dean, Bihar Veterinary College, Patna, for the interest the took in the research problem and valuable suggestion.

I appraise my cordial thanks to Dr. S. D. Singh, Principal, Bihar Veterinary College, Patna, for his co-operative behaviour and constant encouragement during the research period.

I extend my profound thanks to the Rajendra Agricultural University, Pusa, Samastipur (Bihar) for providing me financial assistance in the form of RAU fellowship.

I have no words to express my feeling towards the love, constant inspiration and everwilling help offered to me Dr. K. A. Ahmed (RA) and his wife Dr. (Mrs.) Anjuman Arrah (Govt. of Bihar) during my curricular and short term stay at IVRI, Bareilly (UP).

It might be, there is no words in the present to express gratitude to my senior colleage Dr. P. K. Singh, Asstt. Prof. cum Junior Scientist, SKUAST, Sri Nagar and Dr. Bikash Sahay, Ph.D. Scholar, Division of Animal Bio-technology, IVRI, Mukteshwar, Uttranchal for their honest advice and update scientific information from the border of the nation.

It is my pleasure to remember my respected seniors Dr. R. K. Dwivedi & Dr. Manish Srivastava for their everlasting inspiration and evergreen thoughts.

It is threshold pleasure to me that I have got Drs. Dhirendra, Shambhu and Niraj like animal geneticist friends and all concerned non-teaching staffs, whose critical help carry words but there could be scarse of papers.

I wish to extend eulogy and whole hearted endearment to my colleagues Drs. Asthana, Harish, Narendra, Dharmendra, Nirbhay, Bhagirath, Shankar, Sunil, Manoj Tiger, Alok, Sanjiv Sharma, R. Bharti, K. Kusum, Babloo,

B. G. Roy, Chandan & Pankaj Pandey, for their intimate and cheerful company, constant co-operation and timely help.

I consider myself lucky to have been in the cheerful company of my juniors like Drs. Balwant, Sudhakar, Nirala, Akhileshwar, Jyotindra, Manoj Bharti, Mithilesh, Amit, Krishna, Ashish & Prince.

It is my pleasure to acknowledge the true friends who have shared the moments of laughter and sorrow can never be forgotten. I can never thank enough to Ranju, Ajay, Navendu, Amardeep, Jayant, Subodh Singh, and Anil Raj.

I extend my feeling towards the heartfelt gratitude to respected Smt. Tara chachi jee and lovely Shipu and Shalu for giving homely atmosphere and great affection to me, whenever I was close to them.

It seems, I am only fortunate in the veterinary college campus to get Mrs. Pratima didi and Mr. Sudhir Kumar (Astt., SGIDT) like didi and jeejaji, who kept me as like as their own brothers.

"Where emotions are involved, words cease to mean". I admit my inability to put into words for the depth of my gratitude and indebtness to my Mammi and Daddy. The sufferings they faced for me are the reasons for bring at present stage. I am deeply indebted to all family members for their moral support, affection and unflagging inspiration during the course of this study. I will never forget to express my heartfelt gratitude to Lal Chacha and Chachi jee for their great affection, parental care and valuable guidance during crucial phase of my career.

Working would have been very tedious without love and affection of elder brother Dr. Pritam Pankaj (M.D.), Sister-in-law Dr. Trisala (BDS) and Younger brothers Er. Happy, Tinku, Appu, Sisters Rakhi, Tullu, Minoo, Sonal and twinkling nephew Avi.

It will be extravagant if I could not keep in mind the names of Mr. Samresh ji, Cyber Zone, SAM Computers, Ashiana Road, Patna, for giving me an opportunity to present a manuscript before you, at the cost of their comfort.

Writing this chapter, though non-taxing was perhaps more difficult than compiling the entire manuscript. To acknowledge individually, who extended their helping hands in various ways during the study was not practically feasible. I wish to express my sincere thanks to all those who couldn't be mentioned above.

Above all, I thank to "Almighty God" for the blessing throughout my life.

Rajiv Ranjan.
11.12.03

Patna *(Rajiv Ranjan)*

LIST OF CONTENTS

LIST OF TABLES

ABBREVIATIONS

%	-	*percent*
Ab	-	*Antibody*
AMI	-	*Antibody mediated immunity*
As	-	*Aseel*
CMI	-	*Cell-mediated immune*
Cn	-	*Cari Nirbheek*
CoB	-	*Commercial broiler*
Con-A	-	*Concanavalin-A*
CRBC	-	*Chicken red blood cells*
Cs	-	*Cari Shyama*
CV	-	*Co-efficient of variation*
Df	-	*Desi fowl*
DMRT	-	*Duncan's multiple range test*
dpv	-	*day post vaccination*
DR	-	*Dahlem Red*
ELISA	-	*Enzym linked immuno sorbent assay*
Ext.	-	*Extensive system of management*
FI	-	*Foot-index*
FPV	-	*Fowl pox-virus*
g	-	*gram*
HA	-	*Haemagglutination*
HAU	-	*Haemagglutination unit*
HI	-	*Haemagglutination-inhibition*
Hit	-	*Hitcari*
IgG	-	*Immunoglobulin – G*
IgM	-	*Immunoglobulin – M*
Int.	-	*Intensive system of management*
Kn	-	*Kadaknath*

lb	-	*pound*
Log	-	*Logarithm*
MAS	-	*Marker assisted selection*
MD	-	*Marek's disease*
µg	-	*Microgram*
mg	-	*Milligram*
µl	-	*Microlitre*
ml	-	*Millilitre*
mm	-	*millimeter*
ND	-	*Newcastle disease*
NDV	-	*Newcastle disease virus*
°C	-	*degree centigrade*
PBS	-	*Phosphate buffer saline*
PHA-P	-	*Phytohaemagglutinin-P*
PPD	-	*Purified protein derivative*
PV	-	*Post vaccination*
QTL	-	*Quantitative traits loci*
RD	-	*Ranikhet disease*
RDV	-	*Ranikhet disease virus*
RPM	-	*Revolution per minute*
SE	-	*Standard error*
SRBC	-	*Sheep red blood cells*
Up	-	*Upcari*
W/V	-	*Weight/Volume*
wks	-	*Weeks*
WLH	-	*White Leghorn*

❑❑❑

1.
INTRODUCTION

INTRODUCTION

In India, poultry farming, both for egg and meat production, has attained the status of a viable industry. It has become popular among progressive farmers as a form of diversified agriculture for maximizing income through gains of complementary relationship with crop production providing stability in income. It has been undertaken as an important tool to fight against malnutrition as well as poverty in rural India. Advanced technologies of better management, disease control and production system of poultry has now ranked India 4th largest egg producer (Ahlawat, 2003) and 22nd largest broiler producer in the world. More than thirty four billion (34.034) eggs (Balaraman, 2003) and 350 million broiler birds are being produced annually. On national level, poultry sector has shown 20 percent annual growth rate and Rs. 65 billion mega poultry industries are ready to take off to enter into the international market (Verma, 2001). But there is wide gap between demand and supply of eggs and broiler in Bihar. Per capita availability of egg and meat in the state is only 9.62 and 6.52 gms per annum which is much lower than the basal requirements. (Source : Survey section of Animal Husbandry Department, Govt. of Bihar – projected figures for 2000-01). Indeed, Bihar is an egg deficient state and there is great need and scope to intensify layer and broiler production in the state.

Viability of the birds is the primary factor deciding the economic feasibility of poultry production. The birds resistant to environment can only be in sound physique and grow, produce as well as reproduce to its optimum. The immune system is essential to life as it is most important system controlling disease resistance and thus viability. (Nonnecke and Harp, 1989). Immune responsiveness has been suggested as one of the best indicators of disease resistance influencing growth production and viability among animals (Gavora and Spencer, 1983 ; Warner *et al.*, 1987) and variation in this biological trait has been a subject of investigation by several research workers in recent past (Biozzi *et al.*, 1980; Lamont and

Dietert, 1990; Doenhoff and Davies, 1991; Saxena, 1993; Pandey *et al.*, 1996; Kundu, 1997; Haunshi, 1999).

The defence of the body consist of a complex systems of overlapping and interlinked physiological mechanisms capable to destroy or control almost all invaders (Tizard, 1998). Failure of immune response due to destruction of immune systems, inevitably results, in high morbidity and mortality. The highly evolved avian immune system being an important determinant of survivability, may be classified as **"specific"** and **"non-specific"**. The lymphoid cells – lymphocytes and plasma cells are primarily concerned with the specific immune response however, phagocytic cells (macrophages) are concerned with non-specific immune response by removing microorganisms from blood and tissues. The immune response to an antigen, whatever be its nature, can be of two broad types – the Humoral or Antibody Mediated Immunity (AMI) and the Cellular or Cell-Mediated Immunity (CMI). The primary lymphoid organ of bird is bursa of Fabricius, which is a site of lymphocytic proliferation and differentiation. The differentiated cells are B-cells which regulate the humoral immune response. The thymus derived (T) cells regulate the cell-mediated immune response in fowl. (Glick *et al,* 1956; Chang *et al,* 1957; Szenberg and Warner, 1962).

The *in vivo* proliferative response of T-lymphocyte to diversified Mitogens has been used as a tool for assessing the ability of an individual to mount the cell-mediated immune response. Indeed, the delayed type of hypersensitivity occuring as a result of interaction between injected mitogen and T-lymphocytes (sub-population of T-suppressor and T-helper cells) is a measure of cell-mediated immune response. It is a major mechanism to protect individual against viral infection. Out of several methods suggested in the literatures, the skin test is most commonly used because of its simplicity, practicability and considerable reliability (Cheng and Lamont, 1988; Saxena, 1993; Kundu, 1997 and Haunshi, 1999).

Variation in capacity to resist, control and/or reject infections are wide spread and genetically determined to a considerable extent (Hutt, 1958). No single immunological parameter appears to be sufficient in characterizing general immune responsiveness (Biozzi *et al.*, 1979). However, some of the diseases are resisted by the body either through humoral, macrophages activity and/or cell-mediated immune response.

Newcastle disease (ND) or Ranikhet disease (RD) is one of the dreadful and highly contagious viral disease of poultry. It causes severe respiratory distress, nervous disorder, decreased egg production and high mortality (Alexander, 1997). This disease has been a great hazard to poultry industry inspite of regular vaccination (Charan *et al.*, 1983; Rathore *et al.*, 1987). The severity of this disease has been found to vary from time to time and place to place depending upon the virulency of the isolate. Kelly *et al.*, (1994) reported that 27% of the serum samples from 460 chicken maintained in backyard flocks for meat in Zimbabwe were positive for ND virus only. The cell-mediated and humoral immunity play a role in immunity against Newcastle disease in birds (Timms and Alexander, 1977; Chandra Shekhar *et al.*, 1989).

The traditional poultry farming has been overtaken by modern commercial poultry production system. Although it is high cost-input-oriented, but at the same time more remunerated and profitable. In order to generate highly productive stock, introduction of exotic varieties of fowl as well as up-gradation of resistant native stock through gradual replacement of indigenous inheritance by exotic genomes, has resulted in decrease in adaptability of birds in subsequent generations in general and their capacity to resist the microbial infections in particular. The modern managemental practices strive to protect animals by preventing disease causing agents to enter in the flock through Hi-tech management or by induction of active immunity through vaccinations. In one way, it is antagonistic to natural selection for disease resistance and allows genetic susceptibility to increase in a breeding population. Genetic selection for

increased immune responsiveness and disease resistance can bring permanent improvement in fitness and also enhance vaccine effectiveness in livestock and poultry (Lamont, 1994).

Genetic variation in immune responsiveness to a variety of antigens has been reported in cattle, sheep and swine (Nguyen, 1983; Meerker *et al.*, 1987; Burton *et al.*, 1989). However, the variation in responsiveness to an antigen, thus disease resistance, is well known for its **spatio-temporal** entity. Besides that the variation in immune competence in animals and poultry has been reported to be influenced also by various non-genetic factors including age, sex, climate, general managemental practices as well as various other stressors (Benda *et al.*, 1990; Munnes and Lamont, 1991; Leitner *et al.*, 1992).

Identification of major genes as classical markers, defined as stable and inherited variant of morphological origin, and its association with economic traits has been a vital tool for Marker Assisted Selection (MAS) in livestock and Poultry. In this study, an effort has been made to establish, if any, the effect of major genes on Cell mediated and Humoral Immune Responses in Chicken.

The traditional system of poultry keeping, although loosing its importance from day to day under impact of modernization and industrialization, it is still prevalent in rural and tribal areas of the state. About one lakh poultry farms with flock size ranging from 25 to 250 birds exist in rural areas under backyard extensive system of poultry keeping. The indigenous fowl and their crosses still continue to be the main stay of these rural poultry farms. In general, the modern hybrids are exclusively used under intensive production system. It may not be out of place to mention that rapid growth in commercial layer and broiler production during last four decades has remained confined largely to the urban and peri-urban localities of the state and about 75% of the table eggs and poultry meat produced in the country is consumed by the 25% of the population living in urban and peri-urban areas. There is a great

need to conduct a study to identify some genetic lines, relatively resistant and responsive to available vaccines, for backyard poultry keeping under zero-input management system in the rural areas. The above considerations have been pivotal in planning the present investigation to study the relative influence of genetic and different non-genetic factors on general as well as specific humoral immune responsiveness against Newcastle disease in chicken with following objectives :

1. To estimate general and specific immunocompetence in different genetic groups of chicken (layers).

2. To quantify the variation in immune responsivenesses due to genetic and some non-genetic factors in chicken (layers).

3. To evaluate the influence of non-genetic factors on immune responsiveness in commercial broiler.

4. To study the influence of some of the major genes on general and specific immune response in chicken.

❑❑❑

2.
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

When an antigen comes in contact with host immune system, several processes are automatically triggered on within the body of the host in its protection against non-self. The three major systems of the immunological defences are (i) the Humoral system that gives rise to humoral immunity or antibody production. (ii) the Cellular system that stimulates cell-mediated immunity or direct cellular intervention against pathogen and (iii) the non-specific immune system like Complement system, Phagocytic activity etc.

2.1 Cell-mediated general immune response :

Cell-mediated immunity is that which is not dependent on antibody synthesis, rather in response to stimulation with mitogen, large proportion of immune cell undergo blast transformation and proliferation which are used as the measure of the proliferative capacity of certain cell types like T-lymphocytes. This capacity of an individual is taken as an indicator of its general cellular immune responsiveness.

Weber (1975) recommended Phytohaemagglutinin (PHA) and Concanavalin–A (Con–A) as mitogens capable to stimulate T-cells in avian species. The degree of response to these mitogens indicate the degree of general level of cellular immunity influencing T-cell mechanism, restricting or preventing lymphoma formation. In this study Con-A, was used as a mitogen to study the general cell-mediated immune response in eight genetically divergent chicken groups.

Pink and Miggiano (1977) studied the response to Con-A in five genetically different lines of chicken and reported the variation in response to be genetically dependent, the genetic polymorphism of single autosomal locus being the genetic mechanism involved therein.

Miggiano *et al.* (1976) observed genetic constitution of chicken as a significant factor affecting Con-A stimulation in two inbred lines (CB and WA). A single gene control was proposed for such variation. However, the effect of sex on responsiveness to Con-A in CB and WA inbred lines of chicken was statistically not significant. The results from the mating between (CB X WA) F_2 and back cross CB X (CB X WA) indicated that single dominant locus controlled the ability of peripheral lymphocytes to respond to Con-A.

Lassila *et al.* (1979) studied the effect of PHA-P and Con-A on peripheral blood lymphocytes in two partially inbred lines of chicken and their hybrids. A significantly high PHA-P response in the line P, as compared to the line V and hybrid PXV, was recorded. However, no significant line differences were observed in response to Con-A. Such variation was attributed to the difference in lymphocyte count. The workers were of opinion that the variation in mitogenic response was due to gentic polymorphism at single autosomal locus where low PHA response appeared to be dominant as observed in the (PXV) F_1 crosses. They also suggested that mitogenic responses were neither sex linked, MHC nor IgG allotypes.

Hassan *et al.* (1989) studied the lympho-proliferative response to Con-A (Concanavalin-A) as a measure of general cellular immune response in day old chicks. They found that the mitogenic effect of Con-A had an increasing trend up to 4th week after inoculation and thenafter there was a sharp decline.

Saxena (1993) conducted an experiment to assess the mitogenic response to PHA-P and Con-A in Guinea fowl. The change in wing web swelling was taken as wing web index. The overall mean response to Con-A was reckoned to be 1.39 ± 0.32 mm and males were better respondent than females. The Kadaknath and broiler birds had mean Con-A response to be 1.22 ± 0.05 mm and 1.22 ± 0.06 mm respectively.

The effect of genetic group was, however, not significant for both the mitogens.

Haunshi (1999) made a comparative evaluation of *in vivo* T-cell-mediated immune response in seven genetically divergent chicken population viz. Aseel, Kadaknath, White Leghorn, Dahlem Red (pure breeds) as well as KN X DR, AS X DR and WLH X DR (crossbreds). The response to Con-A measured in terms of mean foot index, ranged from 1.93 ± 0.07 to 2.25 ± 0.05 mm. Among the pure breeds the differences for mean response to Con-A were non-significant. However, among the crosses the response was significant at 5% level of probability.

2.2 Humoral Immune Response :

The humoral activity primarily acts through the body fluids molecules, particularly the heterogenous groups of immunoglobulins (IgG, IgM), complement and other immuologically relevant effector substances. The predominant antibody, immunoglobulin – G (IgG) is formed in B-cells which differentiates in bursa. Combination of humoral and cell-mediated immunity and macrophages may be needed for providing optimum immune response to chicken with a complete spectrum of resistance process.

The immune response to non-specific, natural, multi-determinant, complex and non-pathogenic antigens like Sheep Red Blood Cells (SRBC) may provide good indication of natural immune response characteristics and such resistance may be valid for multiple pathogens. But the control of specific immune response is extremely complex. A number of factors, both genetic and non-genetic, have been reported to influence specific immune response in different species of animals and some relevant literatures of recent past are being discussed here.

McDevitt and Landy (1972) opined that in mice, specific immune response genes (Ir) fell into two major classes : (i) the histocompatibility –

linked immune response genes, coding for the bursal type cell antigen receptors and (ii) the immuno globulin allotype-linked (Ir) genes, expressed in bone-marrow derived (bursal type) immuno-competent lymphocytes determining the structure of the bursal type cell antigen receptors.

Kulkarni *et al.* (1973) found an increasing trend in HI-antibody in Ostro white chicks, vaccinated with LaSota virus against ND, up to 3rd week of post vaccination.

Gavora and Spencer (1978) reported that general disease resistance, measured in terms of "total mortality" in a flock of fowl behaved as a quantitative, polygenic character of low heritability ranging between 1 and 5 percent (cited from Dickerson, 1957 ; Morris, 1959). Whereas, the heritability of resistance to "a specific disease" ranged from 7-17 percent in Newcastle disease (Gordon *et al.*, 1971) and up to 67% in Marek's disease (Friars *et al.*, 1972). They suggested that the estimates of heritability of "total mortality" might not be very meaningful without specifying the causes of death.

Heller and Soller (1981) Conducted an experiment to study the immune response to ND virus vaccine in Bedouin, White Leghorn and their reciprocal crossbred chicken. They observed that HI antibody titre attained peak on day 21 PV in all genetic groups except the Leghorn in which the attainment was on day 14 PV. However, the breed difference was not significant. They were of opinion that the genetic variation in immune response to an antigen, found within commercial poultry population, was not a degenerative effect of maintaining the flocks under modern sanitary condition. Probably this genetic variation was maintained as balanced polymorphism through negative effects of the loci involved in fitness.

Heller *et al.* (1981) did not observed any significant difference in haemagglutination-inhibition tires among Bedouin fowl, commercial

White Leghorns and their reciprocal crosses. The chicks were intramuscularly inoculated with either attenuated or inactivated NDV at four weeks of age. The heritabilities for HI titres due to attenuated virus and inactivated virus were 0.31 and 0.60 respectively. They suggested that the HI titre response to attenuated NDV might be the direct result of tissue susceptibility to virus multiplication (Peleg *et al.*, 1976) and the response to inactivated NDV would be more effective in improving genetic resistance to NDV.

Klingensmith *et al.* (1983) studied the effect of sex-linked Dwarfing gene (dw) on immune response of broiler chicken. They observed that Dwarfing gene (dw) did not have significant effect on natural agglutinin titres to 1% rabbit erythrocyte, either in the parental or in second generation birds. The response of parent generation birds to SRBC immunization was also not significant. However, the second generation Dwarf birds had significantly lower SRBC titre than the normal sized or crossbreds. Their findings indicated that the dwarf birds might have more competent T-cell sub-population, but weaker B-cell reactivity than normal birds.

Kulkarni *et. al.* (1983) observed increase in mean HI-antibody titre after LaSota vaccination against ND from 7th to 28th day of vaccination.

Inooka *et al.* (1984) compared several immunological traits in generation 7 to 12 of two lines of Japanese quails, selected for high and low secondary immune response to several inactivated Newcastle disease viruses. Birds of the high-line had a greater primary response to ND viruses, Influenza viruses, sheep erythrocytes and *Salmonella pullorum* than the low line. It was indicative of the fact that specific immune response in Japanese quails was a genetically dependent trait to certain degree.

Bacon *et al.* (1986) reported that when males of WLH strain crosses were mated to rapid feathering (K) females to produce rapid and low

feathering chicks of both sexes, K did not influence either general or specific humoral mediated immunity. Their findings were resubstantiated by Dunnington *et al.* (1987) who also observed non-significant difference for antibody titre against SRBC in early and late feathering chicks of broiler lines from 8 to 35 days of age. The titre value increase to a post vaccinations plateau at 21 days of age, the average antibody titre (log2) ranging between 2.4 ± 0.2 and 6.5 ± 0.0 in all groups of birds.

Martin *et. al.* (1988) observed significantly lower antibody response to SRBC on 5 *dpv* in Dwarf birds as compared to the normal birds in White Plymouth Rock population.

Petrovsky *et al.* (1988) reported genetic differences in specific humoral response among hens of 4 commercial lines of White Leghorn and RIR as well as their twelve crossbred groups crosses. The birds were immunized simultaneously at 14 months of age against Brucellosis. The results showed higher antibody titre against *Brucella abortus* in both primary and secondary responses in all the groups, the differences being significant between the lines within different breeds.

Takahashi *et al.* (1988) found significantly different antibody titre between Japanese quail lines selected for high and low antibody response to ND virus vaccine through 35 generations. The mean titre values ($\log_2$) were 8.2 and 0.80 in high and low lines respectively in the maximum divergent birds of 24th generation. As the selection process continued, some of the reproductive trait such as hatchability, egg production and egg weight declined, but the viability improved in subsequent generations.

Hassan *et al.* (1989) studied the effect of Levamisole hydrochloride on the immune response to Newcastle disease virus in day old Hubbard chicks. They conducted an experiment on 100 chicks divided into 4 equal groups and vaccinated intraocularly with Newcastle disease vaccine (LaSota strain; 109-1 egg infective dose). They also conducted experiment

for the lymphoproliferative response to 10 μg/ml of Phytohaemagglutinin–P (PHA-P), Concanavalin–A (Con-A) and purified protein derivative (PPD). It was recorded that in Hubbard chicks, vaccinated against ND, the HI antibody titre increased up to week 3-4 PV and thenafter it declined.

Berrio *et al.* (1990) vaccinated a group of chicken, showing the Naked neck trait (Na), of 21 days of age against Newcastle disease with an objective to study the genetic influence of Na genes on the immune response to the LaSota strain virus. The titres of HI antibodies were evaluated at 7, 14, 21, 28, 36, 42 and 56 days after vaccination by the Haemagglutination Inhibition (HI) test. The titres of HI-antibodies were significantly higher in birds with Na genes and they concluded that Na genes had favourable effect on the immune response to the LaSota strain of Newcastle disease virus.

Jhala *et al.* (1990) observed an increasing trend in HI-antibody titre against Newcastle disease in chicken from 1st to 3rd day post vaccination.

Saini *et al.* (1990) estimated humoral immune response in chicks vaccinated with 'F' strain of ND virus and recorded that HI-antibody titre increased from 1st week post vaccination, attained peak in 3rd week post vaccination and thenafter it had a decreasing trend.

Chao and Lee (1991) reported higher immune response to SRBC in country chickens than White Leghorn.

Dunnington *et al.* (1993) observed significant differences in responses in high and low lines to combinations of antigens (SRBC, NDV, a both or none) among 4 populations of chickens from diverse genetic backgrounds viz., White Leghorn selected for high (HA) or Low (LA) antibody response to SRBC, White Plymoth rocks selected for high (HW) or Low (LW) body weight at 8 weeks. They reported that the antibody response at 6 *dpv* was the highest in the birds receiving single Antigen

and there was significant difference between lines for immune response at 27 *dpv*. They also concluded that antibody response to various antigens depended on the genetic constitution of the stock into which it was introduced, the time elapsed after antigen administration as well as the presence of other antigens administered concurrently.

Karnatak *et al.* (1993) recorded an increase in HI-antibody titre in the birds vaccinated with RD vaccine till the 20th day post vaccination. The peak titre level remained maintained till the 30th day post vaccination and thenafter it declined.

Zulkifli *et al.* (1994) recorded that variation in general immune response in Dwarf (dw/dw) and Normal (+/+) fowls put under feed restriction was statistically not significant.

Al–murrani *et al.* (1995) compared the specific humoral and cellular immune response to *Salmonella typhimurium* in local Iraqi and White Leghorn chicks. They measured HI antibody titre 24 hrs. before and 9 and 36 days after vaccination. The wattle test was used to measure the cellular immunity. Study revealed higher antibody titre and greater wattle thickness in native Iraqui chicks than White Leghorn chicks. High within breed variability was also reported. The superior performance of the local chickens was attributed to the accumulation of resistant genes through natural selection.

Jayawardane *et al.* (1995) reported that serum HI antibody titre in chicken vaccinated against V4 ND virus vaccine increased gradually up to 10th *dpv*, the level was maintained up to 17 *dpv* and thenafter it showed a declining trend.

Shobharani and Rao (1995) conducted an experiment to asses the humoral as well as cell-mediated immune response against Newcastle disease in male Babcock chicken with or without the influence of other viral vaccination. In both the treatment groups viz., birds receiving ND

vaccine alone and the birds receiving ND vaccines with MD and Fowl pox vaccines, the HI antibody titre attained peak level on 20th day post vaccination.

Barman *et al.* (1996) conducted an study to evaluate immune response of combined NDV and FPV vaccines given through aerosol route in 7 day old chicks. They observed that maternal antibodies against NDV and FPV were persistent in 7 day old chicks. After first week of vaccination there was drop in antibody titres in ND vaccinated birds, which was attributed to neutralization of NDV maternal antibodies. Chicks vaccinated with NDV vaccine attained peak HI antibody titre at 2nd week and declined gradually up to 6th week of vaccination.

Demey *et al.* (1996) evaluated the immunocompetence status of major genes of Dahlem Red chicken in combination with major genes for Naked neck (NaNa) and Dwarf (dw). Serum samples of NaNa DW, nana dw, Nana Dw and Nana dw genotypes, against ND virus vaccine (NDV-HB1), Sheep RBC, Phyto-haemagglutinin-P (PHA-P) and *Eimmeria tenella* antigens were tested. They did not observed any significant difference either in specific or complement responses after vaccination against NDV in the birds of different genotypes.

Foliste *et al.* (1998) conducted an experiment to evaluate the comparative efficacy of killed-in-oil-emulsion and live NDV vaccine in chicken. The results indicated that concurrent administration of oil emulsion and live NDV induced the best antibody response, but there was not significant different in protection among the birds vaccinated with the two types of vaccines. They recorded a general trend of increase in geometric mean of HI titre (GMT) up to 28th *dpv.*

Subramaniam *et al.* (1998) conducted a study to find out efficacy of the Oil adjuvanted vaccine of NDV vaccine in field condition by estimating the specific humoral response in chicken of Babcock BV layer hybrid strain. The experimental birds were divided into two treatment

groups and maintained alongwith a control group. The birds of one of the treatment groups were vaccinated using oil adjuvanted live lentogenic and mesogenic vaccine, whereas the birds in another treatment group received conventional live vaccine without oil-adjuvant. The serum samples of 5% of the birds from each group were collected and tested by HI-test at the interval of 7 days after immunization. The HI-antibody titre in the birds vaccinated with lentogenic vaccine increased gradually upto week 3 PV and then after it showed a declining trend up to 9th week PV.

Kalita and Dutta (1999) reported that in broiler chicks maternal antibody against NDV was persistent during the first week of age. There was a gradual rise in HI antibody titre in the birds vaccinated against ND from the day of vaccination, reaching its peak by the 2nd week PV and thenafter it had a declining trend.

Pokric *et al.* (1999) reported that both light and heavy weight chicken vaccinated with a vaccine containing surface protein of Newcastle disease virus showed a progressive increase in antibody titre, attaining peak level between weeks 4 and 6 after primary vaccination. The minimum antibody titre was observed in week 18 to 23 and 14 to 18 after primary vaccination in light and heavy weight chicken respectively.

Saravanabava *et al.* (1999) reported that HI-antibody titre in chicken vaccinated against ND had an increasing trend up to week 3 PV and thenafter it declined.

Bozorghmehrifard and Mayahi (2000) made a comparison of ELISA and HI test for the detection of antibody against ND vaccine in broiler chicks. They found ELISA and HI responses almost similar and in the both the cases sero-conversion was detected at 7 *dpv*. The HI-Ab titre rose moderately and attained peak on 9 *dpv*.

Mani *et al.* (2000) conducted a study to find out the influence of Aflatoxin on ND immunity in commercial broilers and reported that the

HI antibody titre in control group of birds, without any aflatoxin supplement in their feed was, the lowest (1.618 ± 0.05) on 5th day post immunization. There was a gradual increase in the titre up to 42nd day (2.389 ± 0.04) post immunization and thenafter it declined. However, fortnightly measures for HI-antibody titre from 5th to 56th day did not differ significantly.

Mishra *et al.* (2000) inoculated three groups of chicken with three ND virus strain, isolated from Chicken, Guinea fowl and pigeon. Precipitating antibodies was detected at 7 *dpv* in all the three groups and birds remained positive till the end of observation period i.e up to 28 *dpv*. The HI antibody titre started rising after 7 *dpv* and the maximum titre was detected in between 15 and 21 *dpv*.

Aydin *et al.* (2001) estimated the serum antibody titres against Newcastle disease virus in vaccinated chicken flocks as well as selected birds using Haemagglutination-Inhibition test and ELISA. The results of HI and ELISA were found to be positively correlated in both the experimental and test groups.

Sheela and Rao (2002) observed that in commercial broilers of Giriraja genetic origin, vaccinated against Newcastle disease using 'F' strain of NDV, attained the peak HI antibody titre after 3 week PV. However, the birds vaccinated with LaSota strain in Hubchicks, maintained at two other different farms, showed relatively poor humoral response without any definite trend.

❑❑❑

3.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

4.1 Experimental Materials

Serum samples from 239 chicken belonging to Kadaknath, Aseel, Upcari Aseel (Cari Nirbheek), Upcari Kadaknath, (Cari Shyama), Upcari Frizzle (Upcari), Upcari Naked Neck (Hitcari) (all egger strains), HS-260 (Broiler strain) as well as Desi (non-descript) genetic groups constituted the experimental materials for this investigation. Altogether 240 birds of eight different genetic groups were included in this study out of which one died prior to start of the experiment.

4.2 Genetic constitution of experimental birds :

4.2.1 Kadaknath :

The original name seems to be "Kalamasi" meaning the fowl having black flesh. However, the present name "Kadaknath" is more popular. This breed is native to Western Madhya Pradesh and mostly bred by the tribals of Jhabua and Dhar districts. The peculiarity of this breed lies in the fact that most of the internal organs of the birds show intense black colouration, which is pronounced in the trachea, thoracic and abdominal air sacs, gonads, elastic arteries at the base of heart and mesentery. Varying degree of blakish colouration is also found in the skeletal muscles, tendons, nerves, meninges, brain and bone-marrow.

4.2.2 Aseel :

The home tract of this indigenous breed is Bastar district of Madhya Pradesh, Khanam district of Andhra Pradesh and Koraput district of Orissa. It is also known as Indian Game Fowl. Aseel means "real or true" and the name appears to have

been given to this breed because of its inherent qualities of fighting. The birds as a rule present an upright material gait suggestive of strength and alertness.

3.2.3 Upcari Aseel (Cari Nirbheek) :

It is cross of Indian native breed Aseel with Cari Red. Birds are active, larger in built, pugnacious in nature with high stamina and majestic gait. They are able to save themselves from their predators due to its fighting characters as well as activeness and are adapted to all climatic zones of the country.

3.2.4 Upcari Kadaknath (Cari Shyama) :

It is cross of Kadaknath breed of Indian native chicken with Cari Red. Birds have plumage of various colours dominated by black. The skin, beak, shank, toes and soles are dark grey in colour. The peculiarity of this breed is that most of the internal organs show the characteristic black pigmentation. Varying degree of blackish colouration is also found in skeletal muscles, tendons, nerves, meninges, brain and bone marrow. The black colour of muscles and tissues is due to deposition of melanin pigment, which causes increase in protein and decrease of fat in muscle fibre.

3.2.5 Upcari Frizzle (Upcari) :

These fowls are the cross of native Aseel breed of chicken with frizzle plumage and Cari Red. These multi coloured birds have single comb and medium body size. Presence of frizzle plumage helps in fast heat dissipation due to which birds are better adapted to tropical climate, specially in arid zones.

3.2.6 Upcari Naked Neck (Hitcari) :

These fowls are the cross of Naked neck Aseel breed of chicken and Cari Red, which are adapted to tropical climate specially for hot and humid coastal region of the country. These are multi coloured, have single as well as pea comb and birds are larger in built.

3.2.7 Commercial broiler :

The Commercial broiler utilized in this study were obtained from Hissarghatta, Bangalore and were a high-line stock, denoted as HS-260.

3.2.8 Desi fowl :

This fowl group consisted of non-descript indigenous birds.

3.3 The experimental units :

The birds were maintained at three different poultry units located at two places viz. Pusa (Samastipur) and Patna.

3.3.1 Animal Production Research Institute (APRI), Pusa :

Out of 240 experimental birds, 180 birds, 30 of each of the six egger genetic groups, were maintained at University Poultry Farm at APRI, Pusa (Samastipur). Pusa is located 25°59' North (latitude), 85°40' East (longitude) and 171 meter high (altitude) from mean sea level. It is surrounded from three sides by river Gandak and the climate of this place is hot-humid. The climatological details of Pusa is summarized in table 3.1.

Table 3.1

Climatological Profile of PUSA (Samastipur), Bihar*

Months	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)
	Max. (Av)	Min. (Av)	Morning (Av)	Afternoon (Av)	Average
January	21.20	4.10	88.50	48.00	3.75
February	24.95	10.55	86.50	44.50	0.50
March	35.75	14.25	79.50	36.50	0.50
April	35.80	20.45	73.50	37.00	15.00
May	34.35	24.55	82.00	58.00	123.75
June	34.50	26.30	88.00	69.00	194.65
July	33.45	27.45	87.50	77.50	226.25
August	33.80	25.60	89.50	73.00	166.50
September	31.70	25.70	90.50	74.50	347.50
October	32.15	24.15	85.00	59.50	136.25
November	29.30	17.10	91.50	51.00	0
December	23.15	10.30	91.00	55.00	0
Overall	**33.48**	**19.57**	**86.04**	**56.96**	**101.22**

* Based on the records of last 10 yrs. of Deptt. Of Climatology, RAU, Pusa.

3.3.2 Poultry Units of Patna :

(a) **Central Poultry Farm (CPF), Patna** – Out of 240 experimental birds, 30 birds belonging to commercial broiler (HS-260) strain were maintained at Central Poultry Farm, Patna.

(b) **Private Poultry unit at BVC Campus, Patna** – Out of 240 experimental birds, 30 birds belonging to Desi fowl group were maintained in a private house at B.V.C. Campus, Patna. Patna, the Capital of Bihar State, is a city of glorious historic significance, located 25°36' North (latitude), 85°06' East (longitude) and 60 meter high (attitude) from mean sea level. The city is on the southern bank of river Ganges and the climate of this place is relatively hot-dry as compared to that of Pusa (Samastipur). The climatological details of Patna is presented in table 3.2.

3.3.3 A brief note on general managemental practices of the experimental birds :

The experimental birds housed at APRI, Pusa and CPF, Patna were reared on deep litter system. In rearing pens, the birds were served fresh and clean drinking water ad-libitum, through fountain system. The birds were offered feed *ad-libitum* in the linear feeders. All mash system of feeding was followed. During experimental period the chicks were subjected to uniform condition of housing including brooding, feeding, watering, lighting and other managerial practices.

The Desi fowl kept at BVC campus, Patna were put under backyard poultry keeping system. Birds were fed daily

Table 3.2

Climatological Profile of Patna, Bihar*

Months	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)
	Max. (Av)	Min. (Av)	Morning (Av)	Afternoon (Av)	Average
January	23.3	9.2	76	57	18.9
February	26.5	11.6	66	45	10.7
March	32.6	16.4	50	30	11.4
April	37.7	22.3	44	23	7.6
May	38.9	25.2	56	32	33.3
June	36.7	26.7	70	54	134.2
July	33.0	26.2	83	74	305.8
August	32.4	26.1	84	77	274.4
September	32.3	25.4	82	76	226.9
October	31.5	21.8	75	68	93.8
November	28.8	14.7	70	60	8.9
December	24.7	9.9	75	60	4.1
Overall	31.5	19.6	69.25	55	1130.0

* Sources from Deptt. Of Meteorology, Govt. of Bihar, Patna.

with some amount of maize grains in the shelter provided during night hours.

3.4 Experimental details :

Serum samples of pre and post-vaccinated birds against ND were the experimental material for this investigation. The details of the procedure adopted to estimate the antibody titre in the serum samples have been summarized as follows :

3.4.1 Vaccines :

(a) **R_2B strain RDV Vaccine** – A commercially available R_2B or Mukteshwar strain of RDV, manufactured by Bio-Med Private Limited, Ghaziabad (UP) was used for the vaccination of 8 weeks old layer birds after proper reconstitution.

(b) **La-Sota RDV Vaccine** – A commercially available LaSota strain of RDV, manufactured by Bio-Med Private Limited, Ghaziabad (UP) was used for vaccination of all the chicken at the age of 4 weeks after proper reconstitutions.

3.4.2 Reagents :

(a) Phosphate Buffer Saline (Aziz, 1985) –

NaCl	–	2.0 g
KCl	–	0.05 g
$Na_2HPO_4.2H_2O$	–	0.14 g
KH_2PO_4	–	0.05 g

Double distilled water 250 ml.

pH	–	7.2 to 7.4

This solution was autoclaved at 15 lb pressure for 15 minutes and stored at refrigerated temperature till use. This buffer was used for reconstitution and preparation of red blood suspension.

(b) Alsever's Solution : -

Dextrose	-	5.125 g
Sodium Citrate	-	2.0 g
Sodium Chloride	-	1.05 g
Citric Acid	-	0.137 g
Distilled Water	-	250 ml

This solution was autoclaved in running steam for one hour and kept at 4^0C for 1 – 2 days.

(c) Concanavalin – A - 500 mg

3.4.3 Preparation of Antigen

Velogenic form of NDV in the form of 10% bursal suspension in phosphate buffer saline (PBS) solution was inoculated into three to four weeks old broiler chicks (free from antibody to NDV), at the dose rate of 0.5ml of suspension per chick by intramuscular route. The chicks were reared in the Deptt. of Veterinary Microbiology, B.V.C., Patna, in standard feeding and managemental condition. The symptoms of Newcastle disease shown after 5 to 6 days. The chicks were sacrificed and bursa, lungs, liver and

spleen were collected aseptically and homogenized in a sterile mortar using glass wool as an abrasive.

The homogenate was diluted in the ratio of 1:1 (W/V) in PBS (pH 7.4) and treated with 10,000 units of penicillin and 10 mg streptomycin per ml of suspension. The suspension was frozen and thawed thrice and centrifuged at 4000 rpm for 15 minutes. The supernatant was collected and tested for the presence of NDV antigen by Haemagglutination Test (HA test). Thereafter, it was distributed in small aliquots and stored at 0^0C as antigen.

3.4.4 Preparation of Chicken red blood cell suspension

Two adult chicken were used as donor of blood. One to two ml of blood was collected from each bird in Alsever's solution (1:1). Supernatant fluid was removed after centrifugation at 500 rpm for 10 minutes. The packed cells were washed three times with PBS and stored at refrigerated temperature (4^0C). This CRBC suspension was used only for four days from the day of preparation and further fresh CRBC suspension was prepared.

3.4.5 Collection of serum samples from chicken

Two to three millilitre (ml) of blood was taken from the wing vein of each bird with the help of 5 ml sterilized disposable syringe using 23 or 24 gauze needle. The blood was transferred immediately to sterilized test tube which thereafter was kept in slanting position and left for one to two hours at room temperature. The separated serum was collected in a clean and sterilized glass vials of 2 ml capacity and preserved by adding sodium azide (1:1000) and stored

at 0^0C until used. This procedure was conducted at weekly interval from the date of start of vaccination till 35 day.

3.4.6 Haemagglutination (HA) Test were conducted as per Buxton and Frazer (1977) method with slight modification :

Equipments : ,

- Centrifuge
- Micro titre plate with 96 'U' shaped wells.
- Micropipette
- Centrifuge tube etc.

Reagents :

- 0.8% CRBC
- Alsever's solution
- Newcastle disease virus
- PBS (pH 7.2)

Procedure for HA Test :

i. Micro titre plate having 96 'U' shaped well were taken.

ii. 50 µl of PBS dispensed into 12 wells of the first row with the help of micropipette.

iii. 50 µl of the virus from stock solution was added to the first well of the row. Virus was mixed in diluent and 50 µl fluid was transferred to the next well. This mixing and transfer of fluid was continued till the 11th well and 50 µl fluid from 11th well was discarded. 12th well received only diluent and served as contest.

iv. 50 µl of 0.8% (V/V CRBC suspension in PBS was added in each well. The ingradient were mixed and plate was incubated at room temperature.

v. Assessment was made by examining the pattern of cells formed on the bottom of well at intervals of 15, 30, 45 and 60 minutes.

vi. The end point of HA activity of virus was the highest dilution of the virus that produced complete agglutination.

The virus titre expressed as HA units contained in undiluted sample is the reciproçal of the end point dilution.

3.4.7 Preparation of 4 HA unit (HAU) antigen

- The dilution factor for stock virus was determined by HA titre by four to make 4 HAU antigen.

- Accuracy of 4 HAU was checked such that 1:4 sample gives the end point.

3.4.8 Haemagglutination – Inhibition (HI) test were conducted as per Buxton and Frazer (1977) method with slight modifications :

HI Test (Beta-procedure) –

Equipments –

- Centrifuge
- Waterbath
- Micropipette
- Microtitre plate with 96 'U' shaped well
- PBS (pH 7.2)
- Centrifuge tube etc.

Reagents –

- 0.8% CRBC
- Newcastle disease virus containing 4 HAU/0.05 ml.

- Anti-Newcastle disease serum, collected at weekly interval from 0th day up to 35th day and heat inactivated at 56^0C for 30 minutes.

HI Procedures -

i. 50 µl PBS (pH 7.2) was placed in well 1 to well 12 of the first row.

ii. 50 µl of heat inactivated serum was added in 1st well. The fluid was mixed properly and 50µl of fluid was transferred to the next well. The mixing and transfer was continued till the 11th well. 50 µl fluid from 11th well was discarded and 12th well was kept as control.

iii. 50 µl of virus antigen containing 4 HAU was added in each well.

iv. 50 µl of 0.8% CRBC in PBS was added and mixed to each well.

v. The plates were incubated at room temperature and read after 15 to 30 minutes or until clear pattern of haemagglutination.

Assessment :

The HI titre is expressed as the reciprocal of the highest dilution of serum, inhibiting agglutination of the CRBC.

3.4.9 Evaluation of *in vivo* T-cell mediated immune (CMI) response :

The *in vivo* T-cell mediated response to concanavation-A (Sigma) was evaluated by the method of Cheng and Lamont (1988).

Reagents –

Concanavalin A –	500 mg
PBS	100 ml

Procedure –

i. The birds were injected intradermally between 3rd and 4th toe of the right foot with 0.5 mg Con-A in 0.1 ml of PBS (5 mg Con-A per ml of PBS).

ii. The left foot received 0.1 ml of PBS and served as control.

iii. The thickness of interdigital skin was measured by using cutimeter type Harpenden skin fold calliper at 0 and 24 hour reaction.

iv. The skin swelling was calculated by substracting the skin thickness at 0 hour from that of after 24 hour of injection. The foot index (FI) was determined as difference between interdigital swelling values of Con-A injected and control foot.

FI (in mm) = (post Con-A inj – pre Con-A inj) – (post PBS – pre PBS).

Where,

post Con-A Inj	=	Thickness of test foot 24 hour post injection of Con-A.
pre Con-A Inj	=	Thickness of test foot pre injection.
post PBS	=	Thickness of control foot 24 hour post injection of PBS.
pre PBS	=	Thickness of control foot before injection of PBS.

3.5 Classification of data :

The data were promptly recorded and classified on the basis of location of the experimental flocks, genetic constitution of the birds, age of the birds on the day they were vaccinated (0 day of vaccination) as well as farming systems.

3.5.1 Location of the experimental flocks :

(i) PUSA (Bihar)

(ii) Patna (Bihar)

3.5.2 The genetic groups :

(i) Kadaknath

(ii) Aseel

(iii) Upcari Aseel (Cari Nirbheek)

(iv) Upcari Kadaknath (Cari Shyama)

(v) Upcari Frizzle (Upcari)

(vi) Upcari Naked Neck (Hitcari)

(vii) Commercial broiler

(viii) Desi fowl

3.5.3 Age of the birds on the day of vaccination :

(i) 4 weeks of age.

(ii) 56 weeks of age.

3.5.4 Farming systems :

(i) Intensive

(ii) Extensive

3.6 Statistical methodologies :

The data pertaining to the results of the experiment on General Immune Responsiveness in terms of Foot-Index (FI) were absolute in values. But the results on experiment on Specific Immune Responsiveness i.e. HI-Antibody titre values were recorded in ratios and as such were first transformed into their $\log_2(n+1)$ values.

In order to study the nature as well as the magnitude of variation in immune response, the data were subjected to **Least squares analysis** without interaction, outlined by Harvey (1987) for LSMLMW PC-1 version, utilizing the following linar mathematical model :

$$Y_{ijklm} = \mu + L_i + G_j + A_k + M_l + e_{ijklm}$$

Where,

Y_{ijklm} = The value of mth individual belonging to i^{th} location of the experimental unit, j^{th} genetic group, k^{th} age group and l^{th} system of management;

μ = population mean ;

L_i = Effect of ith location of the exp.... Unit (i=1, 2);

G_j = Effect of jth genetic group (j = 1, 2, ... 8)

A_k = Effect of kth age group (k = 1, 2);

M_l = Effect of lth management system (l = 1, 2) and

e_{ijklm} = The random error associated with m^{th} individual normally distributed with mean zero and variance δ^2_e.

The restriction $\Sigma L_i = \Sigma G_j = \Sigma A_k = \Sigma M_l = 0$ was imposed.

The statistical significance of various fixed effects were tested by "F" test. Wherever the 'F" value was significant, **D.M.R.T., as modified by Kramer (1957),** was used to examine the pair-wise comparisons among Least squares means at 5% level of probability. The inverse element of co-efficient matrix and error mean squares were used for such comparisons.

If the value of

$$\overline{Y_i} - \overline{Y_j} = \sqrt{\frac{2}{C^{ii} + C^{jj} - 2C^{ij}}}$$ was greater than O-e Z_p, n_e, the difference was said to be significant;

where,

$\overline{Y_i} - \overline{Y_j}$ = Difference, between the least-squares means;

c^{ii} = Corresponding i^{th} diagonal element of c-matrix,

c^{ii} = Corresponding j^{th} diagonal element of c-matrix,

c^{ii} = corresponding off diagonal element;

Z_p, n_e = Studentized ranged value in Duncan's table (0.05) at n_e (error) degree of freedom;

P = Number of means choosen in the range, and

O-e = Standard deviation of error mean square.

❑❑❑

4.
RESULTS

4. RESULT

The results of this study have been presented in paras 4.1 and 4.2, dealing respectively with **general** and **specific** immune responsiveness in chicken. Besides the genotype of the birds; the location of the flocks, age of the birds on '0' day of vaccination/inoculation as well as the management system, under which they were reared during the period of investigation, were the non-genetic factors under consideration to study their effects on general and specific immune responses in fowl.

4.1 GENERAL IMMUNE RESPONSE :

The results of Least squares analysis of variance of CMI response to Con-A, measured in terms of Foot-Index (FI) and determined as difference in skin thickness of inter-digital space between 3rd and 4th toe of control and mitogen injected groups of birds have been presented in tables 4.1 and 4.2. The Least squares mean (μ) for Foot-Index was estimated to be 2.021 ± 0.011 mm, the co-efficient of variation being 11.128 percent (Table 4.2).

4.1.1 Factors affecting *in vivo* CMI response to Con-A :

The results of Least squares analysis of variance showing effects of genetic and the non-genetic factors, included in this study, on CMI response to Con-A revealed that the genetic group, age of the birds on the day of innoculation as well as the management system had significant ($P \leq 0.01$) influence on CMI response to Con-A. But the location of the flocks did not affect the response significantly (Table 4.2).

4.1.1.1 Location of the flocks :

The mean FI (Table 4.1) for the birds maintained at Patna was higher (2.110 ± 0.011mm) as compared to the birds maintained at Pusa (1.932 ± 0.010mm). The difference, however, was statistically not significant (Table 4.2, Fig. 4.1).

Table 4.1

Least squares analysis of variance for cell-mediated immune response to Concanavalin-A in various chicken populations :

Source of Variation	d.f.	Corrected sum of squares.	Mean sum of squares	F	R^2(%)
Location of the flock	1	0.541	0.541	2.540	0.493
Genetic groups	7	12.215	1.745	8.192**	11.132
Age on the day of inoculation	1	5.321	5.321	24.981**	4.858
Management systems	1	4.913	4.913	23.066**	4.478
Error	408	86.732	0.213	-	79.039

** = $P \leq 0.01$

Table 4.2

Least squares means for genetic and non-genetic factors affecting cell-mediated immune response to Concanavalin-A in various chicken populations.

Factors	No. of Obs.	Mean (mm) ± SE	CV(%)
Overall means (μ)	418	2.021 ± 0.011	11.128
Location of the flock			
1. Pusa	358	1.932 ± 0.010	9.793
2. Patna	60	2.110 ± 0.011	4.038
Genetic group			
1. Kadaknath	58	2.060^{a} ± 0.012	4.436
2. Aseel	60	2.114^{a} ± 0.012	4.397
3. Upcari Aseel (Cari Nirbheek)	60	1.867^{b} ± 0.010	4.149
4. Upcari Kadaknath (Cari Shyama)	60	1.840^{b} ± 0.012	5.058
5. Upcari Frizzle (Upcari)	60	2.005^{a} ± 0.011	4.249
6. Upcari Naked Neck (Hitcari)	60	2.101^{a} ± 0.011	4.055
7. Commercial broiler	30	1.792^{b} ± 0.011	3.362
8. Desi fowl.	30	2.392^{c} ± 0.014	3.206
Age on the day of inoculation			
1. 4 Weeks	239	1.831^{a} ± 0.010	8.443
2. 56 Weeks	179	2.211^{b} ± 0.012	7.261
Management System			
1. Intensive (High-input)	388	1.681^{a} ± 0.011	12.889
2. Extensive (Low-input)	30	2.361^{b} ± 0.013	3.059

Values superscripted by different letters were significantly different from each other.

Fig.4.1 Variation in CMI response to Con-A (FI) in chicken due to location of the flock.

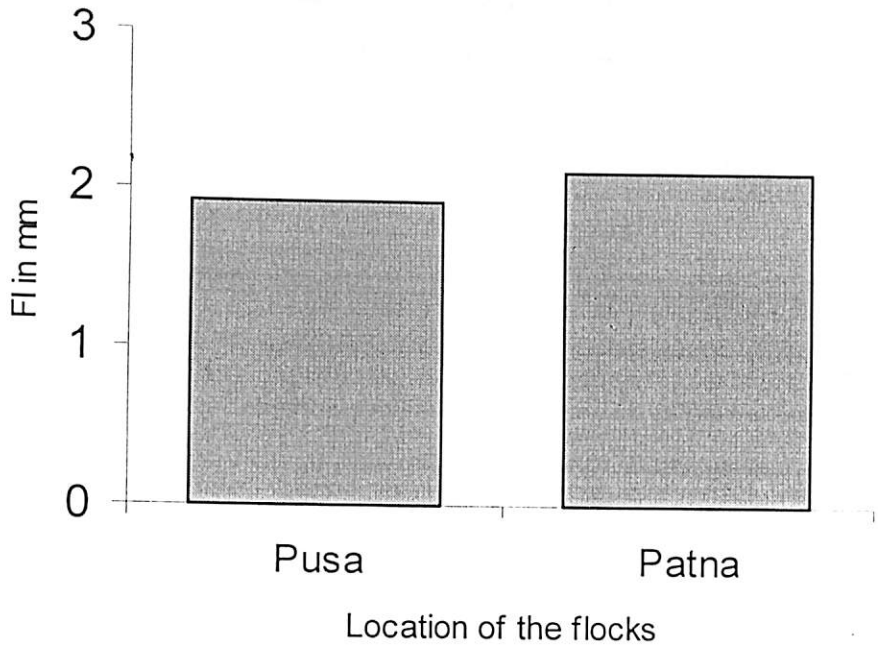
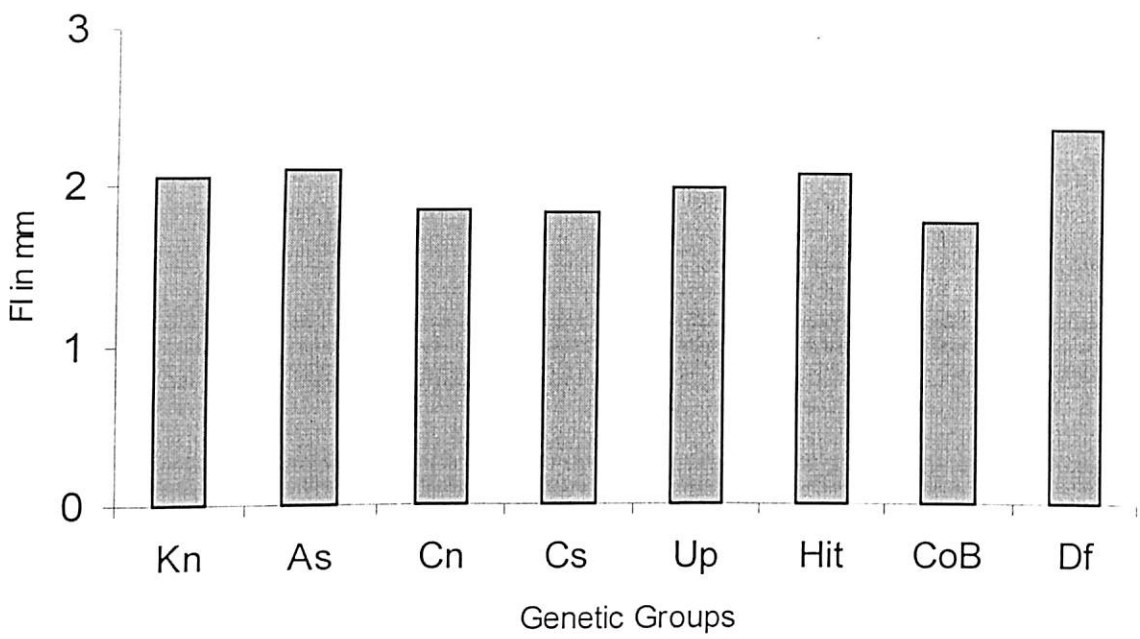


Fig.4.2 Variation in CMI response to Con-A (FI) in chicken due to Genetic groups.



4.1.1.2 Genetic group :

The genetic constitution of the fowl had significant ($P \leq 0.01$) influence on CMI response to Con-A, its contribution to the total variation in the trait being 11.132 percent (Table 4.1). Among the birds of different genetic groups, (Table 4.2, Fig 4.2) Commercial broiler had the lowest mean FI (1.792 ± 0.011mm) followed by Cari Shyama (1.840 ± 0.012mm), Cari Nirbheek (1.867 ± 0.010mm), Upcari (2.005 ± 0.011mm), Kadaknath (2.060 ± 0.012mm), Hitcari (2.101 ± 0.011mm), Aseel (2.114 ± 0.012mm) and Desi fowl (2.392 ± 0.014mm). The pair-wise comparison of Least squares means for the birds under different genetic groups revealed that the birds under Kadaknath, Aseel, Upcari and Hitcari genetic groups did not differ significantly among each other in CMI response to Con-A. The birds under Cari Nirbheek, Cari Shyama and Commercial broiler genetic groups were also not significantly different among each other but they differed significantly from Aseel, Kadaknath, Upcari and Hitcari birds. However, the Desi fowl, having the highest mitogenic response to Con-A were significantly different from the birds of all other genetic groups.

4.1.1.3 Age on the day of inoculation :

The effect of age of the birds on the day of inoculation of Con-A contributed significantly (4.858%) to the total variation in CMI response (Table 4.1). The average FI for older birds, on the day of inoculation was significantly higher (2.211 ± 0.012mm) as compared to that for younger birds (1.831 ± 0.010mm) (Table 4.2, Fig. 4.3)

4.1.1.4 Management Systems :

The influence of management system on CMI response to Con-A was statistically highly significant ($P \leq 0.01$), its contribution to the total variation in FI being 4.478 percent (Table 4.1). The mean FI value (Table 4.2, Fig. 4.4) was significantly higher (2.361 ± 0.013 mm) for the birds

Fig.4.3 Variation in CMI response to Con-A (FI) in chicken due to Age on the day of inoculation.

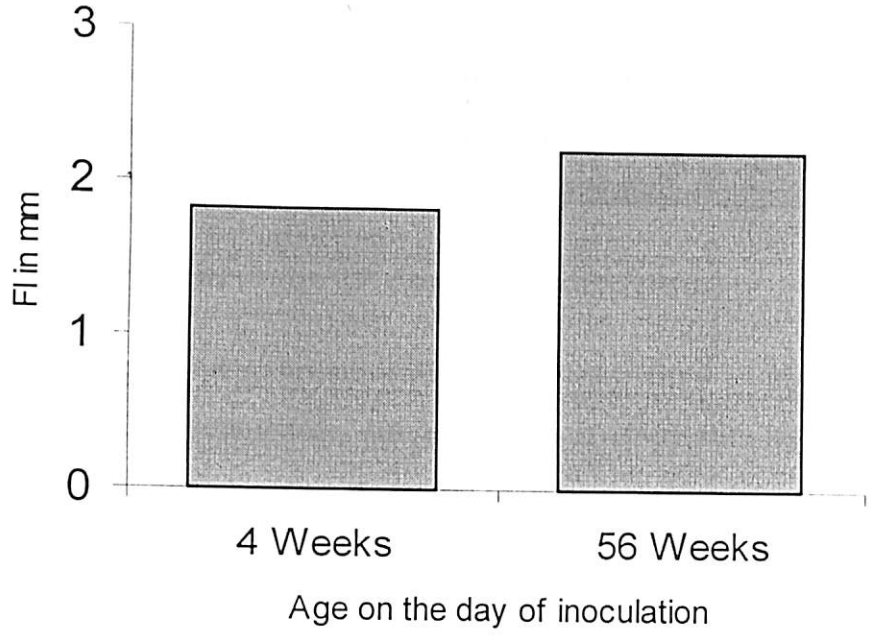
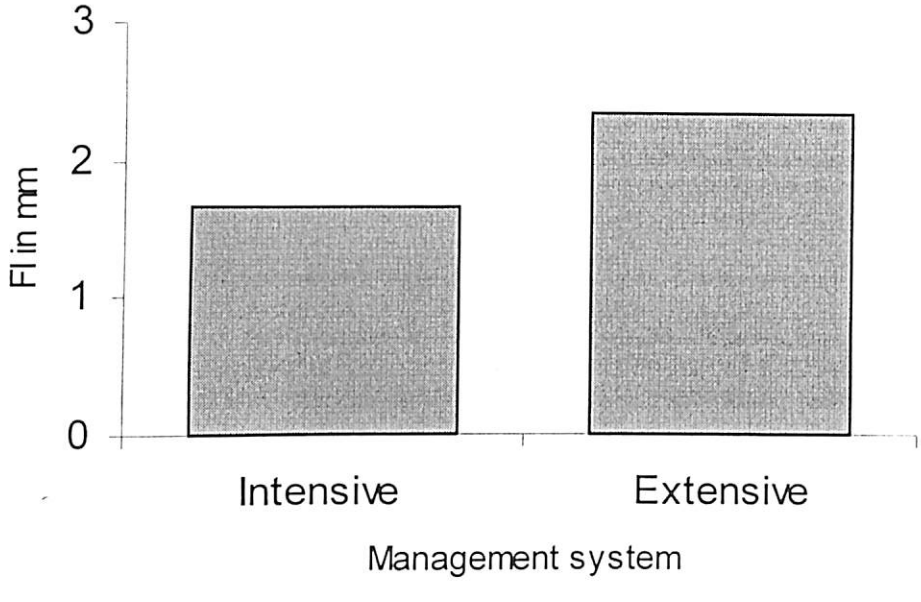


Fig.4.4 Variation in CMI response to Con-A (FI) in chicken due to Management system



under extensive system of management as compared to that for the birds under intensive care (1.681 ± 0.011mm).

4.2 SPECIFIC IMMUNE RESPONSE :

In this investigation, the HI-antibody titre values on 0, 7, 14, 21, 28 and 35 days of vaccination against Newcastle disease was taken as the measure of specific immune response in chicken.

4.2.1 HI antibody titre level on '0' day of vaccination :

The results of Least squares analysis of HI-Ab titre in chicken on 0 day i.e. prior to their vaccination, have been summarized in tables 4.3 and 4.4. The overall Least squares mean for HI-Ab titre level was estimated to be 3.3233 ± 0.0342, the co-efficient of variation being 21.040 percent (Table 4.4).

4.2.1.1 Factors affecting HI-Ab titre on '0' day :

The results of Least squares analysis of variance (Table 4.3) revealed that in pre-vaccinated birds under investigation, the location of the flock and the genetic constitution of the birds did not have significant influence on HI-Ab titre. However, the effects of age the birds on '0' day of inoculation as well as the management system were highly significant ($P \leq 0.01$).

4.2.1.1.1 Location of the flocks :

The contribution of location-effect to the total variation in HI-Ab titre level was statistically not significant (Table 4.3). The Least squares mean (Table 4.4, Fig. 4.5) for HI-Ab titre of the birds maintained at APRI, Pusa was 3.3431 ± 0.0343 which was slightly higher than that for the birds maintained at Patna (3.3035 ± 0.0344) but, the difference was statistically not significant.

Table 4.3

Least squares analysis of variance showing effects of genetic and non-genetic factors on HI antibody titre level on 0 day in chicken vaccinated against Newcastle disease.

Source of Variation	d.f	Sum of squares	Mean squares	F	R^2%
Location of the flocks	1	0.230	0.230	1.949	0.352
Genetic groups	7	2.410	0.344	2.915	3.688
Age at 0 day of inoculation	1	8.027	8.027	68.025**	12.282
Management system	1	6.480	6.480	54.915**	9.915
Residual	408	48.210	0.118	-	73.763

** = $P \leq 0.01$

Table 4.4

Least squares means for genetic and non-genetic factors affecting HI antibody titre level on O day of vaccination in chicken vaccinated against Newcastle disease

Factors	No. of Obs.	Day	CV(%)
		Mean ± SE	
Overall means (μ)	418	3.3233 ± 0.0342	21.040
Location of the flock			
1. Pusa	358	3.3431 ± 0.0343	19.413
2. Patna	60	3.3035 ± 0.0334	7.832
Genetic group			
1. Kadaknath	58	3.3407 ± 0.0982	22.387
2. Aseel	60	3.3050 ± 0.0966	22.640
3. Upcari Aseel (Cari Nirbheek)	60	3.3615 ± 0.0966	22.268
4. Upcari Kadaknath (Cari Shyama)	60	3.2872 ± 0.0966	22.763
5. Upcari Frizzle (Upcari)	60	3.3061 ± 0.0966	22.633
6. Upcari Naked Neck (Hitcari)	60	3.3755 ± 0.0966	22.167
7. Commercial broiler	30	3.2886 ± 0.0966	16.089
8. Desi fowl.	30	3.3218 ± 0.0966	15.928
Age at '0' day of inoculation			
1. 4 Weeks	239	2.6733^{a} ± 0.0965	55.806
2. 56 Weeks	179	3.9733^{b} ± 0.0976	32.864
Management System			
1. Intensive (High-input)	388	2.9631^{a} ± 0.0966	64.217
2. Extensive (Low-input)	30	3.6835^{b} ± 0.0966	14.364

Values superscripted by different letters were significantly different from each other.

Fig.4.5 Effect of Location of the flocks on HI-Ab titre level on '0' day of vaccination in chicken vaccinated against ND.

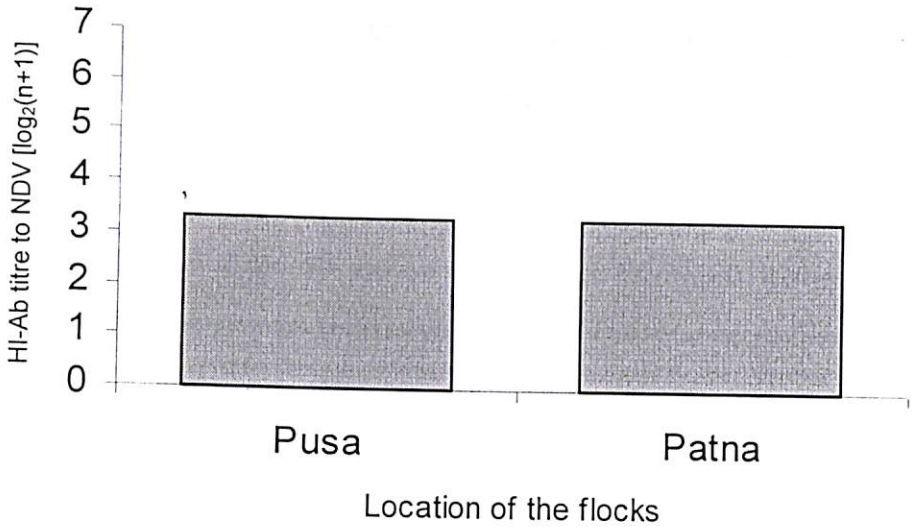
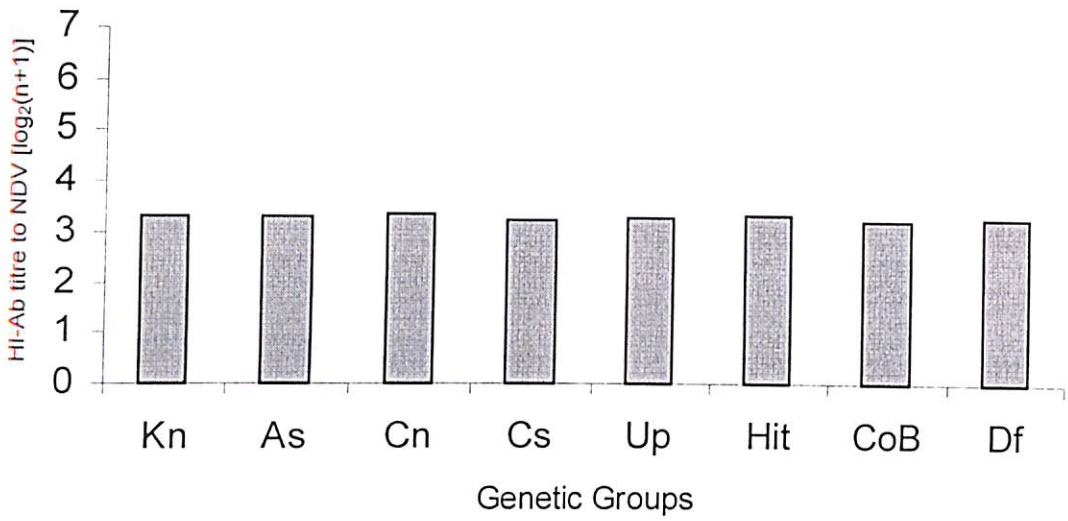


Fig.4.6 Effect of Genetic Groups on HI-Ab titre level on '0' day of vaccination in chicken vaccinated against ND.



4.2.1.1.2 Genetic Groups :

The genetic group-effect contributed 3.688 percent to the total variation in HI-Ab titre level (Table 4.3). Among the birds of different genetic groups the average HI-Ab titre level was the highest in Hitcari birds (3.3755 ± 0.0966) followed by that in Cari Nirbheek (3.3615 ± 0.0966), Kadaknath (3.3407 ± 0.0982), Desi fowl (3.32181 ± 0.0966), Upcari (3.3061 ± 0.0966), Aseel (3.3050 ± 0.0966), Commercial broiler (3.2886 ± 0.0966) and Cari Shyama (3.2872 ± 0.0966) birds (Table 4.4, Fig. 4.6). However, the average HI-Ab titre in the pre-vaccinated birds of different genetic make up did not vary significantly. Relatively lower magnitude of the co-efficient of variation for Commercial broiler and Desi fowl as compared to those for the birds under other genetic groups may be attributed to relatively lower number of observations in Commercial broiler and Desi fowl groups.

4.2.1.1.3 Age at '0' day of inoculation :

Age-effect contributed significantly (12.282%) to the total variation in HI-Ab titre level in pre-vaccinated birds (Table 4.3). The Least squares mean for HI-Ab titre for the birds of 56 weeks of age on '0' day of inoculation was significantly higher (3.9733 ± 0.0976) than the birds of 4 weeks of age (2.6733 ± 0.0965). The co-efficient of variation for the birds under two age groups were 55.806 and 32.864 percent respectively (Table 4.4, Fig. 4.7).

4.2.1.1.4 Management Systems :

Management system contributed significantly (9.915%) to the total variation in HI-Ab titre level in pre-vaccinated birds (Table 4.3). The mean HI-Ab titre was significantly higher (3.6835 ± 0.0966) in the birds under extensive system of management as compared to the birds maintained under intensive system (2.9631 ± 0.0966), the co-efficient of variation being 14.364 and 64.200 percent respectively (Table 4.4, Fig. 4.8).

Fig.4.7 Effect of Age at '0' day of inoculation on HI-Ab titre level on 0 day of vaccination in chicken vaccinated against ND.

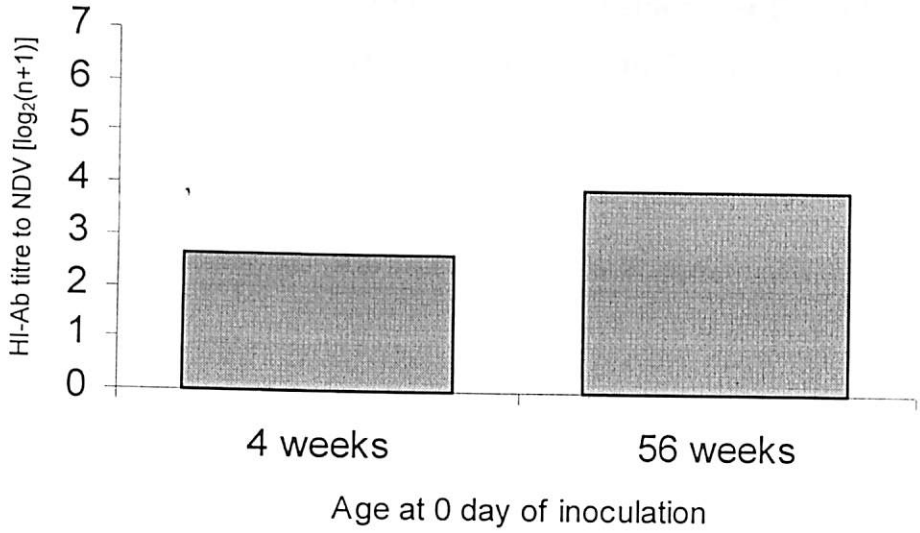
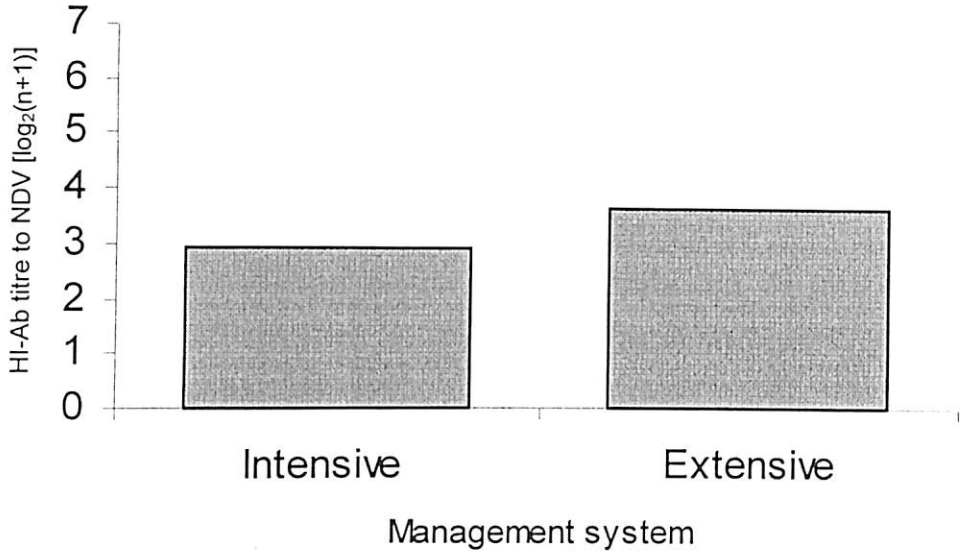


Fig.4.8 Effect Management system on HI-Ab titre level on '0' day of vaccination in chicken vaccinated against ND.



4.2.2 HI-antibody titre level on 7 *dpv* :

The findings pertaining to the Least squares analysis of variance of HI-Ab titre on 7th day of vaccination in chicken, vaccinated against Newcastle disease, have been presented in tables 4.5 and 4.6. The overall Least squares mean for HI-Ab titre level on 7 *dpv* was estimated to be 4.0929 ± 0.0343, the percent co-efficient of variation being 17.092 (Table 4.6).

4.2.2.1 Factors affecting HI–Ab titre level on 7 *dpv* :

Table 4.5 revealed that the genetic group, age at '0' day of inoculation as well as the management system had significant ($P \leq 0.01$) influence on HI-Ab titre level on 7 *dpv*. However, the effect of location of the flocks did not have any significant influence on the immunological trait under reference.

4.2.2.1.1 Location of the Flocks :

The flock's location-effect contributed only 0.502 percent to the total variation in HI-Ab titre level on 7 *dpv* and Least squares mean for the birds at Pusa was slightly higher (4.1044 ± 0.0343) than those at Patna (4.0814 ± 0.0344). However the differences were statistically not significant (Table 4.6, Fig 4.9).

4.2.2.1.2 Genetic groups :

The genetic constitution of the fowl contributed 21.065 percent to the total variation in HI-Ab titre level on 7 *dpv*, the contribution being the highest among the different sources of variation under consideration (Table 4.5). Hitcari birds have the highest (4.6980 ± 0.0966) HI-Ab titre level on 7 *dpv* followed by Desi fowl (4.2199 ± 0.0966), Cari Nirbheek. (4.1804 ± 0.0966), Upcari (4.1548 ± 0.0966), Aseel (4.1534 ± 0.0966), Kadaknath (4.0243 ± 0.1000), Cari Shyama (3.9993 ± 0.0966) and Commercial broiler which has the lowest (3.5414 ± 0.0966) titre level (Table 4.6). The results on DMRT indicated that the HI-Ab titre on 7 *dpv*

Table 4.5

Least squares analysis of variance showing effects of genetic and non-genetic factors on HI antibody titre level on 7 day in chicken vaccinated against Newcastle disease.

Source of Variation	d.f	Sum of squares	Mean squares	F	R^2%
Location of the flocks	1	0.351	0.351	3.441	0.502
Genetic groups	7	14.738	2.105	20.637**	21.065
Age at 0 day of inoculation	1	7.260	7.260	71.176**	10.377
Management system	1	6.470	6.470	63.431**	9.248
Residual	406	41.143	0.102	-	58.808

** = $P \leq 0.01$

Table 4.6

Least squares means for genetic and non-genetic factors affecting HI-antibody titre level on 7 day of vaccination in chicken vaccinated against Newcastle disease.

Factors	No. of Obs.	Day Mean ± SE	CV(%)
Overall means (μ)	416	4.0929 ± 0.0343	17.0923
Location of the flock			
1. Pusa	356	4.1044 ± 0.0342	15.722
2. Patna	60	4.0814 ± 0.0344	6.5286
Genetic group			
1. Kadaknath	56	4.0243^{a} ± 0.1000	18.595
2. Aseel	60	4.1534^{a} ± 0.0966	18.016
3. Upcari Aseel (Cari Nirbheek)	60	4.1804^{a} ± 0.0966	17.899
4. Upcari Kadaknath (Cari Shyama)	60	3.9993^{a} ± 0.0966	18.718
5. Upcari Frizzle (Upcari)	60	4.154^{a} ± 0.0966	18.018
6. Upcari Naked Neck (Hitcari)	60	4.4698^{b} ± 0.0966	16.740
7. Commercial broiler	30	3.5414^{c} ± 0.0966	14.940
8. Desi fowl.	30	4.2199^{a} ± 0.0966	12.547
Age at '0' day of inoculation			
1. 4 Weeks	238	3.4533^{a} ± 0.0966	43.155
2. 56 Weeks	178	4.7325^{b} ± 0.0966	27.233
Management System			
1. Intensive (High-input)	386	3.9957^{a} ± 0.0966	47.498
2. Extensive (Low-input)	30	4.1901^{b} ± 0.0966	12.927

Values superscripted by different letters were significantly different from each other.

Fig.4.9 Effect of Location of the flocks on HI-Ab titre level on 7 *dpv* in chicken vaccinated against ND.

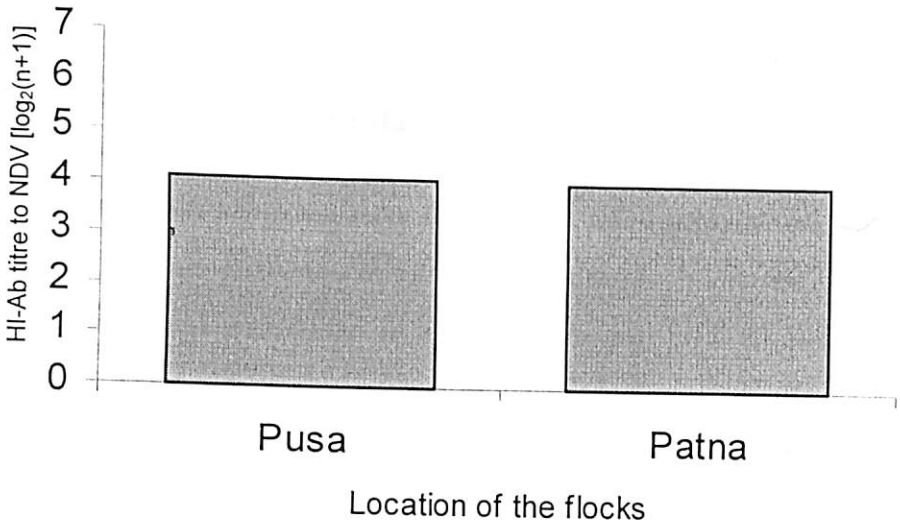
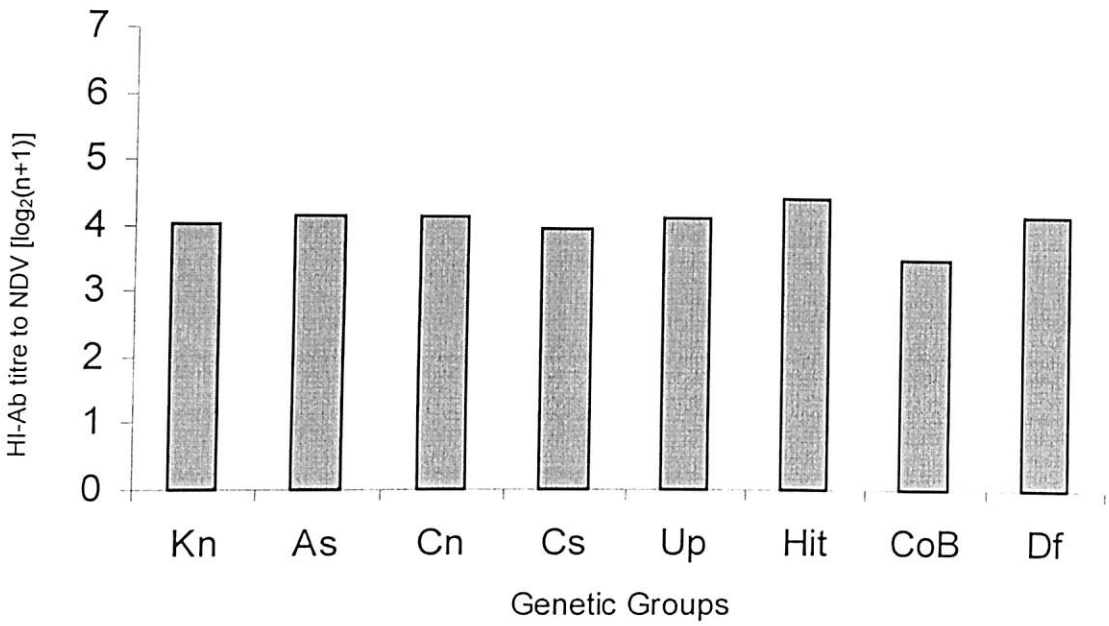


Fig.4.10 Effect of Genetic Groups on HI-Ab titre level on 7 *dpv* in chicken vaccinated against ND.



in Hitcari birds was significantly higher than the birds of all other genetic groups which didn't differ among themselves in this regard, except the Commercial broiler which had significantly the lowest HI-Ab titre on 7 *dpv* (Table 4.6, Fig. 4.10).

4.2.2.1.3 Age at '0' day of inoculation :

The effect of age of the bird on 0 day of vaccination had highly significant ($P \leq 0.01$) influence on HI-Ab titre level on 7 *dpv*, its contribution to the total variation in the trait being 10.773 percent (Table 4.5). Further, the older birds on '0' day of inoculation had significantly higher HI-Ab titre on 7 *dpv* as compared to the younger birds, the corresponding Least squares mean for the birds of two age groups being 4.7325 ± 0.0966 and 3.4533 ± 0.0966. The co-efficient of variation in the data for the young and adult birds were reckoned to be 43.155 and 27.233 percent respectively (Table 4.6).

A critical analysis of the result revealed that the gain in HI-Ab titre on 7 *dpv* due to vaccination was almost the same in the birds of both age groups and it was the difference in basal HI-antibody titre levels which made the birds of two age groups significantly different from each other in respect to HI-Ab titre on 7 *dpv* (Table 4.6 and Fig. 4.11)

4.2.2.1.4 Management System :

The effect of Management system, to which the birds were subjected to during the period of study, contributed significantly ($P \leq 0.01$) to the total variation in HI-Ab titre level on 7 *dpv*. Its contribution to the total variation in titre level was 9.248 percent (Table 4.5). The Least squares mean for HI-Ab titre on 7 *dpv* for the birds under extensive system of management was estimated to be 4.1901 ± 0.0966 which was significantly higher than the corresponding mean for the birds under intensive system of management. The co-efficient of variation for low and high input systems of management were estimated to be 12.627 and 46.498 percent respectively (Table 4.6, Fig. 4.12).

Fig.4.11 Effect of Age at '0' day of inoculation on HI-Ab titre level on 7 *dpv* in chicken vaccinated against ND.

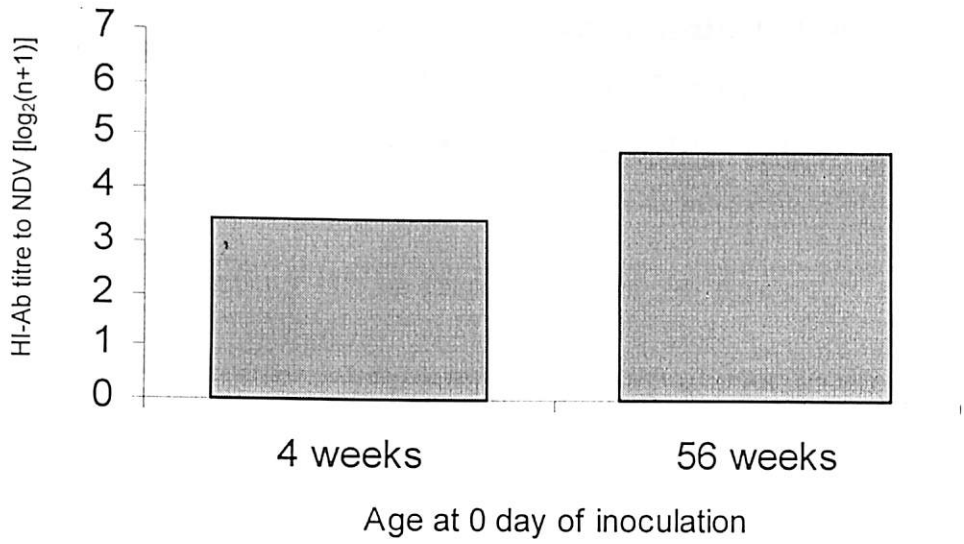
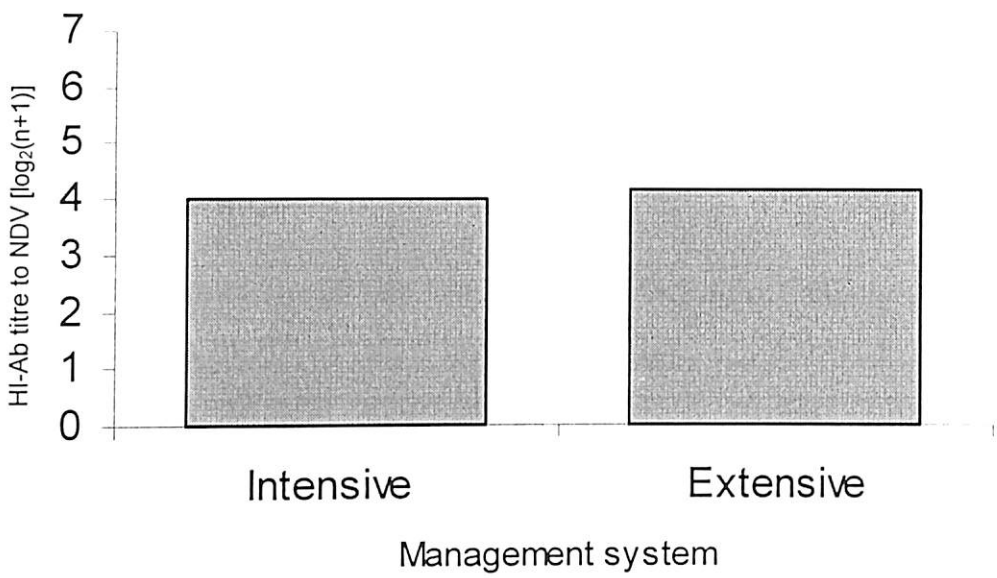


Fig.4.12 Effect of Management system on HI-Ab titre level on 7 *dpv* in chicken vaccinated against ND.



4.2.3 HI-antibody titre level on 14 *dpv* :

The results of Least squares analysis of variance of HI-Ab titre on 14th day of vaccination in chicken vaccinated against Newcastle disease have been presented in tables 4.7 and 4.8. The overall Least squares mean for HI-Ab titre level on 14 *dpv* was estimated to be 4.8633 ± 0.0346, the co-efficient of variation being 14.406 percent (Table 4.8).

4.2.3.1 Factors affecting HI-Ab titre level on 14 *dpv* :

The effect of genetic groups, age at '0' day of inoculation as well as the management system on HI-Ab titre level on 14 *dpv* were statistically significant ($P \leq 0.01$). However, the effect of location of the flocks did not have any significant influence on the immunological trait under reference (Table 4.7).

4.2.3.1.1 Location of the Flocks :

Location of the flock contributed only 0.383 percent to the total variation in HI-Ab titre level on 14 *dpv* which was stastistically not significant. Although the difference was not significant, the Least squars means for the birds at Pusa and Patna were estimated to be 4.8535 ± 0.0342 and 4.8731 ± 0.0350 respectively (Table 4.8, Fig. 4.13).

4.2.3.1.2 Genetic groups :

The genetic make up of the fowl contributed significantly (19.721%) to the total variation in HI-Ab titre level on 14 *dpv*, the contribution being the highest among all the sources of variation under consideration (Table 4.7). The Least squares means for the birds of different genetic groups (Table 4.8) revealed that the Hitcari birds have the highest (5.3137 ± 0.0982) HI-Ab titre on 14 *dpv* followed by Desi fowl (5.0483 ± 0.0982), Upcari (4.9497 ± 0.0966), Kadaknath (4.9087 ± 0.1000), Aseel (4.8460 ± 0.0982), Cari Nirbheek (4.8214 ± 0.0966), Cari Shyama (4.6320 ± 0.0966) and Commercial broiler (4.3867 ± 0.0982). The pair-wise comp-

Table 4.7

Least squares analysis of variance showing effects of genetic and non-genetic factors on HI antibody titre level on 14th day in chicken vaccinated against Newcastle disease.

Source of Variation	d.f	Sum of squares	Mean squares	F	R^2%
Location of the flocks	1	0.299	0.299	2.623	0.383
Genetic groups	7	15.412	2.201	19.307**	19.721
Age at 0 day of inoculation	1	9.360	9.360	82.105**	11.977
Management system	1	7.540	7.540	66.140**	9.648
Residual	400	45.54	0.114	-	58.271

** = $P \leq 0.01$

Table 4.8

Least squares means for genetic and non-genetic factors affecting HI antibody titre level on 14 day of vaccination in chicken vaccinated against Newcastle disease.

Factors	No. of Obs.	Day	CV(%)
		Mean ± SE	
Overall means (μ)	410	4.8633 ± 0.0346	14.406
Location of the flock			
1. Pusa	352	4.8535 ± 0.0342	13.220
2. Patna	58	4.8731 ± 0.0350	5.478
Genetic group			
1. Kadaknath	56	4.9087^{a} ± 0.1000	15.245
2. Aseel	58	4.8460^{a} ± 0.0982	15.433
3. Upcari Aseel (Cari Nirbheek)	60	4.8214^{a} ± 0.0966	15.520
4. Upcari Kadaknath (Cari Shyama)	60	4.6320^{a} ± 0.0966	16.154
5. Upcari Frizzle (Upcari)	60	4.9497^{a} ± 0.0966	15.117
6. Upcari Naked Neck (Hitcari)	58	5.3137^{d} ± 0.0982	14.074
7. Commercial broiler	29	4.3867^{b} ± 0.0982	12.055
8. Desi fowl.	29	5.0483^{d} ± 0.0982	10.475
Age at 'O' day of inoculation			
1. 4 Weeks	234	4.1876^{a} ± 0.0982	35.871
2. 56 Weeks	176	5.5390^{b} ± 0.0982	23.520
Management System			
1. Intensive (High-input)	381	4.1067^{a} ± 0.0966	45.914
2. Extensive (Low-input)	29	5.6199^{b} ± 0.0982	9.4098

Values superscripted by different letters were significantly different from each other.

Table 4.8

Least squares means for genetic and non-genetic factors affecting HI antibody titre level on 14 day of vaccination in chicken vaccinated against Newcastle disease.

Factors	No. of Obs.	Day	CV(%)
		Mean ± SE	
Overall means (μ)	410	4.8633 ± 0.0346	14.406
Location of the flock			
1. Pusa	352	4.8535 ± 0.0342	13.220
2. Patna	58	4.8731 ± 0.0350	5.478
Genetic group			
1. Kadaknath	56	4.9087^{a} ± 0.1000	15.245
2. Aseel	58	4.8460^{a} ± 0.0982	15.433
3. Upcari Aseel (Cari Nirbheek)	60	4.8214^{a} ± 0.0966	15.520
4. Upcari Kadaknath (Cari Shyama)	60	4.6320^{a} ± 0.0966	16.154
5. Upcari Frizzle (Upcari)	60	4.9497^{a} ± 0.0966	15.117
6. Upcari Naked Neck (Hitcari)	58	5.3137^{d} ± 0.0982	14.074
7. Commercial broiler	29	4.3867^{b} ± 0.0982	12.055
8. Desi fowl.	29	5.0483^{d} ± 0.0982	10.475
Age at '0' day of inoculation			
1. 4 Weeks	234	4.1876^{a} ± 0.0982	35.871
2. 56 Weeks	176	5.5390^{b} ± 0.0982	23.520
Management System			
1. Intensive (High-input)	381	4.1067^{a} ± 0.0966	45.914
2. Extensive (Low-input)	29	5.6199^{b} ± 0.0982	9.4098

Values superscripted by different letters were significantly different from each other.

Fig.4.13 Effect of Location of the flocks on HI-Ab titre level on 14 *dpv* in chicken vaccinated against ND.

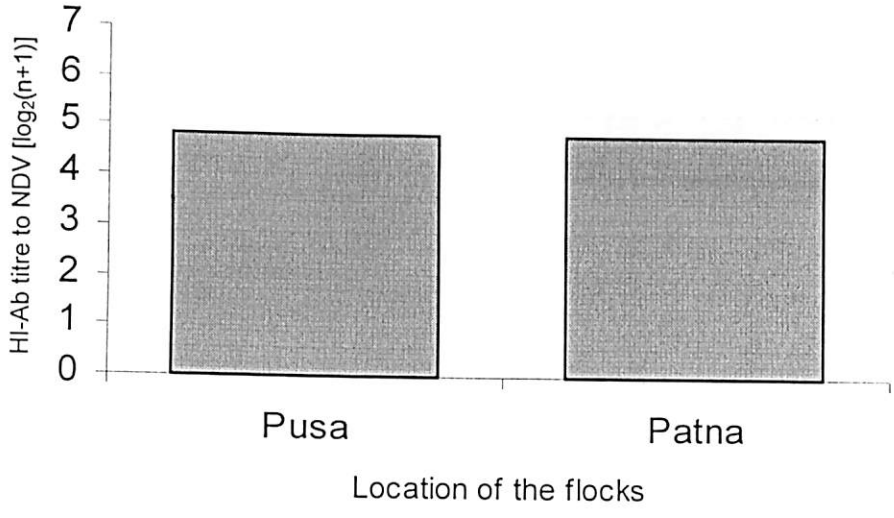


Fig.4.14 Effect of Genetic Groups on HI-Ab titre level on 14 *dpv* in chicken vaccinated against ND.

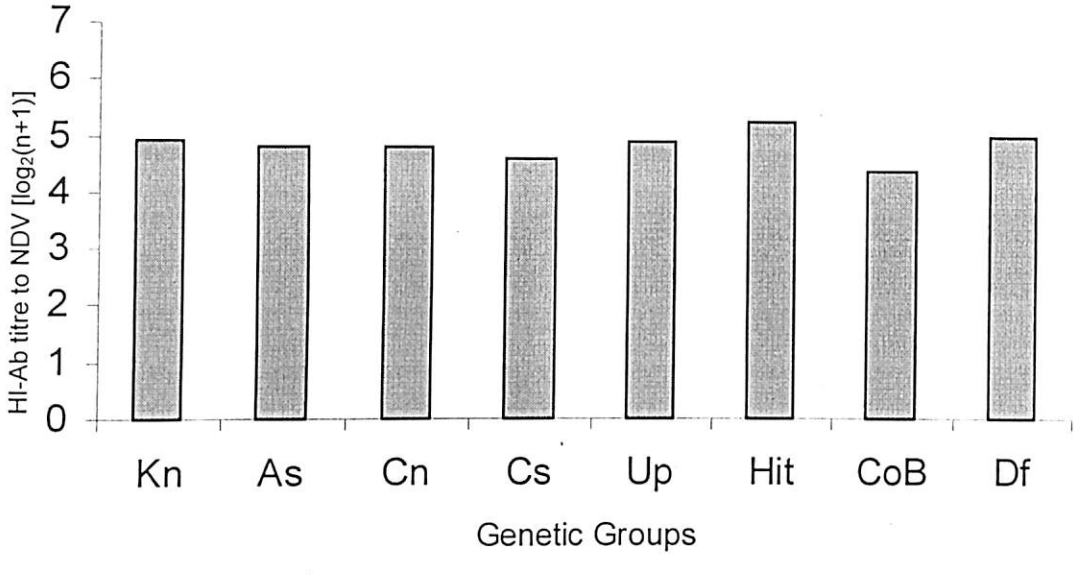


Fig.4.15 Effect of Age at '0' day of inoculation on HI-Ab titre level on 14 *dpv* in chicken vaccinated against ND.

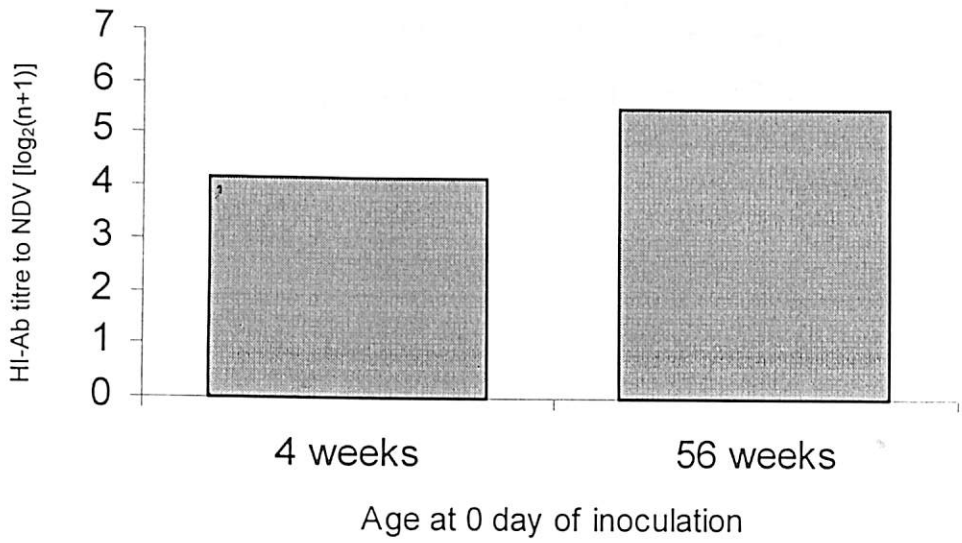
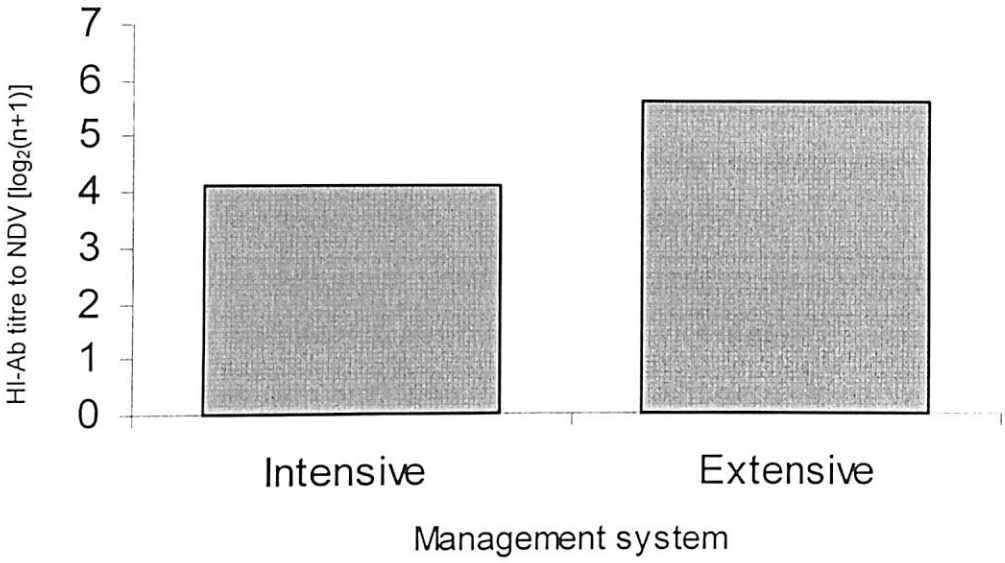


Fig.4.16 Effect of Management system on HI-Ab titre level on 14 *dpv* in chicken vaccinated against ND.



4.2.4.1 Factors affecting HI-Ab titre level on 21 *dpv* :

The effects of genetic group of the birds, their age at '0' day of inoculation as well as the management system on HI-Ab titre level on 21 *dpv* were statistically significant ($P \leq 0.01$). However, the effect of location of the flocks did not have any significant influence on the trait (Table 4.9).

4.2.4.1.1 Location of the Flock :

Variation in HI-Ab titre level on 21 *dpv* due to difference in location of the flocks was only 0.498 percent and statistically not significant. The Least squares means for the birds managed at Pusa and Patna farms were recorded to be 5.6321 ± 0.0352 and 5.6723 ± 0.0349 respectively (Table 4.10, Fig 4.17).

4.2.4.1.2 Genetic groups :

The genetic constitution of the fowl contributed 15.234 percent to the total variation in HI-Ab titre level on 21 *dpv*, the contribution being the lowest among the sources of variation influencing the trait significantly (Table 4.9). The Least squares mean for the birds of different genetic groups, tabulated in table 4.10, revealed that the Desi fowl recorded the highest (5.9905 ± 0.0982) HI-Ab titre on 21 *dpv* followed by Hitcari (5.9556 ± 0.0982), Kadaknath (5.7210 ± 0.1038), Aseel (5.6510 ± 0.0982), Upcari (5.6389 ± 0.1000), Cari Nirbheek (5.6377 ± 0.1000), Cari Shyama (5.4081 ± 0.1018) and Commercial broilers (5.2147 ± 0.0982). The results of DMRT indicated that among all the genetic groups of the birds, Commercial broiler had the lowest HI-Ab titre level on 21 *dpv* which differed significantly from the birds of all other genetic groups except Cari Shyama which did not differ significantly from Commercial broilers. Desi fowl recorded the highest HI-Ab titre on 21 *dpv* which didn't differ significantly from Kadaknath, Aseel and Hitcari birds. The birds belonging to Aseel, Cari Nirbheek, Cari Shyama and Upcari didn't differ significantly among each other in respect to the HI-Ab titre level on

Table 4.9

Least squares analysis of variance showing effects of genetic and non-genetic factors on HI antibody titre level on 21st day in chicken vaccinated against Newcastle disease.

Source of Variation	d.f	Sum of squares	Mean squares	F	R^2%
Location of the flocks	1	0.289	0.289	3.853	0.498
Genetic groups	7	8.837	1.262	16.827**	15.234
Age at '0' day of inoculation	1	10.764	10.764	143.52**	18.556
Management system	1	9.258	9.258	123.44**	15.960
Residual	382	28.859	0.075	-	49.752

** = $P \leq 0.01$

Table 4.10

Least squares means for genetic and non-genetic factors affecting HI antibody titre level on 21 day of vaccination in chicken vaccinated against Newcastle disease.

Factors	No. of Obs.	Day	CV(%)
		Mean ± SE	
Overall means (μ)	392	5.6522 ± 0.0353	12.365
Location of the flock			
1. Pusa	334	5.6321 ± 0.0352	11.422
2. Patna	58	5.6723 ± 0.0349	4.686
Genetic group			
1. Kadaknath	52	5.7210^{a} ± 0.1038	13.084
2. Aseel	58	5.6510^{ab} ± 0.0982	13.234
3. Upcari Aseel (Cari Nirbheek)	56	5.6377^{b} ± 0.1000	13.274
4. Upcari Kadaknath (Cari Shyama)	54	5.4081^{bc} ± 0.1018	13.832
5. Upcari Frizzle (Upcari)	56	5.6389^{b} ± 0.1000	13.270
6. Upcari Naked Neck (Hitcari)	58	5.9556^{a} ± 0.0982	12.557
7. Commercial broiler	29	5.2147^{c} ± 0.0982	10.141
8. Desi fowl.	29	5.9905^{a} ± 0.0982	8.828
Age at '0' day of inoculation			
1. 4 Weeks	225	4.9481^{a} ± 0.0982	29.769
2. 56 Weeks	167	6.3563^{b} ± 0.1018	20.697
Management System			
1. Intensive (High-input)	363	4.4918^{a} ± 0.1018	43.180
2. Extensive (Low-input)	29	6.8126^{b} ± 0.0982	7.762

Values superscripted by different letters were significantly different from each other.

Fig.4.17 Effect of Location of the flocks on HI-Ab titre level on 21 *dpv* in chicken vaccinated against ND.

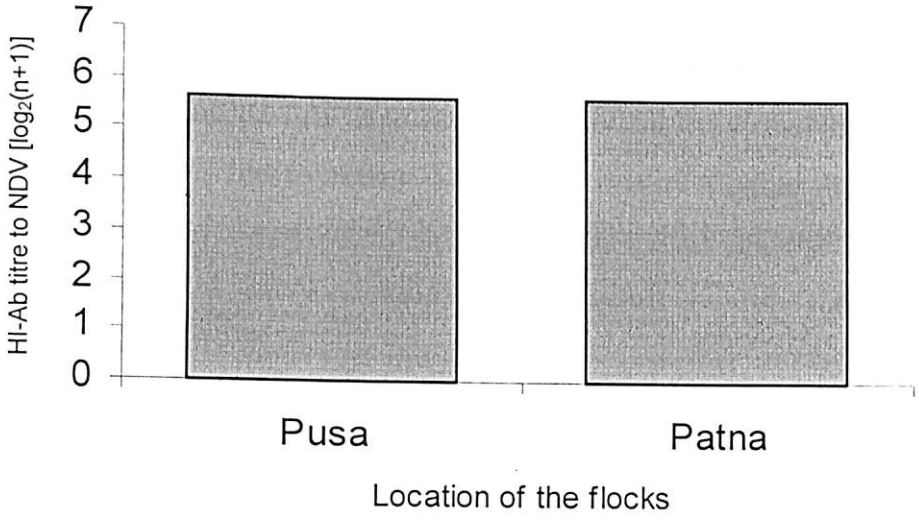
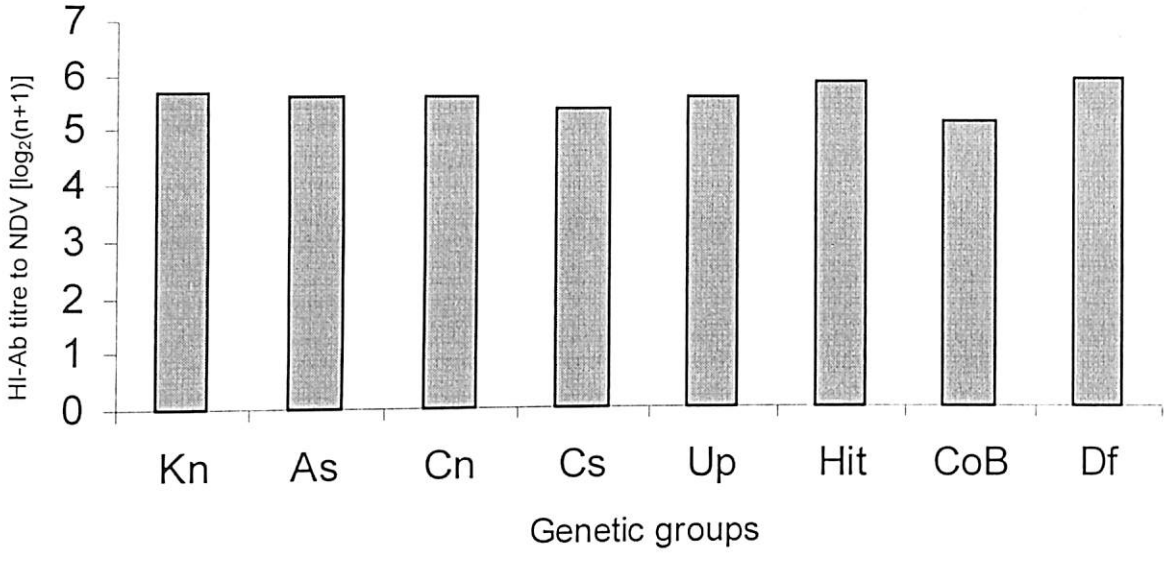


Fig.4.18 Effect of Genetic Groups on HI-Ab titre level on 21 *dpv* in chicken vaccinated against ND.



21 *dpv*, but differ significantly from the birds under Kadaknath, Hitcari, Commercial broiler and Desi fowl genetic groups (Table 4.10, Fig. 4.18).

4.2.4.1.3 Age at '0' day of inoculation :

The effect of age of the birds on 0 day of inoculation had significant influence ($P \leq 0.01$)) on HI-Ab titre level on 21 *dpv*, its contribution to the total variation in the trait being 18.556 percent (Table 4.9). Results revealed that as in the case of HI-Ab titre on 7 and 14 *dpv*, on 21 *dpv* also the older birds on have significantly higher HI-Ab titre (6.3563 ± 0.1018) as compared to the younger (4.9481 ± 0.0982) birds (Table 4.10, Fig. 4.19).

4.2.4.1.4 Management Systems :

The effect of management system on HI-Ab titre on 21 *dpv* had the trend similar to that for 14 *dpv* (ref. para 4.2.3.1.3). The birds under extensive management system had significantly higher HI-Ab titre (6.8126 ± 0.0982) as compared to that for the birds under intensive management system (4.4918 ± 0.1018). The percent contribution of this effect on HI-Ab titre on 21 *dpv* was 15.940 which was more in magnitude to that in case of 7 and 14 *dpv* (Table 4.10). The estimates of co-efficient of variation for the extensive and intensive management systems were 7.762 and 43.180 percent respectively which were lower in magnitude as compared to that in case of 7 and 14 *dpv* (Table 4.10, Fig. 4.20).

4.2.5. HI-Ab titre level on 28 *dpv* :

The result of Least squares analysis of the data on HI-Ab titre level on 28th day of vaccination in chicken, vaccinated against Newcastle disease, have been presented in tables 4.11 and 4.12. The overall Least squares mean (μ) for HI-Ab titre level on 28 *dpv* was estimated to be 6.2703 ± 0.0362, the co-efficient of variation being 11.149 percent (Table 4.12).

Fig.4.19 Effect of Age at '0' day of inoculation on HI-Ab titre level on 21 *dpv* in chicken vaccinated against ND.

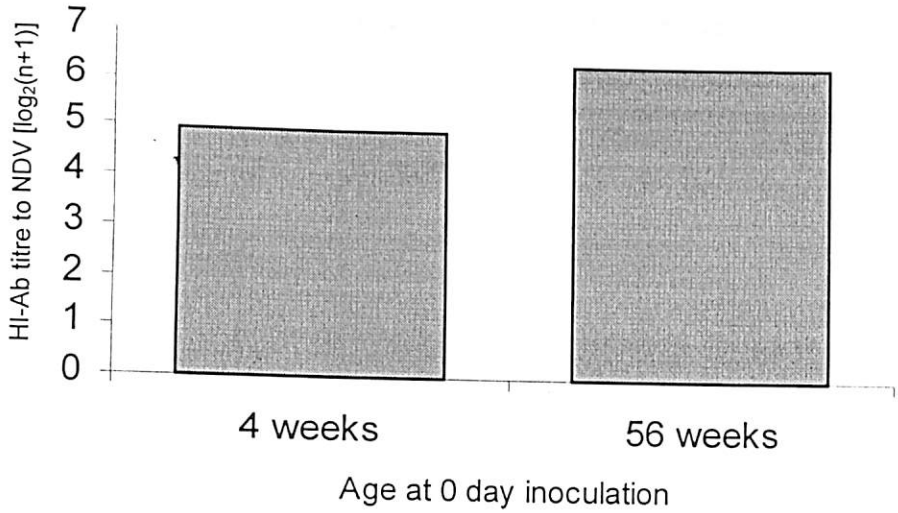


Fig.4.20 Effect of Management system on HI-Ab titre level on 21 *dpv* in chicken vaccinated against ND.

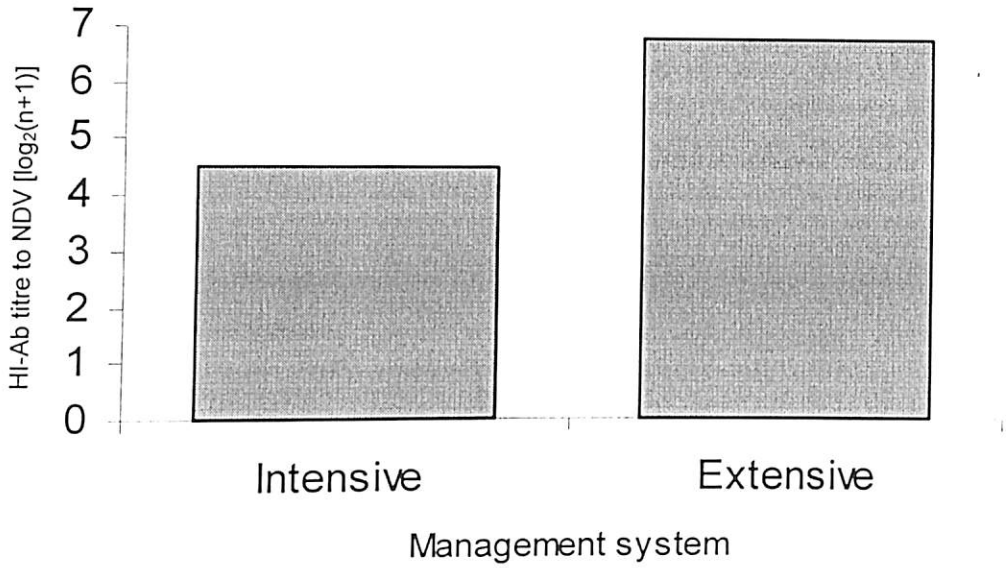


Table 4.11

Least squares analysis of variance showing effects of genetic and non-genetic factors on HI antibody titre level on 28th day in chicken vaccinated against Newcastle disease.

Source of Variation	d.f	Sum of squares	Mean squares	F	R^2%
Location of the flocks	1	0.351	0.351	2.324	0.420
Genetic groups	7	13.281	1.897	12.562**	15.926
Age at '0' day of inoculation	1	7.847	7.847	51.966**	9.410
Management system	1	6.954	6.954	46.052**	8.339
Residual	363	54.955	0.151	-	65.905

** = $P \leq 0.01$

Table 4.12

Least squares means for genetic and non-genetic factors affecting HI antibody titre level on 28 day of vaccination in chicken vaccinated against Newcastle disease.

Factors	No. of Obs.	Day	CV(%)
		Mean ± SE	
Overall means (μ)	373	6.2703 ± 0.0362	11.149
Location of the flock			
1. Pusa	320	6.2698 ± 0.0360	10.271
2. Patna	53	6.2708 ± 0.0363	4.214
Genetic group			
1. Kadaknath	50	6.3384^{a} ± 0.1058	11.802
2. Aseel	56	6.3752^{a} ± 0.1000	11.738
3. Upcari Aseel (Cari Nirbheek)	54	6.1693^{b} ± 0.1018	12.126
4. Upcari Kadaknath (Cari Shyama)	52	6.0236^{b} ± 0.1038	12.426
5. Upcari Frizzle (Upcari)	52	6.2134^{ab} ± 0.1030	11.954
6. Upcari Naked Neck (Hitcari)	56	6.4113^{ac} ± 0.1000	11.672
7. Commercial broiler	26	5.9856^{b} ± 0.1038	8.843
8. Desi fowl.	27	6.6460^{c} ± 0.1018	7.959
Age at 'O' day of inoculation			
1. 4 Weeks	213	5.9568^{a} ± 0.1038	25.432
2. 56 Weeks	160	6.5838^{b} ± 0.1058	20.327
Management System			
1. Intensive (High-input)	346	5.5918^{a} ± 0.1038	34.529
2. Extensive (Low-input)	27	6.9488^{b} ± 0.1018	7.612

Values superscripted by different letters were significantly different from each other.

4.2.5.1 Factors affecting HI-Ab titre level on 28 *dpv* :

The genetic group of the birds, their age at '0' day of inoculation as well as the management system to which they were subjected during the experimental period, had significant influence ($P \leq 0.01$) on HI-Ab titre level on 28 *dpv*. The effect of location of the flocks did not have any significant influence on the immunological trait under reference (Table 4.11).

4.2.5.1.1 Location of the Flock :

Location-effect contributed very little (0.420%) to the total variation in HI-Ab titre level on 28 *dpv* (Table 4.11). The Least squares means for the birds at Pusa and Patna were estimated to be 6.2698 ± 0.0360 and 6.2708 ± 0.0363 respectively which did not differ significantly from each other (Table 4.12, Fig. 4.22).

4.2.5.1.2 Genetic groups :

The genetic make up of the fowl contributed 15.926 percent to the total variation in HI-Ab titre level on 28 *dpv*, the contribution being the highest as compared to the contributions of the other sources of variation under consideration (Table 4.11). Results (Table 4.12, Fig. 4.21) revealed that the Desi fowl had the highest (6.6440 ± 0.1018) Least squares mean for HI-Ab titre on 28 *dpv* followed by the averages for Hitcari (6.4113 ± 0.1000), Aseel (6.3752 ± 0.1000), Kadaknath (6.3384 ± 0.1058), Upcari (6.2134 ± 0.1030), Cari Nirbheek (6.1693 ± 0.1018), Cari Shyama (6.0236 ± 0.1038) and Commercial broiler (5.9856 ± 0.1038). The Duncan's Multiple Range Test revealed that among all the genetic group of birds, Desi fowl, with the highest Least squares mean for HI-Ab titre level on 28 *dpv*, didn't differ significantly from Hitcari birds in this regard. The Commercial broiler having significantly the lowest Least squares mean for HI-Ab titre on 28 *dpv* didn't differ significantly from Cari Nirbheek, Cari Shyama, and Upcari birds, but differed significantly from the birds of Kadaknath, Aseel and Hitcari genetic groups. However, Kadaknath,

Fig.4.21 Effect of Location of the flocks on HI-Ab titre level on 28 *dpv* in chicken vaccinated against ND.

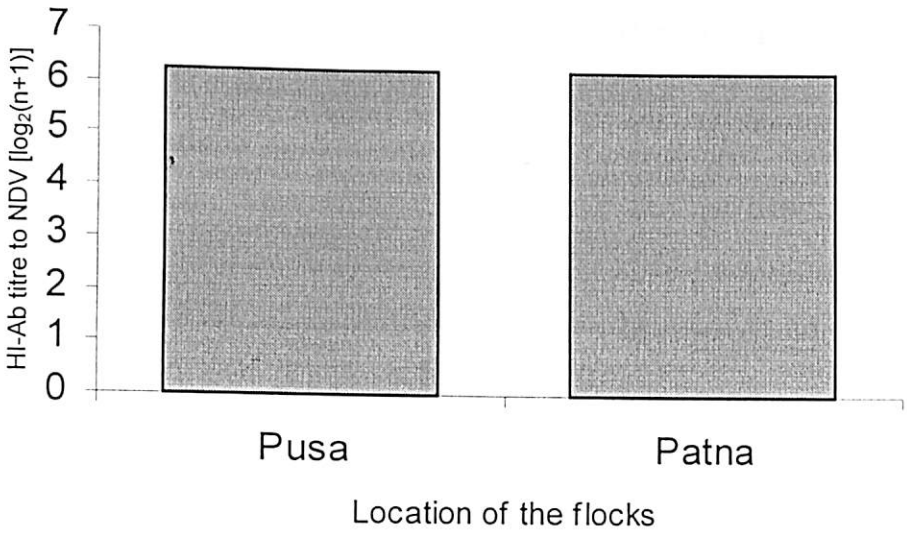
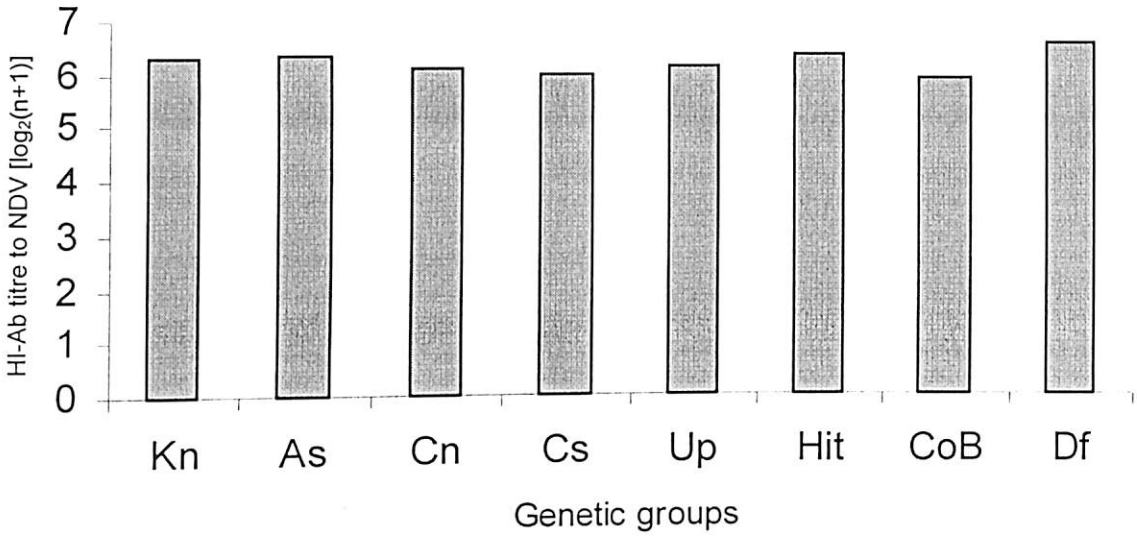


Fig.4.22 Effect of Genetic Groups on HI-Ab titre level on 28 *dpv* in chicken vaccinated against ND.



Aseel, Upcari and Hitcari birds didn't differ significantly among each other.

4.2.5.1.3 Age at '0' day of inoculation :

The effect of age of the birds on '0' day of inoculation had significant influence ($P \leq 0.01$) on HI-Ab titre level on 28 *dpv*. The percent contribution of this effect to the total variation in HI-Ab titre on 28 *dpv* was 9.410 (Table 4.11). The older birds on '0' day of inoculation have significantly higher HI-Ab titre level (6.5838 ± 0.1058) as compared to the younger birds (5.9568 ± 0.1038) the trend being similar to that in the case of 0, 7, 14 and 21 *dpv* (Table 4.12, Fig. 4.23).

4.2.5.1.4 Management Systems :

The influence of management system on HI-Ab titre level on 28 *dpv* had the trend similar to that in the cases of 7, 14 and 21 *dpv* and it contributed 8.339 percent to the total variation in the trait under reference (Table 4.11). The birds under extensive management system had significantly higher Least squares mean for HI-Ab titre level (6.9488 ± 0.1018) as compared to that for the birds under intensive management system (5.5918 ± 0.1038). The estimates of co-efficient of variation in the data on the titre level for the birds under the extensive and intensive management system were 7.612 and 34.529 percent respectively (Table 4.12, Fig. 4.24).

4.2.6 HI antibody titre level on 35 *dpv* :

The results of the Least squares analysis of the data on HI-Ab titre level in the chicken vaccinated against Newcastle disease, on 35th day of vaccination, have been presented in tables 4.13 and 4.14. The overall Least squares mean for HI-Ab titre level on 35 *dpv* was estimated to be 5.5886 ± 0.0368, the co-efficient of variation being 12.511 percent (Table 4.14)

Fig.4.23 Effect of Age at '0' day of inoculation on HI-Ab titre level on 28 *dpv* in chicken vaccinated against ND.

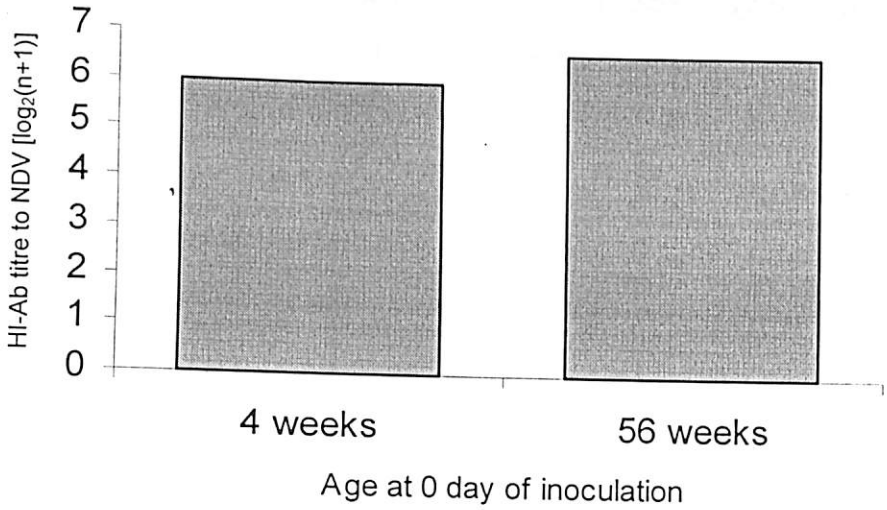


Fig.4.24 Effect of Management system on HI-Ab titre level on 28 *dpv* in chicken vaccinated against ND.

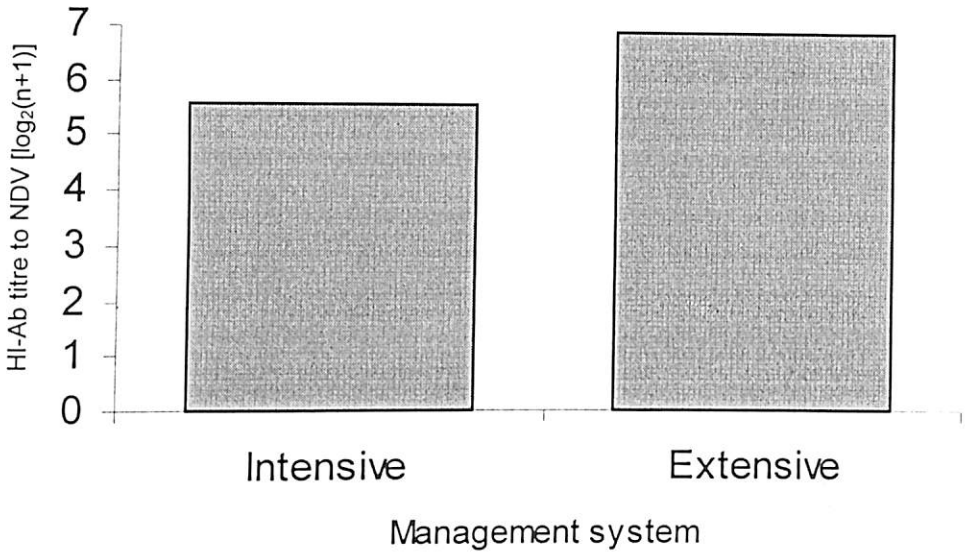


Table 4.13

Least squares analysis of variance showing effects of genetic and non-genetic factors on HI antibody titre level on 35th day in chicken vaccinated against Newcastle disease.

Source of Variation	d.f	Sum of squares	Mean squares	F	R^2%
Location of the flocks	1	0.359	0.359	3.121	0.524
Genetic groups	7	11.319	1.617	14.060**	16.526
Age at '0' day of inoculation	1	8.466	8.466	73.617**	12.359
Management system	1	7.692	7.692	66.886**	11.229
Residual	351	40.662	0.115	-	59.362

** = $P \leq 0.01$

Table 4.14

Least squares means for genetic and non-genetic factors affecting HI-antibody titre level on 35 day of vaccination in chicken vaccinated against Newcastle disease.

Factors	No. of Obs.	Day	CV(%)
		Mean ± SE	
Overall means (μ)	361	5.5886 ± 0.0368	12.511
Location of the flock			
1. Pusa	310	5.5879 ± 0.0364	11.469
2. Patna	51	5.5893 ± 0.0361	4.612
Genetic group			
1. Kadaknath	50	5.7881^{ab} ± 0.1058	12.925
2. Aseel	52	5.6838^{a} ± 0.1038	13.169
3. Upcari Aseel (Cari Nirbheek)	54	5.2975^{c} ± 0.1018	14.121
4. Upcari Kadaknath (Cari Shyama)	50	5.3574^{c} ± 0.1058	13.964
5. Upcari Frizzle (Upcari)	50	5.5525^{a} ± 0.1058	13.474
6. Upcari Naked Neck (Hitcari)	54	5.6597^{a} ± 0.1018	13.218
7. Commercial broiler	26	5.3449^{b} ± 0.1038	9.902
8. Desi fowl	25	6.0246^{c} ± 0.1058	8.780
Age at 'O' day of inoculation			
1. 4 Weeks	206	5.1579^{a} ± 0.1058	29.440
2. 56 Weeks	155	6.0193^{b} ± 0.1038	21.469
Management System			
1. Intensive (High-input)	336	5.0051^{a} ± 0.1058	38.747
2. Extensive (Low-input)	25	6.1721^{b} ± 0.1018	8.247

Values superscripted by different letters were significantly different from each other.

4.2.6.1 Factors affecting HI-Ab titre level on 35 *dpv* :

Analysis of variance (Table 4.13) revealed that variation in genetic group of the birds, their age at '0' day of inoculation as well as the management system had statistically significant ($P \leq 0.01$) influence on HI-Ab titre level on 35 *dpv*. However, the effect of location of the flocks didn't have any significant effect on the variation in this immunological trait (Table 4.13).

4.2.6.1.1 Location of the Flocks :

The location of the flocks did not influence HI-Ab titre level on 35 *dpv* significantly. The Least squares mean for the birds at Pusa and Patna were estimated to be 5.5879 ± 0.0364 and 5.5893 ± 0.0361 respectively. Its contribution to the total variation on 35 *dpv* was 0.524 percent (Table 4.14, Fig. 4.25).

4.2.6.1.2 Genetic groups :

The genetic constitution of the birds contributed the highest (16.526%) to the total variation in HI-Ab titre level on 35 *dpv* in the fowl vaccinated against Newcastle disease (Table 4.13). The Least squares means for the birds of different genetic groups (Table 4.14) revealed that the Desi fowl had the highest (6.0246 ± 0.1058) HI-Ab titre on 35 *dpv* followed by Kadaknath (5.7881 ± 0.1058), Aseel (5.6838 ± 0.1038), Hitcari (5.65997 ± 0.1018), Upcari (5.5525 ± 0.1058), Cari Shyama (5.3574 ± 0.1058), Commercial broiler (5.3449 ± 0.1038) and Cari Nirbheek (5.2975 ± 0.1018). Among all the genetic groups of the birds, Cari Nirbheek had the lowest HI-Ab titre level on 35 *dpv*. It did not differ significantly from Cari Shyama, and Commercial broiler birds, but differed significantly from Kadaknath, Aseel, Upcari, Hitcari and Desi fowl which were not significantly different among each other. The Desi fowl with significantly the highest HI-Ab titre level on 35 *dpv* were significantly different from the birds of all other genetic groups (Table 4.14, Fig. 4.26).

Fig.4.25 Effect of Location of flocks on HI-Ab titre level on 35 *dpv* in chicken vaccinated against ND.

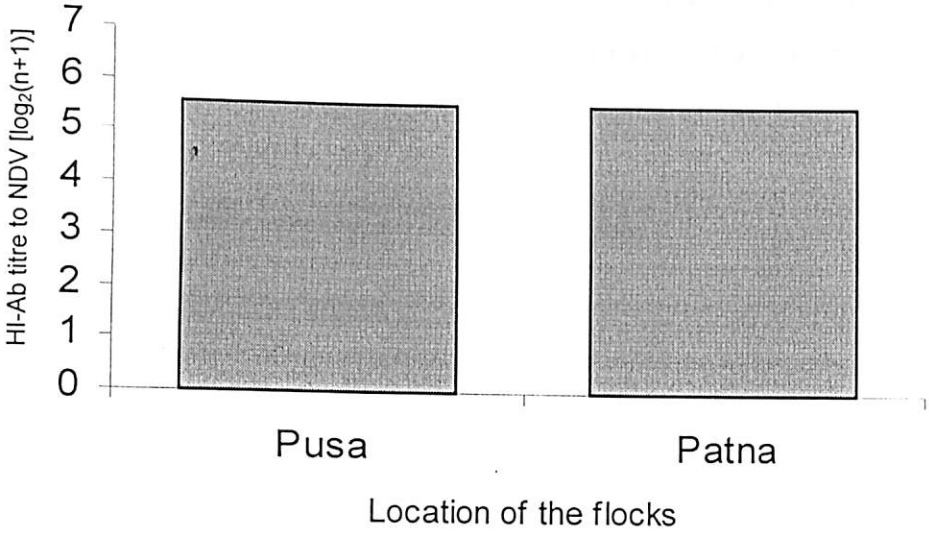
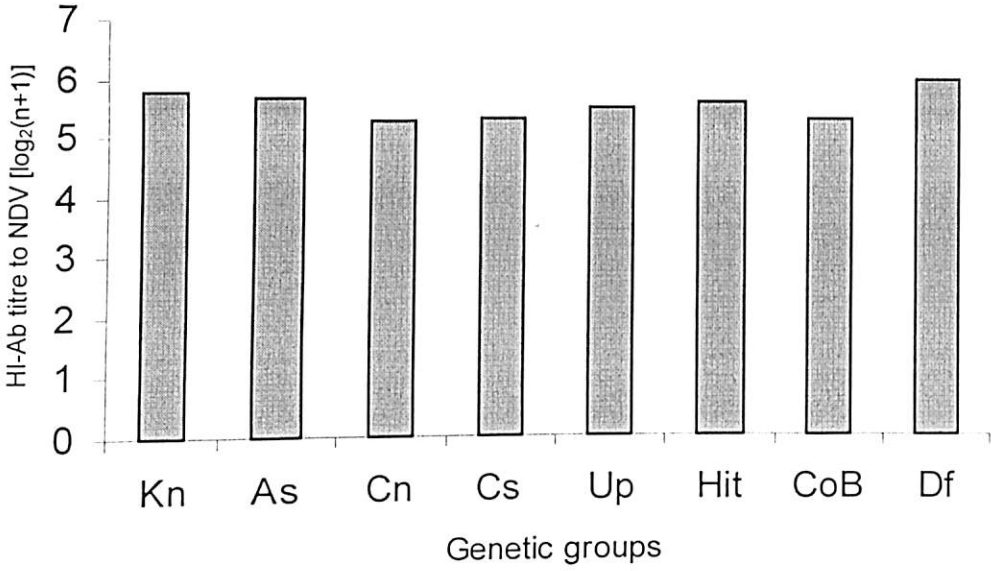


Fig.4.26 Effect of Genetic Groups on HI-Ab titre level on 35 *dpv* in chicken vaccinated against ND.



4.2.6.1.3 Age at '0' day of inoculation :

The results of Least squares analysis (Table 4.13) revealed that the effect of age of birds on '0' day of inoculation had significant influence ($P \leq 0.01$) on HI-Ab titre level on 35 *dpv*, its contribution to the total variation in the trait being 12.359 percent. As evident from the table 4.16 and fig. 4.27, the older birds on '0' day of inoculation had significantly higher Least squares mean for HI-Ab titre level (6.0193 ± 0.1038) as compared to the younger birds (5.1579 ± 0.1058).

4.2.6.1.4 Management Systems :

The effect of management system on HI-Ab titre level on 35 *dpv* had the trend similar to that for 7, 14, 21 and 28 *dpv*. Results indicated that the birds under extensive management system had significantly higher Least squares mean for HI-Ab titre level (6.1721 ± 0.1018) as compared to that for the birds under intensive management system (5.0051 ± 0.1058). The estimates of co-efficient of variation for the extensive and intensive management system were 8.247 and 38.747 percent respectively (Table 4.14, Fig. 4.28).

❑❑❑

Fig.4.27 Effect of Age at 0 day of inoculation on HI-Ab titre level on 35 *dpv* in chicken vaccinated against ND.

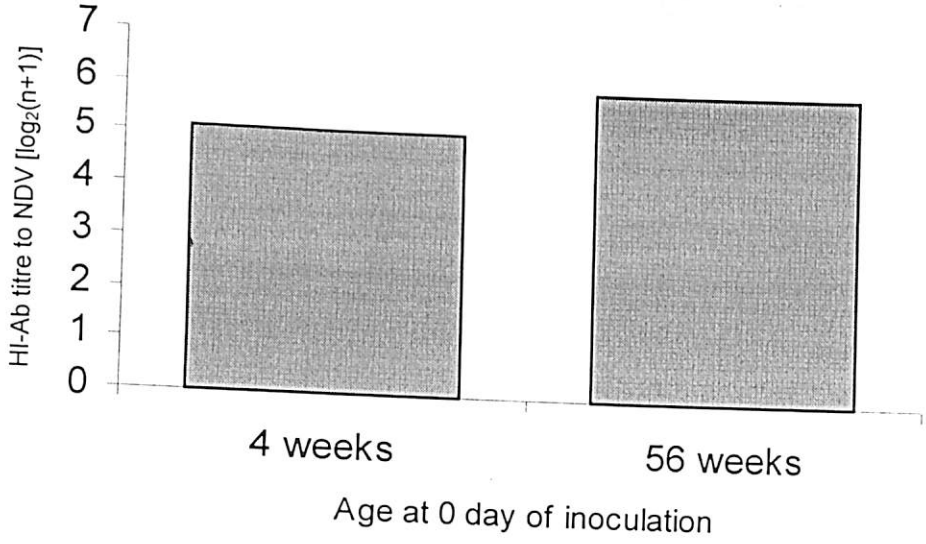
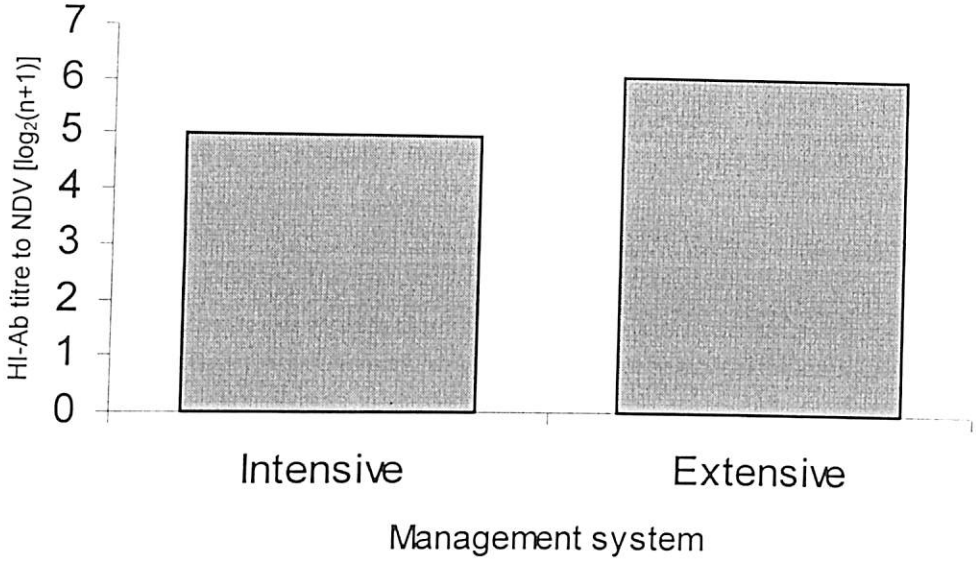


Fig.4.28 Effect of Management system on HI-Ab titre level on 35 *dpv* in chicken vaccinated against ND.



5.
DISCUSSION

5. DISCUSSION

The present investigation was undertaken to study the effect of genetic and some non-genetic factors on general and specific immune response in chicken. Birds belonging to eight genetic groups viz. Kadaknath, Aseel, Cari Nirbheek (Aseel X Cari Red), Cari Shyama (Kadaknath X Cari Red), Upcari (Aseel Frizzle X Cari Red) and Hitcari (Aseel Naked neck X Cari Red as well as Commercial broiler and Desi fowl were included in this study. Besides the effect of genetic make-up of the birds on general and specific immune response, the influence of some non-genetic factors i.e. the location of the farm, age of the birds on '0' day of inoculation of mitogen/vaccine as well as the management systems to which they were subjected during the experimental period, were studied. To study the effect of Major genes on general and specific immune response, the fowl possessing major genes for Frizzle plumage (Upcari) and Naked neck (Hitcari) were included in this study. The immune responsiveness of these two populations having Frizzle and Naked neck major genes was compared with its normal feathered counterpart Cari Nirbheek (Aseel X Cari Red).

The general immune response was measured in terms of foot index for which Concanavalin–A was used as mitogen. The specific immune response was measured in terms of HI-Ab titre on 0, 7, 14, 21, 28 and 35th day of vaccination against Newcastle disease.

5.1 General Immune Response :

The CMI response observed for the birds of various genetic groups varied from 1.792 ± 0.011 to 2.392 ± 0.01mm. The genetic group, age of the birds on the day of inoculation and management system had the significant influence on foot-index. The findings of this study was in close agreement with the results of a classical study conducted by Haunshi (1999) in some specialized chicken populations. They reported the average FI ranging between 1.93 ± 0.07 and 2.25 ± 0.05 mm. Saxena

(1993) observed higher responses against Con–A in Guinea fowl, Kadaknath and broiler chicken, but his study was based on wing-web index. Moreover, the variation in general immune responsiveness to Con-A, recorded in different studies, may be attributed to variation in genetic constitution of the experimental birds, the site of injection, measurement of skin thickness as well as variable environmental condition to which the experimental birds were subjected to.

5.1.1 Effect of Location of Flocks on CMI response :

The contribution of location-effect to the total variation in CMI response was statistically not significant. The Least squares means for CMI response for the birds maintained at Pusa and Patna poultry farms did not differs significantly from each other.

5.1.2 Effect of Genetic group on CMI response to Con–A :

Significant ($P \leq 0.01$) influence of the genetic constitution of fowl on their CMI response to Con-A, recorded in this investigation, was indicative of the fact that the variation in general immune response in chicken was a genetically dependent trait. It was in agreement with the findings of Weber (1975), Pink and Miggiano (1977) and Lassila *et al.* (1979) who opined that Con-A was a mitogen, stimulating the T-cells in avian species, the magnitude of responsiveness to stimulation being genetically dependent. The results also revealed that the birds belonging to conventional indigenous breeds (Aseel and Kadaknath) did not differ significantly from each other in respect to CMI response to Con-A. Miggiano (1977) and Haunshi (1999) also recorded similar results.

It is worth mentioning here that in this study, among the birds of 8 different genetic groups, the Desi fowl had the highest average FI, thus CMI response to Con-A. The superior performance of local birds may be attributed to the accumulation of resistant genes through prolonged natural selections. It was in agreement with the opinion of Al-murrani *et al.* (1995) who recorded superior CMI response measured in terms of

wattle thickness in native Iraqui chicken than WLH birds. Chao and Lee (1991) also recorded similar results.

5.1.3 Effect of age on the day of inoculation on CMI response to Con-A :

The results pertaining to the effect of age on the day of inoculation had the significant ($P \leq 0.01$) influence on CMI response to Con-A. It was indicative of the fact that with advancement in age general immune responsiveness in fowl increased. It may be attributed to the fact that in matured birds, B-cells, T-helper cells and T-suppressor cells are fully matured to interact an antigen/mitogen in relatively better way as compared to that in young birds.

5.1.4. Effect of Management system on CMI response to Con-A :

The results revealed that the management systems i.e intensive and extensive had significant influence ($P \leq 0.01$) on CMI response to con-A. Relatively greater and repeated exposure of the birds under the extensive system as compared to those under intensive system of management might be the probable reason of higher mean FI in the former case. Moreover, the number of observation under extensive group of birds were relatively very limited and the results might have been influenced due to sampling error. A study with comparable and relatively larger number of samples in each group under comparison is further suggested for verification of the findings of this study.

5.1.5 Effect of Major genes on CMI response :

The reports with regard to the influence of major genes such as Naked neck and Frizzle on FI in chicken are lacking in the literature. However, several workers have studied the effect of other major genes like dwarfing and slow feathering on general immune response to Sheep Red Blood cells (Klingensmith *et al.*, 1983, Martin *et al.*, 1983, Bacon *et al.*, 1986, Dunnington *et al.*, 1987, Zulkifli *et al.*, 1994). The findings of these

workers are not quite comparable with the results of this study due to difference in use of mitogen as well as the measure of general immune response. However, Kundu (1997) and Haunshi (1999) have reported that the difference *in vivo* CMI response to Con-A among the Naked neck and Frizzle birds was statistically non-significant. Which was in agreement with the findings of this study. Moreoever, significantly higher cell mediated immunity in the birds possessing major genes belonging to Upcari and Hitcari genetic groups in comparison to the normal birds Cari Nirbheek recorded in this study was in corroboration with the findings of Klingensmith *et al.* (1983). The birds belonging to Aseel, Upcari and Hitcari genetic groups did not differ significantly among each other in respect to CMI response to Con-A. Upcari and Hitcari birds possessed major genes respectively for frizzle and Naked neck plumage and Aseel was one of the parental breeds in their evolution. Further, the birds having major genes had significantly higher average FI than normal feathered birds Cari Nirbheek. As such, on the basis of the findings of this study it may be concluded that the major genes for Necked neck and Frizzle plumage did not have any negative effect on general immunocompetence status of the fowl. Rather, it has shown a positive effect (Table 4.2, Fig 5.1).

5.2 Specific Immune Response :

5.2.1 Effect of Location of the flocks on HI-Ab titre level :

The experimental birds of this investigation were maintained at two different places i.e. Pusa (Samastipur) and Patna. The results of this study revealed that the location of the flocks did not have any significant influence on HI-Ab titre on 0, 7, 14, 21 28 and 35 *dpv.* (Fig. 5.2)

5.2.2 Effect of Genetic Groups on HI-Ab titre level :

The birds of different genetic groups, included in this study, were positive for HI-Ab against NDV prior to vaccination i.e. on '0' day of inoculation, but they did not differ significantly among each other in this

Fig.5.1 Effect of Major genes on Cell-mediated Immune Response to Con-A.

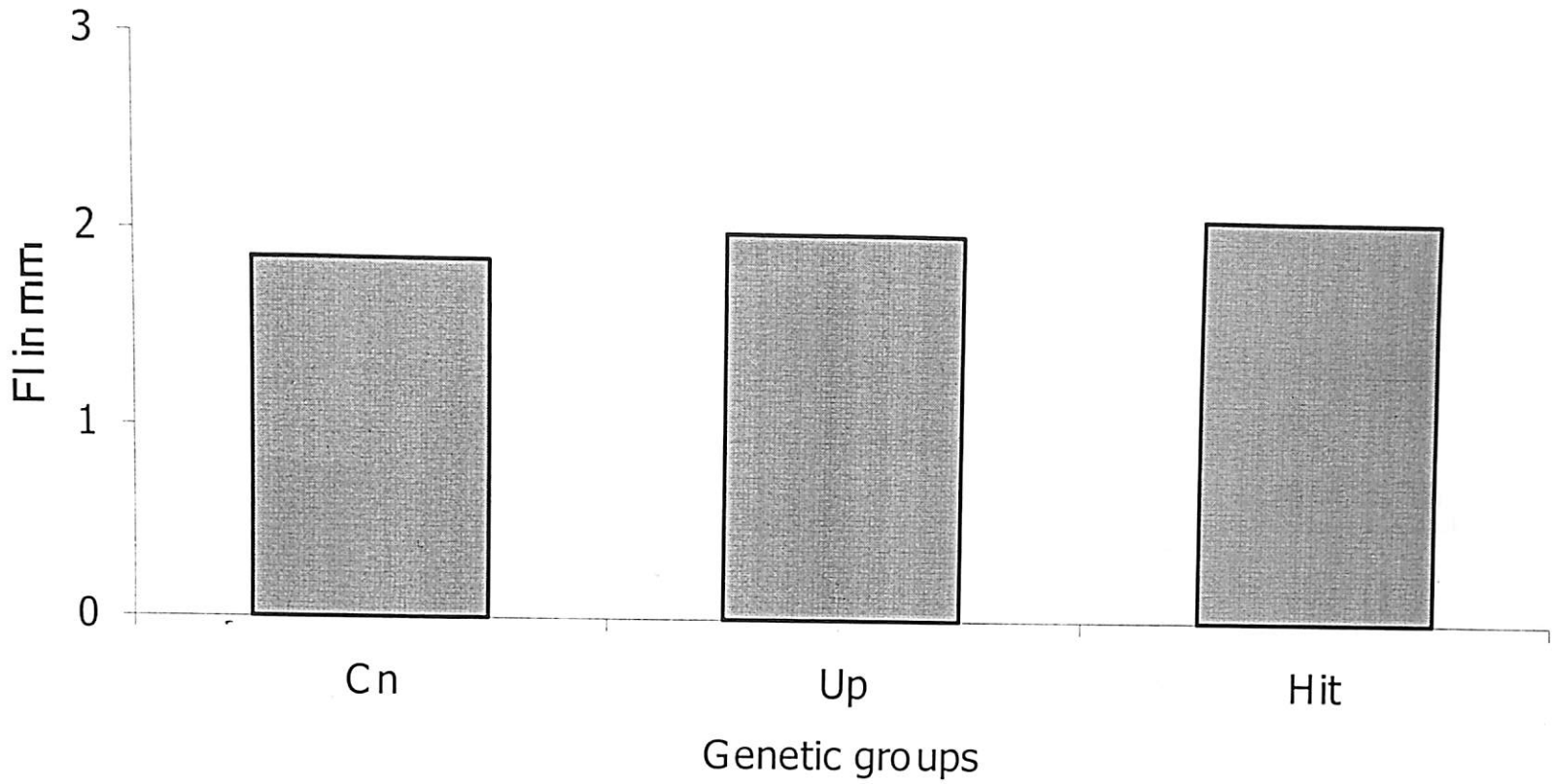
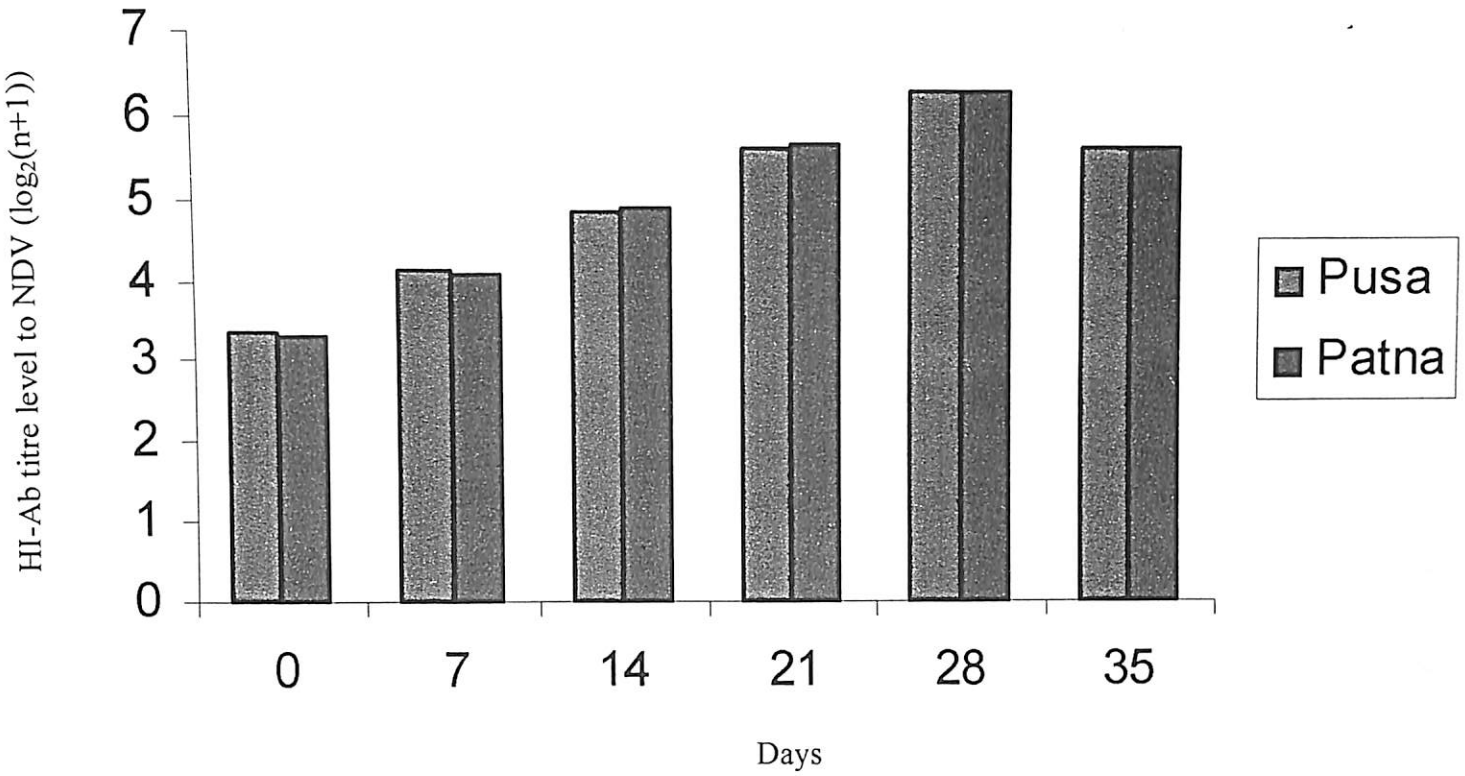


Fig.5.2 Effect of Location of the flocks on HI-Ab titre level on different days after vaccination in fowl vaccinated against ND.

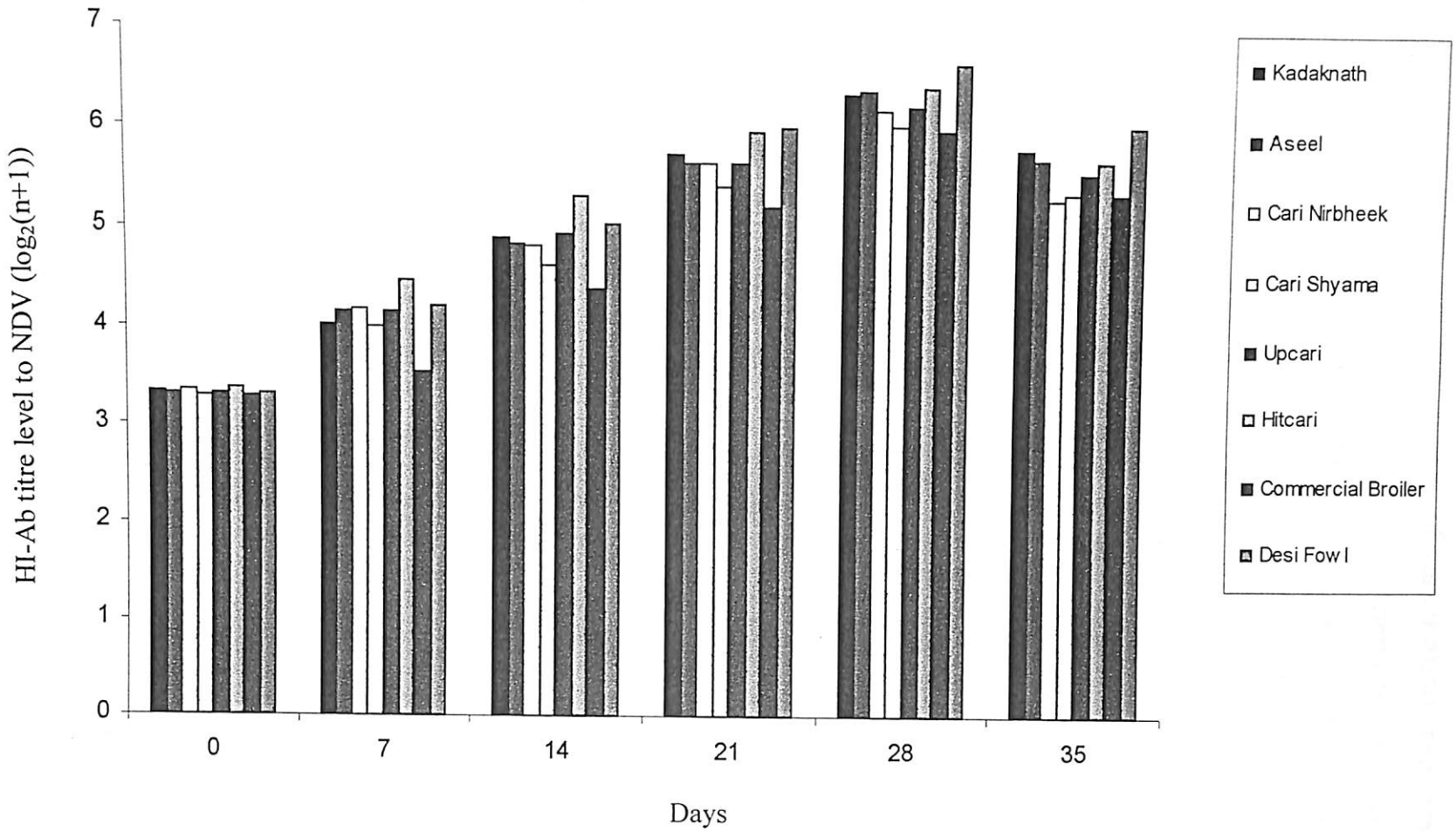


regard. It indicated that the experimental birds were passively immunized with natural antibodies against Newcastle disease virus prior to vaccination. Kalita and Dutta (1999) and Saini *et al.* (1990) also reported passive transfer of antibodies from immunized hen to chicks via yolk material and persistence of HI antibodies in pre-vaccinated birds up to 4 weeks of age. It could also be noted in the present study that although the experimental birds were genetically divergent, the level of natural antibodies or HI-Ab tire level against NDV on '0' day of vaccination was almost the same in the birds of all genetic groups. It indicated that the genetic constitution of the birds did not have any significant influence on the level of maternal antibodies against Newcastle disease transferred passively from hen to chicks.

The genetic constitution of the birds had significant ($P \leq 0.01$) influence on HI-Ab titre level against NDV on 7, 14, 21, 28 and 35 *dpv* (Table 4.4, 4.6, 4.8, 4.10, 4.12, 4.14 and Fig 5.3). It indicated that specific immune responsiveness against Newcastle disease virus in chicken was a genetically dependent trait. The findings of this study was in agreement with the observations of Inooka *et al.* (1984), Martin *et. al.* (1988), Petrovsky *et al.* (1988), Takahashi (1988) and Dunnighton *et al.* (1993) who reported specific immune response to NDV in fowl and Japanese quail to be genetically determined. However, Heller and Soller (1981), Heller *et al.* (1981), Zulkifli *et al.* (1994) reported the breed differences in immune responsiveness in fowl to be non-significant. Heller and Soller (1981) was of opinion that the genetic variation in immune response to an antigen was probably maintained as a balanced polymorphism through negative effect of loci involved in fitness.

It was apparent from (Fig. 5.3) that the HI-Ab titre against NDV increased from 7th *dpv*, attained its peak level on 28th *dpv* and thenafter there was a declining trend. Reports are available to indicate [Kulkarni *et al.* (1973), Heller and Soller (1981), Kulkarni *et al.* (1983), Hassn *et al.* (1989), Jhala *et al.* (1990), Dunnington *et al.* (1993), Karnatak *et al.* (1993), Jayawardane *et al.* (1995), Shobharani and Rao (1995),

Fig.5.3 Effect of Genetic Groups on HI-Ab titre level on different days after vaccination in fowl vaccinated against ND.



Foliste *et al.* (1998), Subramaniam *et al.* (1998), Kalita and Dutta (1999), Pokric *et al.* (1999), Saranbanabara *et al.* (1999), Mani *et al.* (2000), Mishra *et al.* (2000), Sheela and Rao (2002)] that HI-Ab titre level against NDV in chicken starts increasing from 1st day of vaccination, reaches its peak in between 2nd to 6th weeks of vaccination and thenafter, it declines gradually. Such variation in findings of different workers, pertaining to initiation of antibody formation, attainment and maintenance of its peak level and initiation of decline may be attributed to a number of factors including the genetic constitution of the birds into which the antigen was introduced, the type, dose and strain of vaccine used, the time elapsed after antigen administration, the presence of other antigens administered concurrently, the age of the pre-vaccinated birds, the management systems to which the experimental birds are subjected to etc. However, the findings of this study was within the range of the records available in the literature.

It is pertinent to point out here again that among the birds of eight different genetic groups, included in this study, the Desi fowl had the highest average HI-Ab titre level after second week of vaccination i.e. on 21, 28 and 35 *dpv*. As suggested by Al-murrani *et al.* (1995) relatively superior performance of Desi chicken may attributed to the accumulation resistant genes through natural selection. Besides that, repeated exposure of the birds to the external environment may be a plaucible explanation of such results because the Desi birds in this study were put under extensive system of management. But the critical analysis of data revealed that the rate of increase in average HI-Ab titre level against NDV from 0 to 7, 7 to 14, 14 to 21 and 21 to 28 *dpv* varied in the birds of different genetic groups. The quantum of increase in HI-Ab titre level in Hitcari birds was maximum in first week of vaccination and thenafter the rate of increase declined very fastly in subsequent weeks. Except in Kadaknath and commercial broilers, the rate of increase in HI-Ab titre level decreased in 2nd week of vaccination. It may be due to neutralizations of ND maternal antibodies as suggested by Barman *et al.*

(1996). In this study the drop in the amount of increase in antibody titre level in Kadaknath, Upcari and Commercial broilers could be observed in the 3rd week of vaccination. In the birds of rest of the genetic groups i.e. Aseel, Cari, Nirbheek, Cari Shyama and Desi fowl the rate of increase in HI-Ab titre level was more in 3rd week as compared to that in 2nd week of vaccination. However, in the 4th week of vaccination there was general dropping in the quantum of increase in HI-Ab titre level in the birds of all the genetic groups (Table 5.1). Such variation in the quantum of increase in HI-Ab titre level against NDV in subsequent weeks of vaccination in the fowl of different genetic groups may be attributed to variable genetic constitution of the birds. It may be taken as re-substantiation of the fact that specific immune responsiveness in fowl is a genetically determined trait.

5.2.3 Effects of age at '0' day of inoculation :

The effect of age at '0' day of inoculation had the significant ($P \leq 0.01$) influence on HI-Ab titre against NDV throughout the experimentation (Fig. 5.4). The HI-Ab titre level against NDV in younger birds were significantly lower as compared to the older birds. The one of probable reasons of significantly higher HI-Ab titre level against NDV on 7, 14, 21, 28 and 35 *dpv* in older birds as compared to that in young stock, may be the physiological status of bursa of Fabricius which is fully matured in the older birds. Moreover, the older birds included in this study had already been primarily vaccinated against Newcastle disease in its early age and as such the relatively higher HI-Ab titre level in older birds might be a result of amnestic reaction.

5.2.4 Effect of management system on HI-Ab titre level :

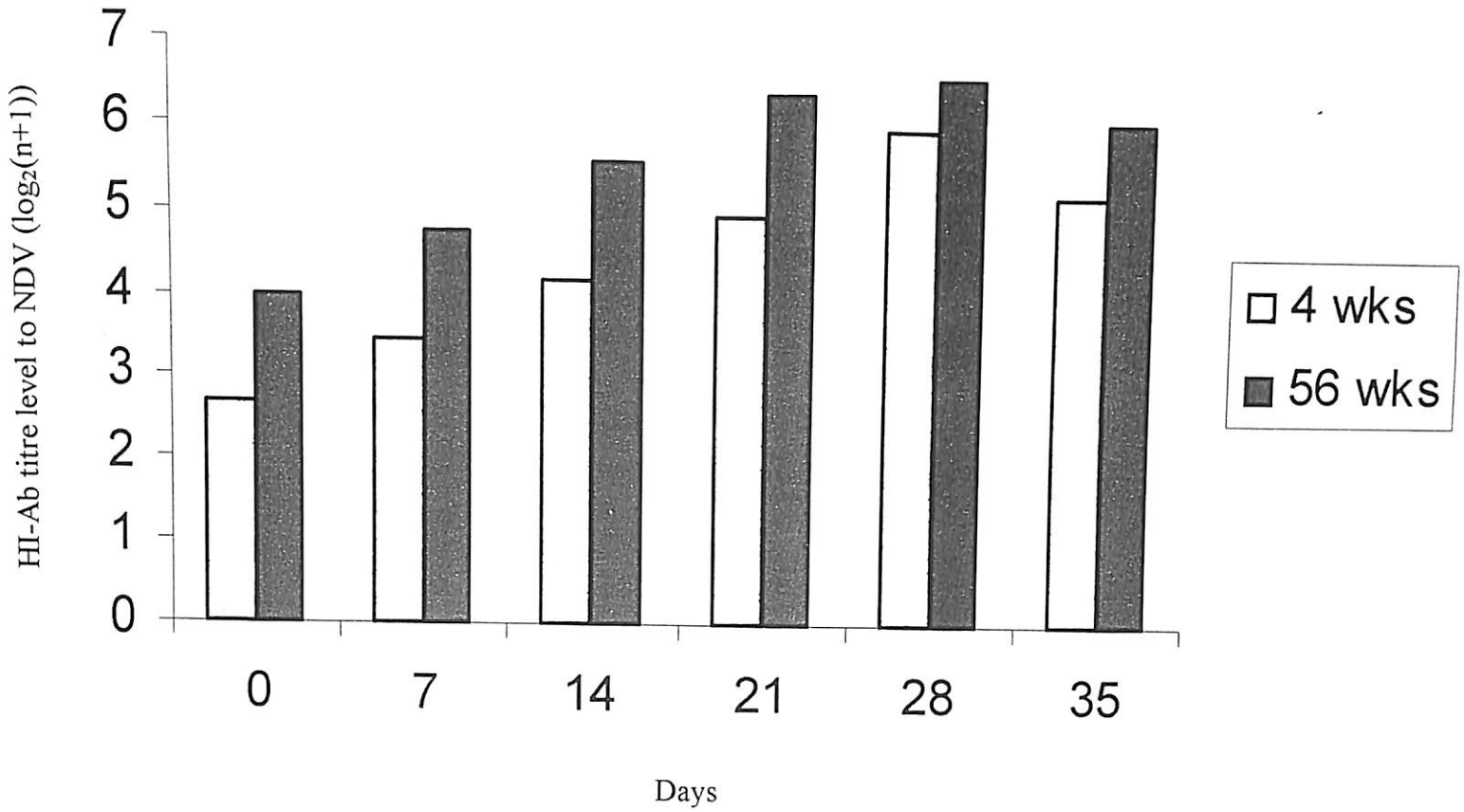
The results revealed that the systems of rearing the fowl had a significant influence ($P \leq 0.01$) on HI-Ab titre level against NDV (Fig. 5.5). Relatively greater and repeated exposure of the birds to external environment under extensive system as compared to that under intensive

Table 5.1

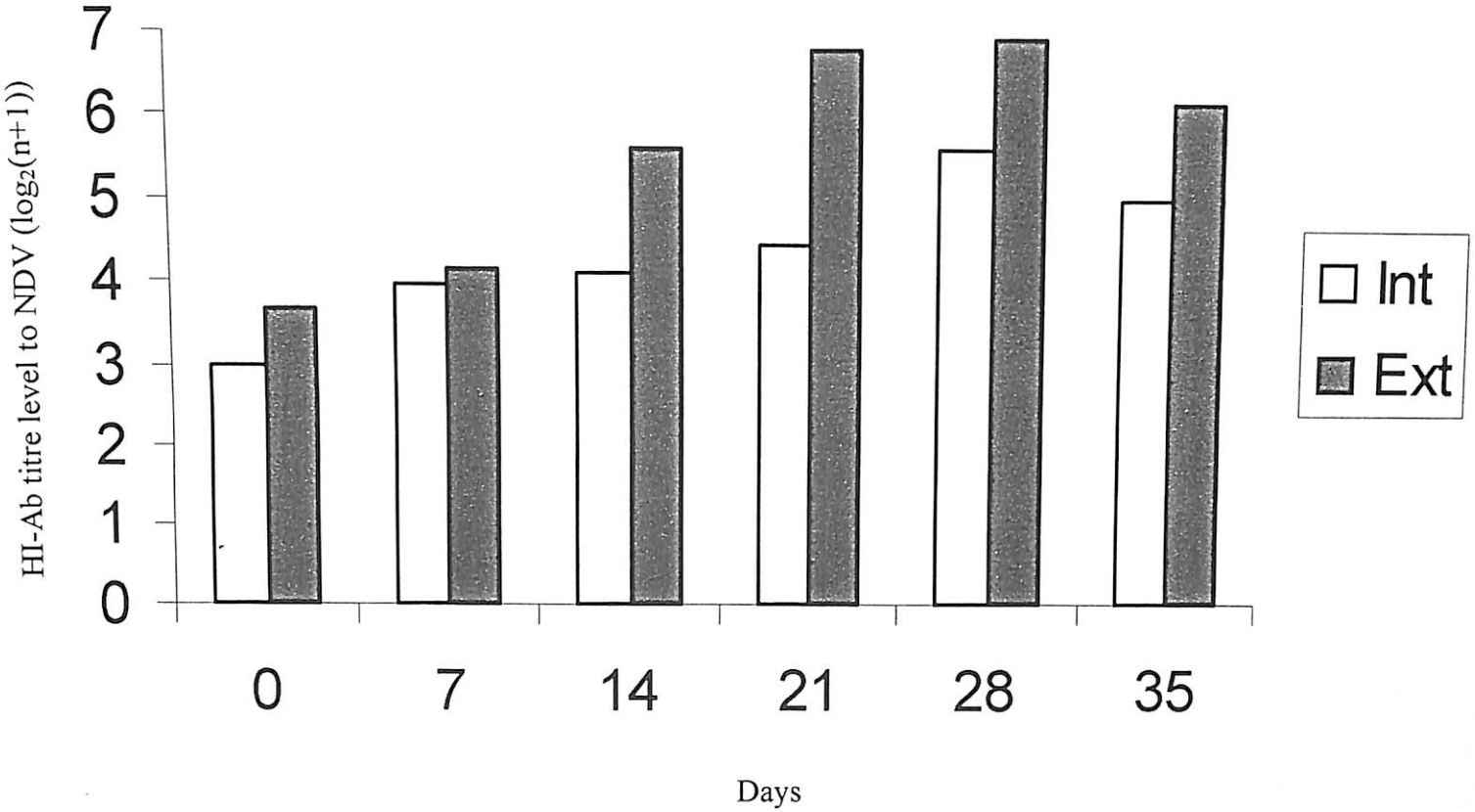
Increase in Average HI-Ab titre level against NDV in sub-sequent *dpv*

Genetic Groups	Quantum of increase			
	0 – 7 *dpv*	**7 – 14 *dpv***	**14 – 21 *dpv***	**21 – 28 *dpv***
Kadaknath	0.7173	0.8844	0.8123	0.6174
Aseel	0.8484	0.6926	0.8050	0.7242
Cari Nirbheek	0.8189	0.6410	0.8163	0.5316
Cari Shayama	0.7121	0.6327	0.7761	0.6155
Upcari	0.8487	0.7949	0.6892	0.5745
Hitcari	1.0943	0.8439	0.6419	0.4557
Commercial broiler	0.2528	0.8453	0.8280	0.7709
Desi fowl	0.8981	0.8284	0.9422	0.6555

Fig.5.4 Effect of Age at '0' day of inoculation on HI-Ab titre level on different days after vaccination in chicken vaccinated against ND.



vaccinated against ND.



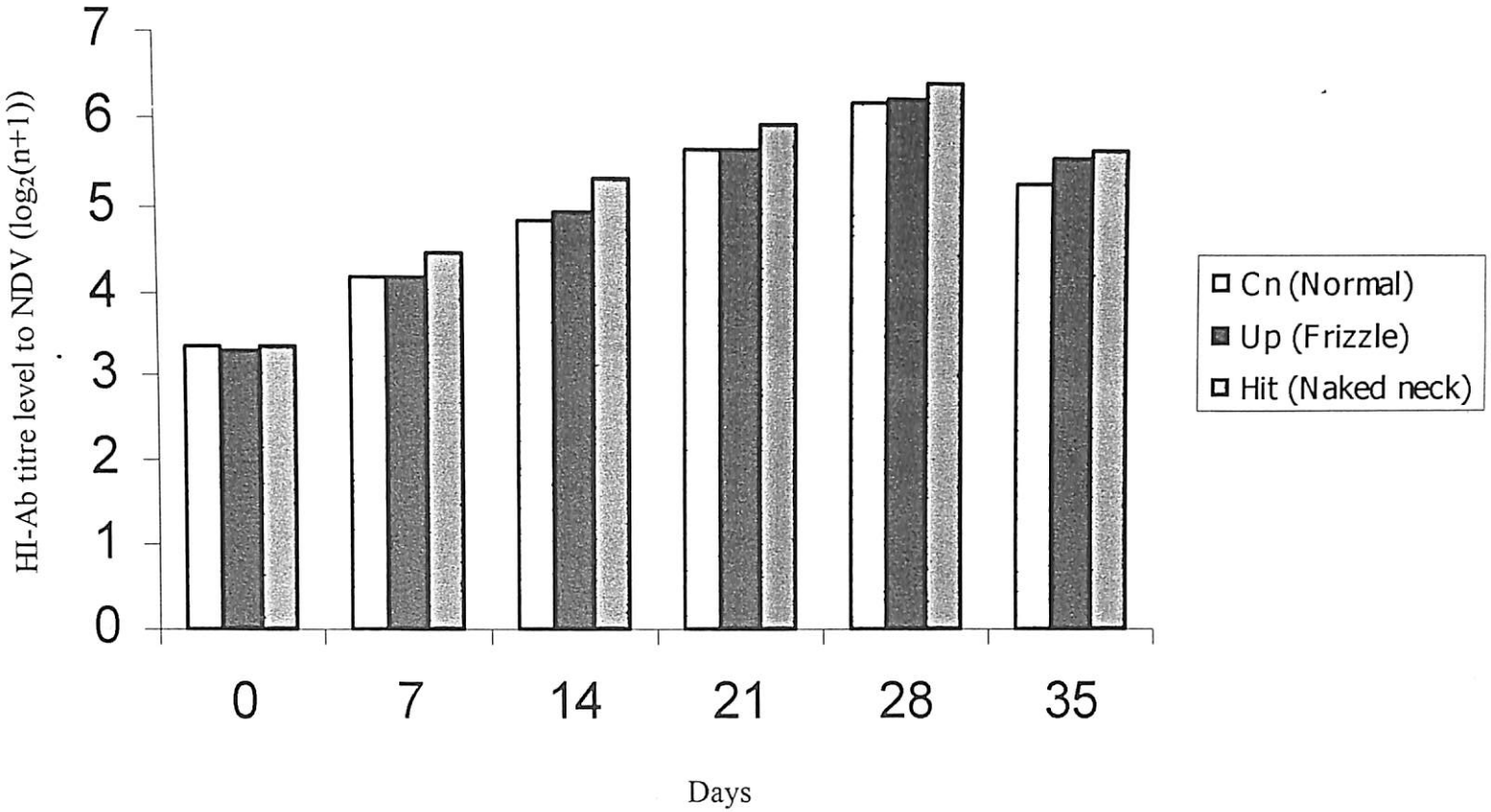
system of management might be the probable reason of higher HI-Ab titre level in the former case. Also, the number of observations under extensive system of birds was relatively very limited and the result might have been influenced due to sampling error. A study with larger and comparable number of samples in both the systems is further suggested.

5.2.5 Effect of Major genes on Specific Immune Response :

In this study, to study the effect of major genes on HI-Ab titre level against NDV in chicken, Frizzle (Upcari) and Naked neck (Hitcari) birds were compared with normal feathered birds Cari Nirbheek. Findings revealed that prior to vaccination i.e. on '0' day of inoculation, the birds belonging to Upcari, Hitcari and Cari Nirbheek genetic groups did not differ significantly among themselves in respect to specific immune response against Newcastle disease. But, pair-wise comparison of the estimates of Least squares means for HI-Ab titre level, measured at weekly interval (Fig 5.6) revealed that the birds having Naked neck major genes had significantly higher HI-Ab titre levels on 7, 14, 21, 28 and 35 *dpv*. It was in agreement with the findings of classical study of Berrio *et al.* (1990) in chicken who concluded that Naked neck genes had a favourable effects on the immune response to the LaSota strain of ND virus. However, Demey *et al.* (1996) did not observe any significant difference either in specific or complement responses against NDV vaccine in the fowl possessing major genes for Naked neck, Dwarfness and their combinations. However, in this study, the mean HI-Ab titre level in the normal feathered (Cari Nirbheek) and Frizzle (Upcari) birds didn't differ significantly from each other during the entire period of experimentation except on 14 *dpv* which could not plaucibly be explained and may be attributed to be a sampling error. Findings of this study indicated that major genes particularly for Naked neck character may be utilized as a classical marker in Marker Assisted Selection Programme for selection of chicken with superior immune responsiveness against Newcastle disease virus subject to the genomic map of the birds are developed and the association of Naked neck lock with Quantitative traits loci (QTL) for immune responsiveness is established.

❑❑❑

Fig.5.6 Effect of Major genes on Specific Immune Response on different days after vaccination in chicken vaccinated against ND.



6.

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

The present investigation was undertaken with the objectives to quantify the variation in general and specific immune responsiveness in chicken due to genetic and some non-genetic factors. To study the effects of some of the major genes on mitogenic response to Con-A and HI-Ab titre level in the fowl vaccinated against NDV was also one of the objectives of this study. Chicken belonging to eight genetic groups viz, Kadaknath, Aseel, Cari Nirbheek (Aseel x Cari Red), Cari Shyama (Kadaknath x Cari Red), Upcari (Aseel Frizzle x Cari Red), Hitcari (Aseel Naked neck x Cari Red), Commercial broiler and Desi fowl were included in this investigation.

The general immune response was measured in terms of Foot-Index (FI) for which Concanavalin–A was used as mitogen. The serum HI-Ab titre levels on 0, 7, 14, 21, 28 and 35th day of vaccination against Newcastle disease were the measures of specific immune response. Besides the effect of genetic make-up of the birds on the cell mediated and humoral immune responses, the influence of some non-genetic factors like location of the flock, age of the birds on '0' day of inoculation as well as the management system to which the experimental birds were subjected to studied. Fowl expressing major genes for Frizzle plumage (Upcari) and Naked neck (Hitcari) were utilized to study the effect of major genes on the general and specific immune responses. The normal feathered Cari Nirbheek (Aseel x Cari Red) were utilized as control while evaluating the effect of major genes.

The CMI response to Con-A measured in terms of Foot-index for the birds of various genetic groups varied from 1.792 ± 0.011 to 2.392 ± 0.014 mm. The genetic group, age of the birds on the day of inoculation and management system had significant influence on foot index. The effect of location of the flock on CMI response to Con-A was statistically not significant.

Among the birds of different genetic groups, Desi fowl and Commercial broiler had respectively the highest (2.392 ± 0.014 mm) and the lowest (1.792 ± 0.011mm) Foot-Index. Older birds on the day of inoculation and the birds reared under extensive system of management had significantly higher foot-index as compared to young stock and the birds put under intensive management.

The overall Least squares means (μ) for HI-Ab titre to NDV were estimated to be 3.3233 ± 0.0342, 4.0929 ± 0.0343, 4.8633 ± 0.0346, 5.6522 ± 0.0353, 6.2703 ± 0.0362 and 5.5886 ± 0.0368 respectively for 0, 7, 14, 21, 28 and 35 *dpv*. The location of the flock and the genetic constitutions of the birds did not have significant influence on HI-Ab titre in pre-vaccinated birds. However, the effect of age on the '0' day of inoculation and management system influenced the basal Ab titre on 4 weeks of age significantly.

Results further revealed that the genetic group, age of the birds on '0' day of inoculation and management system had significant influence on HI-Ab titre levels on 7, 14, 21, 28 and 35 *dpv*. The titre level increased from 7 *dpv*, attained its peak level on 28 *dpv* and thenafter there was a declining trend. The increase in the quantum of HI-Ab titre level against NDV in subsequent weeks of vaccination showed different trends in the fowl of different genetic groups.

The Upcari and Hitcari birds having major genes, respectively for Frizzle plumage and Naked neck, had significantly higher average FI than normal feathered Cari Nirbheek birds. It was indicative of the fact that major genes did not have any negative effect on general immunocompetence status of the fowl. Results pertaining to specific immune responsiveness revealed that the birds having Naked neck had significantly higher HI-Ab titre level on 7, 14, 21, 28 and 35 *dpv*. However, the normal feathered Cari Nirbheek birds did not differed significantly from Frizzle (Upcari) in this regard during the entire period of experimentation except on 14 *dpv*.

On the basis of the findings of this study the following conclusions were drawn :

1. Genetic constitution of the fowl, age of the birds on the day of inoculation and management system had significant influence on general and specific immune responses in chicken, it measured respectively in terms of mitogenic response to Con-A (FI in mm) and HI-Ab titre level against NDV.

2. Variation in CMI response of Con-A (general immune responsiveness) and HI-Ab titre level against NDV (specific humoral immune response) in chicken were genetically determined traits.

3. With the advancement of age, the potency of fowl to express general and specific immune responsiveness increased.

4. The birds reared under extensive management system had significantly better mitogenic response to Con-A as well as higher HI-Ab titre level to NDV on 7, 14, 21, 28 and 35 *dpv* as compared to the birds under intensive system of management.

5. In the fowl of 4 weeks of age, their genetic constitution did not have significant influence on the level of maternal antibodies against NDV, transferred passively from hen to chicks.

6. The HI-Ab titre level to NDV in chicken, increased from first week of vaccination, attained its peak level in fourth week of vaccination and thenafter there was a declining trend.

7. The major genes for Naked neck and Frizzle plumage did not have any negative effect on mitogenic response to Con-A whereas, the birds with Naked neck genes had significantly higher HI-Ab titre level to NDV on 7, 14, 21, 28 and 35 *dpv* in chicken.

□□□

BIBLIOGRAPHY

BIBLIOGRAPHY

Ahlawat, S. P. S. (2003). Status of Animal Genetic Resources in India – A report presented at the 2nd workshop at U.A.S., Bangalore on 5 – 6 February, 2003.

Alexander, D. J. (1997). Newcastle disease and other avian paramyxo-virus infections. In : Disease of poultry, 10th ed. B. W. Calnek, ed. Iowa State Univ. Press, Ames, IA 541 – 570.

Al-murrani, W. K., Hameed, H. K., Abdulgani, Z. G. and A1-omran, A. H. (1995). Some aspects of genetic resistance to *S. typhimurium* in native Iraqui and White Leghorn Chickens. Dirasat. Series B, Pure and Applied Science., **22** : 867 – 875.

Aydin, N. Yardimci, H., Gumussoy, K. S. and Erdem, B. (2001). The diagnosis of antibodies against Newcastle disease virus by HI and ELISA tests in vaccinated chickens. *Turk Veterinerlik Ve Hayvancilik Dergisi.*, **25** : 1 – 5.

Aziz, S. S. (1985). Studies on infectious bursal disease in relation to seroepidemiology, virus isolations and immunosuppression. M.V.Sc. Thesis submitted to university of Agricultural Science, Bangalore.

Bacon, L.D., Fadly, A. M. and Crittenden, L. B. (1986). Absence of influence on immune response on immune competence by the sex linked gene (K) determining slow feathering in White Leghorn chickens. *Avian Dis.*, **30** : 751 – 760.

Balaraman, N. (2003). Status and Prospects – Milk Production Systems. *Indian Dairyman*, **55** : 30.

Barman, N. N., Mahanta, K. Dutta, T. C., Kalita, N. and Kalita C. C. (1996). Combined aerosol vaccination against Newcastle disease and Fowl Pox. *Indian Vet. J.*, **73** : 496 – 500.

Benda, V., Hampl, A. and Trtkova, K. (1990). *Acto. Vet. Brno.*, **59** : 151-156.

Berrio, I., Viamontes, O., Fraga, I.M. and Cardenas, M. (1990). A study of the dyanamics of HI antibodies of White Leghorn chicken carriers of the Naked neck gene (NA). *Cuban Journal of Agricultural Science,* **24** : 199 – 204.

Biozzi, G., Mounton, D., Sant' Anna, O.A. Passos, H.C., Gennari, M., Reis, M.H., Ferreira, V.C.A., Heumann, A.M., Bouthillier, Y., Ibanex, O.M, Stiffel, C. and Siqueira, M. (1979). Genetics of immunoresponsiveness to natural antigens in the mouse. Curr. *Topics in Microbiol. Immunology,* **85** : 31 – 36.

Biozzi, G., Siqueira, M. Stiffel, C., Ibanex, O. M, Mouton, D. and Ferreira, V.C.A. (1980). Genetic selections for relevant immunological functions. In : M. Fougereau and Dausset, J. (Ed.) *Progress in immunology IV,* 432 – 457, Academic Press, N.Y.

Bozorghmehrifard, M. H. and Mayahi, M. (2000). Comparison of enzyme linked–immunosorbent assay and haemagglutination inhibition test for the detection of antibodies against Newcastle disease vaccine in broiler chicks. *Indian Journal of Animal Sciences,* **70** : 39 – 40.

Burton, J. K, Burnside, E. B., Kennedy, B. W., Wilkie, B. N. and Burton, J. I. (1980). *Journal of Dairy Sci.,* **72** : 1252.

Buxton, A. and Frazer, G. (1977). *Animal Microbiology,* 1st ed., Blackwell Scientific Publication Ltd. Victoria, Australia.

Chandrasekhar, S., Venkatesan, R. A., Padmanabhan, V. A. and Masillamony, P. R. (1989). Nature of protective immunity in chicken against Ranikhet disease. *Indian Vet.* J., **66** : 801–806.

Chang, T. S., Rheins, M. S. and Winter, A. R. (1957). The significance of the bursa of Fabricius in antibody production in chickens. *Poultry Sci.*, **36** : 735 – 738.

Chao, C. H. and Lee, Y. P. (1991). Immune response to Newcastle disease virus vaccine and sheep erythrocytes in country chickens and White Leghorn. *Journal of Chinese Society of Anim. Sci.*, **20** : 189 – 201.

Charan, S., Grover, Y. P. and Prasad, S. (1983). Newcastle disease outbreak in vaccinated chicken. *Indian J. Comp. Microbiol. Infect. Dis.*, **4** : 259 – 261.

Cheng, S. and Lamont, S. J. (1988). Genetic analysis of immunocompetence measure in a White Leghorn chicken line. *Poultry Sci.*, **67** : 989-995.

Demey, F., Baclmans, R., Pandey, V. S. and Verhutst, A. (1996). Immunocompetence measurements of major genes Dahlem Red chickens to be used to upgrade local chickens. Proceedings of World Poultry Congress, New Delhi, India, 2-5 Sept., 1996, Vol IV, 93.

Dickerson, G. E. (1957) Genetic variation in some economic characters of leghorn-type chickens. *Poultry Sci.*, **36** : 1113 (Abstract).

Doenhoff, M. J. and Davies, A.J.S. (1991). Genetic improvement of immune system possibility for animals. In breeding for disease resistance in farm animals edited by Owen, J. B. and Axford, R.F.E., Redwood Press Ltd., Melksham.

Dunnington, E. A., Gross, W. B., Larsen, C. T. and Siegel, P. B. (1993). Antibody response to combination of antigens in populations of chickens from diverse genetic background. *Archiv-fur-Gerflugelkunde*, **57** : 171 – 174.

Dunnington, E. A., Martin, A. and Siegel, P. B. (1987). Antibody response to sheep erythrocytes in early (K^+) and late (K) feathering chickens in a broiler line. *Poult. Sci.*, **66** : 2060 – 2062.

Folitse, R., Halvorson, D. A. and Sivnandan, V. (1998). Efficacy of combined Killed-In-Oil emulsion and live Newcastle disease vaccines in chickens. *Avian Disease*, **42** : 173-178.

Friars, G. W., Chambers, J. R., Kannedy, A and Smith, A. D. (1972). Selection for resistance to marek's disease in conjunction with other economic traits in chickens. *Avian Diseases*, **16** : 2.

Gavora, J. S. and Spencer, J. L. (1978) Breeding for Genetic Resistance to Disease : Specific or General ? * *World Poultry Science* J., **34** : 137 – 148.

Gavora, S. S. and Spencer, J. Z. (1983). Breeding for immune responsiveness and disease resistance. *Anim. Blood Groups Biochem. Genet.*, **14** : 159 – 180.

Glick, B., Chang, T. S. and Jaap, R. G. (1956). The bursa of Fabricius and antibody production. *Poultry Science*, **35** : 224 – 225.

Gordon, C. D., Beard, C. W., Hopkins, S. R. and Siegel, H. S. (1971). Chick mortality as a criterion for selection towards resistance or susceptibility to Newcastle disease. *Poultry Sci.*, **50** : 783.

Harvey, W. R. (1987) User's guide for LSMLMW (PC-I version). Mixed Model Least–Squares and Maximum Likelihood Computer Program (January 1988).

Hassan, A. B., Atta, A. H., Soliman, R. T. and Afifi, Nehal A. (1989). Effect of levamisole hydrochloride on the immune response to Newcastle disease virus vaccine in chickens. *Indian Vet. Journal*, **66** : 389 – 394.

Haunshi, S. (1999). Studies on general immune competence in specialized chicken population M.V.Sc. Thesis, I.V.R.I., Izatnagar, Bareilly, U.P. (India).

Heller, D. and Soller, M. (1981). Immune response to Newcastle disease Virus Vaccine. Fowl-Pox vaccine, and Escherichia coli vaccine in Bedouin and White Leghorn Chickens. *Poultry Science*, **60** : 34-37.

Heller, D., Soller, M., Peleg, B. A., Ron-Kuper, N. and Hornstein, K. (1981). Immune response to ND virus vaccine, fowl pox vaccine. And Escherichia coli vaccine in Bedouin and white Leghorn chicken. *Poultry science*, **60** : 34 – 37.

Hutt, F. B. (1958). Genetic resistance to disease in domestic animals. 1st edn. Comstock Publishing Associates, Ithaca, New York.

Inooka, S. Takahashi, S., Takahashi, H. and Mizuma, Y. (1984). Immunological traits in generation 7 to 12 of two lines of Japanese quail selected for high or low antibody response to Newcastle disease virus. *Poultry science*, **63** : 1298 – 1302.

Jayawardane, G.W.L. and Spradbrow, P. B. (1995). Mucosal immunity in chickens vaccinated with the V4 strain of Newcastle disease virus. *Veterinary Microbiology*, **46** : 69 – 77.

Jhala, M. K., Kher, H. N. and Choudhari, H. M. (1990). Immunosuppressive effect of infectious bursal disease virus against Newcastle disease vaccination. *Indian Journal of Animal Sciences*, **60** : 1065 – 1066.

Kalita, D. N. and Dutta, G. N. (1999). Immunomodulatory effect of levamisole upon Newcastle disease, pigeon pox and Marek's disease vaccination in broiler chicks. *Indian Vet. Journal*, **76** : 490–492.

Karnatak, B. C., Shukla, S. K., Kumar, M. and Dixit, V. P. (1993). Immunomodulatory effect of levamisole on the antibody response to Ranikhet disease vaccination. *Indian J. Vet. Med.*, **13** : 48-51.

Kelly, P. J., Chitauro, D., Rohde, C., Rukwava, J., Majok, A., Davelaar, F. and Mason, P. R. (1994). Disease and Management of backyard chicken flocks in Chitungwiza. *Avian Disease,* **38** : 626- 629.

Klingensmith, P. M., Donahoe, J. P and Stephens, J. F. (1983). The effect of sex linked dwarfing gene, dw on the immune response of broiler breeder chickens. *Poultry Sci.*, **62** : 733 - 740.

Kramer, C. Y. (1957). Extension of Multiple Range Test to group correlated adjusted means. *Biometrics,* **13** : 3-17.

Kulkarni, D. D., Kulkarni, M. N. and Gujar, M.B. (1983). Serological studies on chicks infected with infectious bursal disease virus and vaccinated for Ranikhet Disease. *Indian J. Anim. Sci.*, **53** : 1281-1285.

Kulkarni, V. B; Mulbagal, A. N.; Paranjape, V. L.; Khot, J. B. and Manda, A.V. (1973). Immunostimulating effect of tetramisole on antibody formation against Newcastle disease virus in chicks. *Indian Vet. Journal,* **50** : 225-227.

Kundu, A. (1997). Comparison among genetically diverse lines of chicken for immuno-competence measures. Ph.D. Thesis, IVRI, Izatnagar, U. P. India.

Lamont, S. J. (1994). *Poultry Science,* **73** : 1044

Lamont, S. J. and Dietert, R. R. (1990). New direction in poultry genetics, In : Poultry Breeding and Genetics. Ed. By R. D. Crawford, Elsevier Publication, New York.

Lassila, O., Nurmi, T. and Eskola, J. (1979). Genetic differences in mitogenic response of peripheral lymphocytes in chickens. *J. Immunogenet.*, **6** : 37 – 43.

Leitner, G., Zhavauni, Cahaner, A., Gutaman, M. and Heller, D. E. (1992). *Poultry Science,* **77** : 21.

Mani, K., Sudaresan, K. and Viswanathan, K. (2000). Effect of aflatoxin B_1 on immunity to Newcastle disease in commercial chicks. *Indian Vet. J.,* **77** : 193-195.

Martin, A., McNabb, F.M.A. and Siegel, P.B. (1988). Thyroid hormones and antibody response to sheep erythrocytes of dwarf and normal chickens selected for juvenile body weight. *Genetic-Selection Evolution,* **20** : 499-510.

McDevitt, H. O. and Landy, M. (1972). Foreword (p.x.v). Genetic control of immune responsiveness, relationship to disease susceptibility. Academic Press, New York and London.

Meerker, D. L., Rothschild, M.F., Christiana, L. E., Warner, C. N. and Hill, H. T. (1987). *J. Anim. Science,* **64** : 409.

Miggiano, V., North, M., Buder, A. and Pink, J.R.L. (1976). Genetic control of responses of chicken leucocyte to a T-cell mitogen. *Nature,* **263** : 61 – 63.

Mishra, S., Kataria, J. M., Verma, K. C. and Mishra, J. P. (2000). Serological response of Chicken to various Newcastle disease virus isolates. *Indian Journal of Animal Sciences,* **70** : 556–558.

Morris, J. A. (1959). Heritability of Chick viability for two breeds of domestic fowl. *Poultry Sci.,* **38** : 481.

Munns, P.I. and Lamont, S. J. (1991). Effects of age and immunization internal on the amamnestic response to T-cell dependent and T-cell independent antigens in chickens. *Poultry science,* **70** : 2371 – 2374

Nguyen, T. C. (1983). *Vet. Immunol. Immunopathol.*, **5** : 237.

Nonnecke, B. J. and Harp, J. A. (1989). *J. Dairy Sci.,* **92** : 1313.

Pandey, V. S. Demey, F. Verhulst, A. (1996). Genetic variation in susceptibility of two hybrid lines of chickens to infection with Eimeria tenella. Proceedings of World's Poultry Congress, New Delhi, India 2 – 5 Sept., Vol. IV : 93

Peleg, B. A., Soller, M., Ron N., Hornstein, K. Brody, T and Kalmer, E. (1976). Familial differenced in antibody response of broiler chickens to vaccination with attenuated and inactivated Newcastle disease virus vaccine. *Avian disease,* **20** : 661.

Petrovksy, E., Benda, V. and Kalova, J. (1988) Genetic differences in humoral immune reactions of domestic fowl (*Gallus domestics*). *Acta Veterinaria Brno,* **57** : 111 – 121.

Pink, J. R. L. and Miggiano, V. (1977). Complimentation between genetic variants affecting the response of chicken leukocytes to Con-A. *J. Immunology,* **119** : 1796 – 1799.

Pokric, B., Bidin, Z., Stambuk, N. and Cajavoc, S. (1999). Humoral immune response of chickens generated by oil-adjuvanted virus vaccines. *Periodicum Biologicum,* **101** : 59 – 64.

Rathore, B. S., Verma, K. C., Singh, S. D. and Khera, S. S. (1987). Epidemiological studies on Ranikhet disease vaccinal failures in chicken. *Indian J. of Comp. Microbiol. Immunol. Infect. Dis.,* **8** : 175 - 178.

Saini , S. S., Sodhi, S. S., Maiti, N. K. and Sharma, S. N. (1990). Immune response of chicks to oral vaccination against Newcastle disease and Fowl-Pox. *Comp. Immunol. Microbiol. Infect. Dis.*, **13** : 1 – 6.

Saravanabava, K., Nachimuthu, K. and Padmanaban, V. D. (1999). Immunopotentiating effect of tuftsin in birds immunosuppressed with infectious bursal virus. *Indian Vet. J.*, **76** : 485 – 489.

Saxena, V. K. (1993). Genetic studies on immune competence in Guinea fowl (*Numida meleagridis*). Ph.D. Thesis, IVRI, Izatnagar, UP, India.

Sheela, P. and Rao, M. S. (2002). Evaluation of Antibody response in commercial eggers against Newcastle disease virus under different vaccination schedules. *Indian Vet. J.*, **79** : 612-613.

Sobharami, M. and Rao, A. S. (1995). Immunization against Newcastle disease with or without the influence of other viral vaccination. *Indian J. Virol.*, **11** : 23-28.

Subramaniam, N., Paul, W.M., Mukhopadhyay, H. K and Dorairajan, N. (1998). Immune status of commercial chicks against Newcastle disease by live-in-oil vaccines. *Indian Journal of Animal Sciences*, **68** : 464 – 466.

Szenberg, A. and Warner, N. L. (1962). Dissociation of immunological responsiveness in fowls with a hormonally arrested development of lymphoid tissues. *Nature*, **194** : 146 – 147.

Takahashi, S., Shimizu, A., Takahashi, H. and Mizuma, Y. (1988). Selection for high and low titre to inactivated Newcastle disease virus vaccine in Japanese quail through 35 generations. Proceedings XVIII World's Poultry Congress, September 4 – 9, Nagoya, Japan : 499 – 501.

Timms, L. and Alexander, D. J. (1977). Cell-mediated immune response of chickens to Newcastle disease vaccines. *Avian Pathology*, **6** : 55 – 59.

Tizard, I. R. (1988). Veterinary immunology : An introduction, 5th Edn., Published in India by Harcourt Brace and Company, Asia, PTE, Ltd. Under special arrangement with W.B. Saunders Company, U.S.A., Indira Printer, New Delhi.

Verma, B. B. (2001). Status and Stratagies of Animal Production and Health (Animal health). Paper presented in symposium on status and strategies of Animal Health and Production held at Bihar Veterinary College, Patna, May 11 – 12.

Warner, C.M., Meerkar, D. L. and Rothschild, M.F. (1987). Genetic control of emmune responsiveness, A review of its use as a tool for selection for disease resistance. *J. Anmal Sci.*, **64** : 394 – 406.

Weber, W.T. (1975). Avian B-lymphocyte sub-populations : Origin and functional Capacities. *Transplant Rev.*, **24** : 2113 – 125.

Zulkifli, I., Dunnington, E. A., Gross, W.B. and Siegel, P. B. (1994). Food restriction early or later in life and its effect on adaptability, disease resistance and immunocompetence of heat-stressed dwarf and non-dwarf chickens. *British Poult. Science*, **35** : 203-213.

□□□