DISTRIBUTION OF ANTIBODY TITRE TO RANIKHET DISEASE VIRUS IN IMMUNOCOMPROMISED CHICKEN

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

(VETERINARY MICROBIOLOGY)

By

Bhagirath Prasad

(Registration No. - M/V MC/52/2000-01)

Department of Veterinary Microbiology

BIHAR VETERINARY COLLEGE

PATNA, BIHAR (INDIA)

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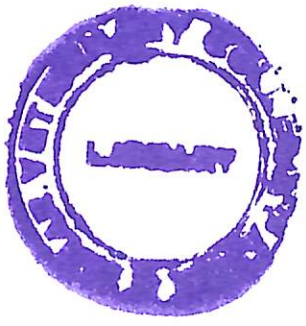

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Dedicated to

My

Parents

Department of Veterinary Microbiology

Bihar Veterinary College, Patna - 800014.
(Rajendra Agricultural University, Bihar)

Dr. B.K. SINHA
M.Sc. (AIIMS), Ph.D.
Associate Prof. & Head
Bihar Veterinary College
Patna-800014

CERTIFICATE-I

This is to certify that the thesis entitled "*DISTRIBUTION OF ANTIBODY TITRE TO RANIKHET DISEASE VIRUS IN IMMUNOCOMPROMISED CHICKEN*" submitted in partial fulfilment of the requirements for **the Degree of Master of Veterinary Science (Veterinary Microbiology) of the faculty of post-graduate studies, Rajendra Agricultural University, Pusa, Bihar** is the record of bonafide research carried out by **Dr. Bhagirath Prasad** 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.

28/1/03
(B.K. Sinha)
Major Advisor

CERTIFICATE – II

We, the undersigned, Members of Advisory Committee of **DR. BHAGIRATH PRASAD**, a candidate for the degree of **Master of Veterinary Science** with **Major in Veterinary Microbiology**, have gone through the manuscript of the thesis and agree that the thesis entitled ***"DISTRIBUTION OF ANTIBODY TITRE TO RANIKHET DISEASE VIRUS IN IMMUNOCOMPROMISED CHICKEN"*** may be submitted by **Dr. Bhagirath Prasad** in partial fulfilment of the requirement for the degree.

(B.K. Sinha)
Chairman
Advisory Committee

***Members of the Advisory Committee*:**

1. Dr. S.P. Verma
   Associate Professor and Head
   Dept. of Veterinary Preventive
   Medicine.

2. Dr. S.R.P. Sinha
   Associate Professor and Head
   Dept. of Veterinary Parasitology &
   I/C Dept. Of Pathology

3. Dr. C. Prasad
   Director, S.G.I.D.T
   Patna
   Nominee, (Dean Post-Graduate Studies)

CERTIFICATE – III

This is to certify that the thesis entitled " ***DISTRIBUTION OF ANTIBODY TITRE TO RANIKHET DISEASE VIRUS IN IMMUNOCOMPROMISED CHICKEN***" Submitted by **Dr. Bhagirath Prasad** in partial fulfilment of the requirement for the Degree of **Master of Veterinary Science (Veterinary Microbiology) of the faculty of post-graduate studies, Rajendra Agricultural University, Bihar** was examined and approved on 15th January 2004.

15/1/04

(B.K Sinha)
Chairman
Advisory Committee

Members of the Advisory Committee:

1. Dr. S.P. Verma — S Verma 15-01-04
2. Dr. S.R.P. Sinha — 15/1/04
3. Dr. C. Prasad — 15/1/04
   (Nominee, Dean Post-Graduate Studies)

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Place: Patna
Date: 27/10/03

Bhagirath Prasad
Bhagirath Prasad

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LIST OF ABBREVIATIONS

Ab	Antibody
AGPT	Agar gel precipitation test
ANOVA	Analysis of variance
b.wt.	body weight
BF	Bursa of Fabricius
0^0C	Degree Centigrade
carbo veg.	Carbo Vegetabilis
Dr.	Doctor
D.F	Degree of freedom
D.W	Drinking water
ELISA	Enzyme Linked Immuno Sorbent Assay
EDTA	Ethylene diamine tetra acetic Acid
edn.	Edition
Fig.	Figure
FCR	Feed Conversion Ratio
gm	gram
gr	group
HA	Haemagglutination
HI	Haemagglutination inhibition
i.o.	Intraocular
i.n.	Intranasal
I.U/Kg.	International unit per kilogram
IBDV	Infectious Bursal disease virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M

Ltd.	Limited
lb	pound
MDA	Maternally derived antibody
MAb	Maternal antibody
M.S	Mean Sum of squares
mg	Miligram
ml	Mililitre
Mr.	Mister
No	Number
NDV	Newcastle Disease Virus
PBS	Phosphate Buffer Saline
PI	Post infection/ post inoculation
PCV	Packed cell volume
Pvt.	Private
RBC	Red blood cell
RD	Ranikhet Disease
RDV	Ranikhet Disease virus
rpm	Revolution per minute
S.E	Standard Error
SPF	Specific pathogen free
vvIBD	very virulent infectious bursal disease
Wt.	Weight
%	percent

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CHAPTER - I

INTRODUCTION

INTRODUCTION

Ranikhet disease (RD) or Newcastle disease (ND) is still a problem for poultry farmers. Among different diseases, it is one which causes major loss in the form of outbreaks. It is caused by virus of paramyxoviridae family. The disease was first time recognized by Kraneveld in 1926. In the same year the disease was reported by Doyle in 1926 at Newcastle in England and in India by Edward in 1927 at Ranikhet in Uttranchal. Today the disease is almost world wide in distribution.

Now a days India ranks fourth in respect of poultry production. Both traditional poultry farming and organized poultry farming are confronted with certain viral diseases, which cause devastating losses. Among the most striking viral diseases of poultry, Ranikhet disease reported from all over India is the most serious threat to the poultry industry.

In rural areas of many countries traditional backyard poultry keeping is widely practiced (Awan *et al.*, 1994). In these backyard poultry, Ranikhet disease is an important limiting factor. Ranikhet disease virus (RDV) is considered to be endemic in the village chicken in most of the developing countries (Spradbrow, 1990; 1993). Thus a constant monitoring would be needed to understand the behaviour of Ranikhet disease.

The severity of the disease has been found to vary depending upon the virulence of the isolate. The clinical features of RD are respiratory, circulatory, gastrointestinal and nervous signs. The particular

set of manifestations depend upon the age and immune status of the host. It is characterized by clinical signs like gasping, cyanosis of comb and wattle, greenish watery diarrhoea and nervous symptoms, which include paralysis of wings and legs, torticollis and ataxia. Gross lesions include pinpoint haemorrhages in the proventriculus and caecal tonsil.

In addition to chicken, turkeys, pheasants guinea fowl, ducks, geese, quails, free flying birds and zoo birds are susceptible and also play a role in the spread of RDV.

Ranikhet disease is controlled by the use of various types of vaccines. The common practice is to vaccinate chick in the first week of age with F-strain by intraocular or intranasal route followed by revaccination between 18 to 25 with LaSota strain through drinking water.

In Bihar, poultry farming is growing rapidly and proving as main resource to meet the quality protein requirement as well as source of employment of the youth in general and poor people. In the poultry sector the broiler farming is becoming more attractive as it gives handsome profit in a short period of 6 to 8 weeks. However, there are certain emerging viral diseases such as RD and IBD are coming on the way of rapid development in the poultry sector. It may be mentioned that the Ranikhet disease, considered to be the number one killer disease of the chicken was well under control till recently due to availability of effective vaccine. But these days the outbreaks of Ranikhet are being reported in the several part of the country in spite of timely vaccination Rathore *et al.* (1987), which is matter of great concern. The study conducted so far in the various part of

country have been able to point to the fact that appearance of the Ranikhet disease in the vaccinated farms is largely because of prevalence of immunosuppressive agents including IBD virus (Faragher *et al.*, 1974 and Rao and Rao, 1992) and aflatoxin (Thaxton *et al.*, 1974 and Mani *et al.*, 2000). Experimentally also both IBD virus and aflatoxin have been found to be immunosuppressive leading to lowering of antibody levels in RD vaccinated flocks. Further it has been found that a special type of vaccine, also called moderate hot strain vaccine or intermediate plus vaccine is being used widely to control the newly emerging very virulent IBD (vvIBD) scenario in the state. Even this vaccine has been found to be immunosuppressive and has caused reduced HI antibody titre after regular RD vaccination. The immunosuppressive nature of aflatoxin and its wide prevalence in the ingredients of the poultry feed warrants detail study of the situation on the ground level (i.e. at the farm level). It is therefore, highly necessary that a planned study be conducted to understand the distribution of HI antibody titre to RD virus in farms which have prior exposure to IBD virus as evidenced by the presence of antibody titre to IBD virus; have received IBD vaccines known to have residual pathogenecity and immunosuppressive effect and such farms which are being given poultry feeds contaminated with aflatoxin as evidenced by emission of bright greenish yellow fluorescence (BGYF) from the feed samples when examined under UV light in UV cabinet.

In the present scenario it is also essential to take up step for improving HI antibody titre to RD vaccine through the use of known immunomodulators namely (i) Charak-E-Sel (a combination of vitamin E

and Selenium (ii)Carbo veg. (a homoeopathic medicine and (iii)Lemasol-p (levamisole hydrochloride). Therefore, the present study has planned with the following objectives:

- Distribution of HI antibody titre to RD vaccine in IBD vaccinated broiler/layer farms.
- Distribution of HI antibody titre to RD vaccine in broiler/layer farms seropositive for IBD virus.
- Distribution of HI antibody titre to RD vaccine in broiler/layer farms being given aflatoxin contaminated feed.
- Improvements of HI antibody titre to RD vaccine in above three categories of selected poultry farms after administration of some immunopotentiating agents.

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Kraneveld (1926) was the first to recognized the Ranikhet disease. In the same year the disease was reported by Doyle (1926) at Newcastle in England. Due to its geographical origin the disease is also known as 'Newcastle disease'.

Ranikhet disease is characterized by various clinical signs like gasping, cyanosis of comb and wattle, greenish watery diarrhoea and nervous symptoms, which include paralysis of wings and legs, torticollis and ataxia. Gross lesions include pinpoint haemorrhages in the proventriculus and caecal tonsil.

Newcastle disease is now reported from all over the countries except Scandinavian countries like Denmark, Norway, Sweden and Finland. In India, the disease was first time reported at Ranikhet in Kumaon hills by Edwards (1927). Since then several workers have reported the incidence of this disease in different part of country (Pazhanivel *et.al.*, 1979; Boro and Chakrabarty; 1981 and Venkata Reddy, 1978).

Pier *et al.* (1971) studied the effect of aflatoxin on immune system. They observed that during aflatoxicosis bursal and generalized immunosuppression takes place as evidenced by impaired vaccinal response, poor body wt. gain and feed conversion ratio.

Allan *et al.* (1972) described an immunosuppressive effect of the infectious bursal agent (IBA) in chicken. In this experiment, chickens were inoculated with IBA at one day of age and vaccinated with

Newcastle disease (ND) vaccine at 23rd and 64th days of age. They observed that the primary and secondary serological responses (NDV HI titres and IgG levels) were significantly reduced in those chickens inoculated with IBA.

Kulkarni *et al.* (1973) reported immunostimulating effect of tetramisole on antibody formation against NDV in chicks. They vaccinated all the chicks on the second day intranasally with LaSota virus and divided into four groups, group I, II, III and IV (control group). Group I, II, and III were treated orally for 3, 7, and 15 days respectively from the day of vaccination with tetramisole at the rate of 1 mg/50 gm body weight. Serum samples from all the four groups were collected and subjected to HI test. The analysis revealed, the chicks, which received tetramisole orally (1mg/50gm body weight) for 3,7 and 15 days showed significant rise of HI antibody titres as compared to the control group. However, no significant difference in HI Ab titres is found between the 3, 7 and 15 days treated groups.

Thaxton *et al.* (1974) reported aflatoxin, a natural contaminant in feedstuffs suppressed hemagglutinin formation in chickens. The primary immune response was reduced in dose-related fashion when aflatoxin was incorporated into the diet at levels as low as 0.625µg per gram. The relative sizes of the bursa of fabricius and the thymus, the primary initiators of immunity were reduced by 30% and 55% respectively, when chicken were kept on diet containing 10µg of aflatoxin per gram of feed. The state of immunosuppression couldn't account for the

carcinogenecity of aflatoxin as well as the enhanced susceptibility to some infectious agents found during aflatoxicosis.

Hirai *et al.* (1974) observed that infectious bursal disease virus (IBDV) depressed the humoral antibody response of chickens to various vaccines. The virus had that effect when injected at the same time or vaccinated on 4^{tn} or 7^{th} day before. The depression was slightly greater when injection was 7 days before and was most pronounced in chickens inoculated with IBDV when 6 weeks old, slight in 4 weeks old and absent in 2 weeks old. Protection against challenge by virulent ND virus 3 weeks after ND vaccination showed the marked differences between IBDV inoculated and uninoculated chickens.

Rao *et al.* (1978) conducted two experiments to study the immunosuppressive effect of infectious bursal disease (IBD) on vaccination against Ranikhet disease. The immunosuppressive effect of IBD and the beneficial effects of IBD vaccination in immunological response to RD vaccine have been experimentally proved for the first time in the country. Two experiments on similar lines were designed. In both, the results confirmed that IBD virus damages the bursa of fabricius and reduces its capacity to supply sufficient number of immunologically competent cells to the system to enable the birds to respond adequately to vaccine stimulus. However, further may bring the RD-HI titre on the par with the titre of the group, which received only RD vaccine as well as the protection level reported in these experiment.

Panigrahy *et al.* (1979) evaluated the effects of antibiotics and levamisole on the immune responses of turkeys. Turkeys which received

three successive antibiotic treatments pre-incubation egg dipping in a solution of tylosin (300 ppm) and gentamycin (500 ppm); day old gentamycin injection (1 mg/poultry); and chlortetracycline in feed (200 gm/ton) and untreated controls were immunized with killed *Brucella abortus, Salmonella pullorum* antigens and Newcastle disease virus. Treated poultry developed significantly less hemagglutination inhibition antibodies than untreated controls. Likewise, the number of poultry that failed to respond primary immunization, was greater in the antibiotic treated than in the control group. In addition, antibiotic treated turkeys raised in batteries gained less weight than the control turkeys. Levamisole restored the immune responses in X-irradiated and antibiotic treated turkeys to a level comparable to that of the controls.

Kumar and Gowda (1979) had chemically analyzed a total 72 feed ingredient samples for aflatoxin contamination. They found that 44 samples containing 0.03 ppm of aflatoxin. They further analyzed and reported that out of 44 samples, 28 samples contained more than 0.2 ppm, 10 samples contained more than 0.6 ppm and 3 samples contained more than 1.75 ppm of aflatoxins, and rest contained 0.03 ppm.

Ajinkya *et al.* (1980) studied on a series of Ranikhet disease outbreaks in a number of poultry farms around Bombay and showed that in all cases of vaccine failure, there was an association of infectious bursal disease.

Boulton *et al.* (1980) studied that corn containing aflatoxin and the same corn ammoniated to inactivate aflatoxin incorporated into layer diets to supply 500 ppb and 2.3 ppb aflatoxin respectively, to

determine whether such diets interfere with immunity to Newcastle disease vaccination. Control diets containing uncontaminated corn both with and without ammoniation were also fed. A trial of 3 months long with 12 birds per treatment was conducted. Vaccination treatments included no vaccination, a single vaccination at the initiation of each trial and monthly vaccination for 3 months. Serum samples for determination of Newcastle disease HI titres by the micro titre method were collected just before vaccination and seven days post vaccination as well as at trial termination. Birds receiving a single initial vaccination and fed a diet containing 500 ppb aflatoxin showed a significant ($p<0.05$) decrease in HI titres whereas birds similarly vaccinated and fed a diet containing inactivated (ammoniated) aflatoxin showed no reduction in titre regardless of dietary treatment.

Mohiuddin *et al.* (1981) studied the effect of aflatoxin @ 100µg daily/chick for 14 days prior to vaccination for Ranikhet disease and a further feeding for 21 days after RD vaccination did not affect the haematological parameters. The effect of feeding 100 and 200µg of aflatoxin daily for 14 days before RD vaccination was similar, but further feeding of aflatoxin @ 200µg for 21 days after RD vaccine resulted in increased serum-alkaline-phosphatase activity, increased RBC fraglity, marked testicular atrophy and a decrease in antibody. Administration of vitamin A gave protection against aflatoxin but failed to enhance the immunity.

Verma *et al.* (1981) investigated an outbreak of infectious bursal disease (IBD) in Andhra Pradesh. Chicks of layer breed of 4 to 9

weeks of age were affected resulting in mortality of 5.5%. At some farms, there were complications with Ranikhet disease, chronic respiratory disease, Aspergillosis, Aplastic Anaemia and tape worm infestations. The IBD was confirmed on the basis of typical lesions, Neutralization test and Agar gel precipitation test and isolation of virus.

Panigrahy *et al.* (1982) observed that the primary NDV HI antibody titre in chicks infected with IBDV at one day of age was lower than those infected at 28 days of age. Infection with IBDV had no influence on secondary immune response to NDV.

Kulkarni *et al.* (1983) studied the haemaggulutination inhibition (HI) antibody titre against Ranikhet disease (RD) in chicks infected with infectious bursal disease (IBD) virus and subsequently vaccinated with RD vaccine. The HI antibody titres followed a decreasing pattern in the chicks infected at 0 and 24 hr after hatching up to sixth week. All the chicks had poor humoral antibody response as compared to vaccinated control.

Edrise *et al.* (1986) observed immunopotentiating effect of ascorbic acid against Newcastle disease in chicken. Diet containing 50 or 250 mg ascorbic acid per kg of feed was fed to one-month-old chicks, starting at the same time as ocular instillation of Hitchner B_1 Newcastle disease vaccine. They challenged with virulent virus 7 weeks later, all vaccinated birds survived. Antibody titre were highest in those receiving the large dose of ascorbic acid. Vitamin treatment also improved growth and feed utilization.

Sharma *et al.* (1986) investigated between 1975 and 1984, the annual number of confirmed outbreaks of Newcastle disease ranged from 2 to 205, with 38 being reported in 1984. Peak occurance was in hot, dry season and the hot humid season. Most of the outbreaks affected in unvaccinated village hens.

Vyas *et al.* (1987) studied on immunomodulation by levamisole along with vaccination in chicks against RD. They were subjected HI test, Sodium sulfite precipitation test and Agar gel elecrtrophoresis of 560 .serum samples. The chicks were administered levamisole orally after RD vaccination on 5th, 6th and 7th day at the dose rate of 3.75mg, 7.5mg and 15.0mg per kg body weight in three group.. Immunomodulatory effect was observed in chicks with statistically significant difference in HI antibody titres and serum Ig concentration between treated groups and untreated control groups of chicks. No significant variation in different doses of levamisole in chicks was noticed in the treated group in the present study. In the same manner no extension in the immunity period was recorded in the treated chicks.

Rathore *et al.* (1987) studied thirty five poultry farms located in nine states of India for occurance of Ranikhet disease in vaccinated flocks and simultaneously the role played by immunosuppressants such as infectious bursal disease (IBD) and aflatoxicosis in the cases RD vaccination failures. Outbreaks of RD were recorded at five farms, IBD seropositives were detected at 6 farms and the clinical form of IBD in one farm. Aflatoxin content ranging from 0.20 to 0.25 ppm was detected in the feeds of three farms. At five farms having encountered RD outbreaks and

the post outbreak RD HI titres were in the range of 2^6 to 2^8. Serum samples from only 4 farms having history of regular vaccination, but no outbreaks showed RD HI above 2^4 (protective level). Whereas at the remaining 26 farms the RD HI titre were in the range of 2^1 to 2^3 (below protective level). The defective vaccination and non- maintenance of cold chain during various stages of vaccine handling were found most important factors for poor immune response in these vaccinated flock.

Rao *et al.* (1987) observed an outbreak of Newcastle disease in the birds of 5-6 weeks age on a large broiler farm of about 40,000 birds. The first case was reported in July 1985 and despite control measures including use of different vaccines, change of feed and vitamin supplement in drinking water, it gradually spread to the rest of the farm by November 1985. It's persistence was attributed to immunosuppression caused by aflatoxin in feed at 0.5–0.6 ppm and vitamin E deficiency.

Jassar and Singh (1989) reported immunosuppression by aflatoxin and it was dose related. They also reported the immunosuppressive effect was reversed after discontinuation of feeding aflatoxin.

Reddy *et al.* (1989) investigated dietary vitamin A in the manifestation of aflatoxicosis syndrome in female broiler chicks from 3 to 52 days of age employing 3 dose levels of aflatoxin (0,0.5 and 2 ppm) and 3 dose levels of supplement as vitamin A (2,000,10,000 and 100,000 IU/kg) in a factorial design. Aflatoxin at 2 ppm level and supplemental vitamin A at 100,000 IU/kg adversely affected the overall performance of the chicks. There was also no significant ($p<0.05$) interaction between

dietary vitamin A and aflatoxin in any of the biochemical characteristics and tested except in liver vitamin A and serum cholesterol. Liver vitamin A decreased with increasing levels of aflatoxin. The decrease was significant at the higher levels of vitamin A. Serum cholesterol was however, reduced in diet with 2 ppm aflatoxin and 0.1 million IU/kg vitamin.

Hassan *et al.* (1989) studied the effect of levamisole hydrochloride (8.0 mg per kg body wt.) when given before or during vaccination on the immune response to Newcastle disease virus vaccine (LaSota strain). Levamisole hydrochloride increased the macrophage migration inhibition for 2 weeks of post vaccination. Its administration before vaccination increased the antibody titre to Newcastle disease virus vaccine for one week after vaccination. Moreover, levamisole hydrochloride increased the delayed hypersensitivity to concentrated Newcastle disease virus when injected intradermally in wattles.

Ozer *et al.* (1989) observed the birds receiving aflatoxin had lower HI titre and leucocytes count.

Viridi *et al.* (1989) observed loss of weight of bursa of fabricius and thymus to the extent of 25 to 38% in chickens fed on aflatoxin diet at the rate of 10% w/w for 3 weeks.

Mangat *et al.* (1989) conducted an experiment and given aflatoxin B_1 in feed at 0.3mg/kg daily from day old to two groups of 50 chicks. One group (A) was vaccinated with F-strain Newcastle disease vaccine at day old and the other group (B) with R_2B strain vaccine at 8 weeks old. Two weeks after R_2B vaccination all the birds were challenged

with virulent Mukteshwar strain of ND virus. HI titre in group B is consistently lower than A and group B could not resist challenged with the. virulent virus. No mortality was seen in group A. Mortality was 100% in a 3rd group that had been fed aflatoxin but not vaccinated as compared to 94.68% in 4th group that had not been fed aflatoxin or vaccinated.

Jhala *et al.* (1990) studied the effect of IBD virus infection on humoral as well as cell-mediated immunity in chicks vaccinated against ND. They divided broiler chicks into group A, B and C. IBDV isolates of 10% bursal suspension was used to infect the chicks of group A. chicks of groups A and B were immunized with F-strain of ND vaccine at 7 days of age. Chicks of group C served as uninfected and unvaccinated controls. Sera were collected from each bird at weekly intervals up to 3 weeks post vaccination. The HI titres were determined. At weeks 2 and 3 post vaccination, the GMT of group B were significantly higher than those of group A, indicating a definite suppressive effect of IBDV infection on humoral immunity.

Mazariegos *et al.* (1990) conducted the study to test the pathogenicity and immunosuppressive effects of seven commercially available infectious bursal disease vaccines. The vaccine strains were intermediate in their pathogenicity in susceptible specific pathogen free (SPF) chickens. One-day-old and three week old SPF chickens were vaccinated with these vaccines. Two week after IBD vaccination they were vaccinated with Newcastle disease virus. The pathogenic and immunosuppressive effects of the IBD vaccines were evaluated by the antibody response to NDV vaccination, the bursa: body weight index and

histopathological lesions of the bursa. The results revealed that these vaccine strains were highly variable in their virulence and immunosuppressive properties. Three of the strains tested were found to be highly virulent and immunosuppressive, two others were moderate and two could be classified as mild.

Ghosh and Chauhan (1991) investigated significant reduction in the relative weights of bursa of fabricius and thymus and humoral response was also suppressed in broiler chicks, when they were fed a diet containing aflatoxin B_1 @ 1μg/kg for 6 weeks.

Rao and Rao (1992) observed maximum immunosuppressive action of infectious bursal disease virus in chicken against Ranikhet disease vaccination when it was inoculated at 6 weeks of age, comparatively at day-old age or 3 weeks of age when subsequent RD vaccination was done at 8 weeks of age. Infectious bursal disease might explain the current failure of RD vaccine to provide adequate protection in commercial practice.

Dutta *et al.* (1992) studied the immunomodulatory effect of levamisole on antibody production in broiler chicks vaccinated against Newcastle disease vaccine (F-strain). In present study, day old broiler chicks were randomly divided into four groups. The chicks of groups I and II were vaccinated with NDF-strain vaccine while, the chicks of groups III and IV were maintained as unvaccinated controls. The chicks of groups I and III were administered levamisole (1mg /50 gm body weight) in drinking water for five consecutive days immediately after vaccination. After post vaccination GMT at 2nd week were found to rise to 1:24.25 and

1:12.13 in group I and II respectively. At 3rd week the HI titres attained their peak 1:55.72 and 1:24.25 for groups I and II, respectively. The rise in group I was double than in group II and it was attributed to be the effect of levamisole. The present study revealed that levamisole had a significant immunomodulatory effect on antibody production in broiler chicks against ND vaccine with F-strain .

McIlroy *et al.* (1993) reported improved performance in commercial broiler flocks with sub-clinical infectious bursal disease infection when fed with diets containing increased concentration of vitamin E. The economic effects of increased vitamin E supplementations in 79 commercial broiler flock incorporating over 1.5 million birds were observed. Approximately half of the flocks were fed on either a high (178 IU/kg) or normal (48 IU/kg) vitamin containing diet. In addition, approximately in half of the flocks sub-clinical IBD was present. Analysis of the performance data showed that flocks with sub-clinical IBD infection were consistently worse for net income, feed conversion ratio and average weight per bird than flocks without sub-clinical disease. The trial also indicated that the average net income of flocks with sub-clinical IBD and fed a high vitamin E containing diet was of better than that from flocks with sub-clinical IBD and fed a normal vitamin E containing diet. It was suggested that increased improved performance from high vitamin E supplemented diet recorded in flocks with sub-clinical IBD is due to enhanced immunocompetense and increased resistance to disease.

Panda and Rao (1993) observed significantly lower geometric mean of HI titre to RD F-strain vaccination in chickens infected with

IBDV at one day of age as compared with chickens vaccinated with RD F-strain but not infected with IBD. The histological features of bursa fabricius of RD vaccinated chickens and unvaccinated uninfected chickens showed normal appearance. The bursa of only IBD infected chickens and IBD infected RD F-strain vaccinated chickens showed lesions specific for IBD.

Chawak *et al.* (1993) studied to assess the immunomodulation in broilers under stress and RD vaccinated. Day-old chicks were randomly divided into 8 equal groups and a challenge control group. The former were allotted to the test treatments of 2 levels each of stocking density (930 and 465 cm^2/ Bird), temp (24 to 30°c and 40 to 42°c) and levamisole hydrochloride administration on seventh day (0 and 7.5mg/kg body wt.). Body weight, feed consumption and serum HI titre were recorded at weekly intervals. It was seen that average live weights were significantly ($p<0.05$) reduced when the birds were subjected to stress. However no significant improvement was observed in live weights in the birds treated with levamisole. Levamisole treatment also had no significant effect on feed consumption. HI titres of the birds under stress were significantly ($p<0.01$) lower than that of control. The levamisole treatment in normal as well as in birds under stress of high stocking density or high temp or both, significantly ($p<0.01$) increased the HI titres against RD.

Karnatak *et al.* (1993) reported immunomodulatory effect of levamisole in broiler chicks. They divided 100 broiler chicks (one week old) vaccinated with F-strain into 4 groups of 25 each. Group I, II, &III were treated with levamisole @ 3.75, 7.50 and 15.0 mg/kg b. wt

respectively. The birds which received 7.5 and 15.0 mg of levamisole showed significant increase in HI titres, but birds which received 3.75mg of levamisole showed significantly increase in HI titre on 20th day only.

Pande *et al.* (1994) studied the immunomodulatory effect of zeetress in chicken vaccinated against ND (F-strain) virus. Zeetress was administered at the rate of 5gm/1000 chicks through drinking water for first 10 consecutive days and thereafter at the rate of 10gm/1000 birds from 24 to 35 days. On 35-day serum samples were collected for HI test. The result revealed that the antibody titre, body weight gain and feed efficiency were significantly higher than untreated vaccinated control.

Chatterjee *et al.* (1994) reported the immnomodulatory effect of herbal product IMMU-21 (research name) in different laboratory animals. He found that animals treated with IMMU-21 (20mg/kg) significantly increased the microcidal activity of neutrophils in experimental animals, it might be due to its decreasing effect on circulating level of corticosteroids under the basal level. Increase in soluble immune complex in the serum of the experimental animals also indicated immunopotentiating action of IMMU-21.

Vijaya Praveen *et al.* (1995) reported induced immuno-suppression in chicks at day-old, 21days and 42 days of age using virulent IBDV isolate. The immune response in immunosuppressed and control chicks were monitored against ND vaccines at weekly intervals for a period of 7 weeks post vaccination by HI. The results indicated immunosuppression to ND vaccine in IBDV infected chicks of all the age

groups. The order of immunosuppression to ND vaccine was found to be day-old> 42 days > 21 days.

Rao *et al.* (1995) reported immune response due to zeetress in infectious bursal disease vaccinated chicks. 120 kalinga brown chicks (layer) were divided into 8 equal groups, which also included appropriate controls. When Newcastle disease vaccine virus was used as indicator system there was higher level of antibody titres in both IBD vaccinated and unvaccinated birds administered zeetress. There was also a marked increase in rosette forming T-lymphocytes suggesting stimulation of cell mediated immunity which was further strengthened by significantly severe delayed type of hypersensitivity reaction in the DNCB (2, 4-dinitrochlorobenzene) skin sensitivity test. The spleen macrophage activity was increased as shown by increase in number of formazan positive cells in nitroblue tetrazolium test as a result of administration of zeetress. Zeetress was also responsible for significantly higher body weight in IBD vaccinated birds. Histological examination of bursa revealed that majority of the follicles were damaged as indicated by atrophied follicles due to destruction of lymphocyte and intra and inter follicular oedema as a result of live IBD vaccine were partially protected due to administration of zeetress. It was concluded that there was a significant improvement in immune status of IBD vaccinated chickens receiving zeetress.

Franchini *et al.* (1995) reported that vitamin E added to inactivated and emulsified vaccine enhanced the immune response to viral antigens in chickens. Vaccines with vitamin E specially when replacing 20 or 30% of mineral oil, induced a more rapid and higher antibody response

than control vaccines. An adjuvant effect of vitamin E was present in viral vaccine lacking bacterial antigens.

Kurtoglu and Nizamlioglu (1996) studied the effect of vitamin A or E or both on antibody titres and blood T lymphocyte percentage value in chickens vaccinated with Gumboro. Blood immunoglobulin IgG increased considerably after vaccination. Vitamin A excess (80,000 IU/kg diet) suppressed antibody titres and increased T-lymphocyte and IgG values. Vitamin A and E given together reduced the level of increase in T-lymphocyte values.

Das *et al.* (1996) observed IBDV induced immunosuppression in NDV vaccinated broiler chicken. The mean HI titres in the birds vaccinated against NDV at day zero were 1.40 ± 0.45. The antibody titre increased gradually and at three weeks post vaccination the mean HI titre was 30.40 ± 12.10. Duplicate serum samples tested for IBDV antibodies employing CIE and ELISA were negative at day zero of age. They concluded that NDV vaccinated birds developed optimum levels of the antibodies up to 3 weeks post primary vaccination in birds without IBDV infection. After booster NDV vaccination the mean HI titre decline and at 5 and 7 weeks post secondary vaccination strongly suggested immunosuppression due to IBDV infection after a few weeks of primary NDV vaccination.

Rao *et al.* (1996) concluded that zeetress had a sparing effect on the damage to lymphoid follicles of bursa in IBD vaccinated group but of no consequence in the challenged/ virulent IBD virus infected birds. It was concluded that zeetress had stimulated the rosette forming T-

lymphocytes and enhanced the macrophage activity in the spleen. However, it had no beneficial effects in virulent IBD virus infection.

Mahesh and Muniyappa (1996) studied the immunogenicity, pathogenicity and immunosuppressive potential of one less attenuated, three intermediate, one mild and combination of mild and inactivated IBD vaccines strain. They also monitored the maternally derived antibody (MDA) response in both experimental and field conditions. The chicks were vaccinated against IBD according to manufacturer's recommendations and against Newcastle disease on day seventh. The evaluation of immune response and MDA was determined by employing indirect ELISA. The pathogenic and immunosuppressive effect of IBD vaccines was evaluated by bursa: body weight ratio and antibody response to ND vaccination. MDA level decreased below protective level in 14 days and their half-life was 3.6 days and varied among breeds under field conditions. The less attenuated strain and one of three intermediate strains induced significantly lower titre followed by other two intermediate strains and significantly by lower titre for mild (Lukert) and combination of mild and inactivated strains.

Christopher *et al.* (1997) studied the influence of vvIBD on immunity to Ranikhet disease at the field level. They statistically analyzed the seroepidemiological data of Ranikhet disease and infectious bursal disease, before (during 1991–92) and (during 1993-1994) the outbreak of very virulent form of IBD (vvIBD) in Tamilnadu. During 1993-94 the half-life of RD maternally derived antibody was 3.2 days and the IBD-MDA was 4.11 days in clean premises. In the infected premises half-life

of the RD-MDA was 2.69 days the $T_{1/2}$ of RD vaccinal antibodies was 1.85 days. They observed RD vaccinal titres of samples collected during 1993-94 are significantly lower than the statistically predicted HI titres for that age. Mathematical conclusions indicate that vvIBDV could be the cause of this perceived difference in RD-HI titre values.

Prabhakaran *et al.* (1997) studied the effect of sub-clinical infection of broilers with IBD virus, on immune response to RD vaccine. They divided the broiler chicks into 3 groups. The group 1 was vaccinated with IBD vaccine on day 1 and RD vaccine (F-strain) on day 7, group 2 was sub-clinically infected with 25 CID_{50} of IBD virus on day 1 and vaccinated with RD vaccine (F-strain) on day 7. The group 3 was kept as uninfected/unvaccinated control. 50% of chicks in each group were challenged with 1000 CMD_{50} of RD virulent virus and 100 CID_{50} virulent IBD virus on day 36 and similarly the remaining chicks in each groups on day 50. The pre and post challenged antibody titre against RD was assessed by HI and ELISA. The immunosuppressive effect was observed in group 2, sub-clinically infected with IBD virus, as exhibited by reduced antibody response and survival to challenge.

Kalorey *et al.* (1997) reported the effect of graded levels of dietary aflatoxin on development of humoral immune response to Ranikhet disease virus vaccine in poultry. The two levels of dietary aflatoxin (2.5 and 5.0 ppm) had marked, relatively dose dependent immunosuppressive effect with reduction in medullary lymphocyte count of bursal follicle. Persistence of residual effect of high level of dietary aflatoxin (5.0ppm) was also observed. Immunostimulatory effect was

observed in levamisole treated aflatoxin fed chicks. However, the effect was non- significant after three days treatment of levamisole at the rate of 7.5 mg/kg body weight. The study indicated immunostimulatory effect of levamisole in healthy chicks but not in aflstoxicated chicks probably due to severe immunosuppression.

Shadaksharappa *et al.*(1998) evaluated the immunomodulatory effect of vitamin E, vitamin C and levamisole hydrochloride on immune response against IBD vaccination in broilers. He observed that the mean antibody titre were comparatively higher but non-significant in vitamin E, vitamin C treated and levamisole treated than vaccinated control group. The mean antibody titres showed appreciable increase when combined treatment with both vitamin E and levamisole hydrochloride as compared to that of either vitamin E or levamisole hydrochloride alone. This observation indicated the synergistic action of these compounds.

Sadekar *et al.* (1998b) reported immunomodulating effects of *Azadirachta indica* (Neem) dry leaves powder in broilers, naturally infected with IBD virus. They divided broilers into 3 groups at 6 weeks of age. The birds had been vaccinated with NDV (LaSota strain) at one day of age and had survived a natural outbreak of IBD. Group I were control, group 2 were given a booster vaccination (NDV strain R_2B) and group 3 were given booster vaccination and fed with powder neem leaves (125mg/bird) daily for 2 weeks. Treatment with neem leaves significantly enhanced the antibody titres against NDV antigen and also potentiated inflammatory reactions to dinitrochlorbenzene in skin test. It was concluded that feeding neem leaves to immunosuppressed birds increase

their humoral and cell mediated immune responses. It is suggested that neem leaves may be useful for treatment of immunosuppressive diseases, such as IBD in birds.

Sadekar *et al.* (1998a) observed the usefulness of *Ocimum sanctum* (tulsi) dry leaves as immunomodulator in poultry, naturally infected with IBD virus. He found that HI titre against ND vaccination in *Ocimum sanctum* treated group was significantly higher as compared to unvaccinated and untreated control as well as vaccinated untreated control groups. Attainment of significant higher titres at the end of 45 days of *Ocimum sanctum* administration seems to have overcome the immunosuppressive effect of IBD on lymphoid organs and has stimulated antibody production in these birds.

Kolte *et al.* (1999) reported the immunomodulatory effect of dry powder of *Ocimum sanctum* (Tulsi) and leaf gall of *Ficus racemosa* (Gular) leaves in broilers, stunted and immnosuppressed by IBD virus. The HI titre against NDV was lower in all group before drug treatment. It was observed that the titre was significantly raised in drug treated group. Birds which received a combination of both Tulsi and Gular revealed the highest HI antibody titre as compared to other treatment group. These observations were clearly indicated that all the tested plant preparations had specific immunostimulatory effect on humoral immune response. Cellular reaction at the DNCB skin contact site revealed that reaction was intense in *0. sanctum* treated and *0. sanctum* plus gular leaf treated group. This observation indicated that the said plant preparations also potentiate the non-specific cell mediated immune response in IBD affected birds.

Kalita and Dutta (1999) studied the immunomodulatory effect of levamisole upon Newcastle disease, pigeon pox and Marek's disease vaccination in broilers. For the study, day-old chicks were vaccinated against Newcastle disease, Pigeon pox and Marek's disease. Levamisole was given daily for 7 days at 1mg/50gm body weight orally to treated group while levamisole untreated group served as control. Blood samples were assessed for antibodies to the 3 viruses by passive Haemagglutination (PHA) and immunoprecipitation (IP) test respectively on 14, 28, 42 and 56 days. Later MAb were detected in most of the birds in the first week of life, but were not detected again in unvaccinated groups, however, antibody levels rose steadily. Levamisole had an enhancing effect on the HI titres of the Newcastle disease vaccinated birds. Variable effect on PHA tests in the birds given pox virus vaccine and also no observable effects on precipitation tests for MD virus vaccination.

Brewoo and Aning (1999) studied the seroprevalence of Newcastle disease virus (NDV) antibodies in 5 areas in the Achimota area of Ghana, sampled between April 1993 and January 1994. None of the flocks sampled had been vaccinated against ND. Sampling was done on two occasions, from April to October and between November and January when severe outbreaks of the disease are usually observed. HI test results showed that 382 of the 656 (58.2%) chickens sampled between April and October were positive for NDV antibodies with a geometric mean titre (GMT) $\log_2 2.5$. Chickens sampled between November and January showed higher prevalence rate of 79.8% with a GMT of $\log_2 4.6$.

Assuming that NDV antibody titre of $\log_2 3$ and above were conferring immunity. The results indicated that rural chickens in Ghana were naturally exposed to NDV throughout the year.

Prakash *et al.* (2000) conducted an experiment with a completely randomized block design used to evaluate the effects of ochratoxin (OA) (2mg/kg of diet), vitamin E (400mg/kg) and selenium (1mg/kg), both individually and in combination, in 6 weeks old broilers. Addition of selenium alone did not alter any of the parameters like body weight, total protein, and relative weight of kidney and liver. Vitamin E alone and in combination with selenium significantly ($p<0.05$) diminished the growth inhibitory effect of OA, reduced the liver weight, severity of hepatic lesions and reduced activity of GCT to control level. The increase in uric acid and decrease in total serum protein values caused by OA were significantly diminished to differing degree by vitamin E plus selenium. These data suggested that vitamin E alone or in combination with selenium could modulate the toxicity of OA in broilers.

Mani *et al.* (2000) observed the effect of graded levels of aflatoxin B_1 fed broiler chicks for a period of 8 weeks. The broilers were vaccinated with ND primary vaccine at seventh and twenty eighth days of age. Blood samples were collected before the ND primary vaccination and at the end of seventh and eighth weeks of age. The ND immune status was estimated. Aflatoxin B_1 in the feed even at 0.10 ppm level had significantly depressed the immune development against ND irrespective of the age of the broilers. Immunity against ND was inversely proportional of the aflatoxin level in feed.

Bakshi *et al.* (2000) studied the effect of different dietary doses of aflatoxin on humoral immune response against Newcastle disease vaccine in commercial broilers. The chicks were vaccinated against Newcastle disease with F-strain at day old and four weeks of age and again with R_2B strain at 8 weeks of age. At four weeks of age chicks were divided in four treatment groups I, II, III and IV were given feed containing 0.38, 0.75, 1.5 and 3.00 ppm of aflatoxin B_1 respectively from fifth weeks onwards till ten weeks of age. The group V was kept as control. Blood was collected at weekly intervals to observe the level of antibodies against ND by HI test. GMT of group I & V increased gradually and were much above the protective levels. But there were significantly reduced HI titres in group III & IV throughout the experiment. Control group recorded the highest antibody titres at 10 weeks of age. The results indicated that dietary aflatoxin was a potent immunosuppresent in young chickens and that the extent of suppression of HI titres was directly related to the dose of aflatoxin.

Arvind *et al.* (2001) studied the effects of dietary inclusion of organic selenium at two levels 0.10 and 0.15 ppm along with vitamin E at two levels 50 ppm and 75 ppm on growth, FCR, feather score and ELISA titre levels of IBD and ND. Higher vitamin E levels (75ppm) resulted in higher body weight, better feather score and FCR. Overall, a combination of organic Selenium at 0.10 ppm and vitamin E at 75 ppm was found to give consistently superior results for growth traits, while a combination of Selenium at 0.15 ppm and vitamin E at 75 ppm gave superior results for immunological traits.

Mani *et al.* (2001) conducted an experiment for a period of eight weeks to find out the influence of 200 ppb of aflatoxin B_1, other immunomodulators and grówth promoter on the performance of broilers. Aflatoxin B_1 alone had significantly reduced the body weight, feed efficiency and immune development against Newcastle disease in broilers. Supplementation of Lactobacilli and vitamin E and Selenium had improved the body weight, feed efficiency and immune status against ND in broilers. Whereas the supplementation with levamisole had improved only the immune response against ND but not the body weight gain, feed efficiency and carcass yields. .

Saravanabava *et al.* (2001) observed the effect of tuftsin a naturally occurring tetrapeptide (Thr-lys-pro-Arg) on the immune response of chicken vaccinated with different NDV vaccines and in turn of the excretion pattern of virulent NDV following challenge. Significant increase in the serum antibody titres was observed in birds vaccinated along with tuftsin as compared to the birds vaccinated without tuftsin. Birds having serum antibody titres ranging between 2.66 and 9.33 excreted the virus for the period 3 to 9 days post challenge, while those with titres of 11.16 and above no virus could be recovered. Tuftsin produced significant increase in the serum antibody level to NDV vaccination which in turn reduced the virus shedding depending upon the antibody titres. Hence, tuftsin could be used safely and effectively as an immunopotentiator in regular NDVcontrol programme.

Khopde *et al.* (2001) studied the antioxidant activity of Amla. For the study, aqueous Amla extract was examined for its ability to inhibit

γ-radiation induced lipid peroxidation (LPO) in rat liver microsomes and superoxide dismutase (SOD) damage in rat liver mitochondria. For LPO experiment, Amla extract was added as its aqueous solution; and irradiation was carried out at different intervals. The extent of LPO was measured in terms of thiobarbituric acid reactive substance. It was observed that the Amla extract acted as a very good antioxidant against γ-radiation induced LPO. Similarly, it was found to inhibit the damage to anti-oxidant enzyme SOD. The antioxidant activity of the Amla extract was found to be both dose and concentration dependent. It was also found that microsomes containing similar amount of ascorbic acid as was present in Amla, no inhibition in LPO was observed. It was observed that reactivity of both Amla ascorbic acid towards ABTS a stable free radical, were similar. Based on these results it is concluded that Amla is a more potent antioxidant than vitamin C.

Satturwar *et al.* (2002) studied the immunomodulatory effect of a poly herbal formulation Haridradi ghrita, a ghee based formulation claimed to be an immunopotentiator and hepatoprotective. The ingredient in the drug contained cow's ghee, *Embilica officinalis, Terminalaia chebula, Terminalaia bellirica, Azadiracta Indica, Sida cordifolia* and *Glycorrhiza glarbra.* The trial was carried out in wistar rats, where the formulation was fed orally at a dose rate of 100mg/kg and 200mg/kg daily. The assessement of the immunomodulatory action was carried out by testing the haemagglutinating antibody titre (HA titre) for humoral and delayed type hypersensitivity (DTH response) for cellular immune responses to the antigenic challenges with sheep RBCs and by neutrophil

adhesion test increase in both, HA titre and DTH response indicated that the Haridradi ghrita potentiates humoral as well as cellular imminity. The neutrophil adhesion was increased as compared to control. It was concluded that Haridradi ghrita promises strong utility in clinical practice.

Kumar *et al.* (2002) studied immunosuppressive effect of infectious bursal disease virus on immune response to Ranikhet disease virus vaccine. They revealed that local IBD virus isolate proved immunosuppressive when given in 2 days-old WLH chicks and measured in terms of immune response to F-strain RD vaccine. They observed that immunosuppressive effect was most marked when interval between experimental IBD virus infection and F-strain vaccination was shortest. While, the effect was lowest when the interval was maximum. The age of birds at the time of IBD virus infection, timing of RD vaccination and maternal antibody strains to RD vaccine were some of the factors attributable for such effects.

Gupta *et al.* (2003) studied immunosuppressive effect of Aflatoxicosis, IBD and their interaction against Newcastle disease vaccination in broiles. They divided 200 broiler chicks into 4 groups of 50 birds each. Chicks of group A (aflatoxin control) and group AV (aflatoxin and IBD) were given 600 ppb of aflatoxin in feed from day 1 till the end of the experiment. Whereas, the chicks of group C (Control) and group V (IBD Control). On 3rd day of age, pathogenic field isolate of IBD virus was inoculated @ 0.1 ml via occulo-nasal routes, to the chicks of group V and AV. They observed that the group A chicks had reduced HI titres as compared to group C on, 7, 14 and 21 DPV, in group V chicks, the

reduced HI titres were observed on 7, 14 and 21 DPV and remained low even booster, the group AV chicks also had reduced titres as compared to the control on 7, 14 and 21 DPV and the titre remained low on 7 and 14 days post booster.

CHAPTER - III

MATERIALS AND METHODS

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MATERIALS

Eymbryonated eggs:-

10 days old embryonated eggs were obtained from private hatchery, Patna. They were used for propagation of RDV.

RDV antigen: -

A commercially available R_2B vaccine manufactured by Indovax Pvt. Ltd, Siswala Hariyana, India, was used for inoculation in embryonated eggs for preparation of RDV antigen.

Farms:-

A survey of different poultry farms in and around Patna was conducted (table-1).

IBD Antigen:-

Poona strain of IBD virus being maintained in the Department of Veterinary Microbiology, Bihar Veterinary College, Patna in the form of 50% bursal homogenate was used as reference antigen throughout this study.

Chicken red blood cells:-

0.8% suspension of chicken RBC in phosphate buffer saline (PBS) was used for HA and HI test.

4HA unit:-

4 HA unit of antigen was used in HI test.

Buffer:-

(I) Phosphate buffer saline (Aziz 1985):-

NaCl	2.0gm
KCl	0.05gm
$Na_2 HPO_4.2H_2O$	0.14gm
KH_2PO_4	0.05gm
Double distilled water	250ml
pH	7.2 to 7.4

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

II For Agar gel precipitation test (Aziz 1985)

(a)Solution A

$Na_2 HPO_4. 2H_2O$	1.4 gm
Double distilled water	100 ml

(b)**Solution B**

NaH_2PO_4	1.4 gm
Double Distilled water	100 ml

Composition of the agar gel

Solution A	84.1 ml
Solution B	15.9 ml
Sodium chloride	8.0 gm
Agarose (Hi-media)	1.0 gm
Sodium azide	0.01 gm

This mixture was autoclaved at 15 lb pressure for 15 minutes.

Drugs:-

Lemasol – P

A commercially available preparation of Levamisole hydrochloride manufactured by Parth Parental Pvt. Ltd. was used as immunomodulator in the present study.

Charak–E-Sel

A commercially available water soluble combination of Vitamin E and Selenium powder manufactured by "Charak animals health care, Ever green Industrial estate, Shakti Mills Lane Mumbai, India was used as immunomodulator.

Carbo vegatabilis (Carbo Veg.)

Carbo Veg. 200 (B&T original) was used as growth promotor/immunomodulator in the present study.

Methods:-

(I) Preparation of Ranikhet disease virus antigen:-

Ranikhet disease virus was prepared from available R_2B strain of Ranikhet disease vaccine. The R_2B strain of Ranikhet disease vaccine was reconstituted in the PBS and inoculated into 10 days old embryonated eggs by allantoic route. The embryonatd eggs were examined daily for embryo mortality. Generally, mortality was observed after 48 hours post inoculation of Ranikhet disease virus. The allantoic fluid was collected and was tested for the concentration of Ranikhet disease virus by HA test. The stock Ranikhet disease virus was stored at $0^{0}C$ for further use as antigen.

Production of Hyperimmune serum:-

Hyperimmune serum against IBDV was raised in 20 weeks old apparently healthy chickens. Each bird was given Georgia strain of IBDV through occulo-nasal route at weekly intervals. Two weeks after the fourth inoculation the birds were bled and the serum was tested for the presence of IBDV antibody by AGPT. This serum was stored at 0^{0}C for further use.

Screening of feed:-

Feeds from different poultry farms were collected in sterile ploythene. Feeds were kept on white paper and placed in UV cabinet under UV light. The presence of aflatoxin in feed was determined on the basis of Bright greenish yellow fluorescence (BGYF). The feed which gave Bright greenish yellow fluorescence (BGYF) was taken as positive for aflatoxin contamination.

Collection of serum samples from chicken:-

Two to three ml blood was taken from the wing vein of each bird with the help of 5ml sterilized disposable syringe using 24 gauze needle. The blood thus drawn was immediately transferred into sterilized test tube, which was thereafter kept in a slanting position and was allowed to clot. After 4 to 5 hours at room temperature the serum was separated. Serum was collected in clean and sterilized vials of 2ml capacity and was inactivated at 56^{0}C for 30 minutes, in water bath. The sera were preserved by adding sodium azide (1:10,000). The serum samples were stored at 0^{0}C until used.

Chicken red blood cell suspension:-

Two adult chickens were used as donor of blood. 1.5ml to 2ml of blood was collected from each bird in an anticoagulent, disodium salt of ethylene diamine tetra acetic acid (EDTA) at the rate of 1mg per ml of blood. Supernatant fluid was removed after centrifugation at 500 rpm for 10 minutes. The packed cells were washed three times with PBS. Finally 0.8%, RBC suspension was made in PBS and stored at refrigerated temperature (4^{0}C). This RBC suspension was used only for four days after preparation and further fresh RBC suspension was prepared.

Assessment of immune response to RDV

Haemagglutination (HA) Test:-

The HA test was performed in Perspex plate to prepare 4HA units of RD virus as described by Beard (1980). Taking 0.5ml of virus material two fold serial dilution were made in PBS, except in control well in which only PBS (0.5ml) was added. In next step 0.5 ml of 0.8% RBC suspension was added to all the wells. A known positive and negative control was also included. The plate was stirred gently for mixing and uniform distribution of erythrocytes and left at room temperature for 40 minutes. The RDV produced sheet of agglutinated RBC covering the bottom of the wells. Negative well showed circumscribed compact button at the bottom. The HA pattern was read and result of HA titre was recorded as reciprocal of the highest dilution showing 100% HA.

Haemagglutination Inhibition (HI) Test:-

The HI test was performed in U-shape bottomed microtitration plate as per the method suggested by Beard (1980). 4 HA

units of virus antigen and 0.8% chicken RBC suspension were used in this test. Using 0.25 ml of serum sample two fold serial dilution were made in PBS. To each serum dilution 0.25 ml (4 HA unit of virus) antigen was added. After a reaction time of 20 minutes at room temperature, 0.5 ml of 0.8% RBC suspension was added to each well containing serum virus mixture. The plate was shaken gently to mix the serum and RBC and left at room temperature for 40 minutes. The HI antibody titre was taken as the reciprocal of the highest dilution of serum showing complete inhibition of agglutination of RBCs.

Assessment for IBD seropositive

Agar gel precipitation test

The test was done following the method of Hirai *et al.* (1972) with some modification. The glass microscopic slide (75 mm x 25 mm) were precoted by dipping them in 0.3% agar solution and dried in open air. Approximately 4 ml of molten agar gel was poured on each glass slide with the help of glass pipette and allowed to solidify. After setting, the slides were kept at 4^{0}C for over night to facilitate punching of gels. A hexagonal well pattern consisting of a central well and five peripheral wells of 3.5 mm in diameter, 8mm apart were punched with the help of a template. The central well was charged with the antigen and one of the peripheral wells with the reference antiserum. The remaining 4 wells were used for test sera. The slides were incubated in humidified chamber at room temperature and observed daily for three days.

Experimental Design

A survey was conducted in and around Patna to locate and list a minimum number of five poultry farms each from the following categories: -

(I) Farms having history of regular vaccination against both IBD and RD virus.

(II) Farm which were positive for antibody to IBD virus but having no any history of IBD vaccination and maintaining regular vaccination practice against RD.

(III) Farms receiving poultry feeds grossly contaminated with aflatoxin to be determined on the basis of bright green yellow fluorescence (BGYF) test under UV light in UV cabinet.

105 serum samples from each of the selected farms in each of the three categories were subjected to HI test and the titres were noted and analyzed. On the basis of analysis, conclusion was drawn as to the levels of antibody against RD virus in order to evaluate the continuance of the conventional schedule of the RD vaccination in the area.

On the basis of above study it was also attempted to see the efficacy of known immunomodulators namely; Levamisole, vitamin E and selenium as well as a homoeopathic medicine Carbo veg. For this purpose one farm from each of the three categories was selected on the basis of having lowest HI titre to RD. One of the three drugs noted above was tried in each of the three selected farm. After administration of the drug again 21 serum samples from treated groups (7 from each treatment group) and 21 from control group (7 from each treatment group) were collected after

15 days of last dose of the drug and HI titre was determined. The data were analyzed and relative efficacy of the three drugs were seen in improving antibody level in field condition.

Body Weight gain and feed conversion ratio:-

The body weight gains of chickens were recorded by subtracting the initial live weight of chicks from the final weight at the end of experiment. Feed conversion ratio per bird at the end of experiment was determined as follows:-

$$\text{FCR} \quad \frac{\text{Total feed consumption of chickens (gm)}}{\text{Total weight gain of chickens (gm)}}$$

Statistical analysis:-

Mean values and their standard errors were calculated. The analysis of variance (ANOVA) was performed whenever necessary as per Snedecor and Cochran (1967).

Table - 1:- **Experimental Design**

Categories of farms	No. of poultry farms	Drug admin-istered in farms.	Name of drug	Dose & Route	Period of treatment	Observation planned
1. Farms having history of regular vaccination against both IBD and RD virus.	5	1	Charak-E-Sel	100mg./kg body wt.daily D.W*.	15	1.(a) HI titre of each categories of farms were determined. (b) One farm from each catego--ries was selected on the basis of having lowest HI titre and drugs like Charak- E-Sel, Carbo veg. and Levamisole were tried as immunomodulators. 2. HI titre were monitored after 15 days of drug schedule. Both body weight gain and FCR were determined at the end of experiment. 3. Relative efficacy of three drugs were compared
2. Farms seropositve to IBD virus but having no history of IBD vaccination.	5	1	Carbo veg.	20ml./100 chicken alternate day D.W.	15	
3. Farms receiving poultry feeds apparently contamin--ated with aflatoxin	5	1	Levamisole	10mg per kg body wt.daily D.W.	15	

* D.W. refers to drinking water

CHAPTER - IV

RESULTS

RESULTS

In an effort to study the HI antibody levels under natural conditions where the birds may be confronted with several types of visible or hidden/silent factors having immunodepression effect, a total of fifteen farms were selected. These farms represented three different categories - five farms in one category represented the group where regular IBD and RD vaccinations were in practice; in second category (which also included five farms) was placed such farms which were seropositve to IBD virus but without any history of clinical IBD, where as the five farms in the third category represented the group which were receiving feeds that were apparently contaminated with aflatoxin (as revealed by emission of typical fluorescence). In all one hundred five serum samples (thirty five from each of the category) were collected and subjected to determination of HI antibody level to RD virus. The results are shown in table- (2,3&4). The perusal of table-2 demonstrated the immunosuppressive effect of IBD vaccine as evidenced by wide variations in HI titre between the farms, the HI titre ranged between 3.42 ± 0.202 to 8.71 ± 0.184. Suggesting failure of proper immune response at least on three of the farms (F_1, F_2 & F_4), where the HI titre were below the protective level (2^4). Further, the perusal of table-3 indicated marked variations in HI titre between the farms, the HI titre ranged between 4.85 ± 0.340 to 6.85 ± 0.260. The findings presented in table-4 also reflected the earlier trend with HI titre ranged between 5.42 ± 0.202 to 7.71 ± 0.359.

The influence of Chark-E-Sel (a combination of vitamin E and selenium) on immune response to RD vaccine has been presented

in table–5. The findings are moderate rise in HI titre in the treated group when compared with the value in the control group. This drug also showed significant improvement in the body weight gain and FCR than the body weight gain and FCR in the control group (table–5).

Table-6 depicts the influence of homoeopathic medicine (Carbo veg.) on immune response to RD vaccine. The results are suggestive of the immunopotentiating effect of Carbo veg. as evidenced by higher level of HI titre to RD vaccine in treated group when compared with the corresponding value in the control group. The Carbo veg. also demonstrated it's effect has growth promoter as marked by higher body weight. gain and improved FCR when compared with the corresponding values in the control group.

A critical look on the table–7 revealed a HI titre of 8.28 ± 0.184 in levamisole treated group while the titre was only 4.85 ± 0.340 in untreated control group. However, this drug did not find its place as growth promoter as it failed to record any significant improvement either in body weight gain or FCR.

Table – 2 : HI antibody titre to RD virus in chicken of different farms in which IBD and RD vaccinations were regular features.

Farm/Flock	Type of chicken	No. of chicken	Age of chicken	Vaccination schedule & route	Mena ± S.E. of HI antibody titre log_2
F_1	Cockerel	55	48 days	F-strain (RD) in 1st week, i.n.* IBD (Bursa-B2K) on 14th day i.o**.	$3.42^a \pm 0.202$ (7)
F_2	Cockerel	80	50 days	F-strain (RD in 1st week, i.n. IBD (Georgia strain) on 20th day, i.o.	$3.57^a \pm 0.202$ (7)
F_3	Layer	100	81 days	F-strain (RD in 1st week, i.n. IBD (Georgia strain) on 14th day, i.o. R_2B (RD) at 2 month age, s/c***	$8.71^b \pm 0.184$ (7)
F_4	Broiler	85	19 days	F-strain (RD) in 1st week, i.n. IBD (Georgia strain) on 16th day, i.o.	$3.71^a \pm 0.285$ (7)
F_5	Broiler	75	23 days	F-strain (RD in 1st week, i.n. IBD (Georgia strain) on 14th day, i.o.	$4.42^c \pm 0.202$ (7)

* i.n. indicates intranasal route, ** i.o. indicates intraocular route, s/c *** indicates subcutaneous route.
- Figures in parenthesis indicate number of observation.
- Mean bearing common superscripts (a & b) in individual column did not differ significantly ($P<0.01$)

Table – 3 : HI antibody titre to RD virus in chicken of different IBD seropositive farms.

Farm /Flock	Type of chicken	No. of chicken	Age of chicken	Vaccination schedule & route	Mean ± S.E. of HI antibody titre log_2
F_1	Broiler	80	36 days	F-strain (RD in 1st week, i.n.*	$6.57^a \pm 0.202$ (7)
F_2	Broiler	90	46 days	F-strain (RD in 1st week, i.o.**	$6.85^a \pm 0.260$ (7)
F_3	Broiler	75	32 days	F-strain (RD in 1st week, i.n.	$5.71^b \pm 0.182$ (7)
F_4	Broiler	125	42 days	F-strain (RD in 1st week, i.n.	$6.42^a \pm 0.202$ (7)
F_5	Broiler	75	34 days	F-strain (RD in 1st week, i.n.	$4.85^c \pm 0.340$ (7)

* i.n. indicates intranasal route, ** i.o. indicates intraocular route

- Figures in parenthesis indicate number of observation
- Mean bearing common superscript (a, b & c) in individual column did not differ significantly ($P<0.01$).

Table – 4 : HI antibody titre to RD virus in chicken of different farms receiving feed suspected to aflatoxin contamination.

Farm/ flock	Type of chicken	No. of chicken	Age of chicken	Vaccination schedule & route	Storage facility	Fluorescence	Mean ± S.E. of HI antibody titre log_2
F_1	Layer	100	11 months	F-strain (RD) in1st week, i.n.* R_2B (RD at 2 month s/c***	Feed kept on humid condition & poor ventilation	BGYF showed highly contamination	$5.71^a \pm 0.184$ (7)
F_2	Broiler	65	36 days	F-strain (RD) in 1st week, i.n.*	Feed kept on humid condition & poor ventilation	BGYF showed highly contamination	$5.42^a \pm 0.202$ (7)
F_3	Layer	85	8 months	F-strain (RD) in 1st week, i.n. R_2 B (RD at 2 month, s/c	Feed kept on floor in ventilated room	BGYF showed low contamination	$7.71^b \pm 0.359$ (7)
F_4	Broiler	80	41 days	F-strain (RD) in 1st week, i.n.	Feed kept on floor in ventilated room	BGYF showed low contamination	$6.71^c \pm 0.285$ (7)
F_5	Broiler	85	45 days	F-strain (RD) in 1st week, i.n.	Feed kept on floor in ventilated room	BGYF showed low contamination	$6.85^c \pm 0.260$ (7)

* i.n. indicates intranasal route s/c *** indicates subcutaneous route

- Figures in parenthesis indicate number of observation
- Mean bearing common superscript (a, b & c) in individual column did not differ significantly ($p<0.01$)

Table – 5 : Effect of Charak-E-Sel on HI immune response to RD virus, body wt. gain and FCR.

Treatment	Type of chicken	Age of chicken	No. of chicken	Duration of treatment	Dose & route	Mean ± S.E. of HI antibody titre (log_2)	Mean ± S.E. of initial wt, Final wt. & body wt. gain			
							Initial wt. on 4th day (gm)	Final wt. on 67th day (gm)	Wt. gain on 67 day (gm)	FCR
Charak-E-Sel	Cockerel	52 days	25	15 days	100mg/kg b.wt. daily, D.W*.	$4.85^a \pm 0.260$ (7)	$45.60^a \pm 0.305$ (25)	$1077.00^a \pm 7.135$ (25)	$1031.40^a \pm 7.19$ (25)	$4.22^a \pm 0.029$ (25)
Control	Cockerel	52 days	25	-	-	$3.28^b \pm 0.184$ (7)	$45.12^a \pm 0.307$ (25)	$1022.40^b \pm 3.150$ (25)	$977.28^b \pm 3.12$ (25)	$4.31^b \pm 0.014$ (25)

* D.W. indicates drinking water

- Figures in parenthesis indicate number of observation.
- Mean bearing common superscript (a & b) in individual column did not differ significantly ($P<0.01$)

Table – 6 : Effect of Carbo veg. On HI immune response to RD virus, body wt. gain and FCR.

Treatment	Type of chicken	Age of chicken	No. of chicken	Duration	Dose & route	Mean ± S.E. of HI antibody titre log_2 on 55 days	Mean ± S.E. of initial wt. Final wt. & body wt. gain			FCR
							Initial wt. on 2nd day (gm)	Final wt. on 55 day of age (gm)	Wt. gain (gm)	
Carbo veg.	Broiler	40 days	44	15 days	20ml/100 birds alternate day, D.W*.	$6.75^a \pm 0.368$ (7)	$42.02^a \pm 0.190$ (44)	$1601.59^a \pm 14.19$ (44)	$1559.57^a \pm 14.21$ (44)	$2.31^a \pm 0.021$ (44)
Control	Broiler	40 days	25	-	-	$4.71^b \pm 0.285$ (7)	$41.68^a \pm 0.213$ (25)	$1520.00^b \pm 19.74$ (25)	$1478.32^b \pm 19.77$ (25)	$2.42^b \pm 0.032$ (25)

* D.W. indicates drinking water
- Figures in parenthesis indicate number of observation.
- Mean bearing common superscript (a & b) in individual column did not differ significantly ($P<0.01$)

Table – 7 : Effect of Levamisole to HI immune response to RD virus, body wt. gain and FCR.

Treatment	Type of chicken	Age of chicken	No. of chicken	Duration	Dose & route	Mean ± S.E. of HI antibody titre log_2 on 57 days	Mean ± S.E. of initial wt. Final wt. & body wt. gain			FCR
							Initial wt. on 2nd day (gm)	Final wt. on 57 day of age (gm)	Wt. gain (gm)	
Levamisole	Broiler	42 days	35	15 days	10mg/kg body wt. daily, D.W*.	$8.28^a \pm 0.184$ (7)	$39.51^a \pm 0.250$ (35)	$1059.71^a \pm 15.91$ (35)	$1020.20^a \pm 15.80$ (35)	$2.60^a \pm 0.04$ (35)
Control	Broiler	42 days	25	-	-	$4.85^b \pm 0.340$ (7)	$39.40^a \pm 0.305$ (25)	$1051.60^a \pm 16.53$ (25)	$1012.20^a \pm 16.54$ (25)	$2.62^a \pm 0.04$ (25)

* D.W. indicates drinking water

- Figures in parenthesis indicate number of observation.
- Mean bearing common superscript (a & b) in individual column did not differ significantly ($P<0.01$)

Fig-1, Bar - Diagram showing HI antibody titre to RD virus in chicken of different farms in which IBD and RD vaccinations were regular features

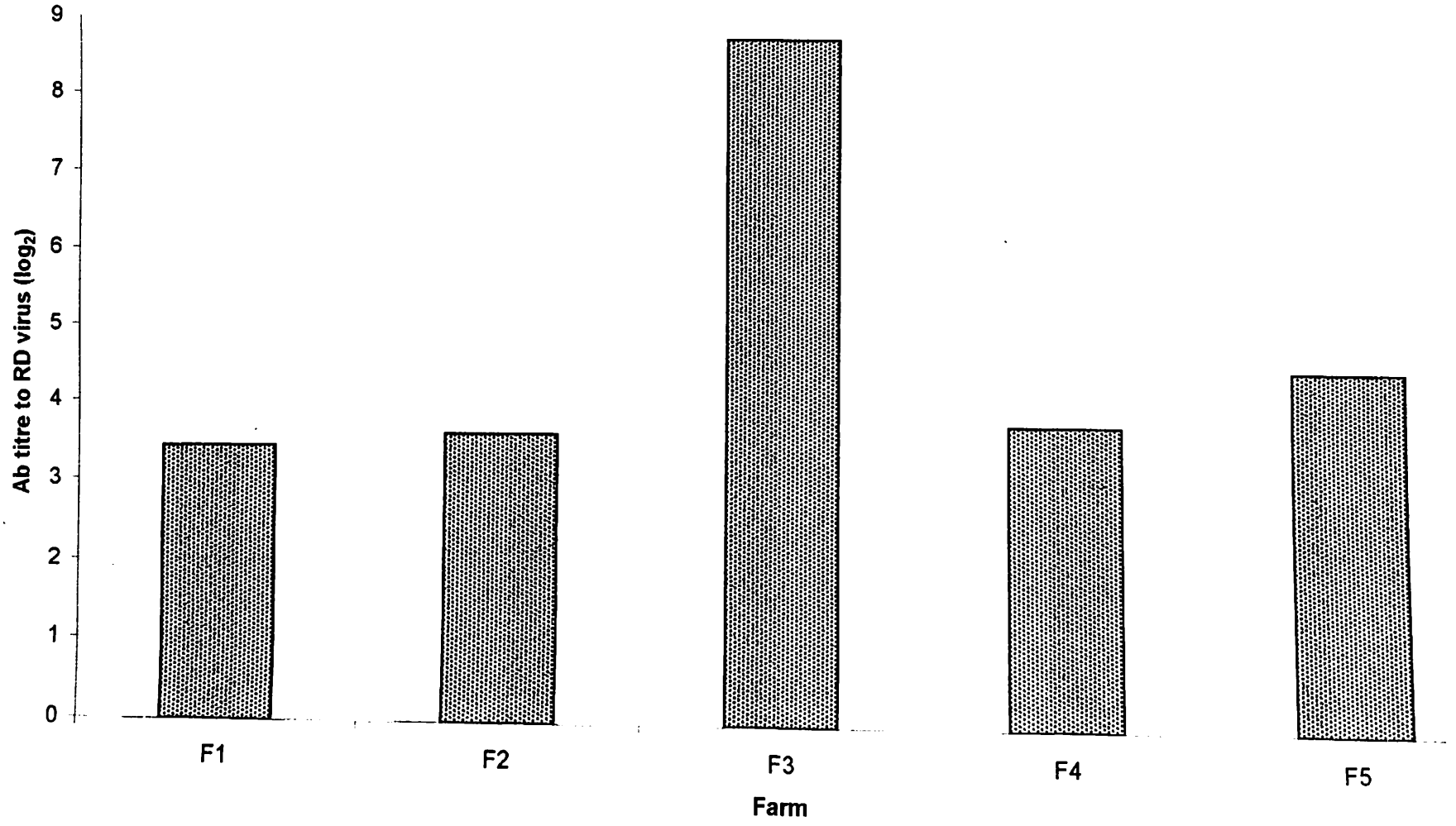


Fig-2, Bar - Diagram showing HI antibody titre to RD virus in chicken of different IBD seropositive farms.

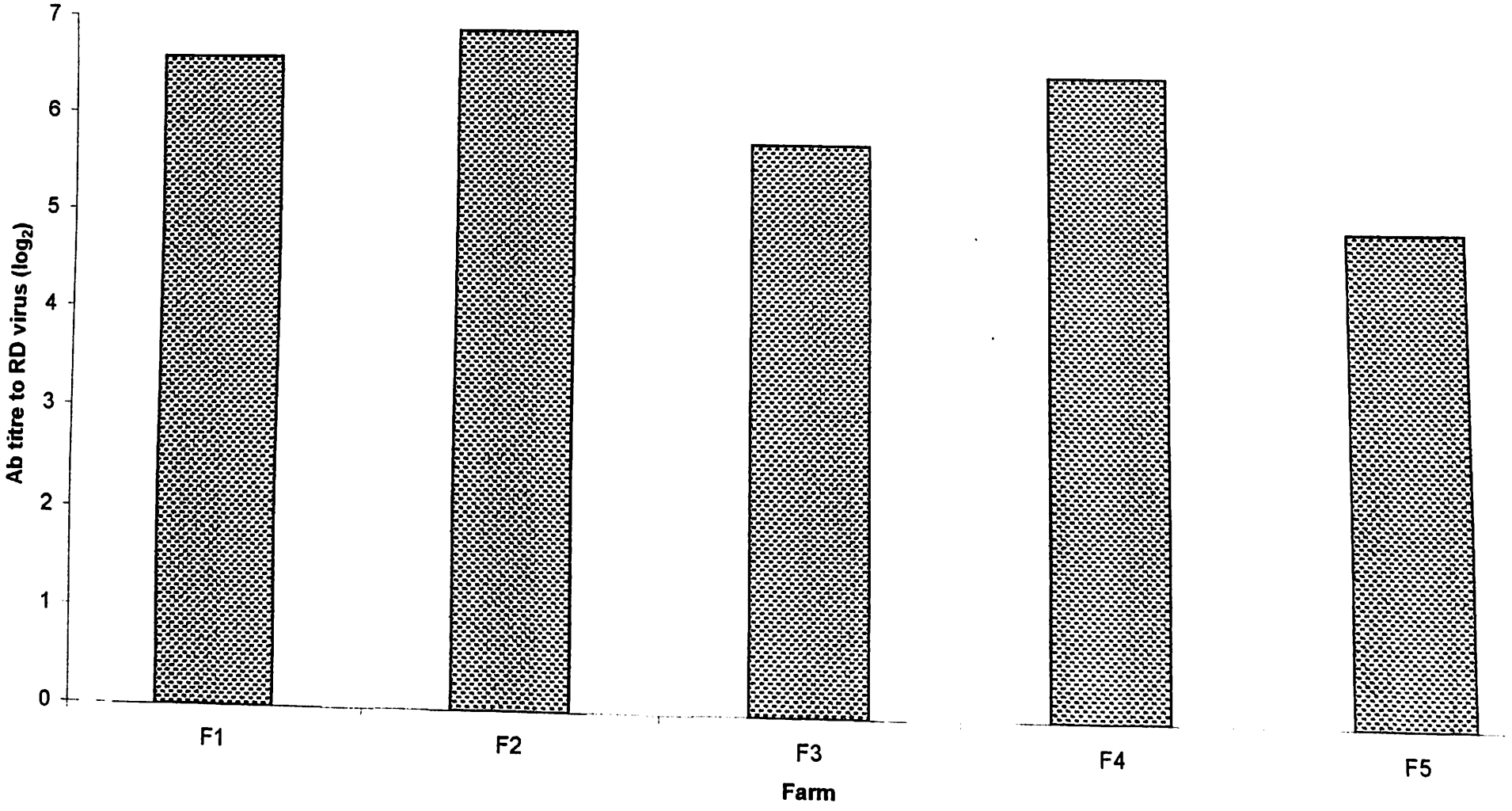


Fig-3. Bar - Diagram showing HI antibody titre to RD virus in chicken of different farms receiving feed suspected for aflatoxin contamination.

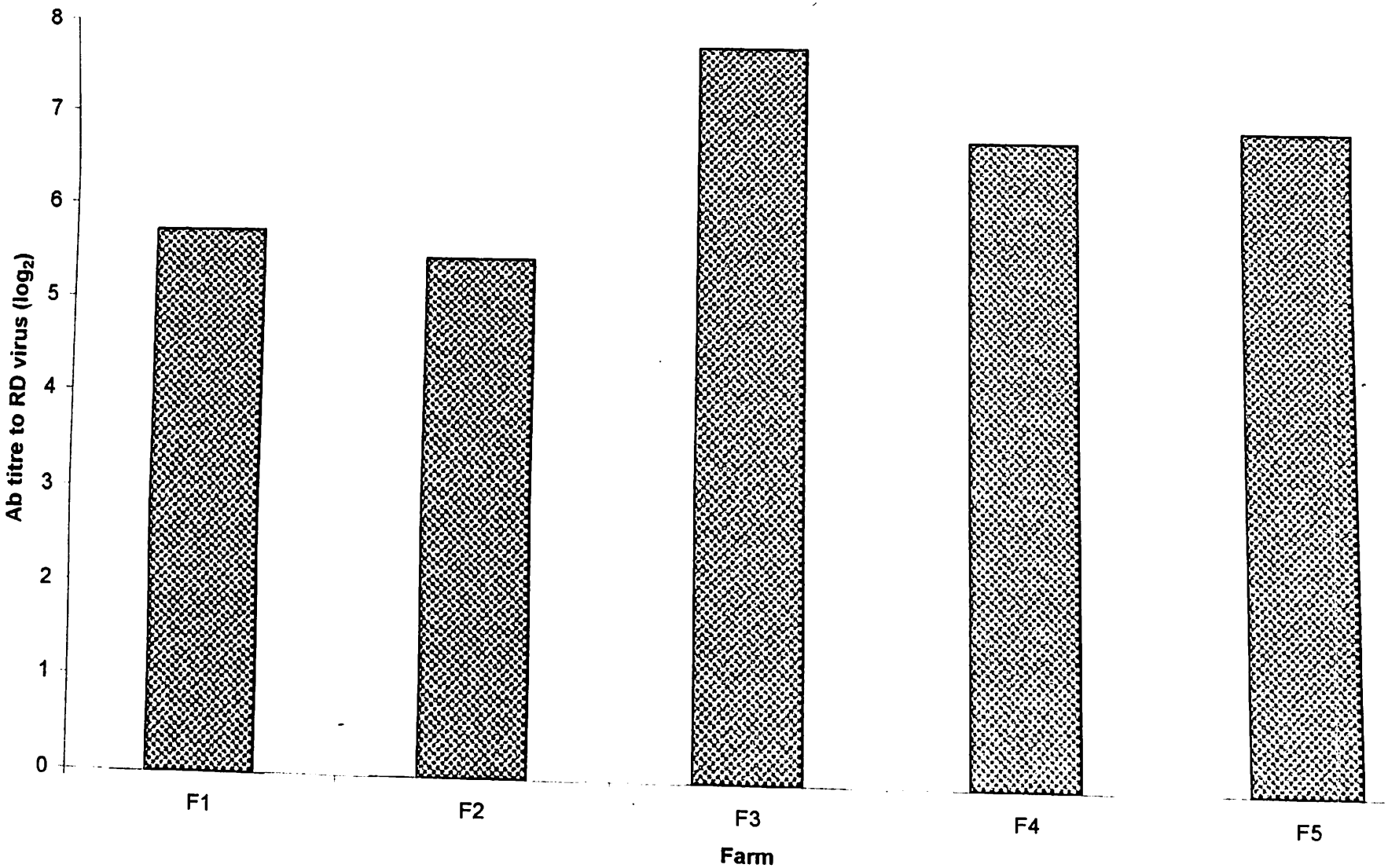


Fig-4. Bar-Diagram showing enhancement of Ab titre to RD virus in chicken of different treatment groups.

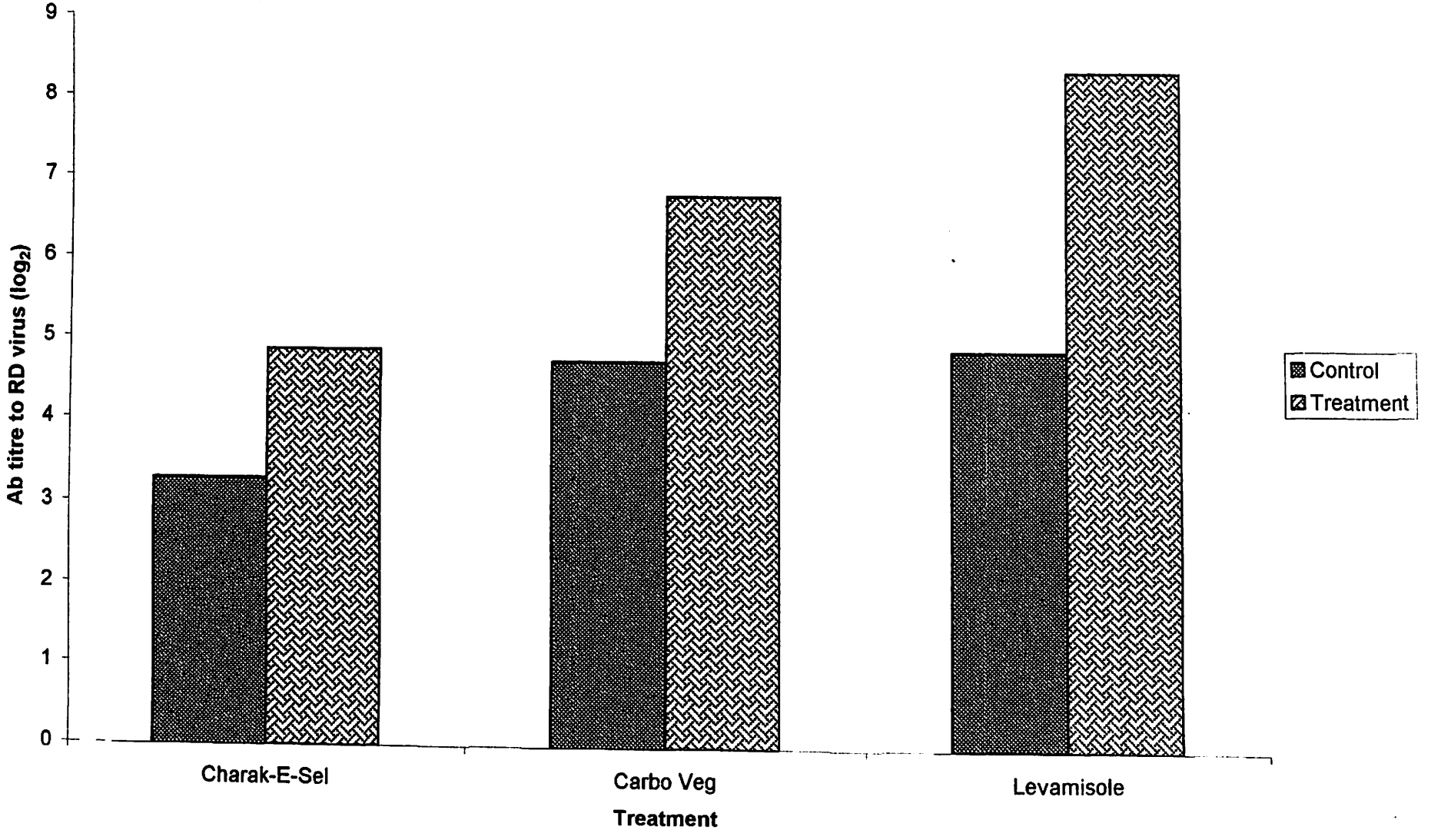


Fig-5. Bar-Diagram showing body wt. gain of chicken of different treatment groups.

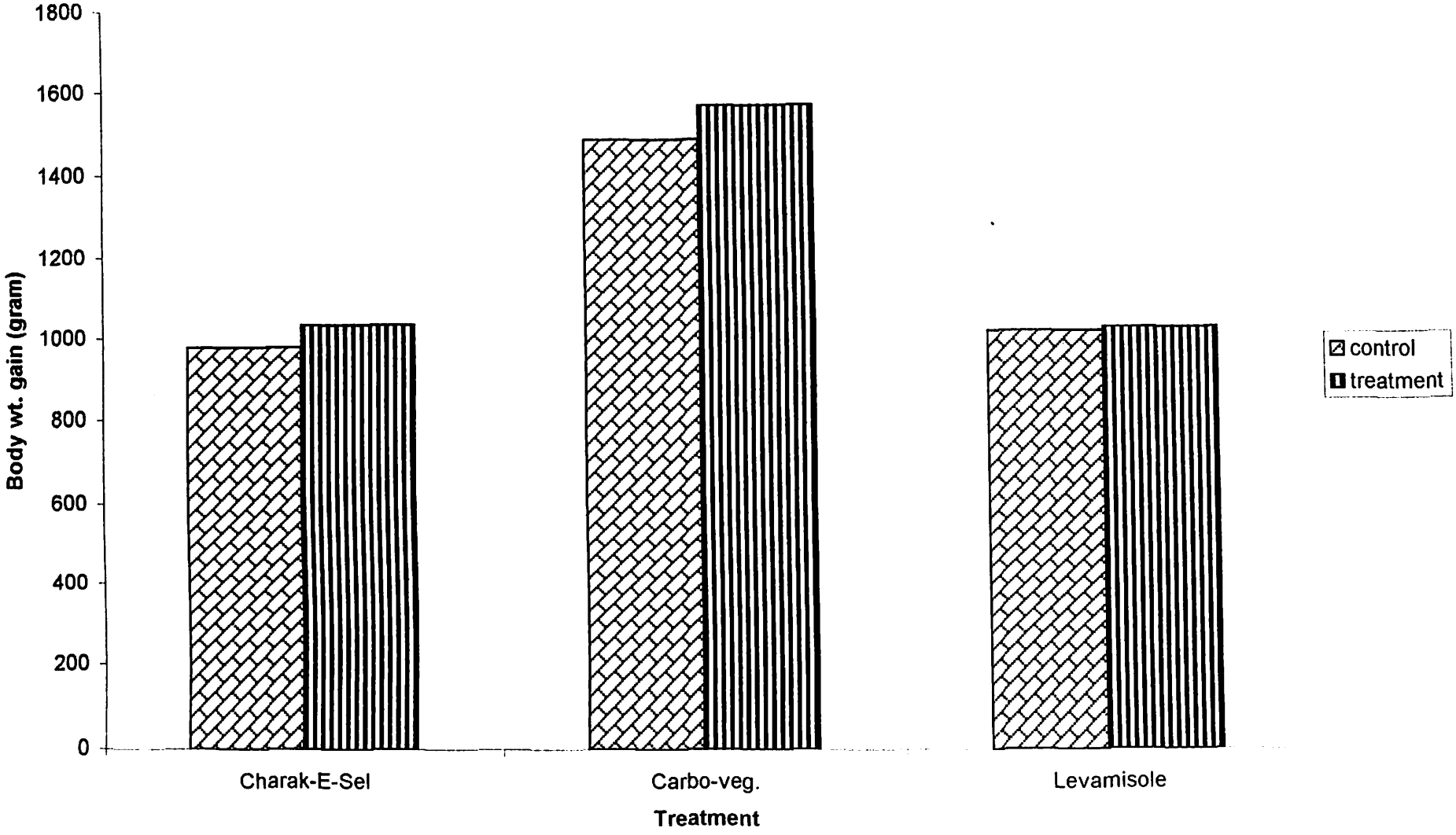
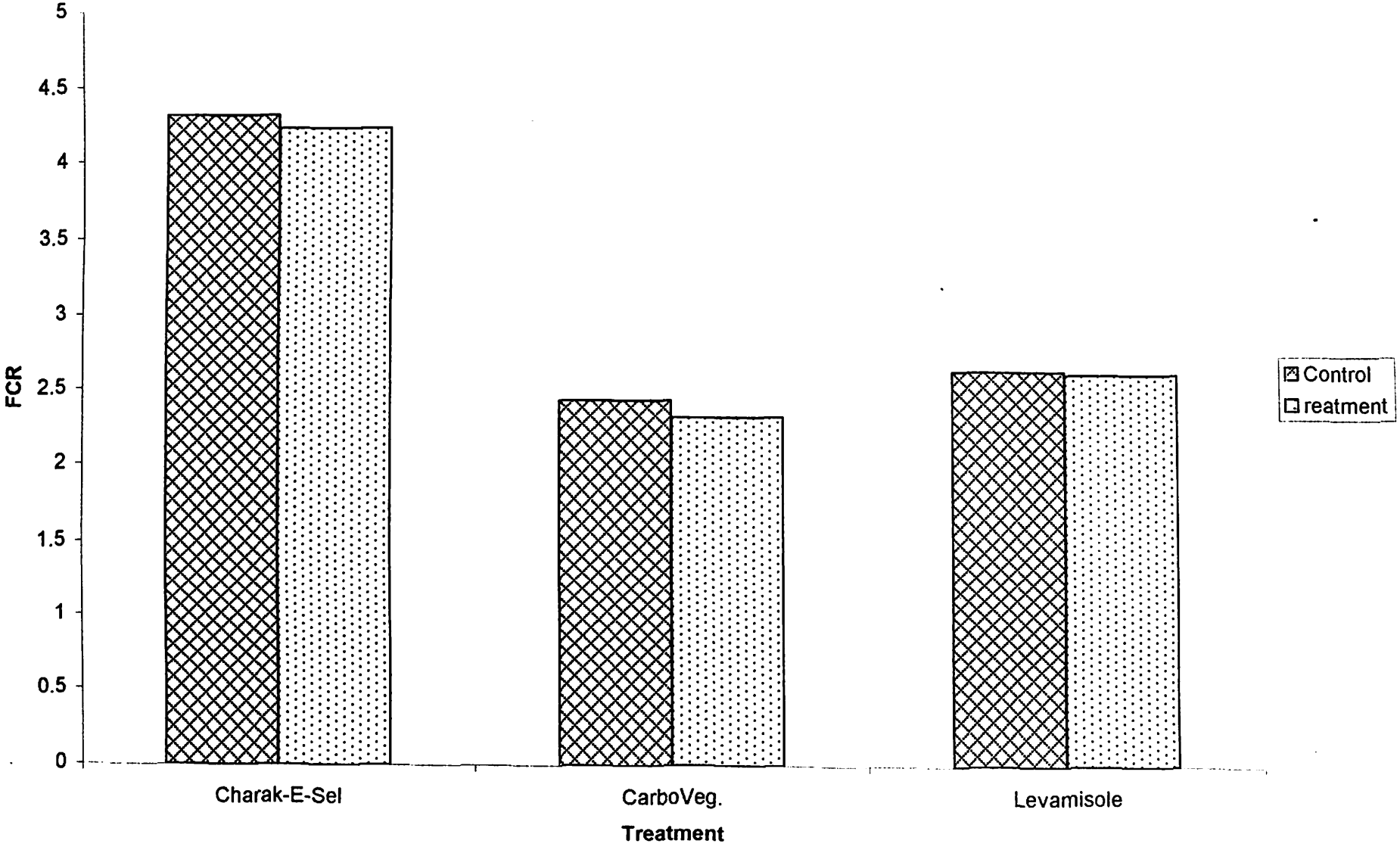


Fig-6. Bar-Diagram showing FCR of chicken of different treatment groups.



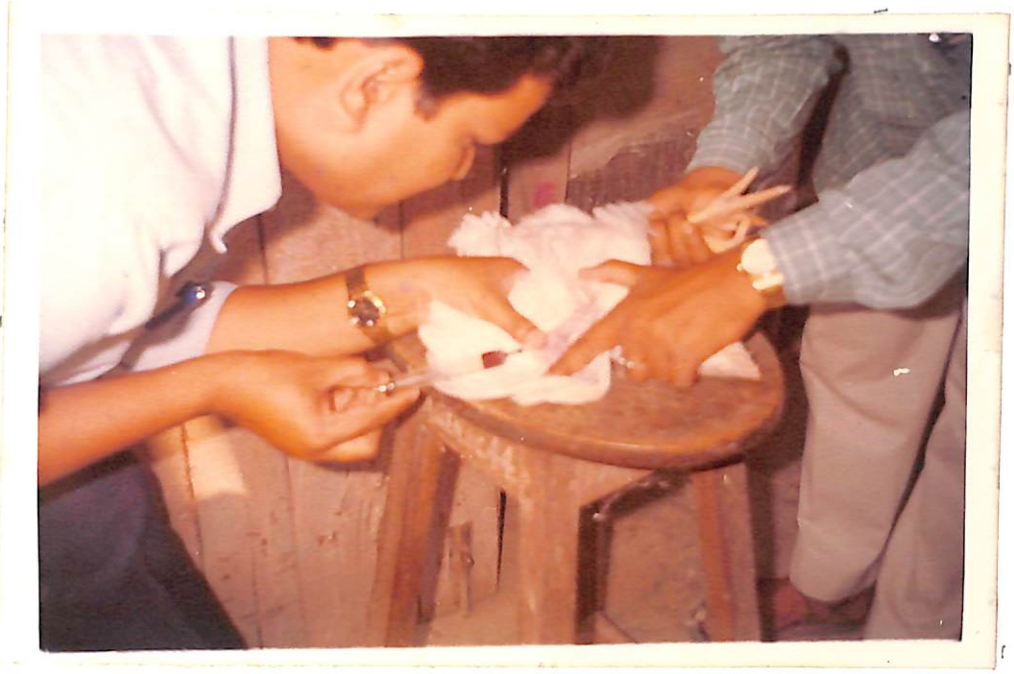


Fig.–7 Photograph of procedure of collection of blood sample from chicken.

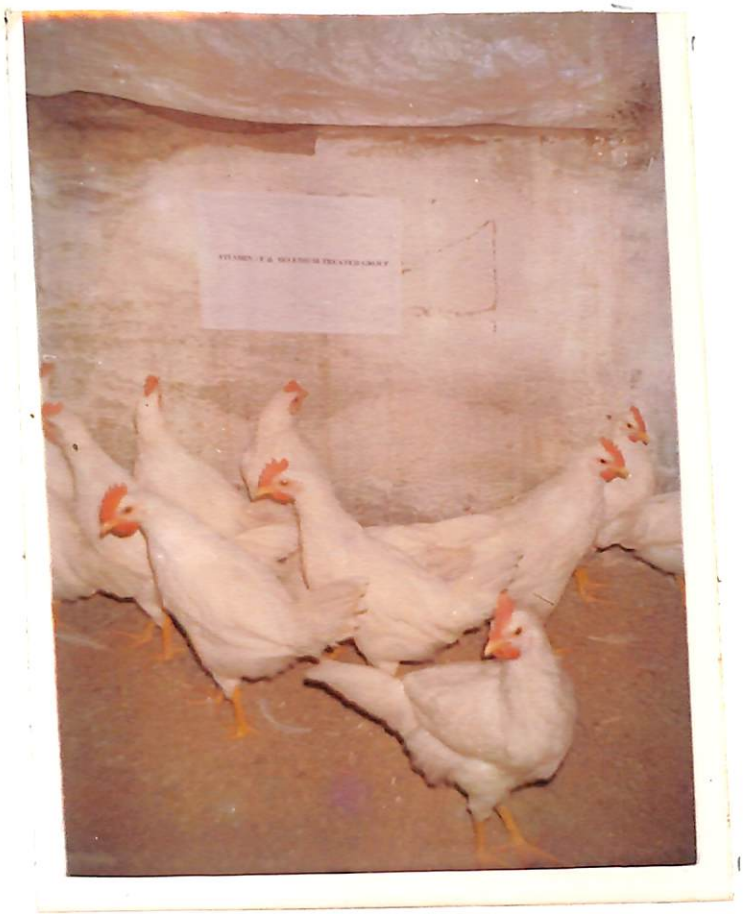


Fig.–8 (a) Photograph of chicken of Charak-E-Sel treated group.

Fig.–8 (b) Photograph of chicken of Charak-E-Sel untreated group (control group).

Fig.–9 (a) Photograph of chicken of levamisole treatment group.

Fig.–9 (b) Photograph of chicken of levamisole untreated group (control group).

CHAPTER - V

DISCUSSION

DISCUSSION

Ranikhet disease is a highly contagious viral infection of chicken of all ages caused by a single stranded RNA virus belonging to the family paramyxoviridae. The disease is widely prevalent in this country and was considered to be a number one killer disease before the development of effective vaccines. After discovery of potent vaccines against RD and strategy for the containment of this deadly infection, it became possible to control the economic losses due to this virus infection. This disease remained successfully controlled and out of scene for many years. However, in recent past this disease has started raising its ugly head due to impaired immune responses to RD vaccines. A number of factors have been attributed to be the cause of lowered responses to available RD vaccines and immunosuppression caused by infectious bursal disease virus has been found to be a commonly encountered cause of the failure of proper immune responses to RD vaccines (Allan *et al.*, 1972; Hirai *et al.*, 1974; Rao *et al.*, 1978; Jhala *et al.*, 1990; Rao and Rao, 1992; Panda and Rao, 1993; Vijaya Praveen *et al.*, 1995; Das *et al.*, 1996; Prabhakaran *et al.*, 1997; and Christopher *et al.*, 1997). The wide spread incidence of IBD virus infections have been found to be followed by outbreaks of RD in the poultry farms in the different parts of the country as well as in the state of Bihar in particular (Rathore *et al.*, 1987; Verma *et al.*, 1981 and Ajinkya *et al.*, 1980). The preliminary study conducted in such outbreaks invariably revealed absence of protective level of HI titre to RD virus (Rathore *et al.*, 1987). Further aflatoxin has been considered as an another immunosuppressive agent commonly reported from different farms

(Thaxton *et al.*, 1974; Viridi *et al.*, 1989; Pier *et al.*, 1971; Ozer *et al.*, 1989; Mohiuddin *et al.*, 1981; Jassar and Singh, 1989; Ghosh and Chauhan, 1991; Bakhsi *et al.*, 2000 and Boulton *et al.*, 1980). It is in this backdrop that it was considered necessary to undertake study to measure the levels of HI antibody against Ranikhet disease as it may happen under natural condition due to the onslaughts of commonly occuring immunodepressents such as IBDV and aflatoxin.

A total of 105 serum samples were collected from the different flocks/farms under three defined categories such as i) farms having history of regular IBD vaccination, ii) farms having seropositive reactor to IBD and iii) farms receiving feeds suspected for aflatoxin contamination. These serum samples consisted of thirtyfive samples collected from each of three categories of flocks as mentioned above. Further, all such serum samples were subjected to HI test for determination of the levels of antibody to RD virus. A number of workers have employed HI test to determine the levels of HI antibody to RD virus (Allan and Gough, 1974 and Beard, 1980). Further HI antibody titre so determined is equivalent to the protective antibody level and in this respect this test (HI test) is invariably used to know the protective level of antibody on the farms (Allan *et al.* 1978; Reetha *et al.*, 2000 and Rathore *et al.*, 1987).

The thirty five serum samples collected from five different flocks in the first categories of farms (i.e farms with history of IBD vaccination) revealed HI antibody titres ranged between 3.42 ± 0.202 to 8.71 ± 0.184 and the lowest HI titre of 3.42 ± 0.202 was demonsterable in F_1 flock (table-2). Therefore, this flock was selected for treatment with a

known immunomodulator namely Charak-E-Sel (a combination of vitamin E and selenium). Interestingly out of the five farms included in this study three (F_1, F_2, & F_4) demonstrated HI titres below protective level (2^4) and the fourth farm (F_5) revealed HI titre which was just above the protective level suggesting the marked immunosuppressive effect of IBD vaccine on these farms. A larger number of workers have demonstrated the immunosuppressive effect of IBD vaccines on immune response to RD vaccines (Mazariegos *et al.*, 1990; Mahesh and Muniyappa, 1996 and Ezeokoli *et al.*, 1990). Further, the findings also revealed that three such farms (F_2, F_4, & F_5) had received Georgia strain of IBD vaccines between 14 - 20 day of age whereas one of the farm (F_1) had been given B2K vaccine on the 14th day of age. Since Georgia strain of IBD vaccine represents intermediate strain of IBD virus, there is nothing unusual if there is impaired immune responses to RD vaccine and as such intermediate strains have been reported to possess residual pathogenicity and immunosuppressive effect (Mazariegos *et al.*, 1990; Mahesh and Muniyappa, 1996; Winterfield and Thacker, 1978; Khaliel and El-Manakhly, 1998). On the other hand, the B2K IBD vaccines represent intermediate plus strain of IBD virus, which normally possess relatively more residual pathogenicity and immunosuppressive effects than intermediate strain vaccines. The manifestation of lowest level of HI titre on F_1 flock is understandable (table-2). However, a very satisfactory level of HI antibody titre to RD virus recorded on F_3 farm which also received Georgia strain of IBD vaccine may be explained on the ground that the birds on this farm had been given R_2B vaccine at 2 months of age in

addition to the primary vaccination with F-strain RD vaccine in the first week of age and hence it is but natural that the birds on this farm showed higher HI titre due to booster effect/secondary immune response. It may also be mentioned that the above mentioned investigation on this farm was conducted in layer birds at 81days of age and hence the effect of age might have also contributed in enhancement of antibody titre.

The immune responses to F-strain RD vaccines in farms which were positive for antibody to IBD virus suggesting prior exposure to IBD virus showed HI titres between 4.85 ± 0.340 to 6.85 ± 0.260, (table-3). The analysis of the result revealed the presence of HI antibody level above protective level on all the five farms surveyed but the titre was lowest and just above border line in case of F_5 and hence this farm was included for the application of immunomodulator. Though, there are several factors which affects the level of antibody such as type of birds, age, nutritional status, exposure to different type of stress factors and strain of virus. But in the present case the time of exposure of birds as well as the degree of multiplicity of infection appeared to be the main cause for variation in antibody titre on different farms (table-3) as there were no clearcut history of time of exposure of birds on these farms. Besides, the outbreaks of IBD were reported to be occuring in the nearby places surrounding these farms. There are number of reports that IBD virus is more immunosuppressive when younger birds below 2 weeks of age are exposed to the virus (Vijaya Parveen *et al.*, 1995 and Kumar *et al.*, 2002). The findings in respect of HI antibody titres to RD vaccines in birds receiving feeds suspected to be contaminated with aflatoxin (table-4) demonstrated HI titres ranging

between 5.42 ± 0.202 to 7.71 ± 0.359. It may be made clear that the assessment in respect of aflatoxin contamination of feed was based on gross appearance of feeds provided to these birds, storage condition, humidity, ventilation and emission of typical fluorescence when the feed samples from these lots were screened in UV light. Since samples from such feeds were not subjected to extraction of aflatoxin and the criteria considered for the presence of aflatoxin as noted above only give tentative idea about the presence of aflatoxin in the feeds. The variation in HI titres as recorded in the present study may be explained on above accounts. The immunosuppressive effect of aflatoxin is well documented (Thaxton *et al.*, 1974 and Bakshi *et al.*, 2000). Further, the immunosuppression brought about by aflatoxin is largely due to direct inhibition of protein synthesis including those with specific function such as immunoglobulins. In addition, aflatoxin is immunosuppressive also by way of its inhibitory effect on degradation of antigen by reticuloendothelial system, increased degradation of antibodies/immunoglobulins by lysosomal enzymes in liver. Besides, several other effects which could ultimately lead to some degree of immune suppression. There are also reported that the level of aflatoxin present in the feed also determines the degree of immunosuppressive effect (Jassar and Singh, 1989; Mani *et al.*, 2000 and Bakshi *et al.*, 2000) and hence the variation in the titre in different farms may be the reflection of the same. Further, the HI antibody level in birds on farm F_3 was 7.71 $\log_2$ followed by 6.85 $\log_2$ (F_5) and 6.71$\log_2$ on farm F_4 and these levels may be considered satisfactory but it would not be prudent to draw specific conclusion unless the feeds are subjected to aflatoxin extraction and

determination of its level. On the other hand HI titres prevalent on the remaining two farms (F_1 & F_2), although above protective level but need further enhancement in order that the birds are kept on highly safer side to counteract the different factors likely to be encountered by these birds and have immunosuppressive effect. Therefore the farm showing the lowest HI titre of 5.42 $\log_2$ (F_2) was selected for application of immunomodulator.

The comparison of HI antibody titre in three categories of farms (table - 2, 3, & 4) revealed wide variations in HI titre suggesting the involvement of several immunosuppressive agents which have gained an alarming proportion and warrant immediate attention of all those involved in poultry business. In a situation where large number of such immunosuppressive factors may be encountered and when several such factors operate concurrently the antibody titres are bound to be reduced to a level, which may render the birds susceptible to RD infection. A number of reports are available which suggest the involvement of various immunodepressent like virus, mycotoxins, climatic conditions, stress, nutritional status, use of drugs specially corticosteroids and antibiotics (Chawak *et al.*, 1993; Panigrahy *et al.*, 1979; Saran and Sharma, 1996 and Ross *et al.*, 2003). In a situation like this where the birds are likely to be confronted with such immunosuppressive factors, it would be wise to consider the inclusion of known and established immune enhancing agents in the schedule of practices to be recommended for running poultry farms as a matter of profit earning venture. Several workers have demonstrated the immune enhancing effect of a number of agents and their application (Kolte *et al.*, 1999; Kalita and Dutta, 1999; Sadekar *et al.*, 1998a; Sadekr *et*

al., 1998b; Dutta *et al.*, 1992 and Saravanabava *et al.*, 2001). A good number of immune enhancers have been studied in poultry also (Shadaksharappa *et al.*, 1998; Rao *et al.*, 1996; Kurtoglu and Nizamlioglu, 1996; Franchini *et al.*, 1995; Chatterjee *et al.*, 1994; Pande and Vijay, 1994; Karnatak *et al.*, 1993; Reddy *et al.*, 1989 and Satturwar *et al.*, 2002). Accordingly three immunopotentiators/growth promoters namely; (i) Charak-E-Sel (a combination of vit. E & selenium) (ii) Carbo veg. (a homoeopathic medicine) and(iii) Lemasol-P (levamisole hydrochloride) were selected to see its effects in birds of the farm which showed lowest HI titre in the particular category.

Many workers have reported that vitamin E as antioxident and prevents the free radicals (peroxides and superoxides) released during disease or vaccinal challenge from the damaged cellular and intercellular structures, which also includes lymphocytic cells of immune system. Whereas, vitamin E is stored in the lipoprotein fraction of cell membrane, selenium forms an integral component of enzyme glutathione peroxides, which is present in cytosol of all cells. The toxicity of free radical to cells is mainly because they attack unsaturated fatty acid component of membrane lipid, thus damaging membrane structure. Vitamin E in the cell membrane and selenium containing enzyme in the cytosol, glutathione peroxidase form vital part of biological antioxidant system in the cells. Vitamin E being the component of cell membrane acts as an efficient scavenger of free radicals and selenium in co-ordination with glutathione peroxidase present in cytosol convert free radicals to inert substances rendering them harmless. Recently both vitamin E and selenium have been found to be responsible

for erythrocyte membrane integrity, since both erythrocytes and lymphoid cells originate from common stem cells, vitamin E and selenium may be associated with membrane fluidity of lymphoid cells, thus affecting immune response mechanism as well. Further, vitamin E stimulate IgG synthesis and selenium promotes the increased synthesis of IgM antibody. Interestingly the level of vitamin E and selenium to be included in the diet for above purpose should be ten to thirty times the recommended dietary level. There are reports that the action of vitamin E is dose dependent and the immunomodulatory effect can be best appreciated when it is given in the amount several times higher than the dietary requirements i.e. 48 IU/kg feed (Tengerdy and Brown, 1977 and Mcllory *et al.*, 1993). In addition vitamin E increases humoral immunity in chicken, turkey and mammal and also increases phagocytosis probably by regulating the biosynthesis of prostaglandin, and their effect on functional activity and proliferative capacity of immune system cells such as B and T lymphocytes, macrophages, polymorpho-nucleated dendritic and plasma cells (Franchini *et al.*, 1995). Again the E type prostaglandin is known to affect immune response Likoff *et al.*, (1978) and supplementation of vitamin E reduces the prostaglandin level in immunopoietic organs and simultaneously improve antibody responses. Therefore, it is obvious that vitamin E has two folds action, first by way of its antioxidant property, thus preventing the release of free radicals and the second by inhibiting the production of prostaglandin E, thereby enhancing humoral immune response. On other hand selenium as a constituent of performed free radicals inert, thus making it harmless.

In the present study efforts were made to study the enhancement of immune response to RD vaccines and other economic parameters after administration of Charak-E-Sel. The result demonstrated that it has potentiating effect on humoral immune response to RD vaccine as evident from higher antibody titre in Charak–E–Sel treated group vis-a-vis titre recorded in control group/untreated group (table-5 & fig.4). Further, the immune enhancing effect of the Charak-E-Sel was of moderate degree only raising antibody level in treated group by 1.5 (log_2) when compared with the corresponding value of HI titre in an untreated control group (table-5 & fig.-4). Though after administration of Charak-E-Sel the HI titre was brought to above protective level of 2^4. even this titre may not be considered safe when there is high multiplicity of infection and outbreaks of RD are occuring in the surrounding locations. Therefore, this drug may be kept in waiting when better drugs are available. Further, this drug has resulted in improvement in body weight gain as evident from the fact that the body weight gain in the treated group was 1031.40 ± 7.19 where as it was only 977.28 ± 3.12 in untreated control group (table-5 & fig.-4). This drug has also led to significant improvement in FCR which is another economic parameter to be considered while evaluating any agent. Since the study in respect of Charak-E-Sel was conducted in cockerel it has to be seen whether similar effects are produced in other types of birds such as broilers and layers. The present findings are in agreement with the observations of (Aravind *et al.*, 2001 and Kujur, 2001).

Homoeopathic medicines have occupied important position in the treatment of various alignments. The applications of this group of

medicine have been advocated to cure various conditions in poultry (Patra, 1983; Bera, 1983; Jagtap *et al.*, 1993 and Samarth *et al.*, 2002). Presently a homoeopathic drug has been employed as immune potentiator, growth enhancer, and antistressor (Patra, 1983 and Jagtap *et al.*, 1993). Though the exact mechanism of action of most of the homoeopathic drugs are yet to be established, it was logical to believe that the homoeopathic drugs with indications of improving general vitality, countering stresses and ability to promote growth should work through a mechanism which may also involve immunological component and defense system of the body. It was in this background that the Carbo veg. was considered for use as immune enhancer, earlier occasion also this drug was employed to study its effect as immune potentiator (Hindustani, 2000 and Kumar, 2000). This drug has established its role as immunomodulator as well as growth enhancer (Kumar, 2000). In the present study also the Carbo veg. demonstrated enhancing effect on immune response to RD vaccines (table-6 & fig.-4), which is incomplete agreement with earlier observations (Kumar, 2000). Further, the HI titre in Carbo veg. treated group was 6.75 $\pm$ 0.368 while it was only 4.71 $\pm$ 0.285 in untreated group (table-6) indicating an increase of HI antibody level by 2 $\log_2$ which gives sufficient margin of safety when the titre is compared with the protective HI titre to RD vaccine (i.e 2^4). There is general concensus that a good immune enhancer should also show encouraging effect on various economic parameters such as body weight gain and FCR. The present findings also suggested the positive effect of this drug on these two accounts. The perusal of the table-6 revealed a body weight gain of 1559.57 $\pm$ 14.21 in Carbo veg. treated group when compared

with the corresponding value in the control group 1478.32 ± 19.77 interestingly, earlier worker has also found Carbo veg. as very good growth promoter along with its positive effect on humoral immune response (Kumar, 2000). They also recommended the use of Carbo veg. in place of various synthetic and herbal preparations already available in the market because they found that Carbo veg. have been able to sustain antibody level above protective titre thereby removing the fear from the minds of the poultry farmers about the imminent risk due to RD virus infection and has proved to be very good growth enhancer, a parameter considered highly desirable for profitable poultry farming. Interestingly, this drug also shows significant improvement. in FCR as compared to its corresponding control value (table-6). Therefore, this drug has emerged as a potential immune enhancer, growth promoter, adaptogenic, antistressor besides being eco-friendly and hence recommended for its use on farms where there is impaired immune responses to vaccines, loss in body weight gain and poor FCR. Since the protocol to study the mechanism of action of homoeopathic medicine is wanting, it will be advisable to keep this option open till the time the procedure for such study becomes available. It is also possible to accept the finding as such in view of the wide application and proven track record of homoeopathic medicines.

Levamisole is a broad spectrum anthelmintic commonly used in veterinary and human medicine. In addition, it has been widely used as an immunopotentiating agent in human, livestock and poultry to enhance the vaccinal response (Shadaksharappa *et al.*, 1998; Kalita & Dutta, 1999; Babuik *et al.*, 1981; Vyas *et al.*, 1987; Hassan *et al.*, 1989; Irwin *et al.*,

1976 and Brunner and Muscoplat, 1980). In the present study levamisole treated birds (table-7 & fig-4) exhibited comparatively higher antibody titre than titre recorded in control group. Several workers have reported the application of levamisole in immunosuppressed chicken and also reported enhancement of immune response in treated groups Shadaksharappa *et al.*, (1998) and Mohanty *et al.*, (2000). It may be mentioned that in most of the cases levamisole is used in livestock and poultry as an immunomodulator to enhance vaccinal responses in immunocompromised animals (Ross *et al.*, 2003 and Panigrahy *et al.*, 1978). Whereas another group of workers reported the rise in humoral response only in healthy birds and not in aflatoxin immunosuppressed birds (Kalorey *et al.*, 1997; Kulkarni *et al.*, 1973 and Vyas *et al.*, 1987). In the present study levamisole led to enhancement of HI titre to RD vaccine in birds which exhibited relatively very low HI titre to RD virus apparently due to the immunosuppressive effect of aflatoxin. Mani *et al.*, (2001) also reported enhancement of immune response in aflatoxin treated birds, which supports the present findings. Hence, further work taking into account the concurrent effect of two or more immunosuppressive against may be helpful in drawing clear-cut conclusion as to the influence of levamisole in aflatoxin treated birds. Till such time the present observation which have the support of a several workers may hold true and can be cited for all practical purposes. Further, the effect of the levamisole at least on two economic parameters such as body weight gain and FCR in experimental birds revealed no significant improvement which may not be considered as a good sign for inclusion of levamisole in routine schedule of poultry farming in spite of the fact that it

has enhancing effect on HI titre to RD vaccine. It is well known that levamisole is also used as anthelmintic at higher doses therefore the dose of this medicine may have significant bearing on various biological effects. Some workers have recorded low effect of levamisole on body weight gain and FCR (Mani *et al.*, 2001 and Chawak *et al.*, 1993) and hence the present findings are in agreement with these workers. Besides levamisole being a synthetic product may have several side effects, which may not be helpful in proper body weight, gain and feed utilization. In addition this drug is not eco-friendly and its cost effectiveness will have to be evaluated before considering its application in the present perspective.

The general impression gathered from this study reflected the different situations under which the various consequence of immunosuppression may be perceptible. In fact during the present study, it has come to the surface that there can be failure of proper immune response to RD vaccines at least under three condition as observed in the present study such (i) lowered HI titre due to immunosuppressive effect of IBD vaccines itself on farms where it is included in the regular schedule of vaccination; (ii) reduced HI titre to RD vaccines due to the after effect of clinical or sub-clinical IBD which is highly likely on farms where the birds are seroreactor to IBD virus and lastly (iii) failure of optimal response to RD vaccines due to suppressive effect of aflatoxin contained in feeds provided to birds as noticed in the farms where the birds are receiving aflatoxin suspected feeds. The present findings are pointer to the fact that in a present scenario where birds are likely to be confronted with any one or more of the prevalent immunosuppressive factors it is rather difficult to

expect proper immune response to any vaccine and there is necessity to consider the incorporation of some effective immunopotentiators which may contribute in restoring optimum vaccinal response as well as ensure proper body weight gain and improvement in FCR. Alternatively, it is also possible to include immunomodulator having enhancing effect on antibody level along with a growth promoter, which may help in proper body weight gain and lowered FCR. In the present study levamisole proved to be very effective enhancer of humoral immune response but it needs supplementation with a suitable growth promoter to augment body weight gain and FCR, the two important economic parameters for profitable poultry raising. The perusal of the result with respect to homoeopathic medicine, Carbo veg. provides very encouraging picture in respect of it's influence on enhancement of HI antibody level as well as in promoting growth rate and improved the FCR. The earlier study conducted in this laboratory on Carbo veg. have yielded almost similar results (Kumar, 2000) and hence the Carbo veg. may be recommended for such farms where there is risk of obtaining proper vaccinal response due to onslaught of commonly available immunosuppressive factors in the surroundings and also where loss in body weight gain and poor FCR are regular feature. The present recommendation of Carbo veg. is also based on its cost effectiveness, availability, minimum side effect and mode of administration.

CHAPTER - VI

SUMMARY

SUMMARY

The poultry farming in the state of Bihar is confronted with the twin problems of breakdown in protective antibody level to RD vaccine on one hand and continuous exposure of the birds to the risk of various immunosuppressive agents operating singly or in combination. It is in this contest the present study was planned to monitor HI antibody responses in three categories of farms such as (i) Farms receiving IBD vaccines known to possess inherent residual pathogenicity and immunosuppressive effect. (ii) Farms which are known seroreactors to IBD virus which ultimately reflex the exposure of these birds to IBD virus at one or another stage and (iii) Farms where the birds are being provided grossly damage feeds apparently contaminated with aflatoxin as screened for emission of typical fluorescence (BGYF). A total of fifteen farms-five from each category were screened for HI antibody titre to RD virus. In all 105 serum samples (35 from each category of farms) were subjected to HI test for determination of antibody level to RD virus. The findings are as under:

(i) The mean HI titres ranged between 3.42 ± 0.202 to 8.71 ± 0.184 in first category of farms, where regular IBD vaccination was in practice.

(ii) In the second category of farms, where the birds were seropositive for IBD virus suggesting prior exposure of these birds to field IBD virus (since IBD vaccination was not in practice on these farms).

The mean HI titres to RD virus varied from 4.85 ± 0.340 to 6.85 ± 0.260.

(iii) In the last category, the farms, which were receiving grossly, damaged feeds found positive for emission of typical fluorescence (BGYF) suggesting apparent contamination with aflatoxin. The mean HI titres of these farms varied from 5.42 ± 0.202 to 7.71 ± 0.359 (iv) In general, the result suggested wide variations in HI antibody titres in each of the three categories of farms which in term reflected variation in degree of immunosuppression due to one or another reason.

(v) The comparison of the results of the fifteen farms taken together representing the above noted three categories demonstrated HI antibody titres ranged from 3.42 ± 0.202 to 8.71 ± 0.184, which suggested the degree of variations in HI titres which leaves the scope for improvement, if suitable measures are taken on time.

Further, from each of the three categories of farms, one farm was selected on the basis of lowest level of HI antibody titre in the respective categories. In order to assess the efficacy of three selected drugs namely; (i) Charak-E-Sel (ii) Carbo veg. and (iii) Lemasol-P (Levamisole hydrochloride) were tried for the immunopotentiating effect. The findings are here under:

(i) The mean HI Ab titres of the birds of farms from the first category (IBD & RD vaccinations were given regularly) after administration of Charak-E-Sel for 15 days was recorded 4.85 ± 0.260, which showed an improvement of more than 1.5 (log_2) value when

compared with the corresponding value recorded in the untreated control. The birds in the treated group showed significantly higher body weight gain at ($p<0.01$) and also significant improvement in FCR at ($p<0.01$) in comparison to the values recorded in untreated control group.

(ii) The findings in respect of influence of Carbo veg. on farm selected from second category (IBD seropositve) revealed the mean HI titre of 6.75 ± 0.368 which reflected an improvement of more than 2($\log_2$), when compared with the value in the untreated control group. Further, the birds in the treatment group also recorded significantly increased body weight gain and FCR at ($p<0.01$).

(iii) The mean HI Ab titres of the farm selected from third category (receiving apparently aflatoxin contaminated feed) after giving levamisole for 15 days was found to be 8.28 ± 0.184 which showed an improvement of about 3.5($\log_2$) in comparison with the corresponding value of the untreated control group. The levamisole treated group of birds showed no significant gain in body weight as well as improvement in FCR.

The comparative evaluation of the immunopotentiating effects of the three selected drugs revealed the following:-

(i) Carbo Veg. proved effective both as an immune enhancer and growth promoter.

(ii) The Charak-E-Sel moderately enhanced the HI Ab titre and its effects on body weight gain and FCR were also of moderate degree.

(iii) The levamisole though brought about marked enhancement in HI Ab titre but it did not show significant increase in body weight gain and improvement in FCR.

The overall pictures suggested that the homoeopathic drug employed in this study proved to be more useful as it acts as both immune enhancer and an ideal growth promoter. The drug is also cost effective, easily available and possess minimum or no side effect and hence the poultry farmers may be advised to include this drug in the schedule of farm operation, specially where the chances of risk to various immunosuppressive factors are high. The levamisole though proved to be an excellent immune enhancer but failed to record significant increase in body weight gain and improvement in FCR. In such situation, it will be advisable to use an additional known growth promoter in addition to levamisole after conducting laboratory and field study. Further, it may also be advisable to see if these drugs show any difference in its efficacy due to different types of immunosuppressive agents.

CHAPTER - VII

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APPENDIX

APPENDIX-1

I. Analysis of variance showing HI Ab titre to RD virus in chicken of different farms in which IBD and RD vaccinations were regular features.

Sources of variation	D.F	M.S	F
Between farms	4	35.04	106.06 **
Error	30	0.33	
Total	34	-	

**** indicates significant at (p<0.01)**

II. Analysis of variance showing HI Ab titre to RD virus in chicken of different IBD seropositive farms.

Sources of variation	D.F	M.S	F
Between farms	4	4.54	10.83 **
Error	30	0.419	
Total	34	-	

**** indicates significant at (p<0.01)**

III. Analysis of variance showing HI Ab titre to RD virus in chicken of different farms receiving feed suspected to aflatoxin.

Sources of variation	D.F	M.S	F
Between farms	4	5.97	12.06 **
Error	30	0.495	
Total	34	-	

**** indicates significant at (p<0.01)**

Appendix- II

Analysis of variance showing effect of Chark-E-Sel on Ab titre, initial body weight., Final body wt. , wt. gain and feed conversion ratio

Sources of Variation	D.F	Ab titre after 15 days treatment		D.F	Initial body wt. on14th		D.F	Final body wt. on 67th day		D.F	Weight gain on 67th day		D.F	Feed conversion ratio	
		M.S	F		M.S	F		M.S	F		M.S	F		M.S.	F
Between treatment	1	8.64	24.20**	1	2.88	0.012^{NS}	1	37264.5	48.99**	1	36612.18	47.62**	1	0.10	10**
Error	12	0.357	-	48	2.34	-	48	760.54		48	768.81		48	0.01	
Total	13	-	-	49	-		49			49	-		49	-	

NS indicates non-significant.

**** indicates significant at ($p<0.01$)**

Appendix III

I. Analysis of variance showing effect of carbo veg. on Ab titre, initial body weight., Final body wt. , wt. gain and feed conversion ratio

Sources of Variation	D.F	Ab titre after 15 days treatment		D.F	Initial body wt. on14th		D.F	Final body wt. on 67th day		D.F	Weight gain on 67th day		D.F	Feed conversion ratio	
		M.S	F		M.S	F		M.S	F		M.S	F		M.S.	F
Between treatment	1	12.07	15.83**	1	1.87	1.30 NS	1	106127.26	11. 56**	1	105030.40	11.40**	1	0.20	10**
Error	12	0.762	-	67	1.43	-	67	9186.36		67	9207.26		67	0.02	
Total	13	-	-	68	-		68			68	-		68	-	

NS indicates non-significant.

**** indicates significant at ($p<0.01$)**

Appendix IV

I. Analysis of variance showing effect of levamisole on Ab titre, initial body weight., Final body wt. , wt. gain and feed conversion ratio

Sources of Variation	D.F	Ab titre after 15 days treatment		D.F	Initial body wt. on14th		D.F	Final body wt. on 67th day		D.F	Weight gain on 67th day		D.F	Feed conversion ratio	
		M.S	F		M.S	F		M.S	F		M.S	F		M.S.	F
Between treatment	1	41.14	78.51**	1	0.19	0.084^{NS}	1	960.19	0.119^{NS}	1	926.67	0.116^{NS}	1	0.01	0.2^{NS}
Error	12	0.524	-	58	2.25	-	58	8025.57		58	7956.15		58	0.05	
Total	13	-	-	59	-		59			59	-		59	-	

NS indicates non-significant.

**** indicates significant at ($p<0.01$)**