EFFECTS OF AFLATOXIN ON IMMUNE RESPONSE TO INVASIVE INTERMEDIATE STRAIN INFECTIOUS BURSAL DISEASE VIRUS VACCINE IN BROILER CHICKEN

Thesis

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE)

In partial fulfillment of the requirements
FOR THE DEGREE OF

Master of Veterinary Science
In
Veterinary Microbiology

By

Anamika

Registration No. M/Vety.Micro/34/1997-98

Department of Veterinary Microbiology
Bihar Veterinary College
Patna, Bihar (India)

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Dedicated to my adorable parents and lovely children

Department of Veterinary Microbiology
Bihar Veterinary College, Patna – 800 014
(Rajendra Agricultural University, Bihar)

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Certificate I

This is to certify that the thesis entitled 'EFFECTS OF AFLATOXIN ON IMMUNE RESPONSE TO INVASIVE INTERMEDIATE STRAIN INFECTIOUS BURSAL DISEASE VIRUS VACCINE IN BROILER CHICKEN", *submitted in partial fulfillment of the requirement 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 bonafied research carried out by* ***Dr. Anamika*** *under my supervision and guidance. No part of this 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.

25/01/02

K. C. P. Singh
(Major Advisor)

Certificate II

We, the undersigned, members of Advisory committee of ***Dr. Anamika,*** *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* "EFFECTS OF AFLATOXIN ON IMMUNE RESPONSE TO INVASIVE INTERMEDIATE STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS VACCINE IN BROILER CHICKEN" *may by submitted by* ***Dr. Anamika*** *in partial fulfillment of the requirement for degree.*

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Certificate III

This is to certify that the thesis entitled "EFFECT OF AFLATOXIN ON IMMUNE RESPONSE TO INVASIVE INTERMEDIATE STRAIN INFECTIOUS BURSAL DISEASE VIRUS VACCINE IN BROILER CHICKEN" *submitted by* ***Dr. Anamika*** *in partial fulfillment 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* 14-11-..... *2002.*

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All may not have been mentioned but none has been forgotten.

Anamika

(Anamika)

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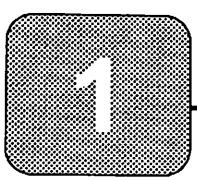
List of Abbreviation

Ab	Antibody
$AFIB_1$	Aflatoxin B_1
AGPT	Agar gel precipitation test
ANOVA	Analysis of variance
BF	Bursa of Fabricius
°C	Degree Centigrade
CS	Course Spray
Co.	Company
Dr.	Doctor
dpv	Days post- IBD vaccination
D.F.	Degree of Freedom
D.W.	Drinking Water
ELISA	Enzyme Linked Immuno Sorbent Assay
EDTA	Ethylene diamine teraacetic acid
edn	Edition
Fig.	Figure
FCR	Feed Conversion ratio
gm	Grams
gr.	Groups
H & E	Haemotoxyline and Eosim
Hb	Heamoglobin
HA	Heamagglutination
HI	Haemagglutination inhibition
hrs.	Hours

i.o	Intraocular
iIELs	Intestinal intraepithelial leucoeytes
IBDV	Infectious Bursal Disease Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Ltd.	Limited
lb	Pound
LDH	Lactate dehydrogenase
µL	Microlitre
MDA	Maternally derived antibody
MAb	Maternal antibody
Micro	Microbiology
M.S.	Mean sum of squares
Mg.	Miligram
Ml	Mililiter
Mr.	Mister
mm^3	Cubic milimeter
No.	Number
NDV	New Castle Disease Virus
P.	Page
PBS.	Phosphate Buffer Saline
PCV	Packed Cell Volume
PI	Post infection / Post inoculation
ppb	Part per billion
ppm	Part per million
Pvt.	Private
QAGPT	Quantitative agar gel precipitation test
RBC	Red Blood Cells

RDV	Ranikhet disease virus
rpm	Revolution per minute
S.E.	Standard Error
SPF	Specific Pathogen free
SGOT	Serum Glutamic oxalic transaminase
SRBC	Sheep red blood cells
TLC	Total Leucocyte count
Uv.	Unvaccinated
v	vaccinated
vvIBD	Very virulent infectious bursal disease
WBC	White blood cells
WLH	White Leghorn
Wt.	Weight
W/V	Weight by volume
%	Percent

CHAPTER



INTRODUCTION

Introduction

Infectious bursal disease (IBD) is a disease of young chickens first reported in 1962 by Albert S. Cosgrove in United States of America from a place named Gumboro near Delware. It is an acute and highly contagious viral disease of the growing chickens. The economic significance of the disease lies in its cosmopolitan distribution, heavy mortality and immunosuppression (Faragher, 1972; Mazariegos *et al.*, 1990; Kouwenhoven and Vanden Bos, 1992; Tsukamoto *et al.*, 1995). The disease produces huge economic losses by direct and indirect effects. The direct effect of this infection is marked by appearance of clinical sign, loss of weight and low to moderate mortality whereas indirect effect is marked by immunosuppression resulting in enhancement of susceptibility to other type of infection, impaired immune response to various vaccines, failure to get optimum weight and poor feed conversion ratio.

The conventional IBD vaccine including mild strain (Lukert type) IBD vaccine used in day old chicks, Intermediate strain (Georgia type) IBD vaccine which is given in 14-18 day old chicks and inactivated IBD vaccine which is largely used in breeder flocks as a booster vaccine, are not sufficiently effective in controlling the newly emerged Pathotype of classical serotype-I IBD virus which causes high morbidity and mortality and is commonly referred to as very virulent infectious bursal disease (vvIBD). This new form of IBD was first reported in 1987 from Netherland (Box, 1989) and subsequently from different parts of European and Asian countries. The disease was for the first time reported from the state of Bihar by (Singh *el al.*, 1994b). vvIBDV outbreaks are being reported even in vaccinated lots. Besides, birds with passive immunity are also susceptible to vvIBD as this virus strain has ability to cross maternal

antibody barrier, such birds causes the disease. Recently introduced intermediate plus strain or invasive intermediate strain or moderate hot strain IBD vaccine appears to be the only vaccine virus that can confer effective protection against the newly introduced vvIBD. This vaccine strain has been shown to have ability to penetrate high level of maternal antibody and incite immune response but is also reported to produce varying degree of immunosuppression. (Kouwenhoven and Vanded Bos, 1994).

Since, this newly introduced dHotp strain vaccine has got more residual pathogenicity than the conventional live IBD vaccine, it is necessary that sufficient measures be taken that the pathogenicity of this vaccine strain does not get precipitated under the influence of various factors operating in nature which have immunosuppressive effects.

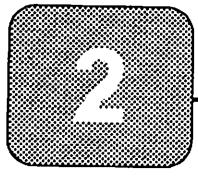
Aflatoxin is one such factors which abundantly available in nature and its production may take place in poultry feed if the feed is not stored properly. The surveys conducted in India and abroad on distribution of aflatoxin in different feed stuffs/grains have revealed the large scale prevalence of aflatoxin in poultry feed ingredients specially maize, groundnut cake (Krishnamachari *et al.,* 1975; Singh *et al.,* 1994) fish meal, wheat etc. Though the level of the aflatoxin in which they are normally found in the feed stuffs may not be harmful by itself but this may act synergistically in combination with the other immunosuppressive agent like IBD virus and precipitate its effect. In a situation, where there is no control on providing aflatoxin free feed to the chickens, interaction of some level of aflatoxin available in the feed provided to the chicken with other immunosuppressive agents can not be ruled out. It is in this context, that study on the interaction of aflatoxin and dHotp strain IBD vaccine is warranted. As IBD virus is a known immunosuppressant care has to be taken whether a very virulent strain of this virus having even little residual pathogenicity can interact with another immunosuppressive agent like aflatoxin

and also whether such interaction between two can enhance the pathogenicity of vaccine virus. This study has got more relevance in our condition where the birds are receiving aflatoxin through feeds continuously even though in harmless concentration specially when intermediate plus strain or hot strain vaccine is used. Therefore, the present study has been planned with the following objectives:

1. To study the immune response of intermediate plus strain infectious bursal disease virus vaccine at different levels of aflatoxin.

2. To study the pathogenicity and immunosuppressive effect of the above vaccine virus in aflatoxin fed chickens.

CHAPTER



REVIEW OF LITERATURE

Review of Literature

Cosgrove (1962) was the first to report this disease as "avian nephrosis" in young broilers of 3-4 weeks ago. He recognized the first outbreak of IBD in United State from a place name Gumboro near Delaware. From its geographical origin the disease is also known as "Gumboro disease".

IBD is characterized by clinical signs like depression, anorexia, retarded growth, white and watery diarrhea and ruffled feathers. Gross lesions include changes like edematous and haemorrhagic bursa with atrophy in later stages, petechial haemorrhages in skeletal muscles and at the juncture of provetriculus and gizzard. Spleen, kidney and thymus are other organs involved (Lukert and Hitchner, 1984).

IBD is now reported from all major poultry producing areas world wide (Okoye, *et al.,* 1984). In India, the disease was first reported from Uttar Pradesh by (Mohanty *et al.,* 1971). Since then several workers have reported the incidence of this disease in different part of country (Chauhan *et al.,* 1980; Ray and Sarkar, 1984; Sulochana and Lalikthakunjamma, 1991; Singh *et al.,* 1994b; Joshi and Shakya, 1996).

Cheville (1967) investigated the cytopathological changes in the bursa, spleen and thymus of chickens following experimental infection with IBDV intraocularly at 28 days of age. Necrosis of lymphocytes in the medulla of bursal follicles was the initial lesion. This was followed by the destruction of all lymphoid tissue in the bursa.

Allcroft (1969) considered that among the different types of Aflatoxins, AFB_1 was the potent hepatotoxin, hepatocarcinogen, immunosuppressant and mutagenic compound for both chickens and human beings. He further reported that AFM_1 had also carcinogenic property.

Pier *et al.* (1971) described 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, poor feed conversion ratio.

Allan *et al.* (1972) found that aflatoxin enhanced the immunosuppressive effect of IBD virus as measured by impaired responses to RD vaccine.

Hirai *et al.* (1974) demonstrated that antigens from IBDV were specific and formed three precipitation lines due to the differences in the diffusion rates of the viral particles, PA-1, PA-2 and PA-3. The precipitating antigens PA-1, migrated most slowly and PA-3 migrated most rapidly.

Thaxton *et al.* (1974) observed immunosuppression in chicken by aflatoxin. They observed that during aflatoxicosis the titre of Abs against IBD during the primary immune response is decreased and the peak titre is delayed in a dose related fashion in chicken.

Cullen and Wyeth (1975) described the quantification of IBDV antibodies by AGPT. Antigen was prepared from the bursa of three to five weeks old chicks. The quantititave agar gel precipitation test (QAGPT) was used to measure the maternal antibody level in chicks from IBDV infected parents. Wyeth and Cullen (1976) and. Wood *et al.* (1979) standardized the QAGPT for determinatioin of IBDV antibodies level in chickens. They reported that the antigen concentration was of no significance within certain limits, but for clarity, high antigen concentration was recommended.

Thronton and Pattison (1975) undertook a comparative study of 9 products from 7 different sources intended for use as vaccines against infectious bursal disease of chickens. No vaccine caused clinical disease after administration to chicks at 7 days of age, but one cause significant impairment of weight gain, and when given to one day old chicks caused some morbidity and deaths. Most vaccines affected the bursa of Fabricius and histological examination of this organ revealed varying degree of tissue damage and reduction in size of this organ. Selected product which differed in their effect on the bursa were tested for their immunosuppressive properties by assessing the response to live Newcastle disease vaccine administered after the IBD vaccine.

Krishnamachari *et al.* (1975) reported the toxic effect of aflatoxin among the people residing in 200 villages in India due to consumption of maize which was heavily contaminated with *Aspergillus flavus*. The maize was containing 6.25 to 15.6 mg aflatoxin per Kg (mg/Kg) and due to its toxicity 106 people died out of a total of 397 affected.

Giambrone *et al.* (1978) reported that birds infected with IBD virus exhibited enhanced mortality and more extensive symptoms if fed aflatoxin. This observation was particularly interesting in view of IBD causing renal impairment, hemorrhages, hypoprotenaemia, depressed antibody production to various vaccines and necrosis of lymphocytes of the thymus, cecal lymphoid tonsil, spleen and particularly the bursa of Fabricius. These effect of IBD are also produced by aflatoxicosis, therefore, these two diseases in combination offer a potentially valuable model for studying interaction between infectious agents and aflatoxicosis.

Winterfield and Thacker (1978) compared the usefulness of AGPT and VN test to study the immune responses of different strains of IBDV applied as

vaccines. They observed that even precipitin negative chickens were often protected, whereas AGPT positive chickens were always protected.

Kumar and Gowda (1979) had chemically analysed a total 72 feed ingredient samples. They found that 44 samples containing 0.03 parts per million (ppm) of aflatoxin. They further analysed 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.

Mohanty *et al.* (1981) reported maximum antigen concentration in the bursa collected at 48 hrs. PI. Bursa collected at 12 hours PI had only faint precipitin lines. Two distinct precipitation lines were seen within bursa collected 24 hours post infection. Whereas those of 48 hrs. PI. had three lines.

Reddy (1981) observed the significant reduction in the level of haemoglobin content in the blood of chickens fed with aflatoxin at a dose rate of 0.50, 0.75 and 1.0 ppm. Further they reported that there was reduction in packed cell volume (PCV) and Red blood cell count at 0.5 ppm and 1.0 ppm dose levels of aflatoxin respectively. He also observed increased in liver lipids resulting in fatty liver and decrease in serum proteins.

Topia *et al.* (1981) showed that in aflatoxin fed (6 ppm) chicks exhibited symptoms of weakness, closed eyes, ruffled feather, loss of appetite and stunted growth. Total plasma proteins, hematocrit, albumin, globulin values were lower as compared with non - aflatoxin fed control chicks.

Chang and Hamilton (1982) proved that during the aflatoxicosis in broiler chickens increased severity and new symptoms of IBD arises. A coagulopathy as indicated by prolonged prothrombin times and by the occurrence of slight hemorrhages in skeletal muscle, a hypoprotenemia, a hypocalcemia, and markedly enlarged kidney were observed in combined aflatoxicosis and IBD, but

not in IBD alone. The size of the bursa of Fabricius following an initial hypertrophy was atrophied 4 days after inoculation and the spleen was enlarged. The effects of aflatoxin and IBD on these two glands were additive. Packed cell volume and hemoglobin values were decreased by aflatoxin only. Thus, aflatoxin had the effects of making IBD a much more severe disease and of changing the symptoms.

Edwards *et al.* (1982) investigated the duration of immunosuppression and the relationship between bursal damage and depression of humoral response caused by an IBD vaccine strain administered at one day old. Examination of bursal sections from chicks seven days post IBD vaccination revealed severe damage with destruction of follicular architecture, depletion of lymphocytes, increased connective tissue, mucous cysts and thickening and corrugation of the epithelium in eight out of ten bursa examined.

Mc Ferram *et al.* (1982) conducted field studies with an inactivated vaccine against infectious bursal disease. Vaccination using an inactivated IBD vaccine stimulated long lasting neutralizing antibodies. Highest titres were produced in the birds which had previously been infected with field strain, but satisfactory titres were achieved after priming with an attenuated vaccines. Bursal lesions were delayed by about 2 weeks in the progeny of vaccinated birds. However no economic advantage could be demonstrated from the use of the vaccine. It is concluded that the depressed feed conversion seen in broiler was not due to IBD virus.

Ley *et al.* (1983) detected serologic, hostopathological and biochemical changes in 35 days old specific pathogen free chickens inoculated with IBDV. A detectable precipitin antibody response occurred between 3 and 5 days post inoculation. Evaluation of pooled serum samples obtained from infectious bursal disease virus infected chicken revealed 58% decrease in potassium

concentration, 63% decrease in cholesterol concentration at day 3 PI, from the pre inoculation value. LDH values increased 52% on day 3 PI and then decreased 45% by day 7 PI. SGOT increased 59% (day 3 PI) above the pre inoculated value. Electrophoratic profile of equal volume pools of pre inoculation and infected group sera showed 67% decreased in albumin and 50% increase in alpha-1 globulin, 53% increase in gamma globulin and 52% decreased in albumin : globulin ratio on day 3 PI comparison to pre inoculated samples. Individual serum samples analysed for uric acid concentration indicated that several IBDV infected chickens have serum uric acid concentration above the normal comparison range.

Histopathological examination of lymphoid and non-lymphoid tissues from IBDV infected SPF chickens affirmed that the predominant lesion was lymphoid necrosis in the bursa of Fabricius. Other lymphoid organs were much less severely affected and possessed greater regenerative potential. Non specific and relatively mild changes were found in the liver and kidney.

Lukert and Hitchner (1984) reported that the histopathological lesions in the bursa commenced with degeneration and necrosis of the lymphocytes in the medullary area of the follicle. The lymphocytes were soon replaced by hetrophils, debris and reticuloepithelial cells. All of the follicles were affected by three to four day PI. As the inflammatory reaction decreased cystic cavity and fibroplasia of the interfollicular connective tissue developed.

Moorthy *et al.* (1985) found pale yellow liver with paravaccular haemorrhages, phlebitis, haematomas and nodules, bile duct hyperplasia in the experimental chickens receiving 6.25 ppm of aflatoxin in feed for 3 weeks.

Shukla and Pachauri (1985) reported that the feeding of aflatoxin contaminated feeds to cockerels caused reduction in intake of feed and water,

loss of general condition, poor growth, ruffled feathers, dullness and paleness of combs.

Mohiuddin *et al.* (1986) reported lowered haematological values (Hb gm%) and decreased in packed cell volume (PCV), total RBC count (TEC), total thrombocyte and total basophilic count. Further they reported that there was slight increase in total white blood cells (WBC) count and considerable increase in heterophil count in poultry feeding on AFB_1 @ 20 ppm for 3 months.

Giambrone and Clay (1986b) compared the efficacy of two intermediate infectious bursal disease vaccines (Clone Vac D-78 and S-706) for immunizing specific pathogen free white Leghorn chickens by coarse spray (CS) against sub-clinical IBD. Both the intermediate IBD vaccines were equally capable of immunizing day old SPF chickens by CS and were safe as evidenced by the absence of morbidity, mortality, or severe gross and microscopic bursal pathology at 28 days of age.

Panigraphy *et al.* (1986) conducted an experiment in which five weeks old chickens were inoculated with IBDV and bled five days post inoculation for analysis of haematological changes and serum biochemical changes. From the experiment they concluded that in IBD infected group there were significant decrease in the total erythrocyte count, packed cell volume, haemoglobin concentration, albumin, albumin : globulin ratio, uric acid and glucose. Serum globulin and cholesterol increased significantly. There was no alteration in the levels of sodium, potassium and calcium in IBD affected birds. Other cellular changes that occur in the acute stage of IBD are lyphocytopenia and panleucopenia (Cheville 1967). Histological lesions in the bursa of Fabricius were characterized by massive cystic degeneration of follicle with depletion of lymphocytes, interfollicular oedema and fibrosis and infiltration by heterophils

and macrophages. No lesions were present in the bursa of Fabricius of control chicken.

Balchandran and Ramakrishnan (1987) noted enlarged yellow coloured, mottled soft and friable liver in the cobb broiler chickens by feeding them with a dietary aflatoxin at a concentration of 1.0 ppm for a period of 4 weeks(0 to 28 days). He found hyperplasia of bile duct and focal necrosis of liver with a prominent regenerative changes of hepatic cells forming a ductular pattern surrounded by a thin layer of fibrous tissue. Further they reported that kidneys were enlarged pale or congested with a few petechial haemorrhage at 1.0 ppm level and were more marked at 3 ppm level. Similarly, extensive epicardial haemorrhages, several spleenomegely, extremely pale breast muscles and copious catarrh of intestinal mucosa were observed due to feeding of aflatoxin at 3 ppm level. They also observed focal necrosis of hyaline degeneration in the thigh muscle and tubular degeneration and haemorrhages and lymphocyte infiltration in the kidneys of broiler chickens fed a diet containing aflatoxin @ 1.0 and 3.0 ppm.

Dafalla *et al.* (1987) reported fatty and studded enlarged liver with haemorrhage and some containing haematoma in the dead hens which fed a diet containing AFB_1 @ 142 ppb. They also reported cytoplasmic vacuolation of hepatocyte, necrotic foci and lymphoid nodules in the peripheral area of liver in hens. The kidney of those hens were enlarged and congested, the epithelial cells of renal tubules were vacuolated, the renal medulla congested and lymphocytic nodules were scattered in the cortex.

Fukal *et al.* (1988) conducted a histopathological study in laying hens restricted in the diet containing 5 mg AFB_1 and 5 mg AFG_1 once daily and they detected the most severe effects of toxin after 3 days as metaplasia of epithelium of bile duct fatty infiltration of hepatocytes whereas, degeneration of

bile duct cells, activation of kupffer cells and infiltration of hepatocytes were observed on 7th day of toxicosis.

O'Koye *et al.* (1988) reported severe degeneration of hepatocytes, bile duct proliferation and depletion of lymphocytes in the lymphoid organs in the broilers feeding aflatoxins @ 2.4 mg/kg level.

Virdi *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.

Mollenhauer et al. (1989) observed hepatocellular lipidosis in the chickens when they were fed aflatoxins at 2.5 and 5.0 mg/gm levels. They also reported enlargement of bile canaliculi, reduction in mitochondrial size, mild lymphocytic infiltration, hepatocellular degeneration and necrosis as a primary lesions in the liver of chicks fed with 5.0 mg aflatoxin in per gram of feed. Further they reported the thickening of glomerular basement membrane at 2.5 and 5.0 mg/gm levels.

Jassar and Singh (1989) reported immunosuppression by aflatoxin is dose related as evidence by lowered HI titre is aflatoxin fed chickens. They also reported the immunosuppressive effect was reversed after discontinuation of feeding of aflatoxin.

Ozer *et al.* (1989) showed the birds receiving aflatoxin had lower HI titre and leucocytes count and fewer pyroninophillic cells in section of the small intestine, spleen, bursa of Fabricius thymes.

Asim *et al.* (1990) studied the occurrence of aflatoxins in poultry liver and associated pathological changes in 110 morbid poultry livers containing 12.3 to 493 ppb aflatoxin B_1 and G_1, and the histopathological changes found were fatty

changes, cellular dissociation, necrosis, cellular infiltration, fibrosis and bile duct hyperplasia.

Ezeockoli *et al.* (1990) evaluated the effect of IBD live virus vaccine of the immune response of chickens by the assessment of the antibody response following vaccination as well as persistence to challenge with virulent virus. Birds were vaccinated at various ages and later challenged with a heterologous vaccine or wild type IBD virus. The Bursa of Fabricius was examined for histopathological changes at regular intervals. Antibody levels to NDV were monitored. Significantly higher mortality rates were observed in birds vaccinated with IBD vaccine than unvaccinated birds following challenge.

Jhala *et al.* (1990) infected four weeks old broiler chicks with infectious bursal disease virus isolate. The birds were sacrificed 48 hours and 5 days after infection. They did not observe any clinical symptoms in experimentally infected birds during the 5 days observation period. All the birds showed significant enlargement of bursa of Fabricius at 48 hours PI and atrophy at 5 days PI. Histopathologically there were necrosis and depletion of lymphocytes in bursal follicles at 48 hours PI. Bursal lesion became severe at 5 days PI, where bursal follicles were found atrophied with regression on size. The corticomedullary epithelium showed formation of cyst severe proliferation of fibrous connective tissue was observed in the interfollicular space. Thymus, spleen and kidney collected at 48 hours and 5 days PI did not reveal any significant histopathological lesion.

Mazariegos *et al.* (1990) conducted the study to test the pathogenicity and immunosuppressive effects of seven commercially available infectious bursal disease vaccine. The vaccine strains were intermediate in their pathogenicity in susceptible specific pathogen free chickens. One day old and three week old SPE chicken were vaccinated with these vaccine. Two weeks

after IBD vaccination they were vaccinated with Newcastle disease virus. The pathogenic and immunosuppresive effect 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 result reveals 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.

Yaman *et al.* (1990) noted the mean haematocrit value of the aflatoxin treated chicks as 24.8% (control 26.2%), red blood cells count 1.97 x 10^6 per cubic millimeter (mm^3) (control 2.21 x 10 per mm^3). White blood cells count 18.19 x 10^3 per mm^3 (control 21.41 x 10^3 per mm^3) and ESR in 1, 2 and 24 hrs as 63.08 millimeter (mm) (control 57.25 mm). 92.83 mm (control 86.0 mm) are 142.58 mm (control 138.66 mm) respectively in the aflatoxin treated chickens at dose rate of 5 kg AFB_1 daily for 2 months in feed.

Vanden Berg *et al.* (1991) isolated a highly virulent strain of IBDV from the field and propagated in SPF chickens, causing upto 100% mortality. Although the virus belonged to the standard serotype 1, serological typing with monoclonal antibodies showed an antigenic drift in this pathogenic strain.

Kumar and Rao (1991) studied the haematological and blood biochemical changes in experimental infectious bursal disease virus infected chickens Experimentally infected birds developed leucocytosis, heterophilia, lymphocytopenia, decreased hemoglobin value and packed cell volume, prolonged clotting time and prothrombin time, raised cholesterol, creatinine and lactate dehydrogenase and decreased glucose, uric acid, urea and acid and alkaline phosphatase concentration.

Somvanshi & Mohanty (1991)studied the pathological changes during aflatoxicosis & IBD infection in chickens and found that, bursa revealed severe necrosis of lymphoid cells, presence of vacuolation or cyst, follicular atrophy, infiltration of heterophils & lymphocytes into interfollicular areas & hyperplasia of mucosal lining of epithelial cells. Thymus showed hyperemia, presence of microcysts, atrophy of lining of cortex. Lungs had variable degrees of hyperemia, haemorrhages, atelactosis and occasional infiltration of mono nuclear cells around peribroncheal areas. It is suggested that severe and certain additional gross and microscopic lesions were due to additive effects of IBDV and aflatoxin

Higashihar *et al.* (1991) observed immunosuppressive effect of infectious bursal disease virus strain of variable virulence.

Berg & Gonze (1991) isolate the highly virulent strains of IBD in poultry where intermediate strain IBD vaccine was used and observed that very virulent IBD virus has ability to penetrate the high level of antibody including maternal antibody level.

Ghosh and Chauhan (1991) observed a significant reduction in the relative weights of both bursa of Fabricius and thymus and a significant increase in the relative weight of spleen in broiler chicks when they were fed a diet containing aflatoxin B_1 @ 1 mg / kg for 6 weeks.

Onkoye *et al.* (1991) found loss of weight of 2.5 times severe in the infectious bursal disease (IBD) and *A. flavus* infected broiler chickens then those infected only with *A. flavus.*

During aflatoxicosis, Bakshi (1991) reported hepatic cell degeneration, necrosis, Periprotal lymphoid cell infiltration around bile ducts. Focal area of

heterophilic and lymphocytic infiltration alongwith the depletion of lymphoid cells from the secondary follicles were also observed during his study.

Arshad *et al.* (1992) found hepatomegaly, mottling and echymotic haemorrhages, microscopic changes as congestion, fatty changes, necrosis, leucocytic infiltration and haemorrhages in liver. Further they found moderately enlarged, congested kidneys with deposits of urates in ureter and degenerated and necrosed kidney tubules with mononuclear cell infiltration in birds fed with higher dose (5310 ppb) of aflatoxin.

Nakamura *et al.* (1992) compared the immunosuppressive effect of highly virulent infectious bursal disease virus on vaccination against New castle disease among 2, 3 and 4 weeks old chickens. The virus suppressed antibody responses to ND vaccination in above three groups.

Nunoya *et al.* (1992) reported the occurrence of acute infectious bursal disease virus with high mortality in Japan. They isolated highly virulent IBDV from field outbreaks and studied the pathogenicity of the field isolates in SPF chickens. The experimentally infected chickens developed severe clinical disease with a high mortality rate. The histopathological changes were marked by bursal and thymic necrosis, aplastic anaemia and acute hepatitis.

Tsukamoto *et al.* (1992) reported isolation of virulent infectious bursal disease virus from field outbreaks with high mortality in Japan. In all cases the parent flock were inoculated with IBD vaccine but their progeny were not. The virulence of the isolate from such outbreaks was investigated in SPF chickens. Infected birds developed diarrhoea within 24 hours of infection and showed depression, trembling, ruffled feathers and were prostate. The 5 isolates caused 30-70% mortality with Yamaguchi strains. Those that survived the disease, lost

weight or showed no weight gain. Atrophy of the bursa of Fabricius was seen in all surviving birds.

Wyeth et al (1992) studied the usefulness of inactivated infectious bursal disease oil emulsion vaccine to control very virulent strain of IBD virus in commercial layer chickens having varying levels of maternal antibodies. The QAGPT titres of M_{Ab} ranged between 2 to 2^5. The chicks were vaccinated at 7, 10, 14 or 28 days old with varying doses of vaccines intramuscularly. The birds were challenged by eye drop with 100 CID_{50} of the CS88 strains of IBD in 0.1 ml of inoculum and sacrificed 56 hours later and their bursa of Fabricius were examined for the presence of viral antigens using the agar gel precipitation test. The partial doses given at 7 or 10 days old gave only partial protection. A full dose given at 10. 14 or 28 days old fail to give full protection but a full dose administered at 7 days old protected all the chicks after each challenge with virulent virus.

Mohiuddin and Vikram Reddy (1993) reported decrease in packed cell volume, haemoglobin concentration and RBC count in the bursectomised chicks which were given aflatoxin @ 1 and 3.0 ppm, respectively.

Jassar and Singh (1993) reported the total serum proteins decreased during aflatoxicosis and this decrease was significant at the level of 6 ppm at all intervals, being most marked on day 14.

Coletti et al. (1994) studied a vaccine strain of infectious bursal disease virus with residual pathogenicity in the fowl. Antibody levels were highest in those chicks which had the lowest maternal antibody levels at the time of vaccination.

Kouwenhoven and Vanden Bos (1994) controlled the very virulent IBD in the Netherlands with more virulent vaccine. The maternal immunity of chicks

hatched from the eggs of vaccinated hens could not withstand infection with a virulent strain of IBD virus which appeared in the Netherlands in 1987, and they developed the disease at 14-28 days of age. Vaccination of broiler at 14-21 days of age solved the problems only partly. Trials of three new more virulent live vaccines the Bursa Vac, LZ228E and Bursa plus were conducted on 29 million birds in 96 replacement layer flocks and 714 broiler flocks between October, 1990 and November 1991 with satisfactory results. However, they found the Bursa Vac is slightly more virulent than the other two hotmvaccines They also observed that hot vaccines were slightly more pathogenic than the Intermediate vaccines.

Singh *et al.* (1994b) reported the occurrence of infectious bursal disease in chickens between February, 1990 to May, 1993 in Bihar. The disease occurred in both acute and sub clinical forms. The acute IBD was marked by high morbidity and high mortality ranging between 35-65%. Three virus isolates were recovered from the affected tissue. Majority of acute IBD outbreaks followed revaccination with RD vaccine

Anjum (1994) described heavy losses inflicted by a severe outbreak of IBD in vaccinated pullet chickens attributed to infection pressure and aflatoxicosis interaction during severe outbreak of IBD that occurred in February 1993, was reported in a flock of 15000 growing pullets. The flock had been vaccinated with a live IBD vaccine at the age of 12 days & revaccinated at 39 days of age. At PM examination severe edematous bursitis, nephrosis and haemorrhages at the proventriculus-gizzard juncture were observed along with liver and kidney damage. In AGPT a sharp line was induced by bursal homogenates of dead birds with known IBDV antiserum.

Devegowda *et al.* (1994) found decrease in body weight and feed conversion and increase in mortality in chickens and ducklings after feeding of aflatoxins @ 500 or 1000 ppb for 6 weeks.

Dimri *et al.* (1994) reported that a dose of 1.0 mg of aflatoxin B_1/Kg of feed caused decrease in serum magnesium, serum calcium and iron level.

Kembi *et al.* (1995) compared the effect of three different routes of administration viz. oral, intramuscular and ocular on the immunogenicity of infectious bursal disease vaccine. They recommended the ocular route as the most effective for vaccination.

Tsukamoto et al (1995) reported the occurrence of highly virulent infectious bursal disease virus in the vaccinated flocks in Japan which produced high mortality. They studied the efficacy of three vaccines available in Japan, two mildly attenuated strains and one intermediate strain in SPF chicks and in commercial chicks with maternal antibody against IBD. Chicks were vaccinated at 20 days old and challenged with highly virulent IBDV 10 days after vaccination. Protection was measured at 7 days after challenge. All the three live vaccines protected SPF chicks; however, only Intermediate strain vaccine protected (100%) commercial chicks against highly virulent IBDV.

Kouwenhoven and Vanden Bos (1996) conducted vaccination trial using conventional intermediate vaccine and more invasive hot vaccines on 95 replacement layer farms and 26 broiler flocks that did not suffer from the disease. They did not find significant difference between the two vaccines on the performance of the vaccinated broilers in respect of mortality, average growth and feed conversion ratio and condemnation percentage.

Mahesh and Muniappa (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, and also monitored the maternally derived antibody response in both experimental and field conditions. The chicks were vaccinated against IBD according to manufacturer recommendations and also 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 were evaluated by bursa : body weight ratio and antibody response to ND vaccination. MDA level decreased below protective level by 14 day and their half-life was 3.6 and varied among breeds under field conditions. The less attenuated strain and one of three intermediate strains induced significantly lower titres followed by other two intermediate strains and significantly lower titres for mild (Lukert) and combination of mild and inactivated strains.

Yamaguchi *et al.* (1996) studied the potency of a new vaccine in controlling highly virulent infectious bursal disease virus (HV-IBDV) infection. They adapted some isolates of HV-IBDV through serial passage in embryonated eggs The embryonated egg and cell culture adapted strains showed reduced pathogenicity and did not kill any young chickens after experimental infection. The bursal lesion of the adapted strain infected chicken were similar to those in classical strains infected chickens. Cross-Virus neutralization analysis showed antigenic diversity between the cell culture adapted HV-IBDV strains and classical strains. In immunization tests, the adapted strain immunized chickens showed good protection against the fatal infection of HV-IBDV. At 3rd day after immunization the adapted strains showed effective immunogenicity against challenge infection.

Zorman Rojs *et al.* (1996) observed the immune responses of two live IBD vaccines (Mild and Intermediate strain) in broiler chicken with maternal antibody in field condition. Two groups of 7060 broiler chickens obtained from a

farm in which a possible infection of IBDV was expected and had low level (ELISA GMT < 500) of maternal antibody were vaccinated with each of the vaccine separately at 8 days of age. The level of specific antibody against IBDV was monitored at weekly interval by immunodiffusion and ELISA test. Practically no antibody was found after 7 day Post vaccination. The increase of antibody was found 14 days after vaccination. No clinical of IBD were seen.

Bekhit (1997) reported highly virulent form of infectious bursal disease virus from Egypt in outbreak of IBD during 1989-1993. He observed severe outbreaks of IBD with usually high moralities (56%). He suggested that the present outbreaks of IBD were attributed to very virulent IBDV belonging to standard Serotype 1. He also suggested that the failure of the different vaccination programmes to give adequate protection against IBDV field challenge may be attributed to many factors other than antigenic variance such as proper timing of vaccination, handling and administration of vaccines, type of vaccine as well as vaccine dose.

Christopher *et al.* (1997) observed the influence of vvIBD on immunity of Ranikhet disease at the field level. They statistically analysed the seroepidemiological data of Ranikhet disease and infectious bursal disease, before (during 1991-92) and after (during 1993-94) 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 $T_{½}$ 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

Bakshi *et al.* (1997) found the dose level of 0.38 ppm of aflatoxin was not found to alter the total serum protein albumin concentration after 2 and 4 weeks of toxin feeding but caused a significant reduction after 6 weeks The serum globulin content was also decreased after 2 weeks of aflatoxin feedings.

Pahar and Rai, (1997) observed the immunogenicity of IBD Virus strain isolated in India. Out of the three isolates given to 7 days old chicks intramuscularly, only strain 394 gave 100% protection, while IBDV strain 494 and IBDV strain 194 gave 88% and 76% protection respectively after challenge with 10^2 CID_{50} IBD virus They opined that IBDV S394 may serve as a prophylactic agent against IBD in poultry without any immunosuppressive effect and mortality in day old chicks.

Vervelde and Davison (1997) characterized leucocytic changes and determined tropism of infectious bursal disease virus following infection of newborn and 3 week old chickens. In the bursa of both age groups rapid depletion of B lymphocytes and an influx of $CD4^+$ TCR $\alpha\beta_1^+$ and $CD8^+$ TCR $\alpha\beta_1^+$ cell was detected within 4 days after inoculation. From 8 days after inoculation and onwards all the lymphoid organs became repopulated with leucocytes and tissue architecture was gradually restored. Virus neutralizing antibodies developed more slowly in newborn birds and 21 days after inoculation the titres was much lower compared to older birds. Lack of clinical signs in newborn chickens was neither due to a failure to respond to the virus nor to a lack of viral replication. It is concluded that age related susceptibility to IBDV in chickens might be due to immunological factors cytokine release, or blood factor.

Zormon-Rojs and Cajavec (1997) reported the efficacy of different vaccination programmes against infectious bursal disease. The trial was conducted with 2 live vaccines (mild and intermediate strain) on 8 commercial

farms in Solvenia. IBD outbreaks were diagnosed in all 8 flocks after vaccination with the mild strain at 8 days of age with mortality of 5.03 percent. After vaccination of 2 flocks with the intermediate strain at 8 day of age, IBD was diagnosed in one flock. IBD was diagnosed in 6 of 8 flocks after administration of intermediate vaccine strains on 15 and 22 of age with mortality of 2.5%. It was concluded that neither vaccine can fully protect broiler against very virulent IBD virus strains.

Al-Afaleq (1998) studied the biochemical and hormonal changes associated with experimental infection of chicks with infectious bursal disease virus. Twenty, 2 weekk old chicks was inoculated with infectious bursal disease virus through the oculonasal route. 12 similar birds kept as uninfected control. All birds were bled 3, 6, 10 and 16 days after inoculation. Infected birds had clinical signs of IBD. These chicks seroconverted and had significantly decreased total protein, lipid and decreased albumin to globulin ratio. There was significant increase in the concentration of corticosterone and thyroxin but not in the levels of tri-iodothyronine in the blood serum of infected birds.

Khaliel and El-Manakhly (1998) observed the pathological, immunocytochemical and immunological studies on a new infectious bursal disease vaccine dntermediate pluspin chickens. One-day old chicks vaccinated against Newcastle disease virus were examined for pathological and immunological effects of 2 types of IBD live vaccine (Intermediate and intermediate plus). Moderate transient bursal atrophy was seen one week after immunization with the intermediate plus vaccine, the highest antibody titres were seen in birds immunized with the intermediate plus vaccine. However, cell mediated response was temporarily reduced. Intermediate plus vaccine showed a slight transient immunosuppressive effect NDV vaccine. It was concluded that, despite the state of immunosuppression and the encountered bursal lesions

following immunization with the intermediate plus IBD vaccine, it provided better protection against IBDV challenge particularly when given after the intermediate vaccine. Both immunosuppressive and immunological effects of the vaccine were transient and within safe limit.

Thangavelu *et al.* (1998) studied the pathogenicity and immunosuppressive properties of two field isolates of IBD virus and five commercial IBD live virus vaccines marketed in India. The pathogenicity of the wild type viruses and vaccines were based on mortality, the bursa body weight ratio and microscopic lesions in the bursa in 3 week old chicks that received these viruses were evaluated by measuring the antibody responses to sheep red blood cells, Brucella abortus plain antigen and NDV vaccine in one day old chicks. One field isolate (N35/93) was found to be more pathogenic and immunosuppressive than the other (N45/92) while none of the commercial mild lukert type vaccines were found to be pathogenic. One of the vaccine strains marked as mild lukert type was highly immunosuppressive, one was moderate and one could be classified as mild. Both the intermediate vaccines tested were highly immunosuppressive.

Gabal and Azzam (1998) reported that ingestion of aflatoxin contaminated feed significantly lowered antibody titre in chickens immunized against ND, IB and IBD compared to nonaflatoxin treated groups. The immunosuppressive effect of aflatoxin has been related to its direct inhibition of protein synthesis, reduction in number of lymphocytes through its toxic effect on the bursa of Fabricius, mortality were increased.

Mohiuddin (1998) found a decrease in total basophils and thrombocyte counts in the broiler chickens feeding a diet containing 200 ppb of aflatoxin over a period of 3 months.

Kim *et al.* (1999) studied the long term effect of IBDV in chickens. Specially the restoration of virus induced bursal lesions and the duration of humoral immunodeficiency were examined. One week old SPF chickens were intraocularly inoculated with an intermediate vaccine strain (IBDV-Vac) or a virulent strain (IM-IBDV). At different intervals post inoculation chickens were examined for histopathologic lesions. The chickens were injected with a mixture of antigens, and primary antibody responses were examined at 10 days postimmunization Initially, the virus caused extensive necrosis of bursal B lymphocytes accompanied by an infiltration of T lymphocytes with time, the necrotic lesion in the bursa was resolved, the follicles became partly repopulated with B lymphocytes The repopulation occurred faster in the chickens exposed to IBDV-Vac than in the chickens exposed to IM-IBDV. Both IBDV-VAC and IM-IBDV caused suppression of the primary antibody response to antigens.

Mani *et al.* (2000) reported that 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. Aflatoxin B_1 had also resected in a significant dose related reduction in bursal weight of broilers.

Bakshi *et al.* (2000) found that the HI titre were significantly reduced through out the experimental period when level of aflatoxin is 0.38 ppm to 3.00 ppm. Control group recorded the highest antibody titre.. They indicated that dietary aflatoxin is a potent immunosuppressant in young chickens and that the extent of suppression of HI titre is directly related to dose of aflatoxin.

Vanden Berg, (2000) reported emergence of new IBDV variants and sequelae to IBD virus infection like imrnunosuppression and vaccinal failures have been found to cause increased number of outbreaks and the disease has

even been recorded in IBD vaccinated flocks resulting into heavy morbidity and mortality losses.. He observed that the virus is extremely lymphocidal with an affinity for immature B-cells resulting in bursal atrophy approximately four days post infection.

Cardoso *et al.* (2000) observed that the initial vaccine developed for controlling IBD though helped is bringing down the moralities due to IBD in chicks but also caused severe immunosuppression and in some cases even frank clinical disease resembling the natural infection.. So, this promoted the . development of attenuated and killed oil emulsified vaccine..

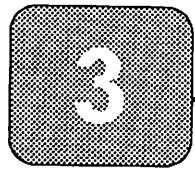
Oguz *et al.* (2000) reported that aflatoxin treatment significantly decreased serum total protein, albumin inorganic phosphorus, uric acid, total cholesterol and the values of hematocrit, red blood cells count, mean corpuscular volume, haemoglobin, thrombocyte counts, percentage of monocyte count and increased values of WBC and heterophil counts

Mani *et al.* (2001) described that 200 ppb of aflatoxin B_1 had significantly reduced the body weight, feed efficiency, carcass yield and immune development against ND in broilers Supplementation of Lactobacilli and vitamin E and selenium had improved the body weight, feed efficiency, carcass yields and immune status against Newcastle disease in broilers. Whereas the supplementation of levamisole had improved the immune development against ND alone but not the body wt. gain, feed efficiency and carcass yield

Mundas and Rao (2001) reported that the liver of aflatoxin (1.0 ppm) fed broiler birds showed hyperplasia of bile ducts epithelium increase the number of bile ducts and individualisation of hepatocytes on 5th week of age, during 6th and 7th week pathological lesions like linear haemorrhages, degenerative changes in hepatocytes, tendency to form microthrombi in the vessels and mild

infiltration of cells by 8th week in addition to these changes periportal infiltration of mononuclear cells and vacuolated appearance of hepatocytes.

CHAPTER



MATERIALS AND METHODS

Materials and Methods

Materials

Chicks

Three to four weeks old apparently healthy broiler chicks unvaccinated against IBD and free from antibodies to IBD, were routinely used for propagation of virus as well as production of antigen. The chicks were obtained from private poultry farm located in Patna.

Antigen

Poona strain of IBD virus being maintained in this department in the form of 50% bursal homogenate was used as a reference antigen throughout this study.

Antiserum

The hyper immune serum against a vaccine strain of IBD virus (Georgia strain, Indovax pvt.Ltd. , Siswala, Hariyana, India) raised in this laboratory was used throughout the study as reference antiserum. The serum was inactivated at 56^0 C for 30 minutes and stored at 0^0C.

Vaccines

F- Strain RDV Vaccine

A commercially available F-strain vaccine, manufactured by Indovax pvt. Ltd. Siswala, Hariyana , India was used for vaccination of day old chickens after

proper reconstitution. F- strain RD virus was further used as antigen in HA and HI tests after propagation in embryonating eggs by allantoic route.

IV 95 IBDV Vaccine

Cell culture adapted live invasive intermediate IBD virus vaccine (IV 95) available in freeze dried form manufactured by Indovax pvt. Ltd. Siswala, Hissar, Hariyana was used during this study. The vaccine was reconstituted in diluent supplied with vial and used within few hours after reconstitution.

Lasota Strain RDV Vaccine

The commercially available Lasotaj Strain vaccine manufactured by Indovax pvt. Ltd. siswala, Hissar, Hariyana was used for vaccination of chickens after proper reconstitutions.

Chicken red blood cells

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

Aflatoxin

Aflatoxin (B_1) available from department of Microbiology under ICAR project with the courtesy of Dr. B. K. Sinha, HOD & Associate Prof. of Microbiology. Calculated amount of aflatoxin was dissolved in 5 ml of chloroform with 5 ml of propylene glycol to provide the desired level of aflatoxin, mixed with feed and given to the chickens.

Buffers

i) For agar gel precipitation test (Aziz, 1985)

(a) Solution A

$Na_2HPO_4 2H_2O$	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

The mixture was autoclaved at 15 1b pressure for 15 minutes.

ii) Phosphate buffer saline (Aziz,1985)

Nacl	2.0 gm
KCl	0.05 gm
$Na_2HPO_4.2H_2O$	0.14 gm
K_2PO_4	0.05 gm

Double distilled water	250 ml
pH	7.2 to 7.4

This solution was autoclaved at 15 1b pressure for 15 minutes and stored at refrigerator temperature till used. This buffer was used for reconstitution and preparation of antigen as well as preparation of red blood cells suspension.

Methods

Preparation of antigen

Poona strain of IBDV in the form of 10 % bursal suspension in PBS, was inoculated into 3 to 4 weeks old broiler chicks at the rate of 0.2 ml of suspension per chicks by intraocular route. The chicks were sacrificed 48 hours post inoculation and the bursa were collected aseptically and homogenized in a sterile mortar using glass wool as an abrasive.

The homogenate was diluted 1:1 (w/v) in PBS, pH7.4 and treated with 10,000 units of penicillin and 10 mg streptomycin per ml of suspension. The suspension were frozen and thawed thrice and centrifuged at 4,000 rpm for 15 minutes. The supernatant was collected and tested for the presence of IBDV antigen by agar gel precipitation test (AGPT). Then it was distributed in small aliquots and stored at 0^0c as antigen. The normal (uninfected) bursal suspension prepared in the same manner served as negative antigen control.

Production of Hyper immune Serum

Hyper immune serum against IBDV was raised in 20 weeks old 6 apparently healthy chickens. Each bird was given Georgia strain of IBDV through oculonasal route at weekly intervals. 2 weeks after the 4th inoculation,

the bird were bled and the serum was tested for the presence of IBDV antibody by AGPT. This serum was stored at 0^0c for further use.

Collection of serum samples from chicken

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

Collection of blood samples from chicken for Haematological study

Two to two and half ml of blood drawn as described above was immediately transferred into clean and sterilized vials of 5 ml capacity.1% solution of EDTA 0.1 ml per ml of blood was kept at 4 ^{0}C until further use.

Chicken red blood cell suspension

Two adult chickens were used as donor of blood. 1 to 1.5 ml of blood was collected from each bird in an anticoagulant, disodium salt of ethylene diamine tetra acetic acid (EDTA) at the rate of 1 mg 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 percent RBC suspension was made in PBS and stored at refrigerator temperature. This RBC suspension was used only for four day after preparation and thereafter the fresh RBC suspension was prepared.

(i) Agar gel precipitation test

The test was done following the method of Hirai et al., (1974) with some modification. The glass microscopic slide (75 mm x 25 mm) were precoated 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 a performed glass pipette and allowed to solidify. After setting the slide were kept at 4^0c for over night to facilitate punching of gels. A hexagonal well pattern consisting of a central well and five peripheral well of 3.5 mm diameter, 8 mm 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.

(ii) Quantitative agar gel precipitation test (QAGPT)

The level of precipitating antibody was determined as per the method of Cullen and Wyeth (1975) with some modifications. A two fold serial dilution of the test serum was made in the same buffer as that was used in the preparation of gel. The central well contained reference antigen and the peripheral well contained two fold dilution of sera. The volume of reagents put in each well was 0.2 ml. Three replicate for each test were carried out simultaneously. The reciprocal of the highest dilution of serum which gave precipitating line was taken as the test of the serum. The mean antibody titre of the positive samples were calculated according to Villegas and Purchase (1980).

(iii) Haemagglutination (HA) Test

The HA test was performed in perspex plate to prepare 4 HA units of RD virus as described by Beard (1980). Taking 0.5 ml of virus material two fold serial dilution were made in PBS, except in control well in which only PBS (0.5 ml) 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 induced. The plate was stirred gently for mixing and uniform distribution of erythocytes 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 with the aid of reading mirror and result of HA titre was recorded as reciprocal of the highest dilution showing 100% HA.

(iv) 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 (Charan *et. al.*, 1981) 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 (4HA units) 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. In each test, a known positive and negative serum samples were also included as controls. The plate was shaken gently to mix the serum virus and RBCs and incubated at room temperature for 40 minutes. The HI antibody titre was taken so the reciprocal of the highest dilution of serum showing complete inhibition of agglutination of RBCs.

Experimental Design

One hundred and twenty five newly hatched chicks were obtained from private poultry farm, Patna. All the chicks in the different groups were housed under identical feeding and manageable condition for 56 days.

Birds in all the five groups were given F-strain RDV vaccine intraocularly at the dose rate of 0.05 ml per bird at zero day of age while all the birds from group I to IV received IBD vaccine (IV95 strain) intraocularly at 14 days of age. Group V remained unvaccinated. At 28 days of age birds in all the five groups were given Lasota strain RDV vaccine through drinking water. The birds of group I to group III were given aflatoxin supplemented feed at the dose rate of 50 ppb, 100 ppb and 200 ppb of aflatoxin from day of hatching to day 49. Group IV and V were given non-aflatoxin supplemented feed.

From each group of chickens Pre IBD vaccinated blood serum samples were collected in 13 days of age and post IBD vaccinated blood serum samples were collected on 21, 28, 35 and 42 and 56 days of age. Blood samples collected were evaluated for haematological changes including differential leucocytic count, Hb, packed cell volume (PCV) and total leucocuytic count. Serum samples were evaluated for determination of antibody titres to IBD vaccine and Ranikhet disease F strain vaccine as well as estimation of serum total protein, serum albumin, globulin and serum calcium and phosphorus level. Five birds from each group were sacrificed by cervical dislocation on 3rd, 7th and 11th day post IBD vaccination. Portion of bursa, Kidney, Liver and spleen were collected in 10% formalin saline for histopathological examination. Bursa : body wt ratio, Spleen : body wt ratio were determined on 3rd, 7th and 11th day post IBD vaccination. Body wt gain and feed conversion ratio (FCR) were determined when the birds were 56 days of age. *(i.e.* the termination of experiment.)

Protein Estimation

Total serum protein, albumin and globulin were determined by biuret method as described by Reinhold (1953), with some modifications.

Serum Calcium Estimation

Serum Calcium was determined by method described by Clark and Collip (1925) with modification of Kramer Tisdall method.

Serum Inorganic Phosphorus Estimation

It was determined by method described by Fiske and Rao (1925).

Haemoglobin Estimation

It was determined by Sahlins Haemoglobin meter method as described by Jain (1986).

Packed Cell Volume

PCV was calculated by employing Wintrobe haematocrit tube as per method described by Jain (1986). The percentage value of PCV was calculated as follows :

$$PCV\,(\%) = \frac{\text{Height of red blood cells (mm)}}{\text{Total height of column (mm)}} \times 100$$

Total Leucocyte Count

It was determined by Haemocytometer which contains one counting chamber (slide and cover), RBC pipette and one WBC pipette, as described by Natt & Herick (1952).

Differential Leucocyte Count

The percent lymphocyte, heterophil, monocyte, basophil and eosinophil were counted by staining the film with Leishman's stain as described by Lucas and Jamroz (1961).

Bursa : Body weight (B : BW) ratio

This was calculated as follows :

$$B : BW = \frac{\text{Bursa weight (gm)}}{\text{Body weight (gm)}} \times 1000$$

Spleen : Body weight (S : BW) ratio

$$S : BW = \frac{\text{Spleen weight (gm)}}{\text{Body weight (gm)}} \times 1000$$

Body weight gain and feed conversion ratio

The body weight gains of chickens were recorded by subtracting the live weight of chicks at day 2 from live weight at day 56. Feed conversion ratio per bird at 56 days of age was determined as follows :

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

Histopathology

The tissue samples for histopathological examination were processed in acetone benzene (Lillie and Fullmer, 1976), wax (melting point 62ºC) and embeded in Paraffin. Five micron thick sections were cut and stained by haemotoxylin and eosin (Drury and Wallington, 1980).

Scoring of bursal lesions

The scoring system suggested by winterfield and Thacker (1978) was followed with slight modifications. Bursal lesioin score was done on a scale of 0 (none), 1 (minimal), 2 (mild), 3 (moderate) and 4 (marked) by the following criteria : Lymphoid necrosis, Lymphoid depletioin, reticulo epithelial hyperplasia, vacuolar degeneration, follicular cyst, Interfollicular edema, epithelial changes, Interstitial fibrosis and cellular infiltration.

Statistical analysis

The mean and standard errors of the values obtained were determined. The percent values of Hb, PCV, DLC and mortality were transformed into Arc Sin inverse data. Analysis of variance (ANOVA) was performed wherever necessary as per Snedecor and Cochran (1967).

TABLE 1 : EXPERIMENTAL DESIGN

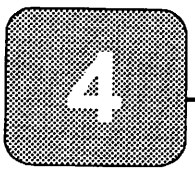
Group	No. of chicks in a group	Level of Aflatoxin (B_1) (ppb)	Period of Aflatoxin treatment (days)	Age at IBD vaccination (days)	Age at RD-F strain vaccination (days)	Age at RD Lasota strain vaccination (days)	No. of chicks sacrificed on days post IBD vaccination			OBSERVATION PLANNED
							3rd	7th	11th	
I	25	50	0 – 49	14 (i,0)*	0 (i,0)	28 (D.W.)**	5	5	5	1. Collection of pre IBD vaccinated blood samples on 13 day of age for determination of maternal Ab level of IBDV and RDV, normal biochemical status of chicks. 2. Collection of blood samples at 7, 14, 21, 28, 35 and 42nd days post IBD vaccination for : i) Determination of Ab titre to IBDV by QAGPT ii) Determination of Ab titre to RDV (F-strain) by HI test iii) Determination of serum total protein, albumin, globulin and Ca & P level. iv) Determination of differential leucocyte count, TLC, PCV and Hb 3. Collection of Bursa of Fabricius, spleen, Kidney and liver in 10% formalin saline for its histopathological examination by H & E staining method. 4. Body wt. gain and FCR recorded on termination of experiment. 5. Determination of bursa : body wt. ratio and spleen : body wt. ratio on 3rd 7th and 11th day post IBD vaccination.
II	25	100	0 – 49	14 (i,0)	0 ((i,0)	28 (D.W.)	5	5	5	
III	25	200	0 – 49	14 (i,0)	0 (i,0)	28 (D.W.)	5	5	5	
IV	25	-	-	14 (i,0)	0 (i,0)	28 (D.W.)	5	5	5	
V	25	-	-	Unvaccin-ated	0 (i,0)	28 (D.W.)	5	5	5	

* i,0 indicate for intraocular route

** D.W. indicate for Drinking Water

- indicate no aflatoxin

CHAPTER



RESULTS

Result

Effect of aflatoxin on immune responses to IBDV (IV 95 strain) in chickens

The immune responses to IBD virus vaccine in different groups of chickens are presented in table - 2. The perusal of the table demonstrated the occurrence of seroconversion by 14 days post IBD vaccination in groups of birds that received IBD vaccine (gr. I-IV). The perusal of this table also revealed progressive rise in precipitating antibody titre till the 28 days of observation (i.e. 28 days post IBD vaccination) and thereafter decrease in antibody titre till the last day of observation (i.e. 42 days post IBD vaccination) in all the vaccinated groups. Further the comparison of QAGPT titres between different groups (gr I - IV) clearly indicated higher titre in groups which received only IBD vaccination than the titre observed in groups which received IBD vaccination and aflatoxin both (gr. I- III) overall the intervals post IBD vaccination (Table-2). Again comparison of QAGPT titres between different groups of aflatoxin treated chickens (gr I-III) revealed significantly lower titre in group III which received 200 ppb of aflatoxin than the titre recorded in group II & I which received 100 ppb and 50 ppb of aflatoxin respectively. No antibody could be detected in the last group (gr-V) which did not receive IBD vaccine.

Effect of aflatoxin on immune responses to RDV (F-strain and Lasota strain) vaccine in IBD vaccinated chickens.

Immune response to RD virus in IBD vaccinated chickens are presented in table-3. The perusal of the table showed that the IBD vaccine strain had immunosuppressive effect as evident from lower HI titre in control group which

TABLE 2 : EFFECT OF AFLATOXIN ON IMMUNE RESPONSES TO A VACCINE STRAIN OF IBD VIRUS IN BROILER CHICKENS.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Pre IBD vaccinated	Mean ± SE of QAGPT titre (log_2) Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	-ve	$1.40^{a} \pm 0.244$ (5)	$2.00^{ab} \pm 0.200$ (5)	$2.60^{ab} \pm 0.200$ (5)	$2.80^{ab} \pm 0.200$ (5)	$2.60^{ab} 0.245$ (5)	$2.40^{ab} \pm 0.374$ (5)
II	14	100	-ve	$1.60^{a} \pm 0.244$ (5)	$2.00^{ab} \pm 0.316$ (5)	$2.40^{ab} \pm 0.400$ (5)	$2.60^{b} \pm 0.400$ (5)	$2.20^{b} \pm 0.374$ (5)	$2.00^{b} \pm 0.374$ (5)
III	14	200	-ve	$1.20^{a} \pm 0.200$ (5)	$1.40^{a} \pm 0.244$ (5)	$1.80^{a} \pm 0.374$ (5)	$1.80^{c} \pm 0.374$ (5)	$1.20^{c} \pm 0.200$ (5)	$1.00^{c} \pm 0.00$ (5)
IV	14	-	-ve	$1.80^{a} \pm 0.374$ (5)	$2.60^{b} \pm 0.245$ (5)	$3.20^{b} \pm 0.200$ (5)	$3.60^{a} \pm 0.245$ (5)	$3.20^{a} \pm 0.245$ (5)	$3.20^{a} \pm 0.374$ (5)
V	Unvaccinated	-	-ve	-ve	-ve	-ve	-ve	-ve	-ve

- Figures in parenthesis indicates number of observation
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$)

TABLE 2 : EFFECT OF AFLATOXIN ON IMMUNE RESPONSES TO A VACCINE STRAIN OF IBD VIRUS IN BROILER CHICKENS.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Pre IBD vaccinated	Mean ± SE of QAGPT titre (log_2)					
				Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	-ve	$1.40^{a} \pm 0.244$ (5)	$2.00^{ab} \pm 0.200$ (5)	$2.60^{ab} \pm 0.200$ (5)	$2.80^{ab} \pm 0.200$ (5)	$2.60^{ab} 0.245$ (5)	$2.40^{ab} \pm 0.374$ (5)
II	14	100	-ve	$1.60^{a} \pm 0.244$ (5)	$2.00^{ab} \pm 0.316$ (5)	$2.40^{ab} \pm 0.400$ (5)	$2.60^{b} \pm 0.400$ (5)	$2.20^{b} \pm 0.374$ (5)	$2.00^{b} \pm 0.374$ (5)
III	14	200	-ve	$1.20^{a} \pm 0.200$ (5)	$1.40^{a} \pm 0.244$ (5)	$1.80^{a} \pm 0.374$ (5)	$1.80^{c} \pm 0.374$ (5)	$1.20^{c} \pm 0.200$ (5)	$1.00^{c} \pm 0.00$ (5)
IV	14	-	-ve	$1.80^{a} \pm 0.374$ (5)	$2.60^{b} \pm 0.245$ (5)	$3.20^{b} \pm 0.200$ (5)	$3.60^{a} \pm 0.245$ (5)	$3.20^{a} \pm 0.245$ (5)	$3.20^{a} \pm 0.374$ (5)
V	Unvaccinated	-	-ve	-ve	-ve	-ve	-ve	-ve	-ve

- Figures in parenthesis indicates number of observation
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$)

Fig. 28 (a) : Histogram showing effect of aflatoxin on Ab titre to IBD vaccine in chicken.

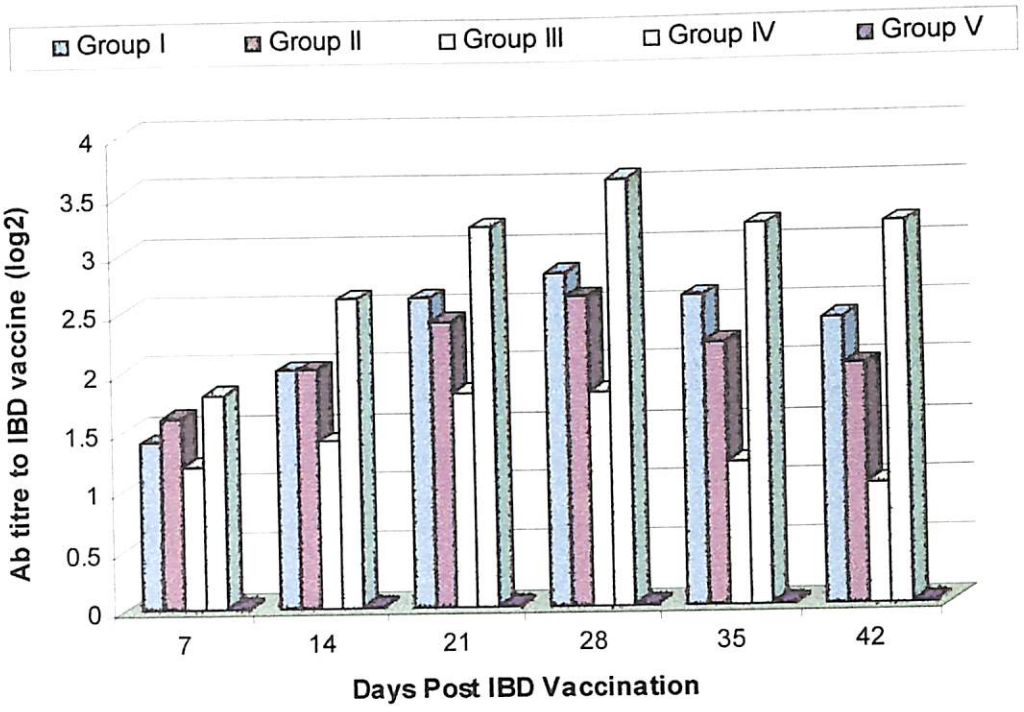


Fig. 28 (b) : Line graph showing effect of aflatoxin on Ab titre to IBD vaccine in chicken.

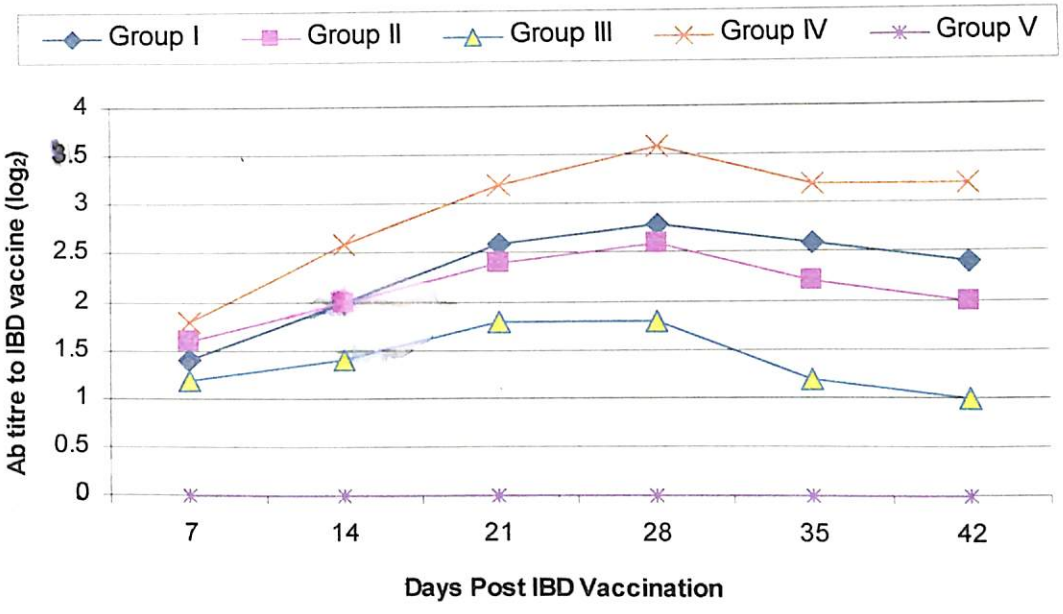


Fig. 29 (a) : Histogram showing effect of aflatoxin on Ab titre to RDV (F – Strain and Lasota strain) vaccine is IBD vaccinated chickens.

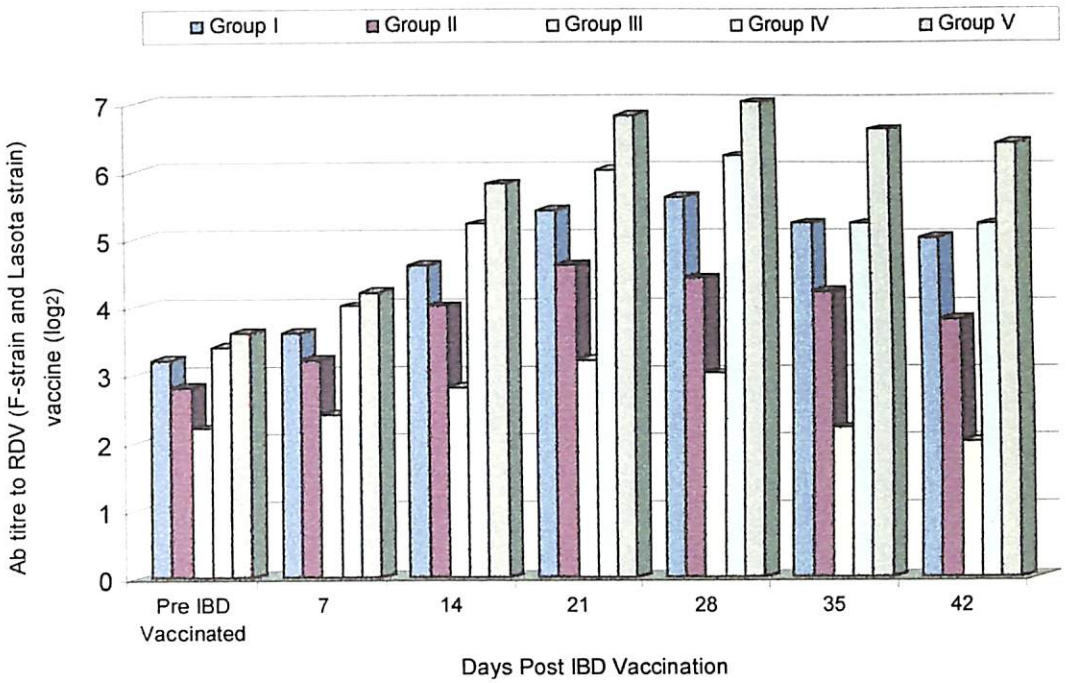


Fig. 29 (b) : Line graph showing effect of aflatoxin on Ab titre to RDV (F – Strain and Lasota strain) vaccine is IBD vaccinated chickens.

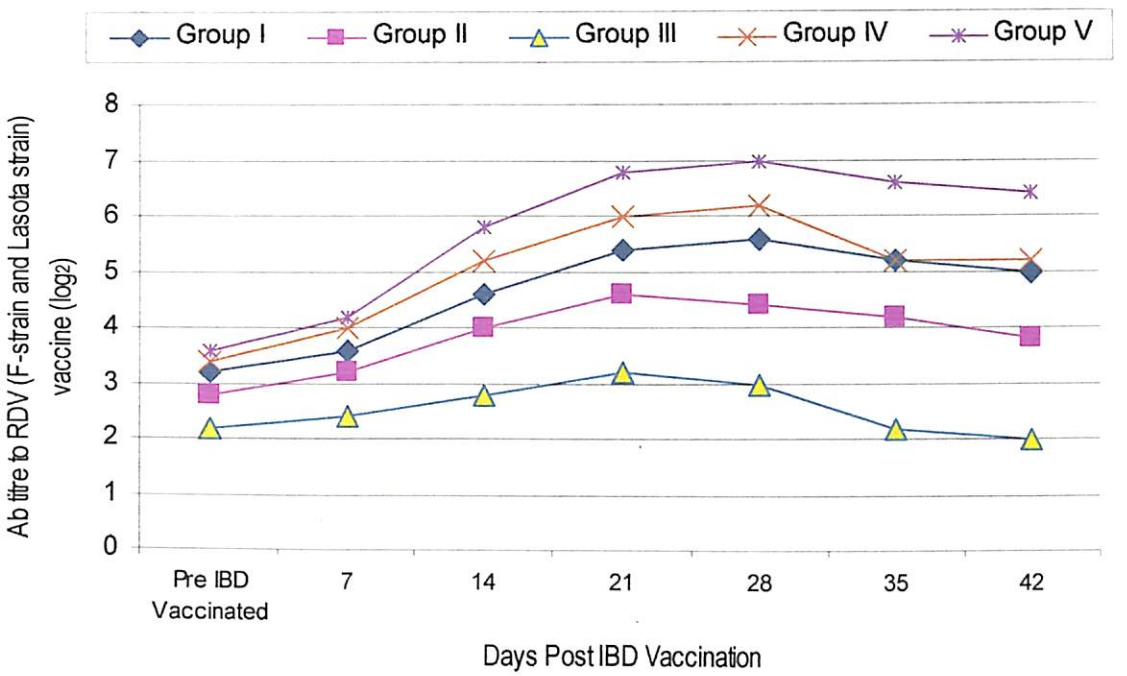


TABLE 3 : EFFECT OF AFLATOXIN ON IMMUNE RESPONSES TO RDV (F-STRAIN AND LASOTA STRAIN) VACCINE IN IBD VACCINATED BROILER CHICKENS.

Group	Age of RDV, F-strain vaccination (days)	Age at IBD vaccination (days)	Age at RDV Lasota strain vaccination (days)	Level of Aflatoxin (ppb)	Mean + SE of HI antibody titre (log_2) to RDV vaccine						
					Pre IBD vaccinated	Days Post IBD Vaccination					
						7	14*	21	28	38	42
I	0	14	28	50	$3.20^{a} \pm 0.200$ (5)	$3.60^{ab} \pm 0.244$ (5)	$4.60^{ab} \pm 0.244$ (5)	$5.40^{a} \pm 0.316$ (5)	$5.60^{a} \pm 0.245$ (5)	$5.20^{a} \pm 0.200$ (5)	$5.00^{a} \pm 0.316$ (5)
II	0	14	28	100	$2.80^{a} \pm 0.374$ (5)	$3.20^{a} \pm 0.200$ (5)	$4.00^{a} \pm 0.316$ (5)	$4.60^{b} \pm 0.200$	$4.40^{b} \pm 0.244$ (5)	$4.20^{b} \pm 0.244$ (5)	$3.80^{b} \pm 0.200$ (5)
III	0	14	28	200	$2.20^{b} \pm 0.200$ (5)	$2.40^{d} \pm 0.244$ (5)	$2.80^{d} \pm 0.374$ (5)	$3.40^{c} \pm 0.374$ (5)	$3.00^{c} \pm 0.400$ (5)	$2.20^{c} \pm 0.200$ (5)	$2.00^{c} \pm 0.316$ (5)
IV	0	14	28	-	$3.40^{a} \pm 0.244$ (5)	$4.00^{bc} \pm 0.316$ (5)	$5.20^{bc} \pm 0.200$ (5)	$6.00^{a} \pm 0.316$ (5)	$6.20^{a} \pm 0.316$ (5)	$5.40^{a} \pm 0.374$ (5)	$5.20^{a} \pm 0.374$ (5)
V	0	Unvaccinated	28	-	$3.60^{a} \pm 0.400$ (5)	$4.20^{c} \pm 0.245$ (5)	$5.80^{c} \pm 0.400$ (5)	$6.80^{d} \pm 0.245$ (5)	$7.00^{d} \pm 0.200$ (5)	$6.60^{d} \pm 0.245$ (5)	$6.40^{d} \pm 0.400$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d) in individual column did not differ significantly ($P < 0.05$).
* Lasota vaccination given here.

received only IBD vaccine but not aflatoxin (gr. IV) over all the periods post IBD vaccination when compared with the HI titre for the corresponding intervals in group of birds which did not receive IBD vaccine (gr. V).

Further it was noted that administration of aflatoxin in IBD vaccinated birds (gr. I j III) resulted in reduction of HI titre over all the intervals post IBD vaccination when compared with HI titre for the corresponding intervals in control group (gr. IV). Again comparison of HI titre of birds in different aflatoxin treated groups (gr. I-III) clearly demonstrated that the HI titres were significantly lower in group III followed by group II and I. It was also observed that the rise of HI titre continued up to 28 dpv and thereafter the titres started declining in group I, IV and V whereas in group II and III the rise of HI titre continued up to 21 dpv only and thereafter the titre started declining.

Effect of aflatoxin on Bursal lesion scores in different groups of IBD vaccinated chickens

The effect of aflatoxin on bursal lesion scores in different groups of chickens sacrificed on 3rd ,7th and 11th day post IBD vaccination are presented in table - 4, 5 and 6. The result demonstrated production of bursal lesion typical of IBD virus as characterized by lymphoid depletion and necrosis, interfollicular oedema, epithelial invagination, interstitial fibrosis, cellular infiltration and vacuolar degeneration. Further perusal of this tables revealed that the lesion score was highest in group III followed by group II and I. The lesion score was lowest in vaccinated control group (gr IV). The lesion score were dose dependent (ie highest when aflatoxin level was 200 ppb followed by 100 ppb and so on) as apparent from the findings recorded in table 5 & 6 (ie 7th & 11th day post IBD vaccination).

TABLE 4 : MEAN ± S.E. OF BURSAL LESION SCORES IN DIFFERENT GROUPS OF CHICKENS SACRFICED ON 3RD DAY POST IBD VACCINATION

Group	Follicular Changes						Epithelial Changes				Interstitial fibrosis	Cellular infiltration	Total lesion score
	Inter follicular oedema	Lymphoid necrosis	Lymphoid depletion	Reticulo epithelial hyperplasia	Vacuolar degene-ration	Follicular cyst	Hyperp-lasia	Epithelia invagin-ation	Cyst formation	Vacuol-ation			
I	0.8 ± 0.37 (5)	1.85 ± 0.60 (5)	2.0 ± 0.40 (5)	0.2 ± 0.20 (5)	0.4 ± 0.24 (5)	0.6 ± 0.24 (5)	0.0 (5)	0.6 ± 0.24 (5)	0.0 (5)	0.0 (5)	0.8 ± 0.20 (5)	0.6 ± 0.31 (5)	0.73^{a} ± 0.037 (60)
II	1.2 ± 0.26 (5)	2.0 ± 0.40 (5)	2.0 ± 0.40 (5)	0.2 ± 0.20 (5)	0.6 ± 0.24 (5)	0.6 ± 0.20 (5)	0.6 ± 0.20 (5)	1.0 ± 0.00 (5)	0.8 ± 0.20 (5)	0.2 ± 0.24 (5)	1.2 ± 0.40 (5)	0.8 ± 0.32 (5)	0.90^{ab} ± 0.038 (60)
III	1.6 ± 0.40 (5)	2.0 ± 0.20 (5)	2.6 ± 0.24 (5)	0.0 (5)	0.2 ± 0.40 (5)	0.0 (5)	0.8 ± 0.24 (5)	1.0 ± 0.32 (5)	0.8 ± 0.40 (5)	0.0 (5)	1.6 ± 0.63 (5)	1.2 ± 0.20 (5)	0.96^{b} ± 0.035 (60)
IV	0.8 ± 0.24 (5)	1.8 ± 0.20 (5)	1.6 ± 0.31 (5)	0.0 (5)	0.0 (5)	0.0 (5)	0.0 (5)	1.2 ± 0.60 (5)	0.4 ± 0.24 (5)	0.0 (5)	0.6 ± 0.24 (5)	0.2 ± 0.40 (5)	0.51^{c} ± 0.039 (60)
V	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$).

TABLE 5 : MEAN ± S.E. OF BURSAL LESION SCORES IN DIFFERENT GROUPS OF CHICKENS SACRIFICED ON 7TH DAY POST IBD VACCINATION.

Group	Follicular Changes						Epithelial Changes				Interstitial fibrosis	Cellular infiltration	Total lesion score
	Inter follicular oedema	Lymphoid necrosis	Lymphoid depletion	Reticulo epithelial hyperplasia	Vacuolar degene-ration	Follicular cyst	Hyperp-lasia	Epithelia invagin-ation	Cyst formation	Vacuol-ation			
I	0.6 ± 0.24 (5)	2.0 ± 0.37 (5)	1.8 ± 0.40 (5)	0.6 ± 0.20 (5)	0.6 ± 0.40 (5)	0.8 ± 0.20 (5)	0.2 ± 0.31 (5)	0.6 ± 0.24 (5)	0.2 ± 0.60 (5)	0.4 ± 0.24 (5)	0.8 ± 0.40 (5)	0.2 ± 0.20 (5)	0.73^{A} ± 0.063 (60)
II	1.2 ±0.60 (5)	1.8 ± 0.31 (5)	2.0 ± 0.40 (5)	0.0 (5)	1.0 ± 0.00 (5)	0.8± 0.36(5)	0.6 ± 0.40 (5)	0.6 ± 0.40 (5)	0.6 ± 0.24 (5)	0.2 ± 0.32 (5)	1.4 ± 0.40 (5)	0.4 ± 0.40(5)	0.83^{b} ± 0.095 (60)
III	1.0 ± 0.20 (5)	2.2 ± 0.60 (5)	2.2 ± 0.24 (5)	0.0 (5)	1.0 ± 0.20 (5)	1.2 ± 0.24 (5)	1.0 ± 0.60 (5)	1.0 ± 0.40 (5)	0.8 ± 0.20 (5)	0.2 ± 0.81(5)	2.0 ± 0.81 (5)	0.8 ± 0.24 (5)	1.11^{C} ± 0.10 (60)
IV	0.6 ± 0.40 (5)	1.8 ± 0.24 (5)	1.4 ± 0.31 (5)	0.2 ± 0.40 (5)	0.6 ± 0.40 (5)	0.4 ± 0.40 (5)	0.2 ± 0.20 (5)	1.0 ± 0.00 (5)	0.4 ± 0.40 (5)	0.0 (5)	0.8 ± 0.23 (5)	0.4 ± 0.32 (5)	0.65^{a} ± 0.092 (60)
V	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$).

TABLE 6 : MEAN ± S.E. OF BURSAL LESION SCORES IN DIFFERENT GROUPS OF CHICKENS SACRIFICED ON 11TH DAY POST IBD VACCINATION.

Group	Follicular Changes						Epithelial Changes				Interstitial fibrosis	Cellular infiltration	Total lesion score
	Inter follicular oedema	Lymphoid necrosis	Lymphoid depletion	Reticulo epithelial hyperplasia	Vacuolar degene-ration	Follicular cyst	Hyperp-lasia	Epithelia invagin-ation	Cyst formation	Vacuol-ation			
I	0.2 ± 0.20 (5)	1.80 ± 0.40 (5)	1.4 ± 0.40 (5)	0.6 ± 0.20 (5)	0.8 ± 0.24 (5)	0.6 ± 0.40 (5)	0.2 ± 0.20 (5)	0.6 ± 0.24 (5)	0.2 ± 0.20 (5)	0.6 ± 0.20 (5)	1.0 ± 0.0 (5)	0.2 ± 0.60 (5)	0.68^{ab} ± 0.087 (60)
II	0.8 ± 0.32 (5)	2.0 ± 0.60 (5)	1.6 ± 0.20 (5)	0.0 (5)	1.0 ± 0.0 (5)	0.6 ± 0.24 (5)	0.4 ± 0.24 (5)	0.8 ± 0.32 (5)	0.6 ± 0.40 (5)	0.4 ± 0.40 (5)	1.8 ± 0.20 (5)	0.4 ± 0.24 (5)	0.86^{a} ± 0.094 (60)
III	0.8 ± 0.37 (5)	2.2 ± 0.31 (5)	1.6 ± 0.24 (5)	0.2 ± 0.24 (5)	1.2 ± 0.20 (5)	1.0 ± 0.20 (5)	1.0 ± 0.20 (5)	0.8 ± 0.37 (5)	0.8 ± 0.23 (5)	0.6 ± 0.24 (5)	2.4 ± 0.36 (5)	0.8 ± 0.40 (5)	1.12^{c} ± 0.12 (60)
IV	0.2 ± 0.20 (5)	1.8 ± 0.10 (5)	0.8 ± 0.40 (5)	0.2 ± 0.24 (5)	0.6 ± 0.40 (5)	0.4 ± 0.20 (5)	0.2 ± 0.40 (5)	0.6 ± 0.23 (5)	0.4 ± 0.24 (5)	0.2 ± 0.24 (5)	0.6 ± 0.20 (5)	0.0 (5)	0.50^{b} ± 0.081 (60)
V	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$).

Effect of aflatoxin on biochemical profile in IBD (IV 95 strain) vaccinated chickens

The effect of IBD vaccine virus on serum total protein values in chickens after supplementation of aflatoxin in feed are presented in table -7. The perusal of the table revealed that the vaccine virus has lowering effect on total serum protein as evident by the values recorded in group IV over different intervals post IBD vaccination when compared with the corresponding values in the unvaccinated control (gr. V). It was also revealed that aflatoxin treatment caused further lowering of total serum protein values over and above the values recorded in group IV (Table-7). The synergistic effect of aflatoxin was dose dependent - higher the dose level higher was the rate of reduction as apparent from the values recorded in different aflatoxin treated groups (gr. I-III) (Table-7).

Mean + SE of serum albumin values are shown in table-8. The perusal of the table showed rising trends of serum albumin values in unvaccinated group (gr. V) with increase in age. On the other hand the group of birds which received IBD vaccines (gr. I - IV) demonstrated decline in serum albumin values on 7, 14 and 21 day post IBD vaccination when compared with their pre-vaccinated value, whereas in group III decline of serum albumin values were observed overall the periods post IBD vaccination. On comparison between aflatoxin treated groups (gr. I - III) the values were lowest in group III followed by group II and I. On perusal of this table, it also revealed that the values of serum albumin of group III and group IV significantly differed by 28, 35 & 42 days post IBD vaccination. In general more was the dose of aflatoxin; reducing effect on serum albumin was of higher degree.

Mean ± SE of serum globulin values are depicted in table j 9. In general, the serum globulin values did not differ significantly at any intervals post IBD

TABLE 7 : EFFECT OF AFLATOXIN ON TOTAL SERUM PROTEIN (GM/100 ML) IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Total Serum Protein (gm/100 ml)						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$3.17^{a} \pm 0.02$ (5)	3.29^{a} 0.01 (5)	$3.19^{a} \pm 0.015$ (5)	$3.28^{a} \pm 0.05$ (5)	$3.26^{a} \pm 0.01$ (5)	$3.48^{a} \pm 0.01$ (5)	$3.62^{a} \pm 0.06$ (5)
II	14	100	$3.10^{a} \pm 0.05$ (5)	$3.17^{a} \pm 0.02$ (5)	$3.07^{a} \pm 0.014$ (5)	$3.21^{a} \pm 0.014$ (5)	$3.14^{a} \pm 0.02$ (5)	$3.11^{a} \pm 0.02$ (5)	$3.05^{b} \pm 0.01$ (5)
III	14	200	$3.07^{a} \pm 0.05$ (5)	$3.00^{a} \pm 0.03$ (5)	$3.01^{a} \pm 0.01$ (5)	$3.23^{a} \pm 0.07$ (5)	$3.14^{a} \pm 0.02$ (5)	$3.03^{a} \pm 0.059$ (5)	$2.97^{b} \pm 0.085$ (5)
IV	14	-	$3.24^{a} \pm 0.07$ (5)	$3.29^{a} \pm 0.01$ (5)	$3.58^{b} \pm 0.016$ (5)	$3.66^{b} \pm 0.06$ (5)	$3.90^{b} \pm 0.06$ (5)	$4.05^{b} \pm 0.02$ (5)	$4.10^{c} \pm 0.02$ (5)
V	Unvaccinated	-	$3.14^{a} \pm 0.01$ (5)	$3.32^{a} \pm 0.05$ (5)	$3.69^{b} \pm 0.06$ (5)	$3.73^{b} \pm 0.06$ (5)	$3.98^{b} \pm 0.046$ (5)	$4.18^{b} \pm 0.056$ (5)	$4.30^{c} \pm 0.065$ (5)

- Figures in parenthesis indicates number of observation
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$)

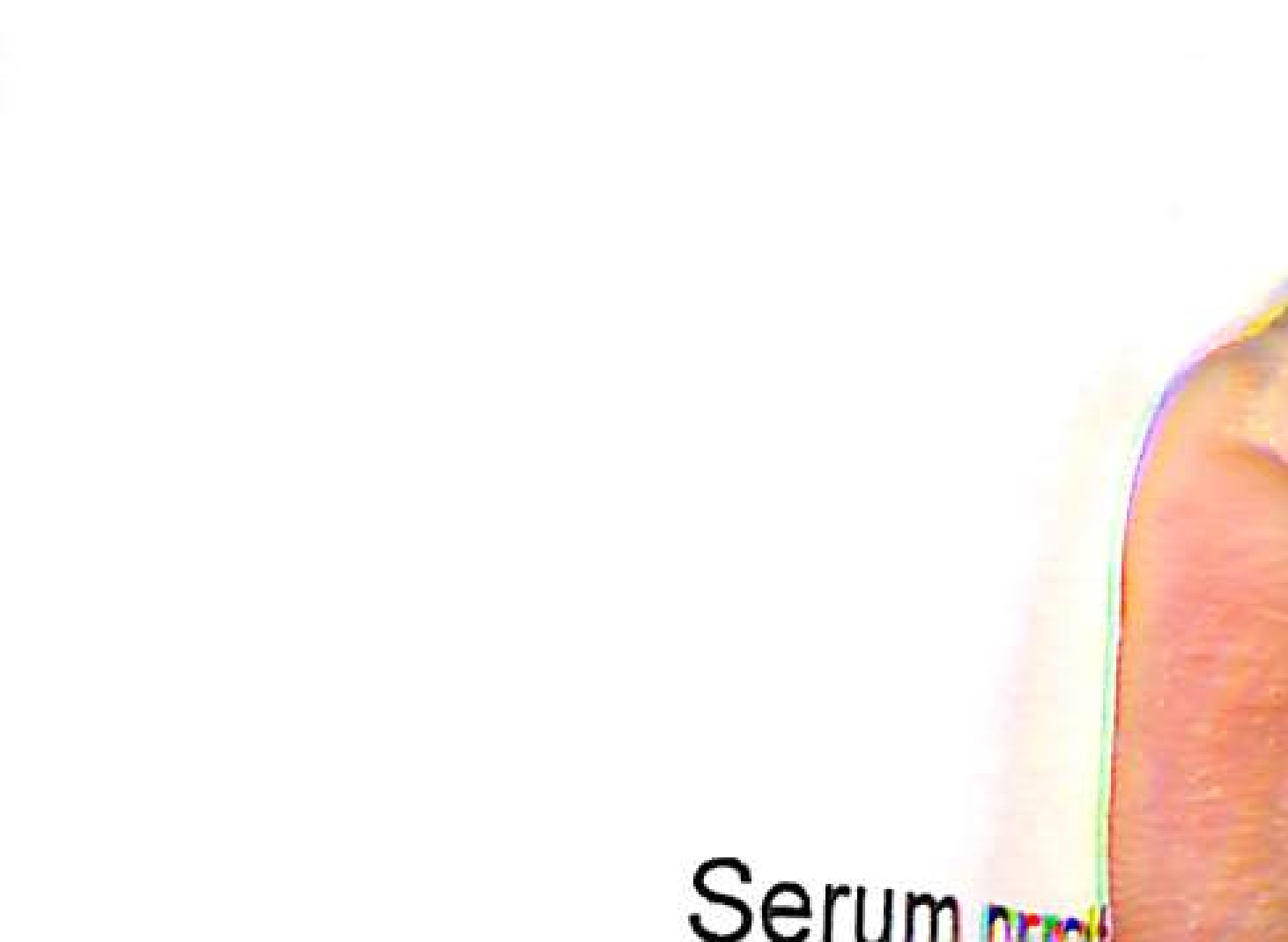

Serum

Fig. 30 (a) : Histogram showing total serum protein level in aflatoxin treated groups Pre & Post IBD vaccination

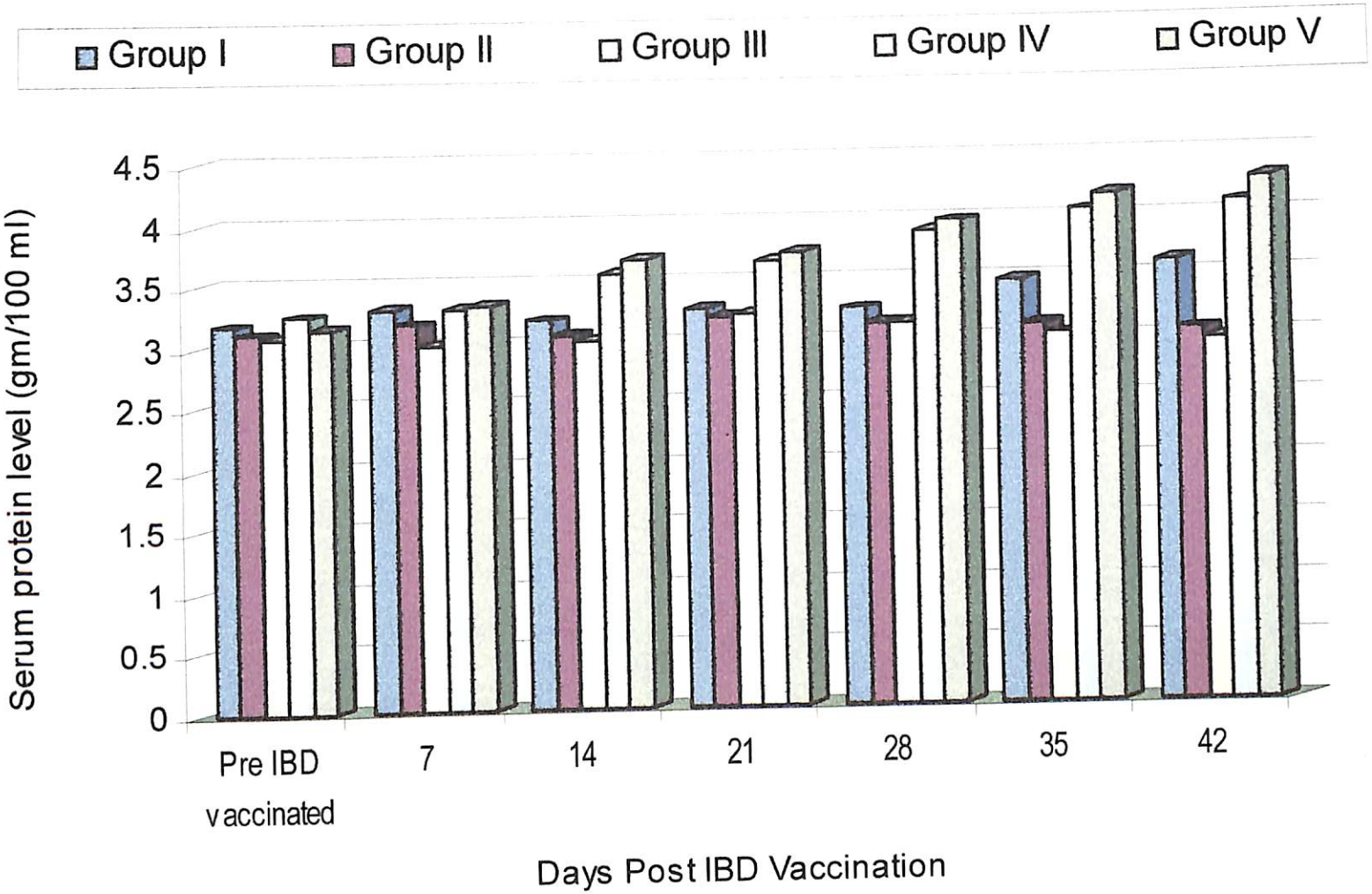


Fig. 31 (a) : Histogram showing serum albumin level Pre & Post IBD vaccination in aflatoxin treated groups of chickens.

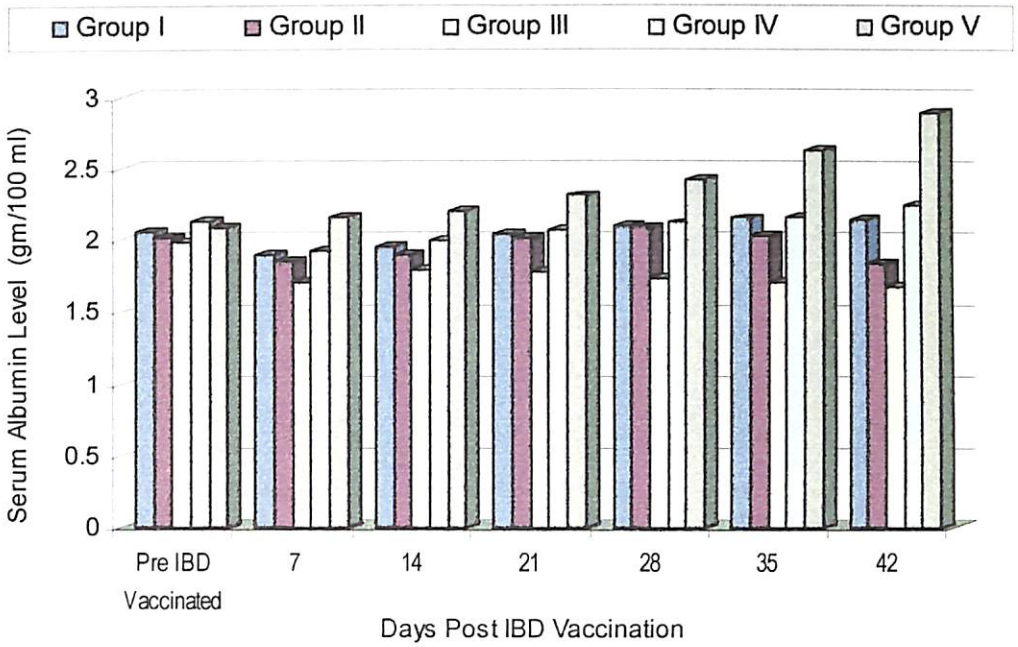


Fig. 31 (b) : Line Graph showing serum albumin level Pre & Post IBD vaccination in aflatoxin treated groups of chickens.

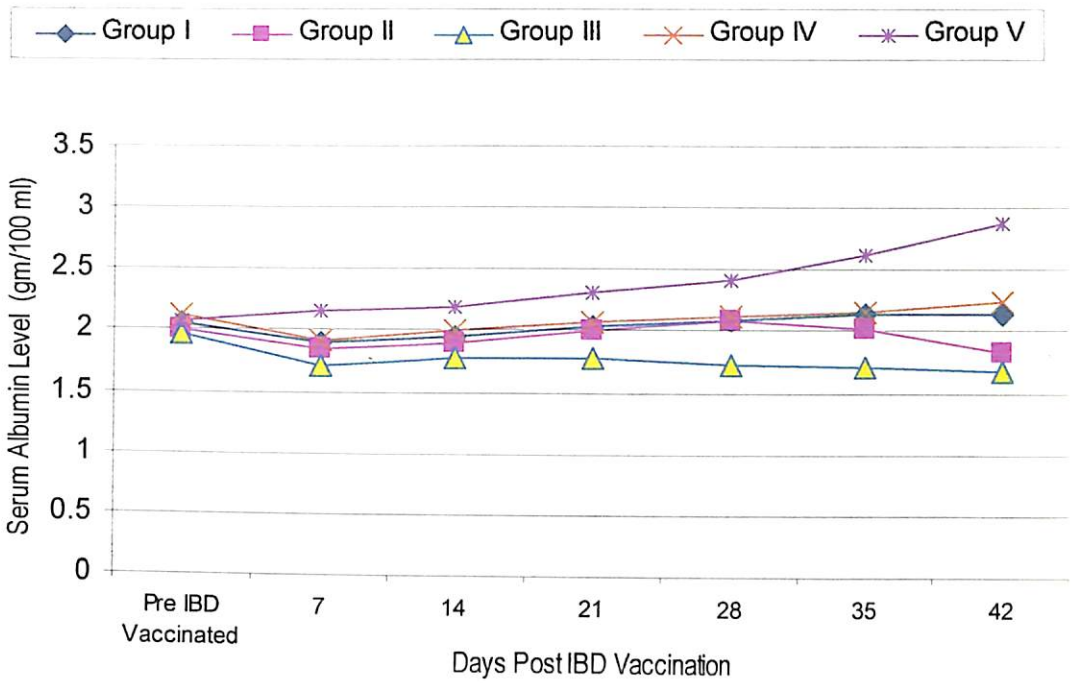


TABLE 8 : EFFECT OF AFLATOXIN ON SERUM ALBUMIN (GM/100 ML) IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Total Serum Albumin (gm/100 ml)						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$2.06^{a} \pm 0.023$ (5)	$1.90^{a} \pm 0.027$ (5)	$1.95^{a} \pm 0.045$ (5)	$2.04^{ab} \pm 0.020$ (5)	$2.10^{a} \pm 0.014$ (5)	$2.15^{ab} \pm 0.031$ (5)	$2.14^{a} \pm 0.016$ (5)
II	14	100	$2.01^{a} \pm 0.029$ (5)	$1.85^{a} \pm 0.031$ (5)	$1.90^{a} \pm 0.042$ (5)	$2.01^{ab} \pm 0.035$ (5)	$2.09^{a} \pm 0.028$ (5)	$2.02^{ab} \pm 0.047$ (5)	$1.83^{b} \pm 0.033$ (5)
III	14	200	$1.98^{a} \pm 0.057$ (5)	$1.71^{a} \pm 0.028$ (5)	$1.79^{a} \pm 0.027$ (5)	$1.78^{a} \pm 0.048$ (5)	$1.73^{a} \pm 0.048$ (5)	$1.71^{c} \pm 0.027$ (5)	$1.68^{b} \pm 0.051$ (5)
IV	14	-	$2.13^{a} \pm 0.051$ (5)	$1.92^{a} \pm 0.039$ (5)	$2.00^{a} \pm 0.023$ (5)	$2.00^{a} \pm 0.023$ (5)	$2.07^{a} \pm 0.013$ (5)	$2.16^{b} \pm 0.018$ (5)	$2.24^{a} \pm 0.021$ (5)
V	Unvaccinated	-	$2.08^{a} \pm 0.028$ (5)	$2.16^{a} \pm 0.080$ (5)	$2.20^{a} \pm 0.029$ (5)	$2.31^{b} \pm 0.036$ (5)	$2.42^{b} \pm 0.033$ (5)	$2.63^{d} \pm 0.013$ (5)	$2.89^{c} \pm 0.036$ (5)

- Figures in parenthesis indicates number of observation
- Mean bearing common superscript (a, b, c, d) in individual column did not differ significantly ($P < 0.05$)

Fig. 32 (a) : Histogram showing effect of aflatoxin on serum globulin level in IBD vaccinated chickens.

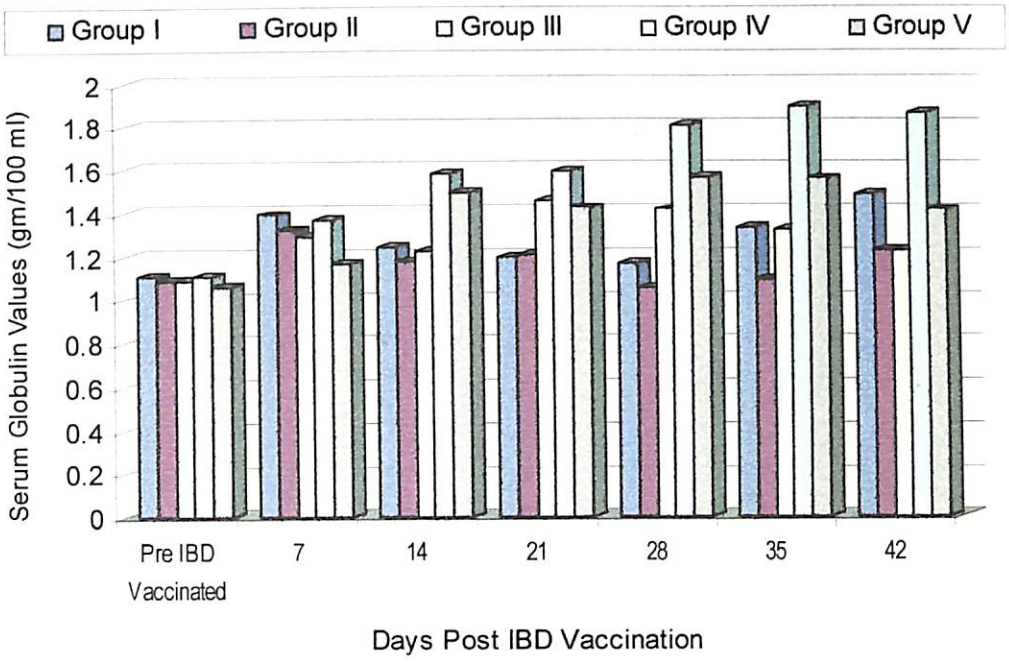


Fig. 32 (b) : Line Graph showing effect of aflatoxin on serum globulin level in IBD vaccinated chickens.

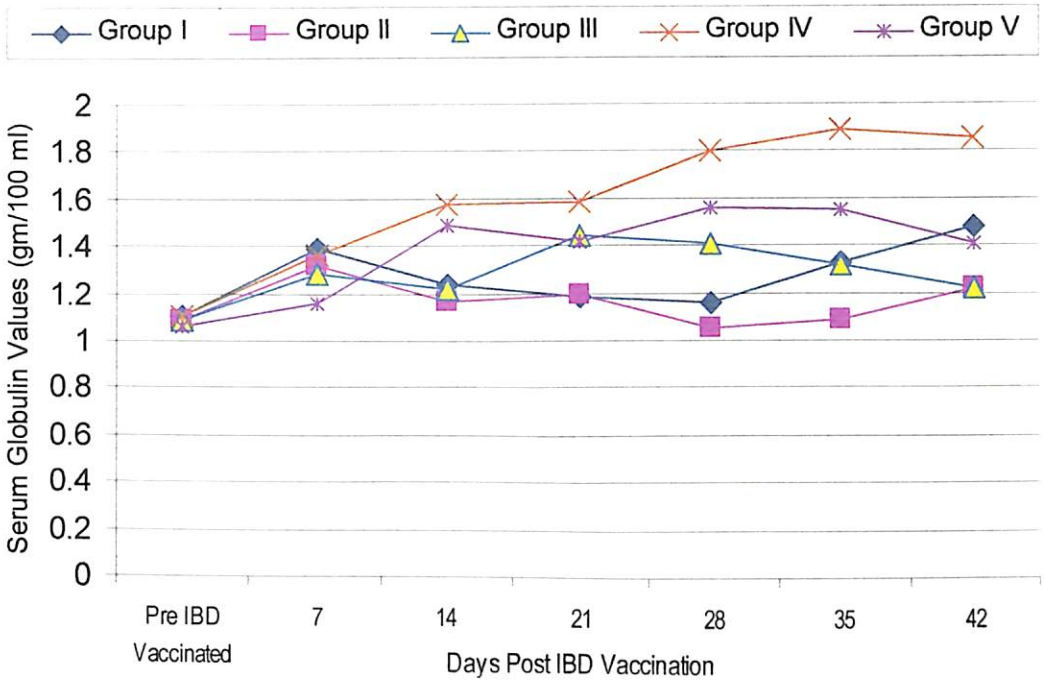


TABLE 9 : EFFECT OF AFLATOXIN ON SERUM GLOBULIN (GM/100 ML) IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Total Serum Globulin (gm/100 ml)						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$1.11^{a} \pm 0.020$ (5)	$1.39^{a} \pm 0.055$ (5)	$1.24^{ab} \pm 0.014$ (5)	$1.19^{a} \pm 0.056$ (5)	$1.16^{ab} \pm 0.016$ (5)	$1.33^{ab} \pm 0.018$ (5)	$1.48^{ab} \pm 0.070$ (5)
II	14	100	$1.09^{a} \pm 0.038$ (5)	$1.32^{a} \pm 0.056$ (5)	$1.17^{a} \pm 0.01$ (5)	$1.20^{a} \pm 0.048$ (5)	$1.05^{a} \pm 0.041$ (5)	$1.09^{a} \pm 0.44$ (5)	$1.22^{a} \pm 0.050$ (5)
III	14	200	$1.09^{a} \pm 0.074$ (5)	$1.29^{a} \pm 0.050$ (5)	$1.22^{ab} \pm 0.039$ (5)	$1.45^{a} \pm 0.074$ (5)	$1.41^{ab} \pm 0.134$ (5)	$1.32^{ab} \pm 0.050$ (5)	$1.22^{a} \pm 0.029$ (5)
IV	14	-	$1.11^{a} \pm 0.037$ (5)	$1.37^{a} \pm 0.039$ (5)	$1.58^{b} \pm 0.030$ (5)	$1.59^{a} \pm 0.029$ (5)	$1.80^{b} \pm 0.084$ (5)	$1.89^{b} \pm 0.059$ (5)	$1.86^{b} \pm 0.005$ (5)
V	Unvaccinated	-	$1.06^{a} \pm 0.088$ (5)	$1.16^{a} \pm 0.018$ (5)	$1.49^{ab} \pm 0.073$ (5)	$1.42^{a} \pm 0.047$ (5)	$1.56^{ab} \pm 0.189$ (5)	$1.55^{ab} \pm 0.159$ (5)	$1.41^{ab} \pm 0.067$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b) in individual column did not differ significantly ($P < 0.05$).

Fig. 33 (a) : Histogram showing effect of aflatoxin on serum calcium level in IBD vaccinated chickens

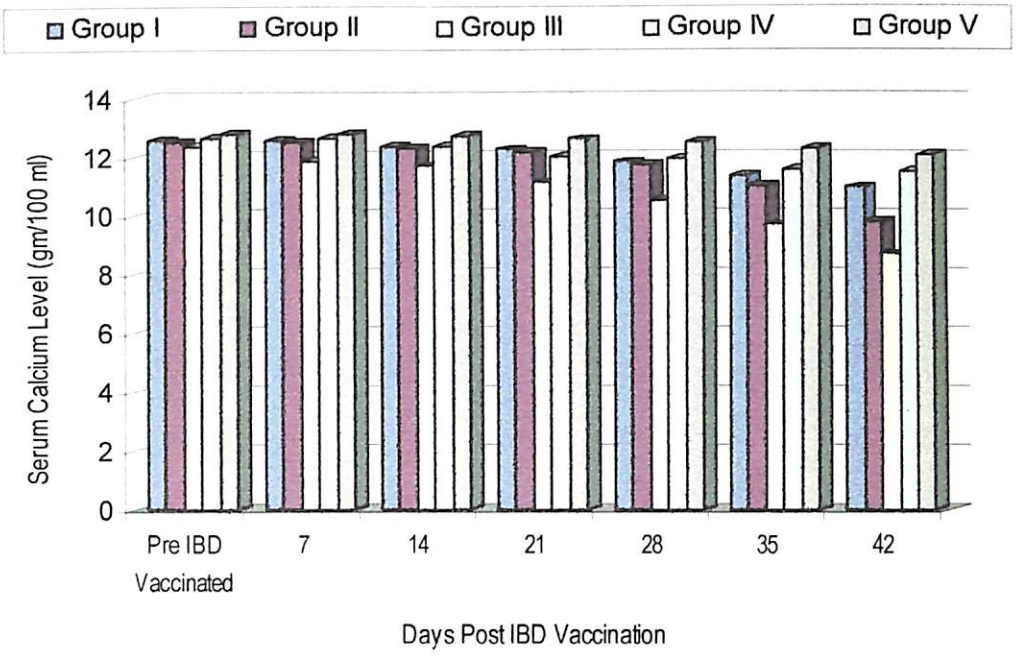


Fig. 33 (b) : Line Graph showing effect of aflatoxin on serum calcium level in IBD vaccinated chickens

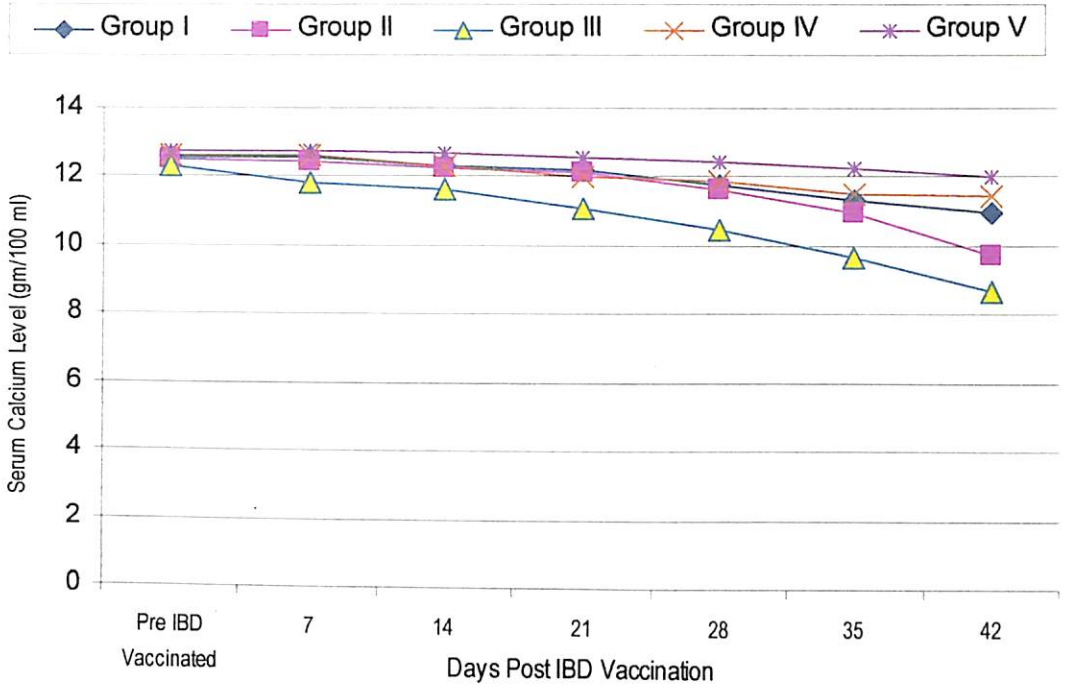


TABLE 10 : EFFECT OF AFLATOXIN ON SERUM CALCIUM (GM/100 ML) IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Total Serum Calcium (gm/100 ml)						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$12.56^{a} \pm 0.014$ (5)	$12.57^{a} \pm 0.044$ (5)	$12.35^{a} \pm 0.02$ (5)	$12.24^{a} \pm 0.026$ (5)	$11.81^{a} \pm 0.022$ (5)	$11.33^{b} \pm 0.240$ (5)	$10.96^{a} \pm 0.440$ (5)
II	14	100	$12.50^{a} \pm 0.026$ (5)	$12.46^{a} \pm 0.044$ (5)	$12.26^{a} \pm 0.055$ (5)	$12.12^{a} \pm 0.012$ (5)	$11.68^{a} \pm 0.260$ (5)	$11.00^{b} \pm 0.300$ (5)	$9.75^{b} \pm 0.014$ (5)
III	14	200	$12.33^{a} \pm 0.025$	$11.86^{b} \pm 0.05$ (5)	$11.67^{b} \pm 0.31$ (5)	$11.09^{b} \pm 0.230$ (5)	$10.47^{b} \pm 0.190$ (5)	$9.67^{a} \pm 0.326$ (5)	$8.67^{c} \pm 0.050$ (5)
IV	14	-	$12.63^{a} \pm 0.08$ (5)	$12.63^{a} \pm 0.06$ (5)	$12.36^{a} \pm 0.11$ (5)	$12.00^{a} \pm 0.014$ (5)	$11.963^{a} \pm 0.200$ (5)	$11.54^{b} \pm 0.230$ (5)	$11.49^{d} \pm 0.020$
V	Unvaccinated	-	$12.77^{a} \pm 0.065$ (5)	$12.79^{a} \pm 0.068$ (5)	$12.70^{a} \pm 0.050$ (5)	$12.59^{a} \pm 0.300$ (5)	$12.48^{a} \pm 0.020$ (5)	$12.26^{c} \pm 0.016$ (5)	$12.05^{e} \pm 0.019$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d, e) in individual column did not differ significantly ($P < 0.05$).

Fig. 34 (a) : Histogram showing effect of aflatoxin on serum inorganic phosphorus in IBD vaccinated chickens

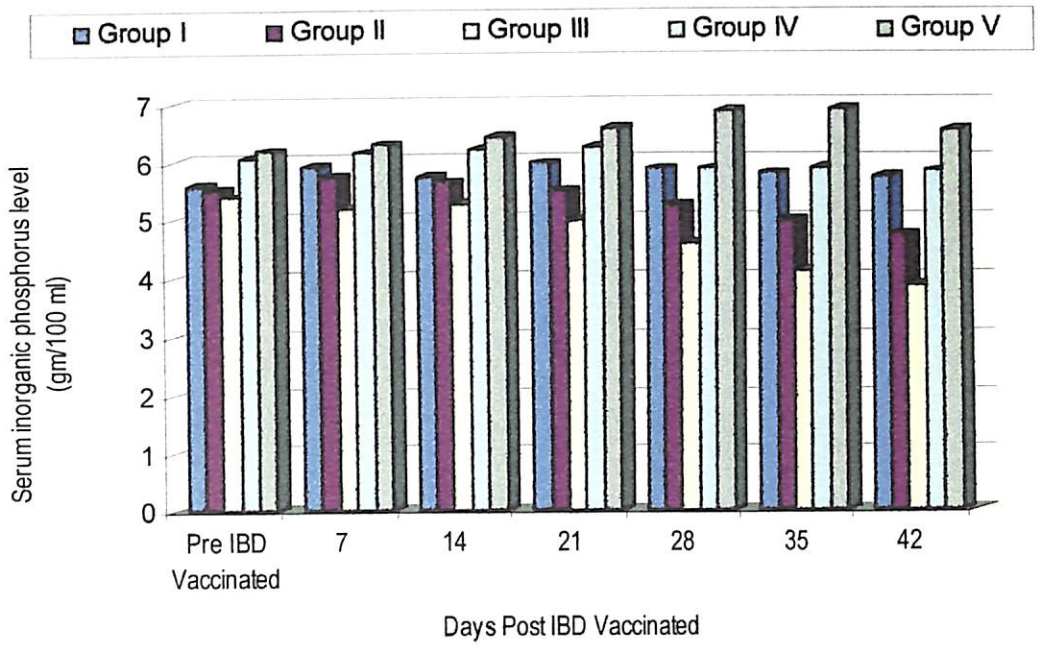


Fig. 34 (b) : Line Graph showing effect of aflatoxin on serum inorganic phosphorus in IBD vaccinated chickens

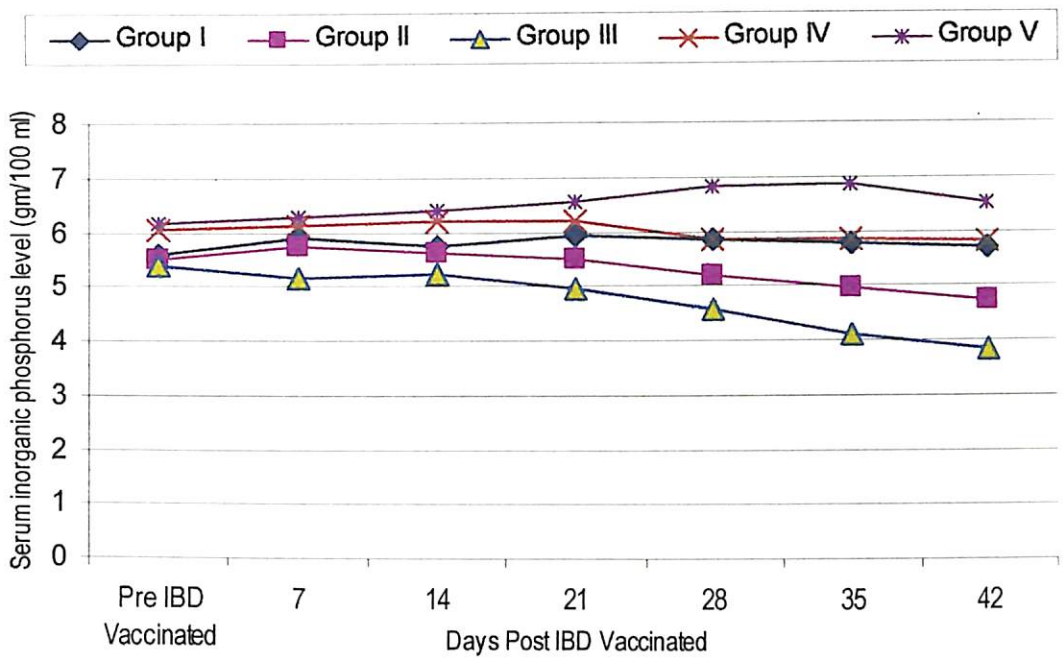


TABLE 11 : EFFECT OF AFLATOXIN ON SERUM INORGANIC PHOSPHORUS IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Total Serum Inorganic Phosphorus (gm/100 ml)						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$5.58^{ab} \pm 0.27$ (5)	$5.90^{b} \pm 0.18$ (5)	$5.74^{ab} \pm 0.19$ (5)	$5.96^{bc} \pm 0.21$ (5)	$5.86^{a} \pm 0.17$ (5)	$5.78^{a} \pm 0.17$ (5)	$5.72^{b} \pm 0.19$ (5)
II	14	100	$5.52^{ab} \pm 0.21$ (5)	$5.74^{ab} \pm 0.18$ (5)	$5.64^{ab} \pm 0.189$ (5)	$5.50^{c} \pm 0.16$ (5)	$5.22^{ab} \pm 0.32$ (5)	$4.98^{b} \pm 0.32$ (5)	$4.74^{c} \pm 0.25$ (5)
III	14	200	$5.39^{a} \pm 0.14$ (5)	$5.18^{a} \pm 0.29$ (5)	$5.26^{a} \pm 0.25$ (5)	$4.98^{a} \pm 0.23$ (5)	$4.58^{a} \pm 0.240$ (5)	$4.12^{b} \pm 0.27$ (5)	$3.86^{d} \pm 0.29$ (5)
IV	14	-	$6.04^{ab} \pm 0.24$ (5)	$6.14^{b} \pm 0.28$ (5)	$6.20^{b} \pm 0.08$ (5)	$6.22^{b} \pm 0.079$ (5)	$5.86^{b} \pm 0.27$ (5)	$5.88^{a} \pm 0.28$ (5)	$5.82^{b} \pm 0.17$ (5)
V	Unvaccinated	-	$6.29^{b} \pm 0.068$ (5)	$6.29^{b} \pm 0.068$ (5)	$6.40^{b} \pm 0.214$ (5)	$6.56^{c} \pm 0.294$ (5)	$6.85^{c} \pm 0.269$ (5)	$6.89^{c} \pm 0.095$ (5)	$6.52^{a} \pm 0.68$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d) in individual column did not differ significantly ($P < 0.05$).

vaccination except in group II and IV when the values differed significantly on 14, 28, 35 and 42 day post IBD vaccination. The perusal of table also revealed that the serum globulin values were highest in vaccinated control group (gr IV) and lowest in group III followed by group II and I.

Table-10 shows the mean ± SE of serum calcium level at different intervals post IBD vaccination in different groups of chickens. On observation it was found that the serum calcium showed declining trend in all groups (gr. I-V). But the values were highest in unvaccinated control group (gr. V) when compared with all other groups (gr. I - IV) overall periods post IBD vaccination. This table clearly indicated that the IBD vaccine and aflatoxin had additive effect on lowering the serum calcium level. Further the lowering effect got deepened with relative increase in the dose of aflatoxin.

The effect of IBD virus and aflatoxin on serum inorganic phosphorus level are shown in table-11. The results were suggestive of lowering effect of IBD vaccine virus on serum inorganic phosphorus (gr. I-IV) when compared with the corresponding values of group V at all intervals post IBD vaccination. Further the administration of aflatoxin showed additive effect on lowering of serum inorganic phosphorus specially when the dose levels were 100 ppb and 200 ppb (gr. II & gr. III)

Effect of aflatoxin on haematological profile of IBD vaccinated chickens

The mean ± SE of haemoglobulin values have been presented in table-12. The perusal of this table revealed that the IBD vaccinated groups (gr. I - IV) showed lower Hb values overall the intervals post vaccination than the values in the unvaccinated control group for the corresponding intervals. Further Hb values demonstrated higher degree of lowering effect in the first three groups of

Fig. 35 (a) : Histogram showing effect of aflatoxin on haemoglobin level in IBD vaccinated chickens

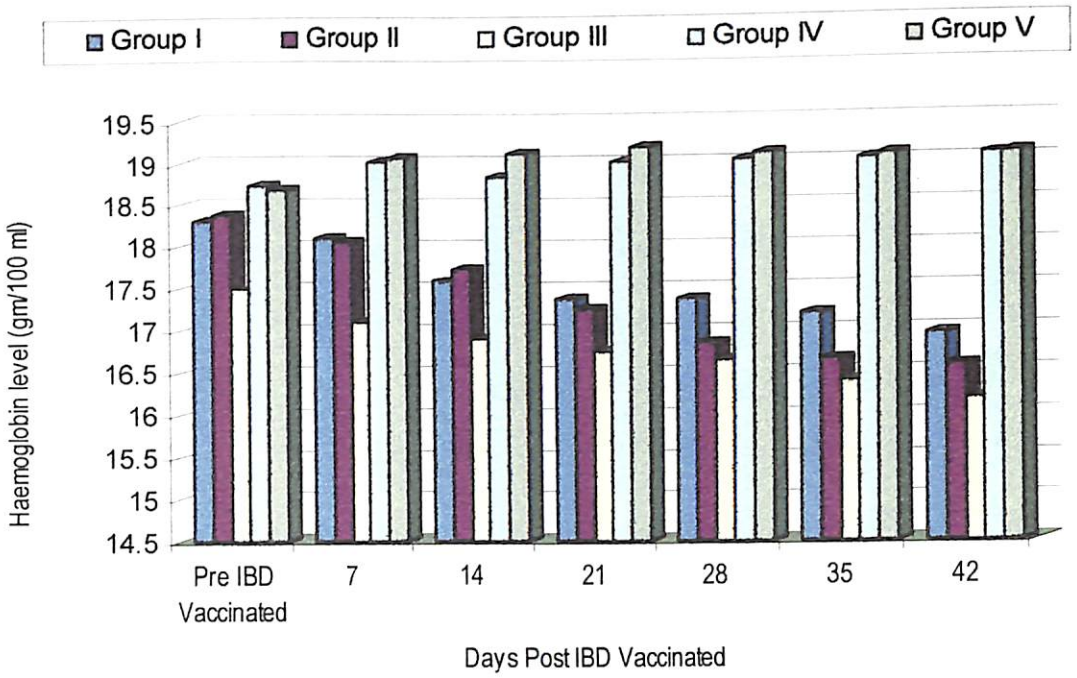


Fig. 35 (b) : Line Graph showing effect of aflatoxin on haemoglobin level in IBD vaccinated chickens

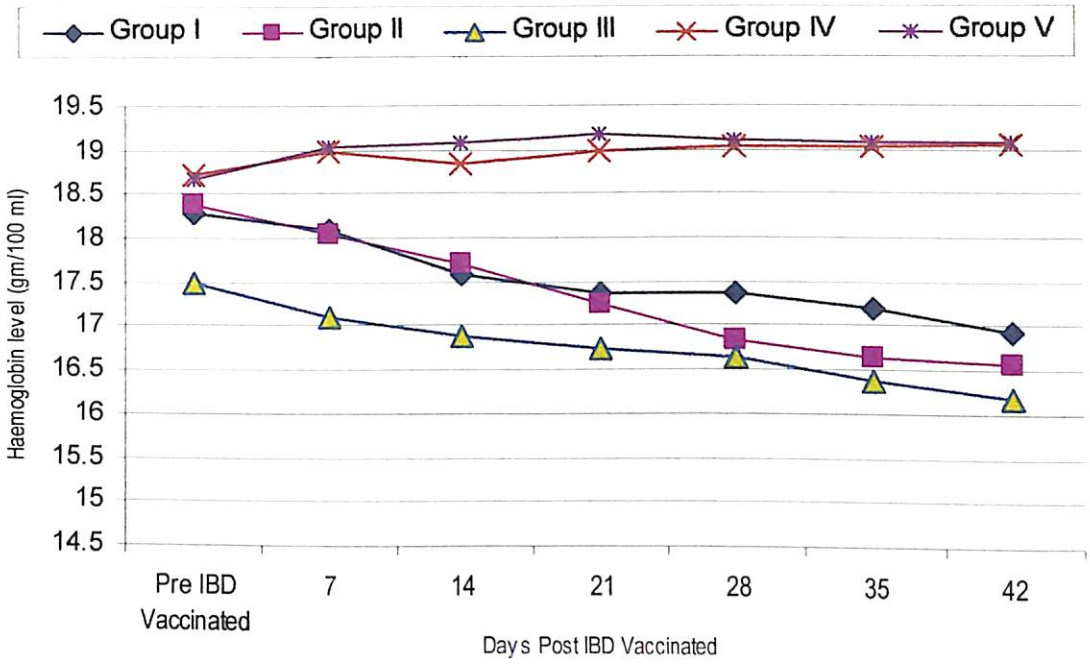


TABLE 12 : EFFECT OF AFLATOXIN ON HAEMOGLOBIN LEVEL (GM%) IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Haemoglobin Level						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$18.30^{ab} \pm 0.247$ (5)	$18.10^{ab} \pm 0.280$ (5)	$17.59^{ab} \pm 0.059$ (5)	$17.38^{b} \pm 0.272$ (5)	$17.38^{a} \pm 0.241$ (5)	$17.21^{a} \pm 0.185$ (5)	$16.95^{a} \pm 0.141$ (5)
II	14	100	$18.38^{ab} \pm 0.280$ (5)	$18.05^{b} \pm 0.456$ (5)	$17.72^{a} \pm 0.156$ (5)	$17.26^{b} \pm 0.353$ (5)	$16.85^{ab} \pm 0.141$ (5)	$16.64^{ab} \pm 0.035$ (5)	$16.58^{a} \pm 0.012$ (5)
III	14	200	$17.49^{a} \pm 0.076$	$17.10^{c} \pm 0.046$ (5)	$16.89^{c} \pm 0.059$ (5)	$16.74^{b} \pm 0.059$ (5)	$16.64^{b} \pm 0.228$ (5)	$16.39^{b} \pm 0.348$ (5)	$16.18^{a} \pm 0.347$ (5)
IV	14	-	$18.72^{b} \pm 0.241$ (5)	$19.00^{d} \pm 0.273$ (5)	$18.84^{d} \pm 0.243$ (5)	$19.00^{a} \pm 0.212$ (5)	$19.03^{c} \pm 0.203$ (5)	$19.03^{c} \pm 0.185$ (5)	$19.07^{b} \pm 0.228$ (5)
V	Unvaccinated	-	$18.69^{b} \pm 0.273$ (5)	$19.05^{d} \pm 0.289$ (5)	$19.10^{d} \pm 0.076$ (5)	$19.18^{a} \pm 0.256$ (5)	$19.11^{c} \pm 0.296$ (5)	$19.08^{c} \pm 0.012$ (5)	$19.08^{b} \pm 0.188$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d) in individual column did not differ significantly ($P < 0.05$).
- Mean ± SE of Arcsin √Percentage

Fig. 36 : Histogram showing effect of aflatoxin on Packed Cell Volume in IBD vaccinated chickens

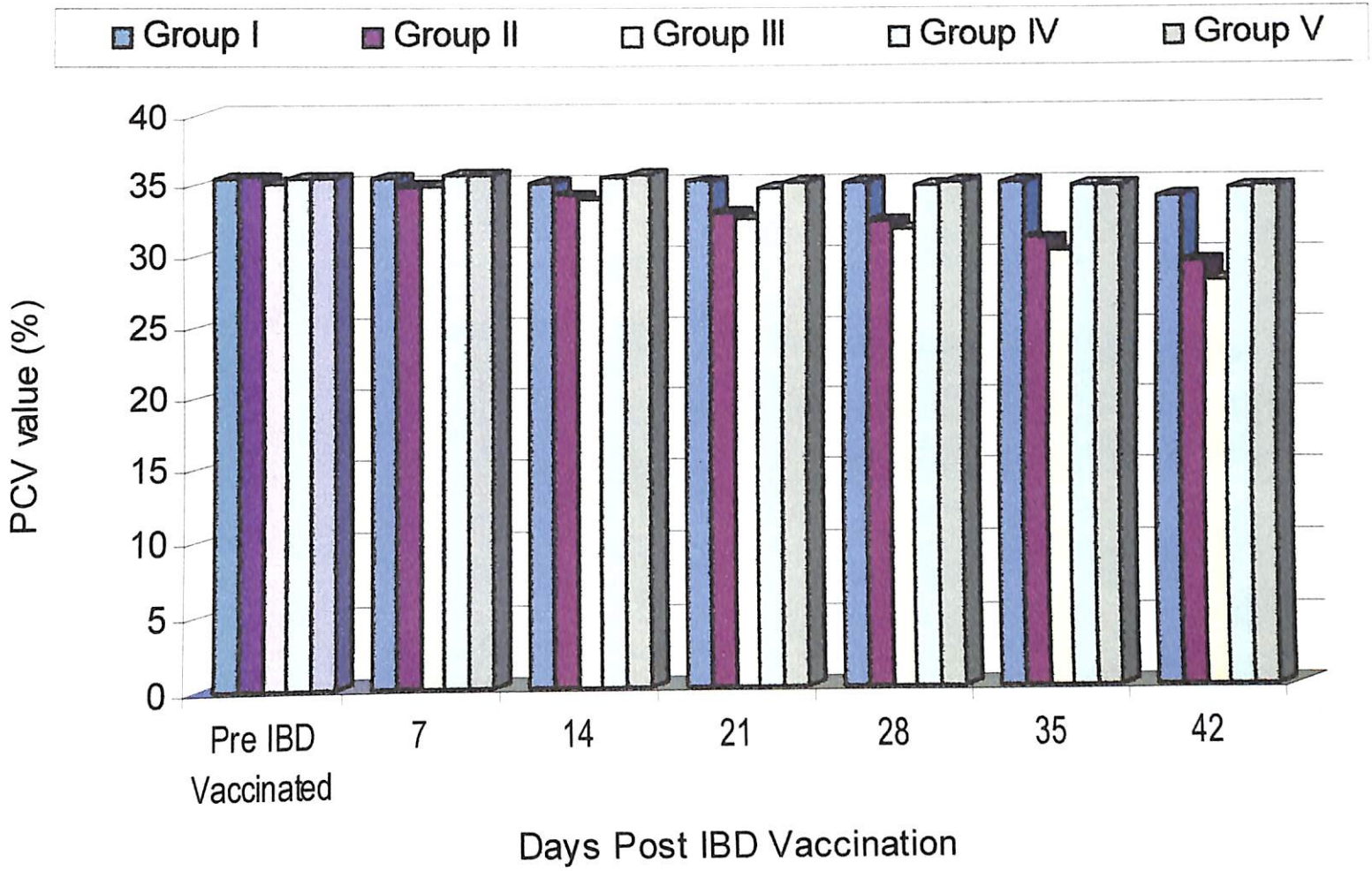


TABLE 13 : EFFECT OF AFLATOXIN ON PACKED CELL VOLUME (%) IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Packed Cell Volume (%)						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$35.44^{a} \pm 0.045$ (5)	$35.41^{a} \pm 0.068$ (5)	$35.07^{a} \pm 0.047$ (5)	$35.13^{b} \pm 0.041$ (5)	$35.09^{bc} \pm 0.028$ (5)	$35.01^{a} \pm 0.017$ (5)	$34.12^{a} \pm 0.01$ (5)
II	14	100	$35.51^{a} \pm 0.892$ (5)	$34.79^{a} \pm 0.467$ (5)	$34.18^{b} \pm 0.412$ (5)	$32.93^{a} \pm 0.345$ (5)	$32.30^{c} \pm 0.327$ (5)	$31.09^{b} \pm 0.526$ (5)	$29.47^{b} \pm 0.472$ (5)
III	14	200	$35.07^{a} \pm 0.602$ (5)	$34.85^{a} \pm 0.461$ (5)	$33.79^{b} \pm 0.672$ (5)	$32.56^{b} \pm 0.327$ (5)	$31.78^{a} \pm 0.516$ (5)	$30.14^{c} \pm 0.250$ (5)	$28.14^{c} \pm 0.337$ (5)
IV	14	-	$35.49^{a} \pm 0.395$ (5)	$35.57^{a} \pm 0.413$ (5)	$35.32^{ab} \pm 0.263$ (5)	$34.59^{b} \pm 0.547$ (5)	$34.82^{d} \pm 0.547$ (5)	$34.78^{d} \pm 0.547$ (5)	$34.60^{d} \pm 0.553$ (5)
V	Unvaccinated	-	$35.49^{a} \pm 0.521$ (5)	$35.58^{a} \pm 0.231$ (5)	$35.62^{ab} \pm 0.151$ (5)	$35.05^{b} \pm 0.678$ (5)	$34.98^{b} \pm 0.568$ (5)	$34.85^{d} \pm 0.263$ (5)	$34.76^{d} \pm 0.543$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d) in individual column did not differ significantly ($P < 0.05$).
- Mean ± SE of Arcsin √Percentage

Fig. 37 : Histogram showing effect of aflatoxin on total leucocyte count in IBD vaccinated chickens

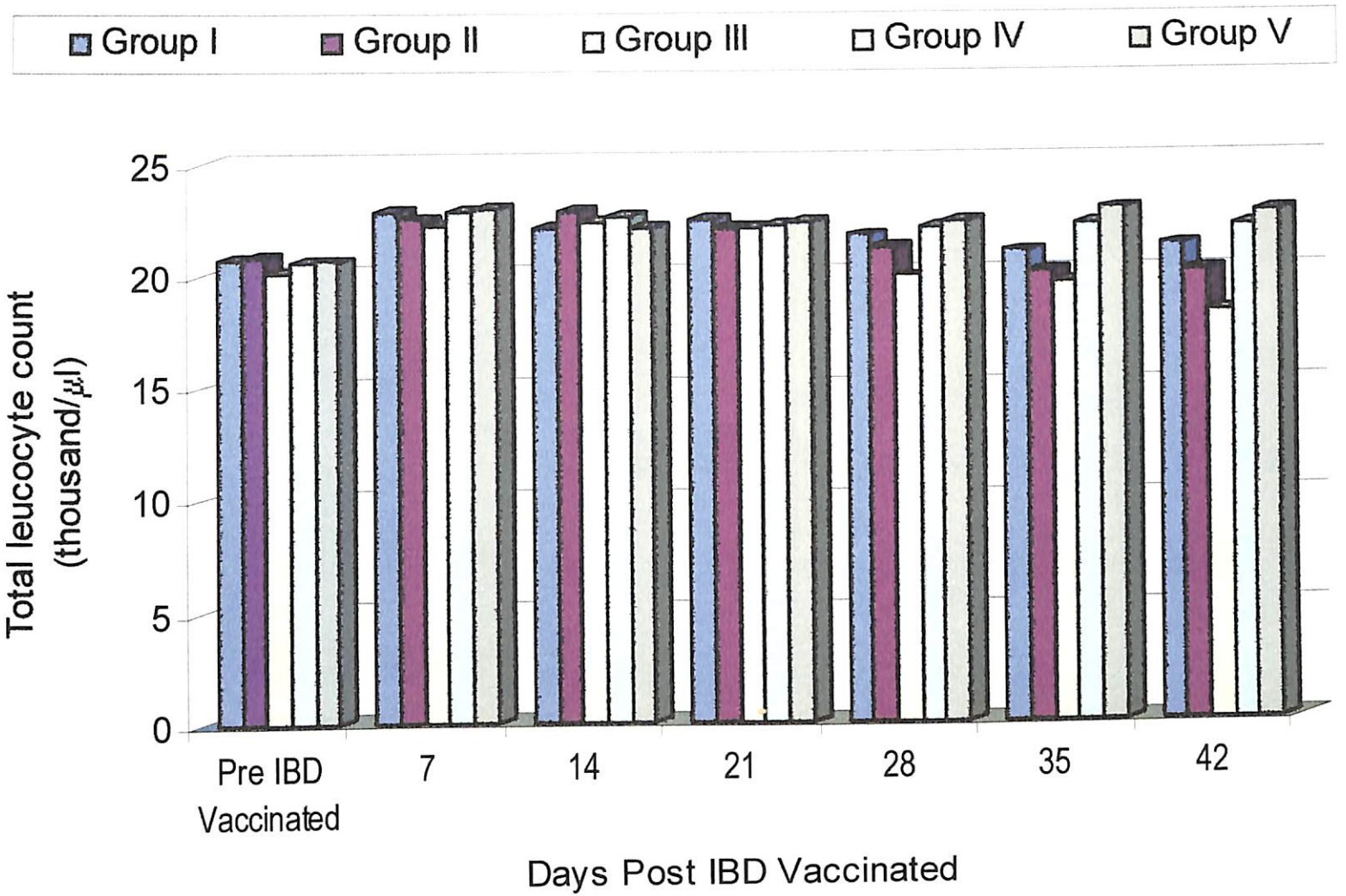


TABLE 14 : EFFECT OF AFLATOXIN ON TOTAL LEUCOCYTE COUNT (IN THOUSAND/µL) IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Total Leucocyte Count (in thousand/µl)						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$20.69^{a} \pm 0.028$ (5)	$22.78^{a} \pm 0.025$ (5)	$22.03^{a} \pm 0.042$ (5)	$22.41^{a} \pm 0.025$ (5)	$21.75^{bc} \pm 0.039$ (5)	$21.00^{a} \pm 0.049$ (5)	$21.24^{a} \pm 0.050$ (5)
II	14	100	$20.78^{a} \pm 0.025$ (5)	$22.50^{a} \pm 0.016$ (5)	$22.78^{a} \pm 0.049$ (5)	$22.07^{a} \pm 0.047$ (5)	$21.19^{c} \pm 0.041$ (5)	$20.07^{b} \pm 0.032$ (5)	$20.04^{b} \pm 0.045$ (5)
III	14	200	$20.04^{a} \pm 0.046$ (5)	$22.17^{a} \pm 0.027$ (5)	$22.32^{a} \pm 0.066$ (5)	$22.03^{a} \pm 0.047$ (5)	$19.89^{a} \pm 0.041$ (5)	$19.61^{c} \pm 0.037$ (5)	$18.20^{c} \pm 0.053$ (5)
IV	14	-	$20.59^{a} \pm 0.041$ (5)	$22.81^{a} \pm 0.045$ (5)	$22.54^{a} \pm 0.044$ (5)	$22.20^{a} \pm 0.036$ (5)	$22.10^{b} \pm 0.049$ (5)	$22.15^{d} \pm 0.052$ (5)	$22.05^{d} \pm 0.047$ (5)
V	Unvaccinated	-	$20.62^{a} \pm 0.048$ (5)	$22.89^{a} \pm 0.062$ (5)	$22.08^{a} \pm 0.041$ (5)	$22.28^{a} \pm 0.046$ (5)	$22.32^{b} \pm 0.068$ (5)	$22.86^{d} \pm 0.032$ (5)	$22.65^{d} \pm 0.029$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d) in individual column did not differ significantly ($P < 0.05$).
- Mean ± SE of Arcsin √Percentage

birds (gr I-III) which had received different doses of aflatoxin in addition to IBD vaccine (Table j 12).

The effects of IBD vaccine and aflatoxin on packed cell volume (PCV) values are depicted in table-13. The PCV values of the group which did not receive IBD vaccine (gr V) were found to be higher than the corresponding values in group which received IBD vaccine alone (gr. IV) overall the periods post IBD vaccination. On comparison between vaccinated control group (gr. IV) and aflatoxin fed group (I -III) the PCV values were found to be lowest in group III followed by group II and I. The result were suggestive of lowering effect of aflatoxin on PCV value when compared with the corresponding values of vaccinated control and unvaccinated control (gr IV and V) specially when the doses of aflatoxin were 100 ppb and 200 ppb (gr II & III).

The mean ± SE of total leucocyte count (TLC) values are depicted in table-14. The perusal of this table revealed that the values of TLC were lower in all vaccinated group (gr I-IV) when compared with corresponding values of unvaccinated control group (gr. V) at all intervals post IBD vaccination. Again comparison between vaccinated control group (gr. IV) and aflatoxin fed groups (gr. I - III) showed that the values were lowest in group III followed by group II , I and IV The decline in TLC values were more marked specially in 100 ppb and 200 ppb dose level groups (gr. II & III).

Differential Leucocytic Count

The lymphocyte percentage are shown in table-15. The perusal of this table revealed that the lymphocyte count in all IBD vaccinated group (gr. I - IV) were significantly lower than the corresponding values of unvaccinated control group (gr.V) overall intervals post IBD vaccination. Again comparison between vaccinated control group (gr. IV) to aflatoxin treated groups (gr. I - III) showed

Fig. 38 : Histogram showing effect of aflatoxin on Lymphocyte count in IBD vaccinated chickens

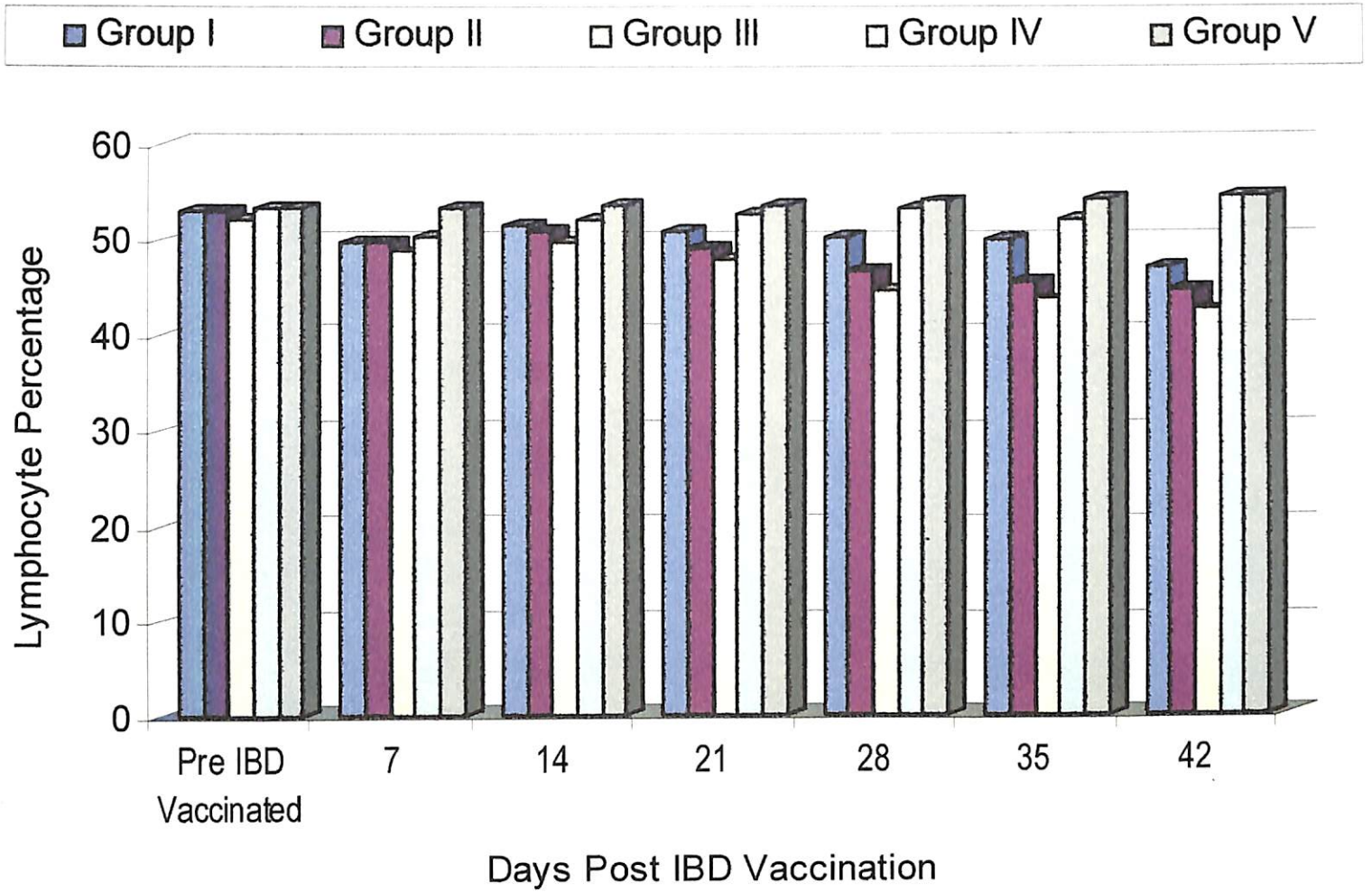
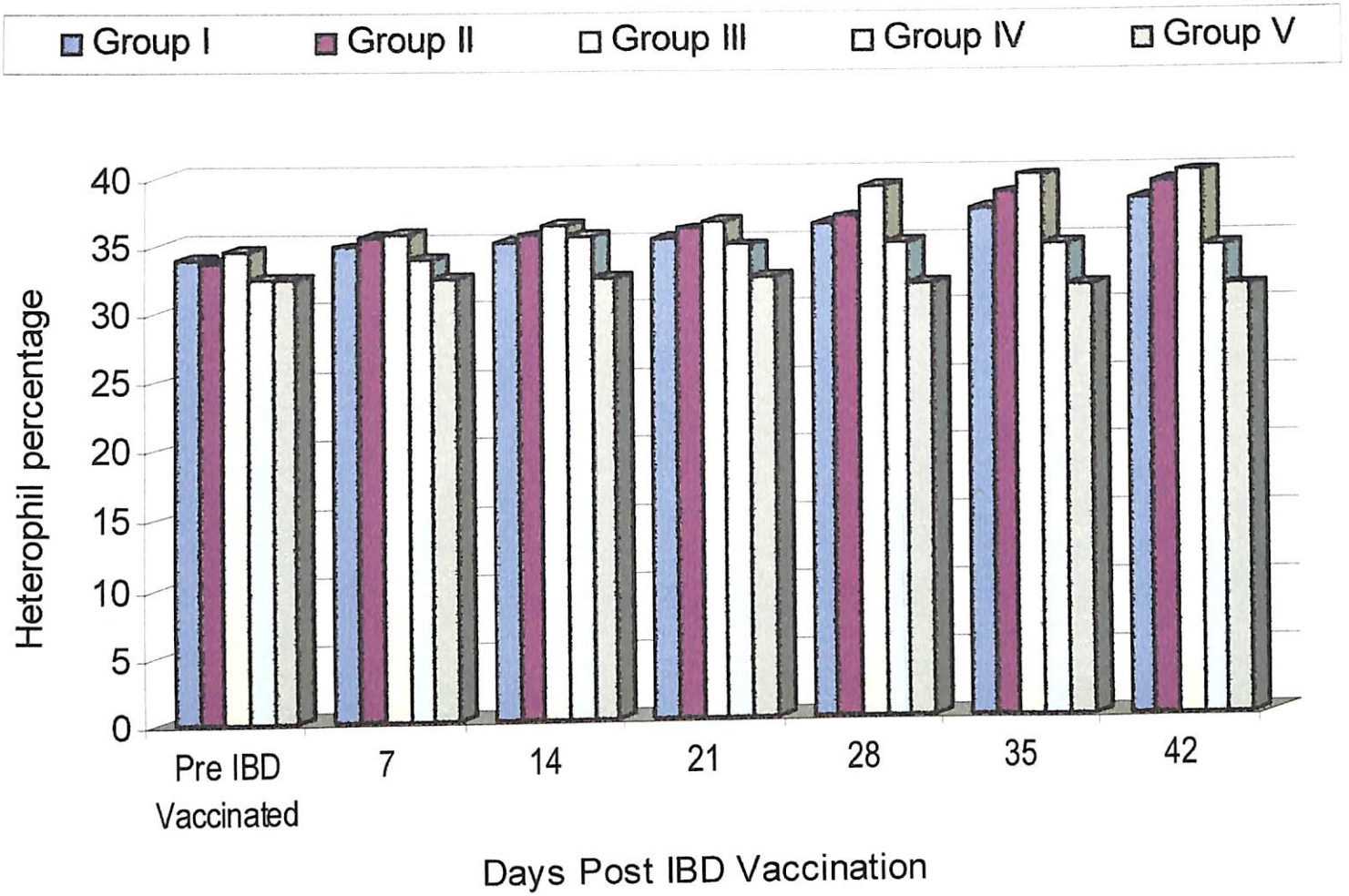


TABLE 15 : EFFECT OF AFLATOXIN ON LYMPHOCYTE COUNT PERCENTAGE IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Lymphocyte Count Percentage						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$52.89^{a} \pm 1.012$ (5)	$49.72^{a} \pm 0.468$ (5)	$51.46^{a} \pm 0.869$ (5)	$50.91^{a} \pm 0.532$ (5)	$50.28^{a} \pm 0.621$ (5)	$49.99^{a} \pm 0.503$ (5)	$47.09^{a} \pm 0.468$ (5)
II	14	100	$52.83^{a} \pm 1.02$ (5)	$49.62^{a} \pm 0.493$ (5)	$50.80^{ab} \pm 0.732$ (5)	$49.06^{a} \pm 0.482$ (5)	$46.61^{b} \pm 0.482$ (5)	$45.55^{b} \pm 0.264$ (5)	$44.64^{b} \pm 0.329$ (5)
III	14	200	$52.06^{a} \pm 1.039$ (5)	$48.61^{b} \pm 0.432$ (5)	$49.72^{b} \pm 0.466$ (5)	$47.97^{c} \pm 0.42$ (5)	$44.68^{c} \pm 0.431$ (5)	$43.57^{c} \pm 0.252$ (5)	$42.34^{c} \pm 0.218$ (5)
IV	14	-	$53.13^{a} \pm 1.038$ (5)	$50.13^{a} \pm 0.598$ (5)	$52.04^{a} \pm 1.015$ (5)	$52.69^{d} \pm 0.898$ (5)	$53.29^{d} \pm 0.932$ (5)	$52.09^{d} \pm 1.083$ (5)	$52.45^{d} \pm 1.304$ (5)
V	Unvaccinated	-	$53.12^{a} \pm 1.056$ (5)	$53.28^{c} \pm 0.895$ (5)	$53.45^{c} \pm 0.946$ (5)	$53.60^{d} \pm 0.532$ (5)	$53.95^{d} \pm 1.035$ (5)	$54.15^{e} \pm 1.012$ (5)	$54.46^{e} \pm 0.106$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d, e) in individual column did not differ significantly ($P < 0.05$).
- Mean ± SE of Arcsin √Percentage

Fig. 39 : Histogram showing effect of aflatoxin on Heterophil Percentage in IBD vaccinated chickens



Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Heterophil Percengage						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$33.79^{a} \pm 0.031$ (5)	$34.77^{a} \pm 0.039$ (5)	$35.01^{a} \pm 0.045$ (5)	$35.36^{a} \pm 0.048$ (5)	$36.25^{a} \pm 0.161$ (5)	$37.33^{a} \pm 0.038$ (5)	$37.92^{a} \pm 0.034$ (5)
II	14	100	$33.60^{a} \pm 0.049$ (5)	$35.48^{ab} \pm 0.0461$ (5)	$35.59^{ab} \pm 0.050$ (5)	$36.04^{a} \pm 0.052$ (5)	$36.84^{a} \pm 0.109$ (5)	$38.49^{b} \pm 0.047$ (5)	$39.27^{b} \pm 0.058$ (5)
III	14	200	$34.52^{a} \pm 0.045$ (5)	$35.69^{b} \pm 0.050$ (5)	$36.25^{b} \pm 0.058$ (5)	$36.61^{a} \pm 0.054$ (5)	$38.97^{b} \pm 0.122$ (5)	$39.74^{c} \pm 0.056$ (5)	$39.94^{b} \pm 0.045$ (5)
IV	14	-	$32.47^{b} \pm 0.028$ (5)	$33.77^{c} \pm 0.041$ (5)	$35.56^{ab} \pm 0.038$ (5)	$34.79^{b} \pm 0.042$ (5)	$34.84^{c} \pm 0.042$ (5)	$34.72^{d} \pm 0.045$ (5)	$34.39^{c} \pm 0.042$ (5)
V	Unvaccinated	-	$32.43^{b} \pm 0.046$ (5)	$32.47^{d} \pm 0.477$ (5)	$32.40^{c} \pm 0.032$ (5)	$32.39^{c} \pm 0.059$ (5)	$31.85^{d} \pm 0.046$ (5)	$31.59^{e} \pm 0.032$ (5)	$31.46^{d} \pm 0.038$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d, e) in individual column did not differ significantly ($P < 0.05$).
- Mean ± SE of Arcsin √Percentage

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Monocyte Percentage						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$9.98^{a} \pm 0.012$ (5)	$10.63^{ab} \pm 0.015$ (5)	$10.52^{a} \pm 0.029$ (5)	$11.27^{a} \pm 0.068$ (5)	$11.36^{a} \pm 0.028$ (5)	$11.43^{a} \pm 0.066$ (5)	$11.24^{a} \pm 0.026$ (5)
II	14	100	$10.47^{a} \pm 0.028$ (5)	$10.88^{a} \pm 0.028$ (5)	$10.63^{ab} \pm 0.015$ (5)	$11.32^{a} \pm 0.060$ (5)	$11.41^{a} \pm 0.125$ (5)	$11.50^{a} \pm 0.012$ (5)	$11.37^{a} \pm 0.024$ (5)
III	14	200	$10.72^{a} \pm 0.152$ (5)	$11.30^{a} \pm 0.062$ (5)	$11.45^{b} \pm 0.028$ (5)	$11.86^{a} \pm 0.054$ (5)	$11.92^{a} \pm 0.158$ (5)	$11.72^{a} \pm 0.059$ (5)	$11.61^{a} \pm 0.042$ (5)
IV	14	-	$9.63^{a} \pm 0.029$ (5)	$10.47^{ab} \pm 0.058$ (5)	$9.72^{c} \pm 0.012$ (5)	$9.90^{b} \pm 0.028$ (5)	$9.94^{b} \pm 0.026$ (5)	$10.01^{b} \pm 0.028$ (5)	$9.90^{b} \pm 0.012$ (5)
V	Unvaccinated	-	$9.48^{a} \pm 0.023$ (5)	$9.62^{b} \pm 0.015$ (5)	$10.12^{a} \pm 0.057$ (5)	$9.85^{b} \pm 0.057$ (5)	$9.84^{b} \pm 0.014$ (5)	$9.76^{b} \pm 0.065$ (5)	$9.69^{b} \pm 0.018$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$).
- Mean ± SE of Arcsin √Percentage

Fig. 40 (a) : Histogram showing effect of aflatoxin on Eosinophil percentage in IBD vaccinated chickens

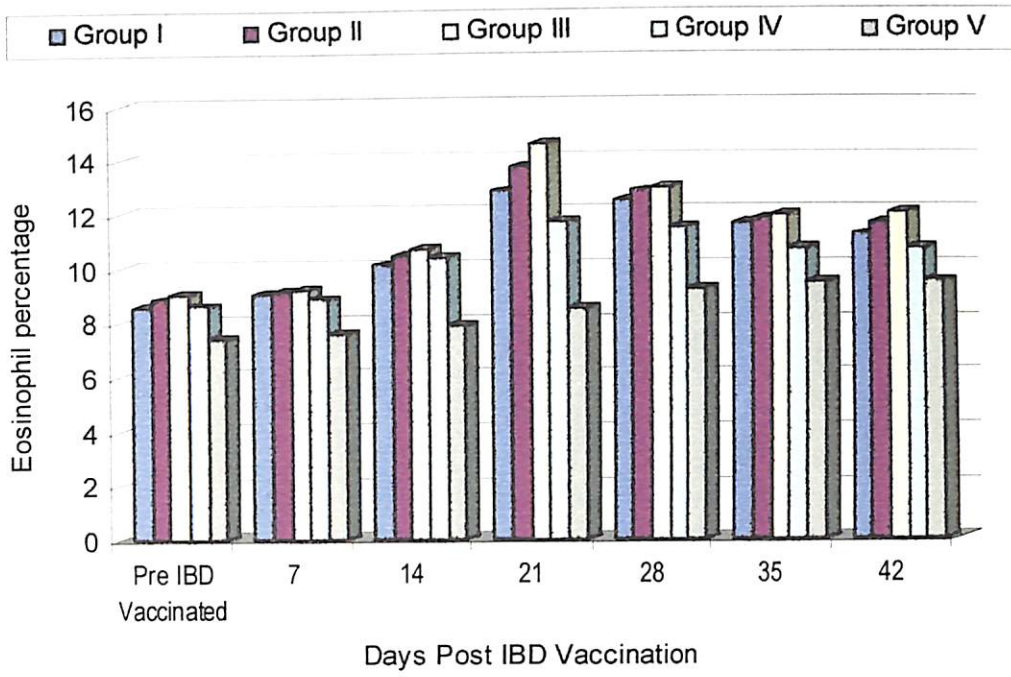


Fig. 40 (b) : Line Graph showing effect of aflatoxin on Eosinophil percentage in IBD vaccinated chickens

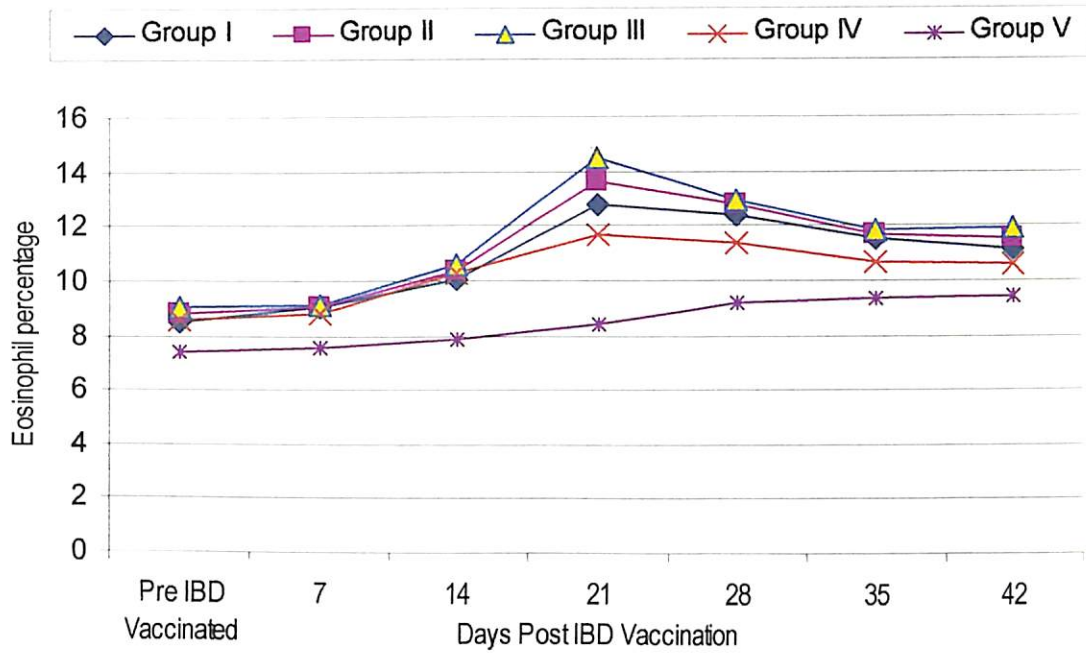


TABLE 18 : EFFECT OF AFLATOXIN ON EOSINOPHIL PERCENTAGE IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Eosinophil Percentage						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$8.53^{ab} \pm 0.025$ (5)	$9.04^{a} \pm 0.013$ (5)	$10.06^{a} \pm 0.022$ (5)	$12.79^{a} \pm 0.024$ (5)	$12.41^{ab} \pm 0.048$ (5)	$11.59^{ab} \pm 0.028$ (5)	$11.15^{ab} \pm 0.87$ (5)
II	14	100	$8.82^{ab} \pm 0.036$ (5)	$9.07^{a} \pm 0.024$ (5)	$10.41^{a} \pm 0.039$ (5)	$13.69^{b} \pm 0.036$ (5)	$12.79^{a} \pm 0.039$ (5)	$11.72^{a} \pm 0.012$ (5)	$11.59^{ab} \pm 0.029$ (5)
III	14	200	$9.04^{a} \pm 0.028$ (5)	$9.15^{a} \pm 0.025$ (5)	$10.63^{a} \pm 0.036$ (5)	$14.54^{c} \pm 0.021$ (5)	$12.92^{a} \pm 0.025$ (5)	$11.88^{a} \pm 0.014$ (5)	$11.97^{a} \pm 0.047$ (5)
IV	14	-	$8.59^{ab} \pm 0.018$ (5)	$8.82^{a} \pm 0.013$ (5)	$10.31^{a} \pm 0.021$ (5)	$11.63^{d} \pm 0.012$ (5)	$11.41^{b} \pm 0.036$ (5)	$10.66^{b} \pm 0.036$ (5)	$10.62^{b} \pm 0.069$ (5)
V	Unvaccinated	-	$7.38^{b} \pm 0.014$ (5)	$7.54^{b} \pm 0.018$ (5)	$7.86^{b} \pm 0.045$ (5)	$8.46^{e} \pm 0.062$ (5)	$9.18^{c} \pm 0.056$ (5)	$9.39^{c} \pm 0.032$ (5)	$9.44^{c} \pm 0.011$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c, d, e) in individual column did not differ significantly ($P < 0.05$).
- Mean ± SE of Arcsin √Percentage

that the aflatoxin treated group were having lower percent lymphocyte values than the control group (gr. IV). The rate of decline of percent lymphocyte value was related to the dose level - higher the dose level more the decline in values.

The mean ± SE of heterophil values are presented in table-16. The values were relatively lower in unvaccinated control group (gr. V) than the corresponding value in all the vaccinated groups (gr. I - IV) over all the periods post IBD vaccination. Again comparison between vaccinated control group (gr. IV) and aflatoxin treated groups (gr. I -III) showed lower values in group IV than the first three groups overall the intervals post IBD vaccination.

The mean ± SE of monocyte percentage are presented in table -17. The perusal of this table clearly demonstrated that the monocyte percentage were relatively lower in unvaccinated group (gr. V) than the IBD vaccinated birds (gr. I-IV) over all the intervals post IBD vaccinatioin. Further perusal of this table revealed that the values were lower in vaccinated control group (gr. IV) than the corresponding values of aflatoxin treated groups (gr. I - III) over all the periods post IBD vaccination. Again comparison between aflatoxin treated groups showed that the values of monocyte percentage were highest in group III followed by group II and I. The values were dose dependent i.e. the higher dose level, higher the monocyte percentage.

Effect of IBD vaccine and aflatoxin on eosinophilic count are presented in table-18. The perusal of this table revealed that the eosinophilic count were lower in unvaccinated control group (gr. V) when compared with the vaccinated groups overall the periods post IBD vaccination. The values were lower in vaccinated control group (gr. IV) when compared with the corresponding values of aflatoxin treated groups (gr. I - III) overall intervals post IBD vaccination. Further the result clearly indicated that the aflatoxin and IBD vaccine together

TABLE 19 : EFFECT OF AFLATOXIN ON BASOPHIL PERCENTAGE IN BROILER CHICKENS AT DIFFERENT INTERVAL POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Basophil Percentage						
			Pre IBD vaccinated	Days Post IBD Vaccination					
				7	14	21	28	35	42
I	14	50	$8.19^{a} \pm 0.694$ (5)	$6.90^{a} \pm 0.498$ (5)	$6.68^{a} \pm 0.40$ (5)	$6.51^{ab} \pm 0.420$ (5)	$6.42^{ab} \pm 0.855$ (5)	$6.80^{ab} \pm 0.429$ (5)	$6.88^{a} \pm 0.468$ (5)
II	14	100	$8.24^{a} \pm 0.568$ (5)	$6.88^{a} \pm 0.625$ (5)	$6.28^{ab} \pm 0.603$ (5)	$6.18^{ab} \pm 0.629$ (5)	$6.11^{ab} \pm 0.468$ (5)	$6.23^{ab} \pm 0.468$ (5)	$6.19^{a} \pm 0.629$ (5)
III	14	200	$8.39^{a} \pm 0.628$ (5)	$6.54^{a} \pm 0.625$ (5)	$6.08^{a} \pm 0.598$ (5)	$5.98^{a} \pm 0.568$ (5)	$5.85^{a} \pm 0.600$ (5)	$5.96^{a} \pm 0.595$ (5)	$6.05^{a} \pm 0.625$ (5)
IV	14	-	$8.14^{a} \pm 0.525$ (5)	$7.38^{a} \pm 0.565$ (5)	$7.14^{b} \pm 0.45$ (5)	$6.84^{ab} \pm 0.394$ (5)	$6.76^{bc} \pm 0.045$ (5)	$6.93^{ab} \pm 0.525$ (5)	$6.98^{a} \pm 0.565$ (5)
V	Unvaccinated	-	$6.76^{a} \pm 0.429$ (5)	$6.89^{a} \pm 0.525$ (5)	$7.03^{b} \pm 0.424$ (5)	$7.26^{b} \pm 0.424$ (5)	$7.38^{c} \pm 0.512$ (5)	$7.22^{b} \pm 0.442$ (5)	$7.26^{a} \pm 0.400$ (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$).
- Mean ± SE of Arcsin √Percentage

had synergistic effect on enhancement of percent values of eosinophil. The mean ± SE of percent basophil values are shown in table - 19. The values did not differ significantly at the termination of experiment.

Effect of aflatoxin on Bursa : Body wt. ratio, Spleen : Body wt. ratio, Body wt. gain and feed conversion ratio (FCR) in IBD vaccinated chickens.

Bursa : Body wt. ratio

The Bursa : Body wt. ratio of IBD vaccinated and aflatoxin treated chickens sacrificed on 3rd, 7th and 11th day post IBD vaccination are shown in table 20, 21 and 22. The ratio were lower in aflatoxin treated groups (gr. I - III) than the vaccinated control group (gr. IV) and unvaccinated control group (gr. V). Further comparison between aflatoxin treated groups (gr. I - III) showed that the higher the dose level of aflatoxin, lower the bursa : body wt. ratio (i.e. the ratio were dose dependent) as apparent from the finding recorded in table 20, 21 and 22.

Spleen : Body wt. ratio:

The Spleen : Body wt. ratio of IBD vaccinated and aflatoxin treated chickens sacrificed on 3rd 7th & 11th day post IBD vaccination are shown in table 23, 24 & 25. The ratio were higher in aflatoxin treated groups (gr. I - III) when compared with vaccinated (gr. IV) and unvaccinated control groups (gr. V). Further comparison between aflatoxin treated groups (gr. I - III) showed higher the dose level of aflatoxin, higher the spleen : body wt. ratio (i.e. the ratio were dose dependent) as apparent from the findings recorded in table 23, 24 and 25.

TABLE 20 : EFFECT OF AFLATOXIN ON BURSA : BODY WEIGHT RATIO ON 3RD DAY POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Bursa Wt., Body Wt. and Bursa : Body Wt. Ratio		
			Bursal Wt. (gm)	Body Wt. (gm)	Bursa : Body Wt. Ratio
I	14	50	0.4753 ± 0.0017 (5)	265.5 ± 2.82 (5)	1.79^{ab} ± 0.021 (5)
II	14	100	0.4453 ± 0.0017 (5)	258.8 ± 3.18 (5)	1.72^{ab} ± 0.031 (5)
III	14	200	0.4281 ± 0.003 (5)	250.9 ± 3.16 (5)	1.69^{b} ± 0.021 (5)
IV	14	-	0.4964 ± 0.0027 (5)	268.9 ± 5.59 (5)	1.85^{a} ± 0.035 (5)
V	Unvaccinated	-	0.4975 ± 0.0032 (5)	272.5 ± 4.48 (5)	1.82^{a} ± 0.062 (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b) in individual column did not differ significantly ($P < 0.05$).

TABLE 21 : EFFECT OF AFLATOXIN ON BURSA : BODY WEIGHT RATIO ON 7TH DAY POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Bursa Wt., Body Wt. and Bursa : Body Wt. Ratio		
			Bursal Wt. (gm)	Body Wt. (gm)	Bursa : Body Wt. Ratio
I	14	50	0.5975 ± 0.0019 (5)	355.1 ± 1.68 (5)	1.68^{ab} ± 0.010 (5)
II	14	100	0.5800 ± 0.0029 (5)	346.1 ± 1.68 (5)	1.67^{ab} ± 0.006 (5)
III	14	200	0.5425 ± 0.0022 (5)	339.1 ± 1.38 (5)	1.59^{b} ± 0.007 (5)
IV	14	-	0.6289 ± 0.001 (5)	369.6 ± 1.50 (5)	1.70^{a} ± 0.012 (5)
V	Unvaccinated	-	0.6292 ± 0.003 (5)	372.2 ± 1.85 (5)	1.69^{ab} ± 0.017 (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b) in individual column did not differ significantly ($P < 0.05$).

TABLE 22 : EFFECT OF AFLATOXIN ON BURSA : BODY WEIGHT RATIO ON 11TH DAY POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Bursa Wt., Body Wt. and Bursa : Body Wt. Ratio		
			Bursal Wt. (gm)	Body Wt. (gm)	Bursa : Body Wt. Ratio
I	14	50	0.8303 ± 0.004 (5)	454.2 ± 7.39 (5)	1.82^{a} ± 0.036 (5)
II	14	100	0.7921 ± 0.003 (5)	442.0 ± 9.73 (5)	1.79^{ab} ± 0.049 (5)
III	14	200	0.7016 ± 0.001 (5)	435.4 ± 11.27 (5)	1.61^{b} ± 0.043 (5)
IV	14	-	0.8939 ± 0.003 (5)	478.4 ± 3.14 (5)	1.86^{a} ± 0.018 (5)
V	Unvaccinated	-	0.8956 ± 0.0032 (5)	485.2 ± 3.73 (5)	1.84^{a} ± 0.027 (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b) in individual column did not differ significantly ($P < 0.05$).

TABLE 23 : EFFECT OF AFLATOXIN ON SPLEEN : BODY WEIGHT RATIO ON 3RD DAY POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Spleen Wt., Body Wt. and Spleen : Body Wt. Ratio		
			Spleen Wt. (gm)	Body Wt. (gm)	Spleen : Body Wt. Ratio
I	14	50	0.1666 ± 0.0002 (5)	265.5 ± 2.82 (5)	0.626^{a} ± 0.002 (5)
II	14	100	0.738 ± 0.0004 (5)	258.8 ± 3.18 (5)	0.672^{a} ± 0.0072 (5)
III	14	200	0.1917 ± 0.0013 (5)	250.9 ± 3.16 (5)	0.764^{b} ± 0.0044 (5)
IV	14	-	0.1504 ± 0.0006 (5)	268.8 ± 5.59 (5)	0.560^{c} ± 0.011 (5)
V	Unvaccinated	-	0.1506 ± 0.0008 (5)	272.5 ± 4.48 (5)	0.572^{c} ± 0.019

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$).

TABLE 24 : EFFECT OF AFLATOXIN ON SPLEEN : BODY WEIGHT RATIO ON 7TH DAY POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Spleen Wt., Body Wt. and Spleen : Body Wt. Ratio		
			Spleen Wt. (gm)	Body Wt. (gm)	Spleen : Body Wt. Ratio
I	14	50	0.4760 ± 0.012 (5)	355.1 ± 1.68 (5)	1.34^{ab} ± 0.032 (5)
II	14	100	0.4910 ± 0.002 (5)	346.1 ± 1.55 (5)	1.42^{a} ± 0.004 (5)
III	14	200	0.5642 ± 0.001 (5)	339.1 ± 1.38 (5)	1.66^{c} ± 0.012 (5)
IV	14	-	0.4556 ± 0.002 (5)	369.6 ± 1.50 (5)	1.23^{b} ± 0.01 (5)
V	Unvaccinated	-	0.4546 ± 0.018 (5)	372.2 ± 1.85 (5)	1.22^{b} ± 0.012 (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$).

TABLE 25 : EFFECT OF AFLATOXIN ON SPLEEN : BODY WEIGHT RATIO ON 11TH DAY POST IBD VACCINATION.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Spleen Wt., Body Wt. and Spleen : Body Wt. Ratio		
			Spleen Wt. (gm)	Body Wt. (gm)	Spleen : Body Wt. Ratio
I	14	50	0.6155 ± 0.0013 (5)	454.2 ± 7.39 (5)	1.35^{a} ± 0.024 (5)
II	14	100	0.6233 ± 0.0025 (5)	442.0 ± 9.73 (5)	1.41^{a} ± 0.034 (5)
III	14	200	0.6526 ± 0.0040 (5)	435.4 ± 11.27 (5)	1.49^{a} ± 0.045 (5)
IV	14	-	0.5347 ± 0.0041 (5)	478.4 ± 3.14 (5)	1.11^{b} ± 0.010 (5)
V	Unvaccinated	-	0.5358 ± 0.0059 (5)	485.2 ± 3.37 (5)	1.10^{b} ± 0.013 (5)

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b) in individual column did not differ significantly ($P < 0.05$).

The value of body weight gain and FCR in different groups of chickens are presented in table 26. The body wt. gain were lower in vaccinated groups (gr. I-IV) than the unvaccinated group (gr. V). Comparison between aflatoxin treated groups (gr. I - III) showed that the body wt. gain was lowest in group III followed by group II and I when compared with the vaccinated control group (gr. IV) and unvaccinated control group (gr. V).

Poor FCR values were noticeable in aflatoxin treated groups (gr. I - III) when compared with the vaccinated control group (gr. IV) and unvaccinated control group (gr. V) but none of the values differed significantly.

TABLE 26 : EFFECT OF AFLATOXIN ON PERFORMANCE OF BROILER CHICKENS.

Group	Age at IBD vaccination (days)	Level of Aflatoxin (ppb)	Mean ± SE of Initial body wt., Final body wt., Body wt. gain & FCR				
			Initial body wt. on day 1 (gm)	Final body wt. on day 56 (gm)	Body wt. gain on day 56 (gm)	Feed conversion ratio	Mortality (%)
I	14	50	$45.06^{a} \pm 0.35$ (25)	$1169.5^{a} \pm 12.77$ (9)	$1142.4^{a} \pm 9.91$ (9)	$2.63^{a} \pm 0.023$ (9)	11.54
II	14	100	$44.6^{a} \pm 0.789$ (25)	$1159.9^{a} \pm 4.05$ (8)	$1115.26^{b} \pm 4.67$ (8)	$2.68^{a} \pm 0.011$ (8)	16.43
III	14	200	$44.3^{a} \pm 0.628$ (25)	$1145.7^{a} \pm 4.64$ (7)	$1101.3^{b} \pm 5.16$ (7)	$2.72^{a} \pm 0.012$ (7)	20.27
IV	14	-	$45.6^{a} \pm 1.19$ (25)	$1224^{b} \pm 14.12$ (9)	$1178.48^{c} \pm 13.23$ (9)	$2.54^{a} \pm 0.029$ (9)	11.54
V	Unvaccinated	-	$44.8^{a} \pm 0.732$ (25)	$1232^{b} \pm 12.89$ (9)	$1187.2^{c} \pm 11.79$ (9)	$2.52^{a} \pm 0.014$ (9)	11.54

- Figures in parenthesis indicates number of observation.
- Mean bearing common superscript (a, b, c) in individual column did not differ significantly ($P < 0.05$).
- Mortality in Arcsin √Percentage

Fig 1 : Experimental chicks.

Fig 2 : Chickens of aflatoxin fed group showing retarded growth, ruffuled feathers at 42 days of age.

Fig 3 : Chickens of control group at 42 days of age.

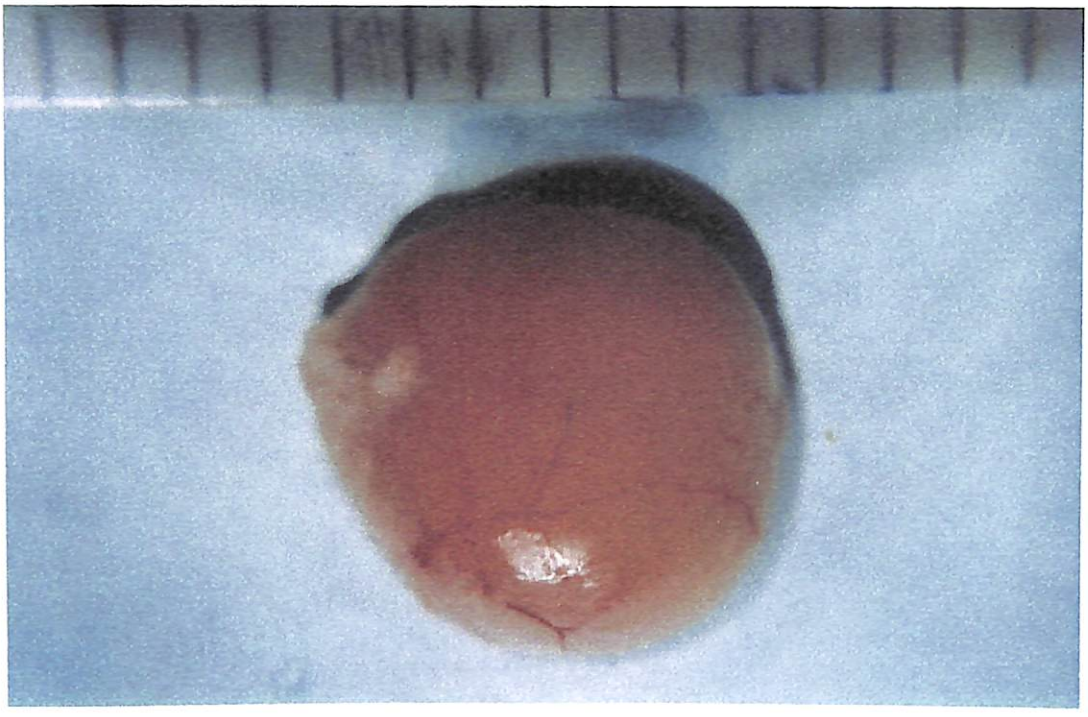


Fig 4 : Enlarged bursa of Fabricius showing haemorrhagic line on the surface of chick of group II sacrificed on 3rd day post IBD vaccination.

Fig 5 : Chicks of group III sacrificed on 3rd day post IBD vaccination showing heamorrhages in thigh muscles.

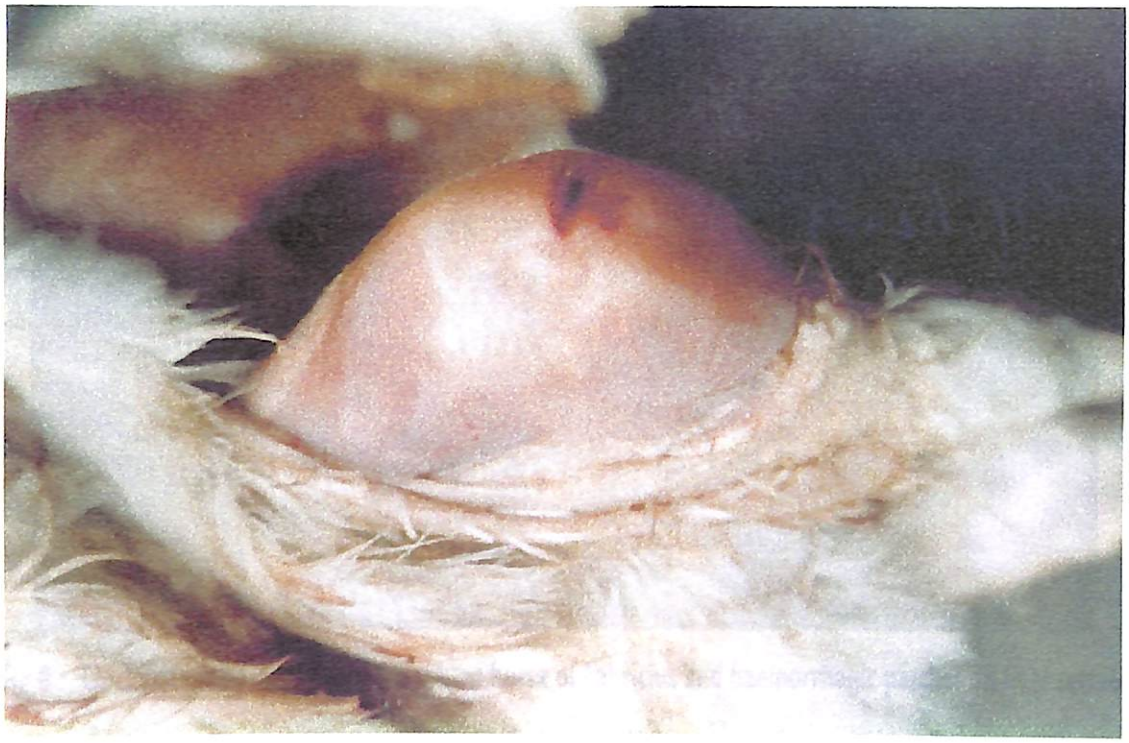


Fig 6 : Chicks of group II sacrificed on on 3rd day post IBD vaccination showing haemorrhages in muscle.

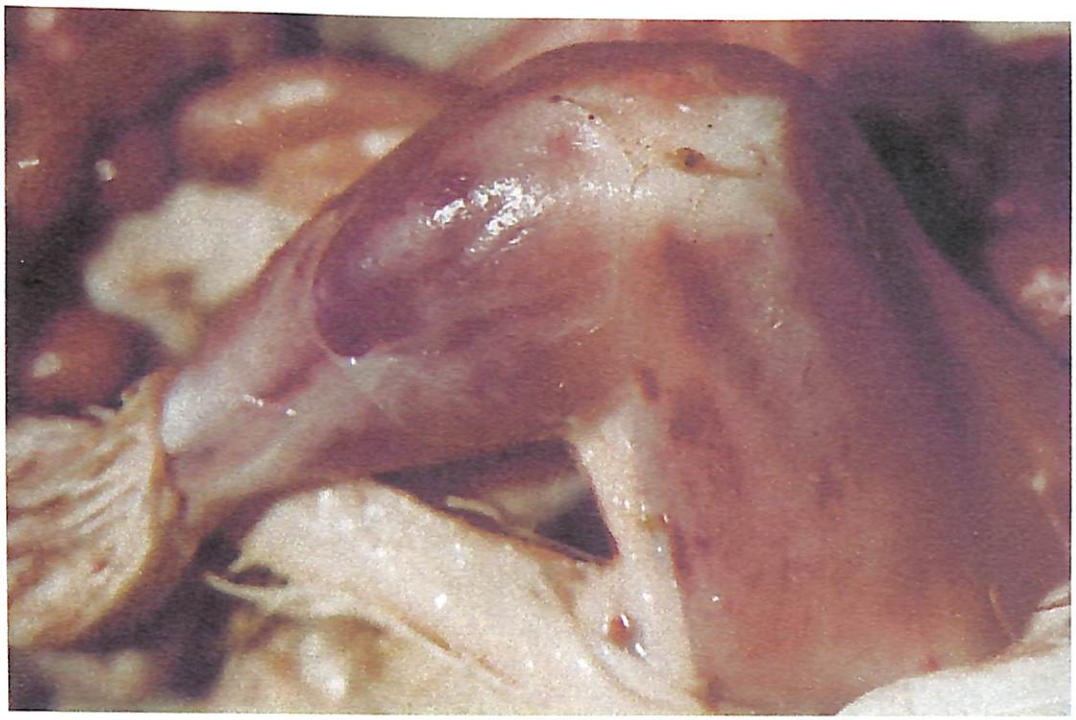


Fig 7 : Chicks of group III sacrificed on 7th day post IBD vaccination showing extensive heanorrhages is thigh muscle post IBD vaccination.

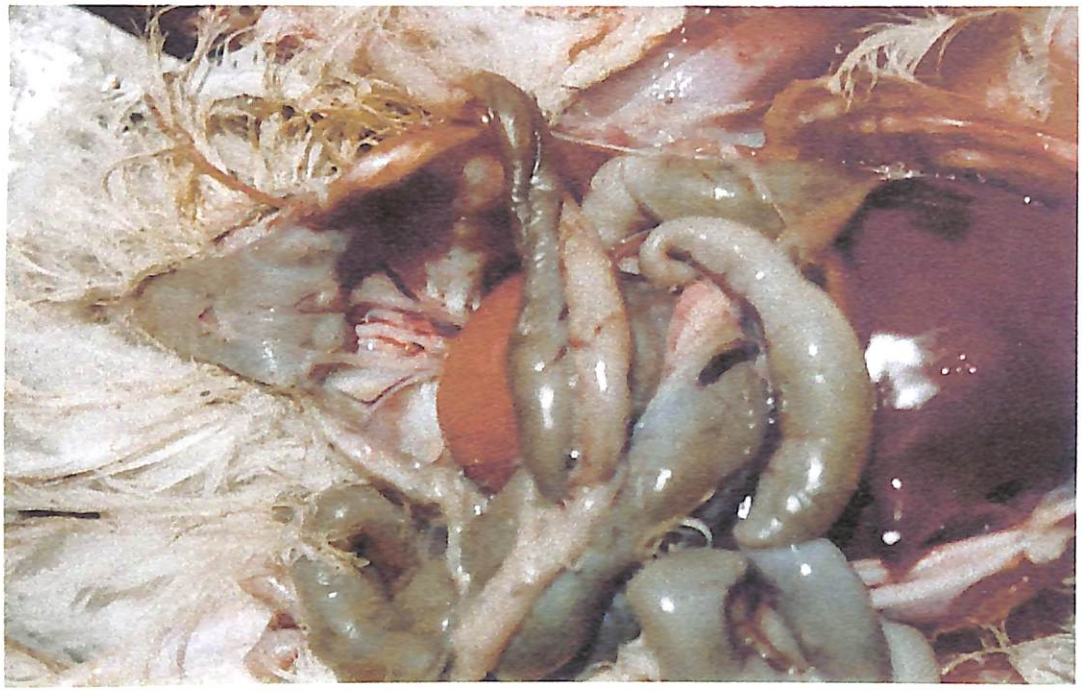


Fig 8 : Chicks of group III showing atrophied bursa of Fabricius and haemorrhagic patches on liver surface sacrificed on 11th day post IBD vaccination.

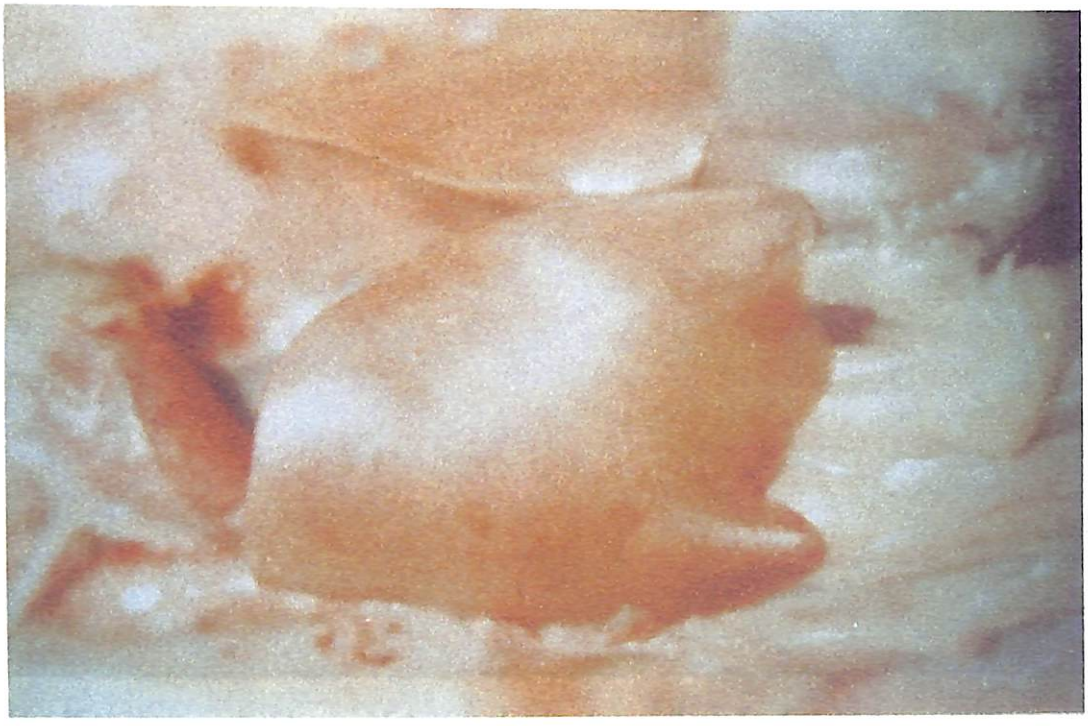


Fig 9 : Chicks of group III sacrificed on 11th day post IBD vaccination showing pale, enlarged liver.

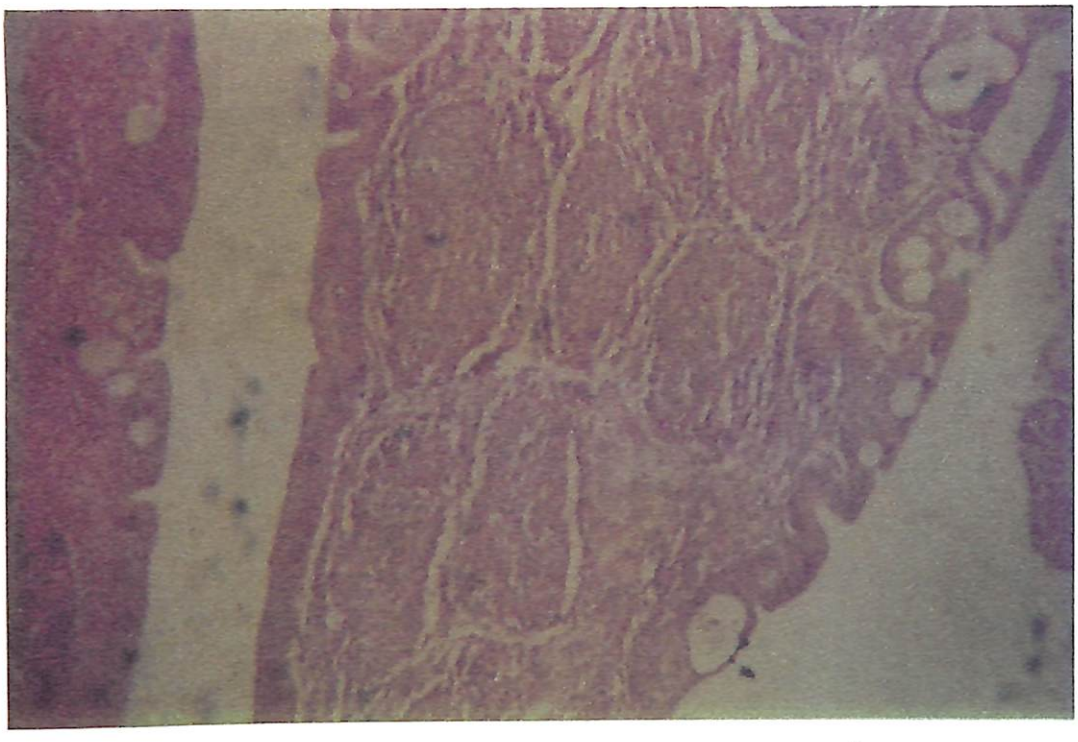


Fig 10 : Microphotograph of bursa of Fabricius of chick sacrificed on 3rd day post IBD vaccination showing marked cystic degeneration in plical epithelium loss of architecture, total degeneration and necrosis of lymphoid cells.

(H&E x 100)

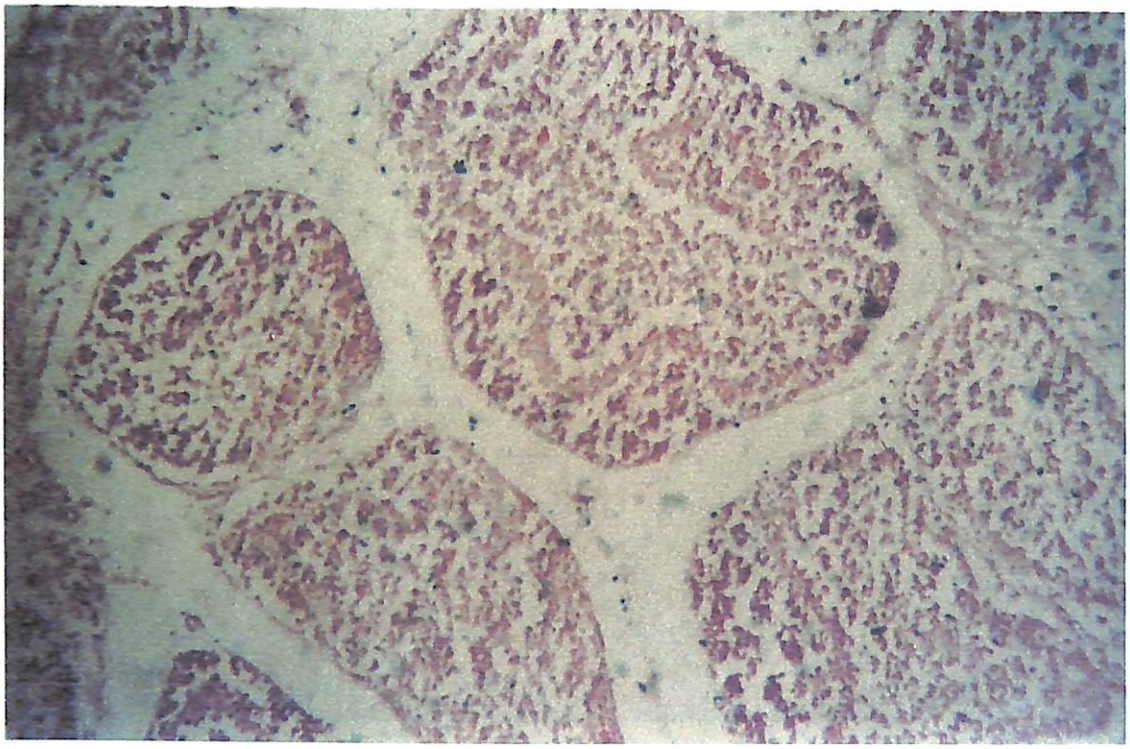


Fig 11 : Microphotograph of bursa of Fabricius of chiks of group II sacrificed on 3rd day post IBD vaccination showing marked interfollicular edema and marked depletion of lymphoid cells in the medullary region.

(H&Ex100)

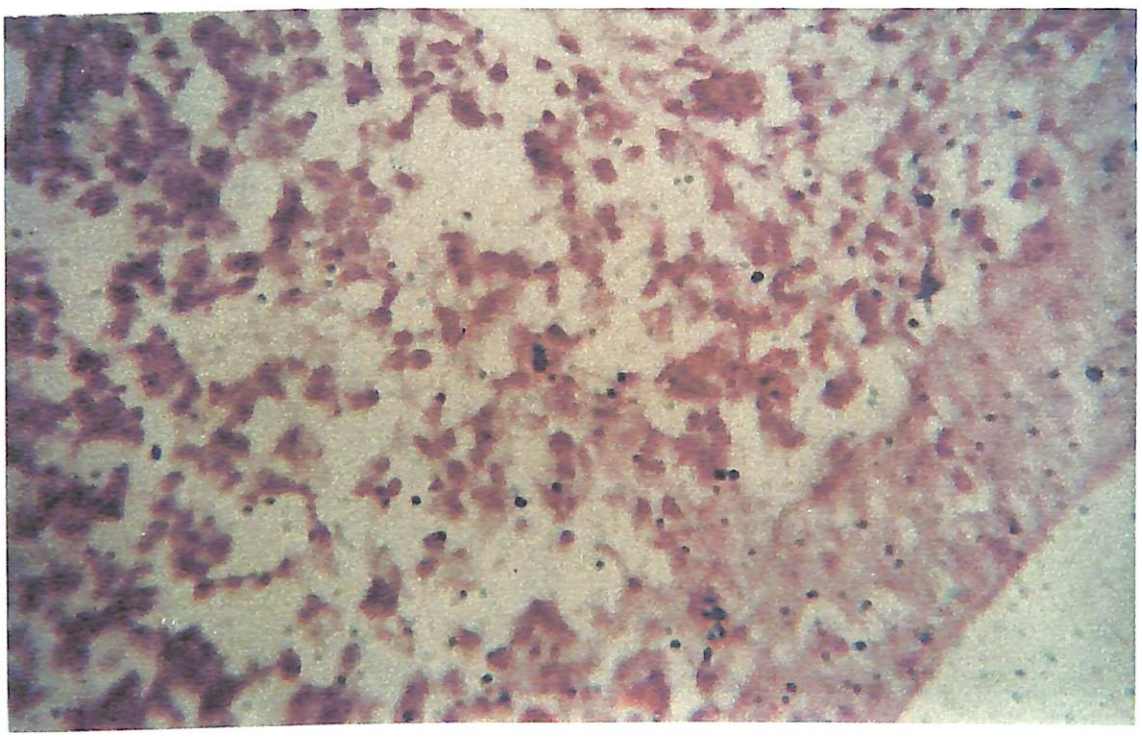


Fig 12 : Microphotograph of bursa of Fabricius of chicks of group III sacrificed on 3rd day post IBD vaccination showing extensive vacuolar degeneration, depletion of lymphoid cells and thickening of plical epithelium.

(H&Ex400).

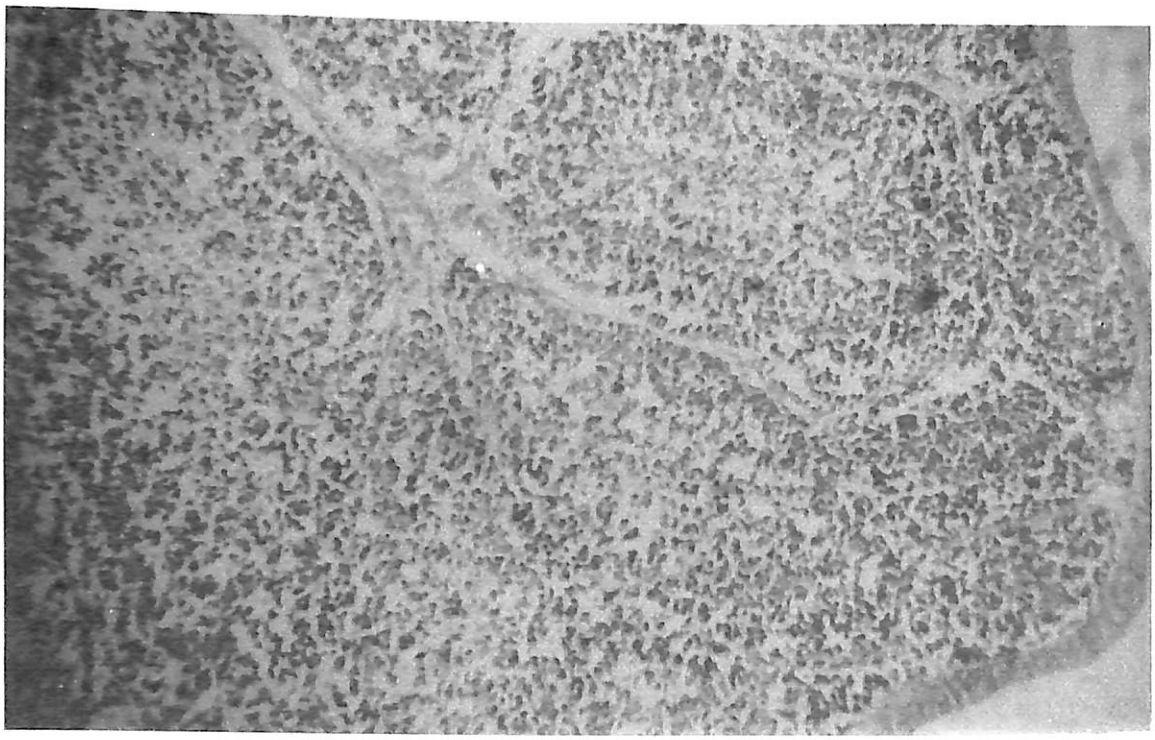


Fig 13 : Microphotograph of bursa of Fabricius of chick of group II sacrificed on 7th day post IBD vaccination showing degeneration and depletion of lymphoid cells edema of interfollicular space in some places and mild proliferative changes. (H&Ex100)

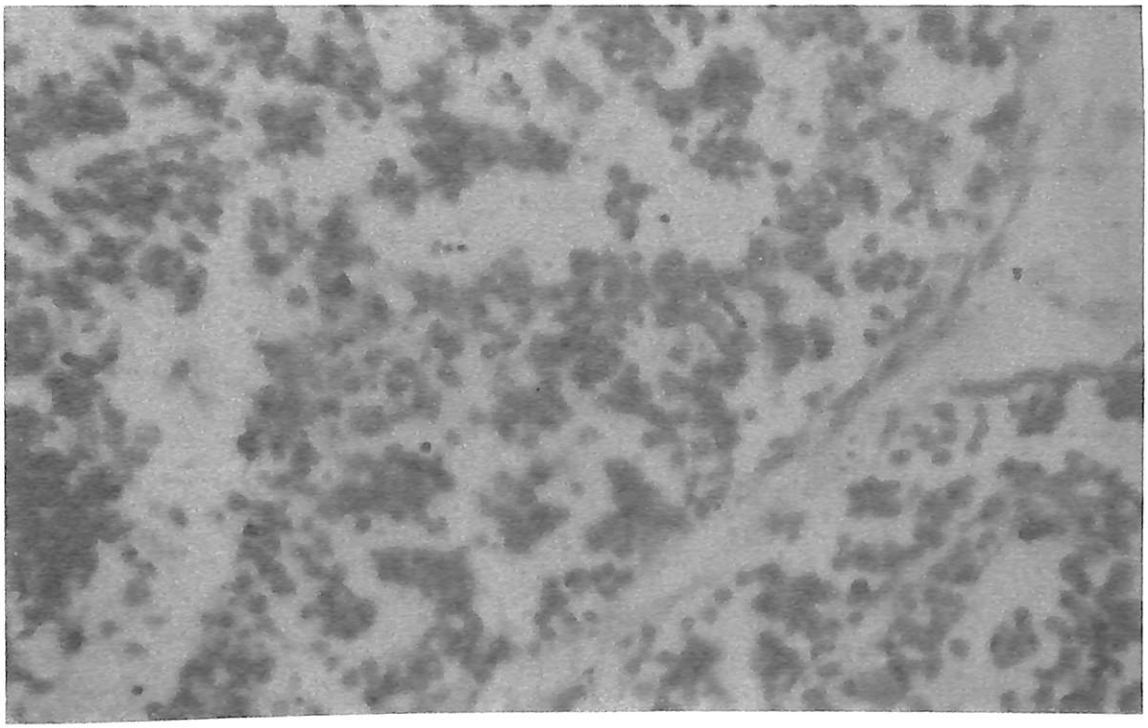


Fig 14 : Microphotograph of bursa of Fabricius of chick of group II sacrificed on 7th day post IBD vaccination showing necrotic mass, interfollicular fibrosis, total degeneration of lymphoid cells only vacuolar network left. (H&Ex400)

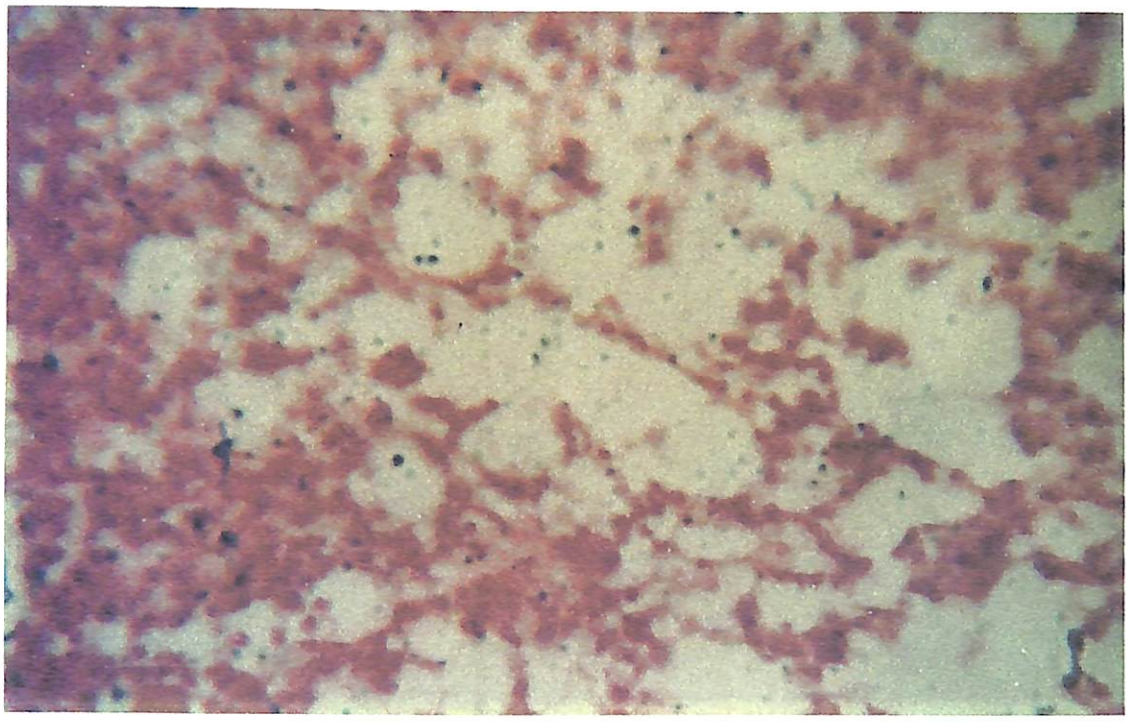


Fig 15 : Microphotograph of bursa of Fabricius of chick of group II sacrificed on 7th day post IBD vaccination showing complete loss of architecture, total degeneration of lymphoid cells only vacuolar network left.

(H&Ex400)

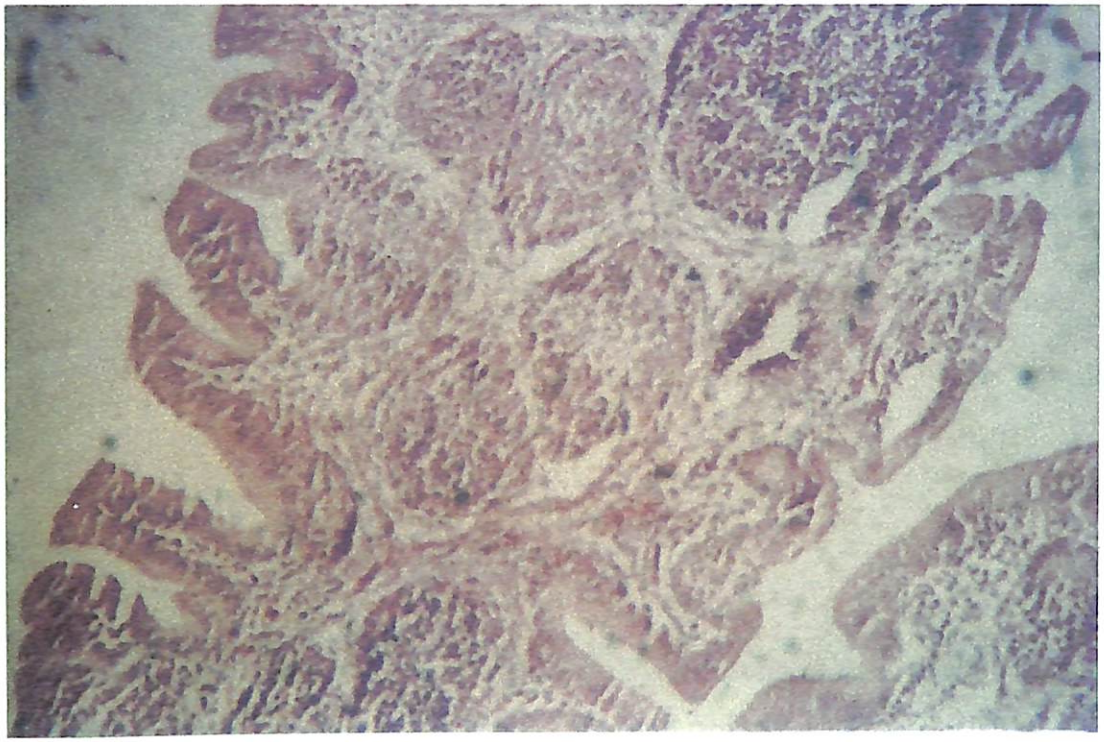


Fig 16 : Microphotograph of bursa of Fabricius of chick of group III sacrificed on 7th day post IBD vaccination showing marked invagination of plical epithelium, degenerative cells with pyknotic nuclei, atrophied follicle, proliferative changes in the interfollicular area. (H&Ex100)

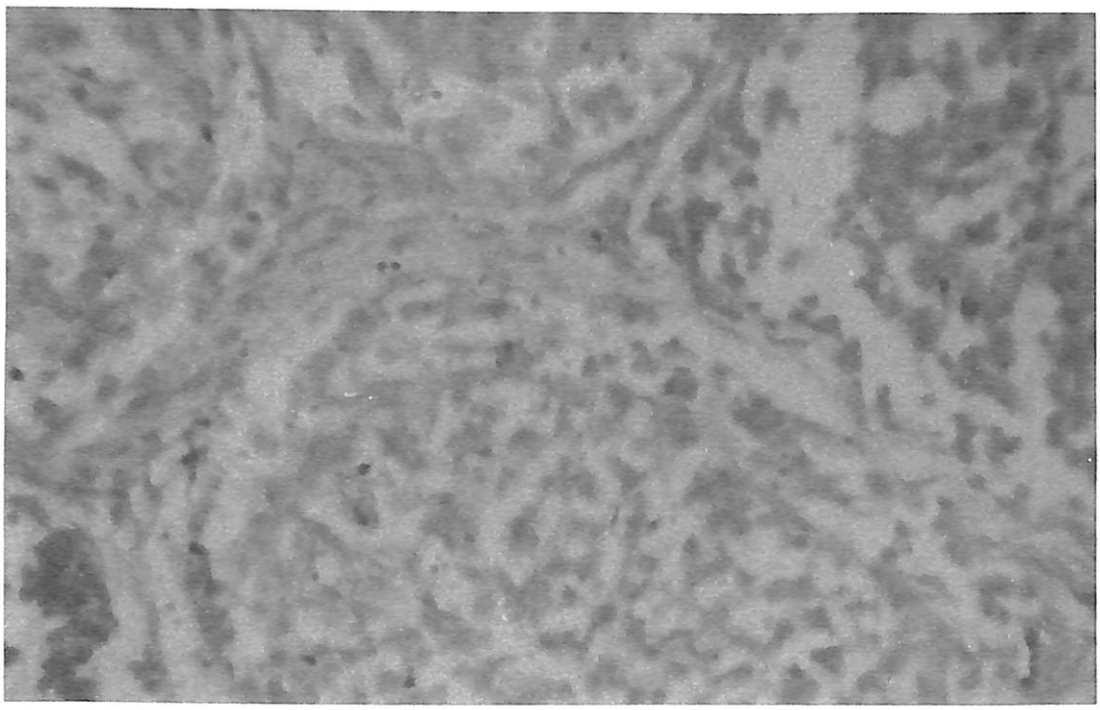


Fig 17 : Microphotograph of bursa of Fabricius of chick of group III sacrificed on 7th day post IBD vaccination showing interfollicular fibrosis, loss of lymphoid cells and vacuolar degeneration of lymphoid cells. (H&Ex400)

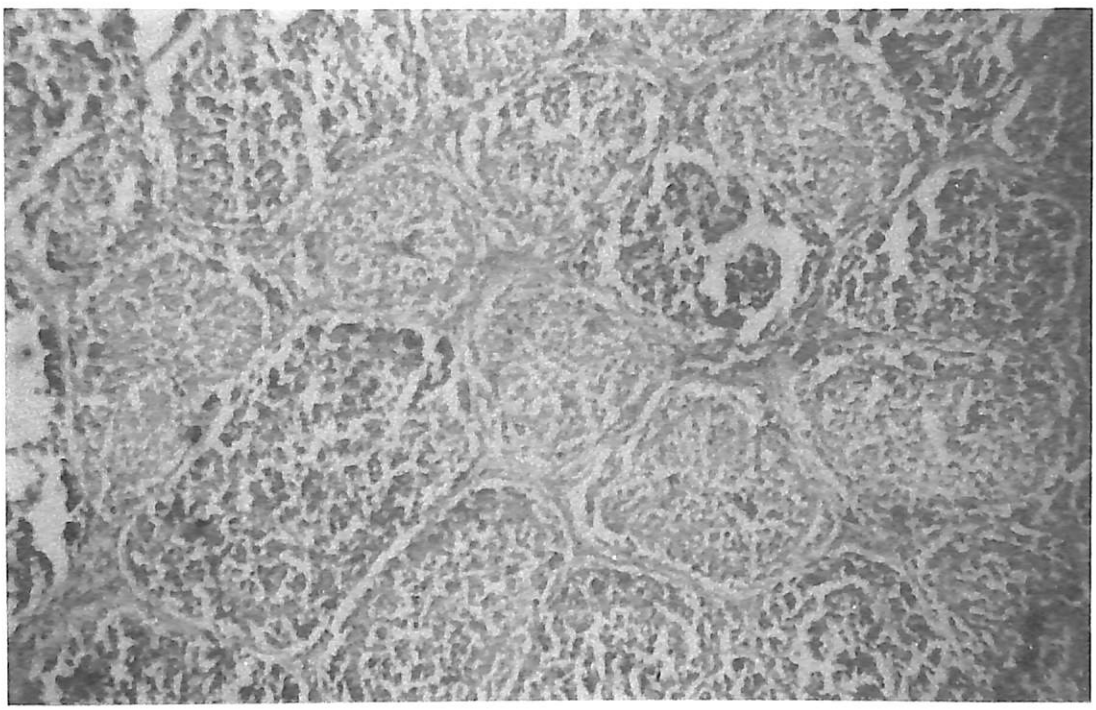


Fig 18 : Microphotograph of bursa of Fabricius of chick of group II sacrificed on 11th day post IBD vaccination showing atrophic follicle, interfollicular fibrosis and degeneration of lymphoid cells. (H&Ex100)

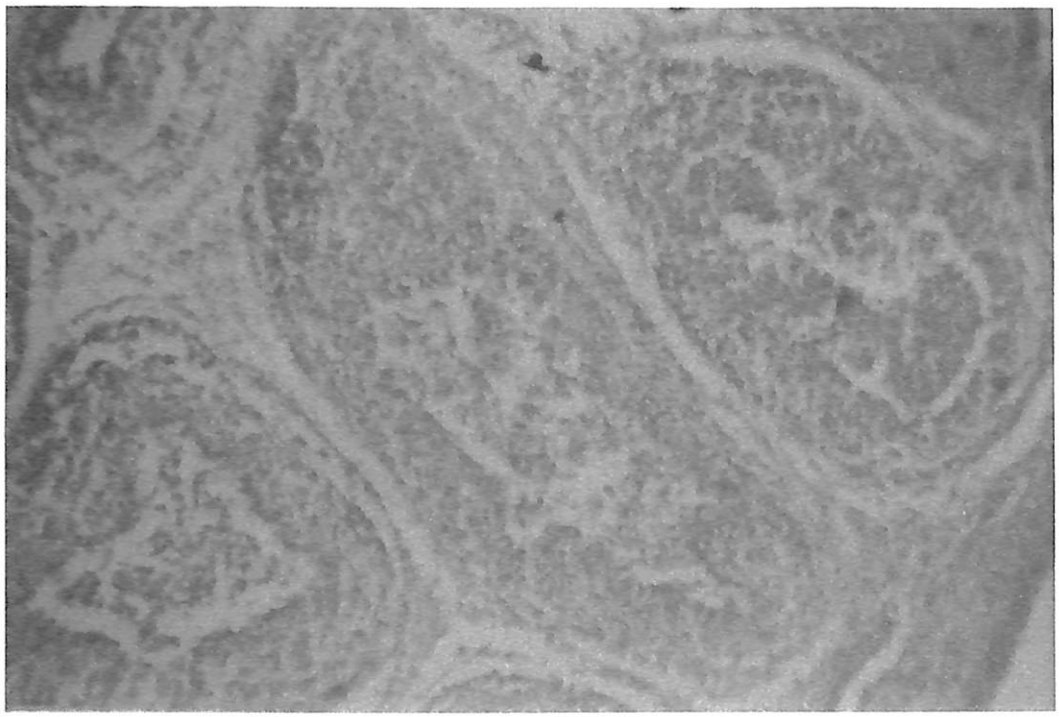


Fig 19 : Microphotograph of bursa of Fabricius of chick of group III sacrificed on 11th day post IBD vaccination showing loss of architecture, depletion and degeneration of lymphoid cells in medullary area and heterophilic infiltration. (H&Ex 100)

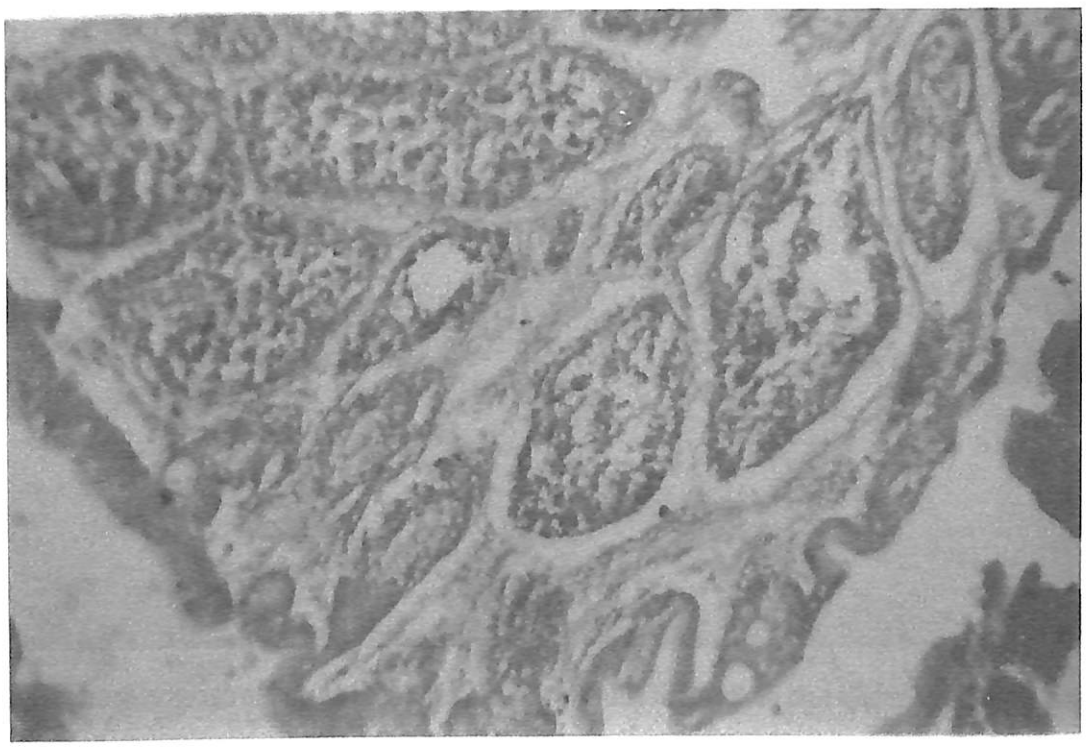


Fig 20 : Microphotograph of bursa of Fabricius of chick of group III sacrificed on 11th day post IBD vaccination, showing invagination & cystic degeneration in plical epithelium, total degeneration of lymphoid cells, atrophic follicle and fibrosis around follicle. (H&Ex100)

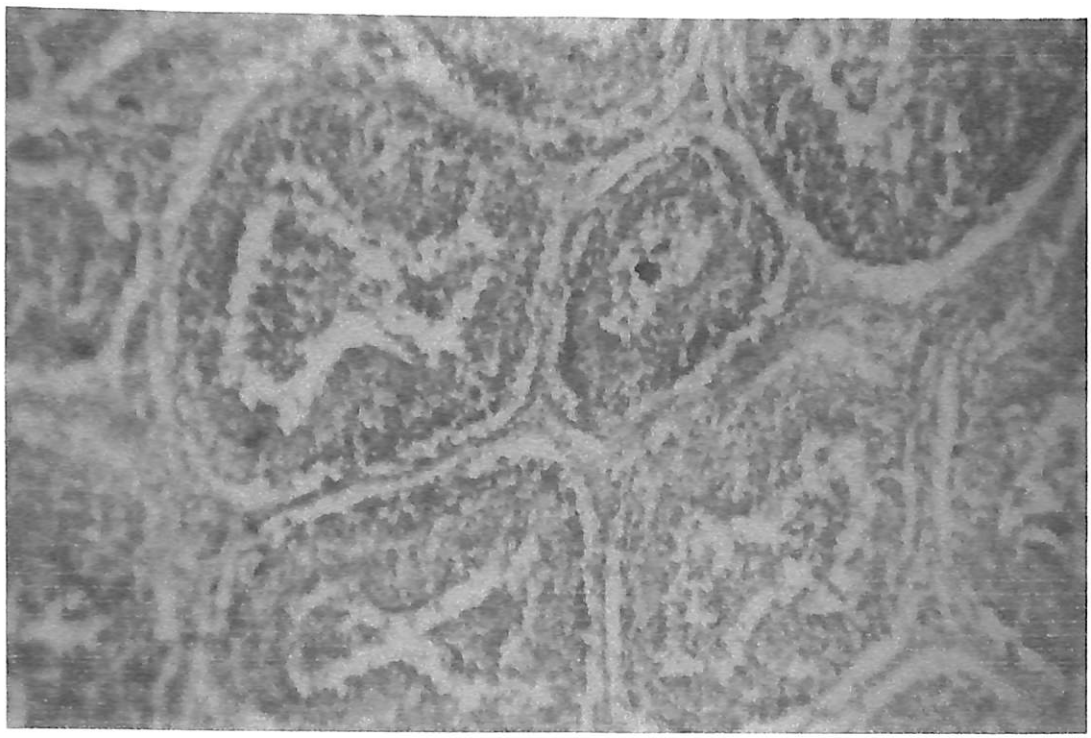


Fig 21 : Microphotograph of bursa of Fabricius of chick of group III sacrificed on 11th day post IBD vaccination showing depletion, degeneration and necrosis of lymphoid cells. (H&Ex100)

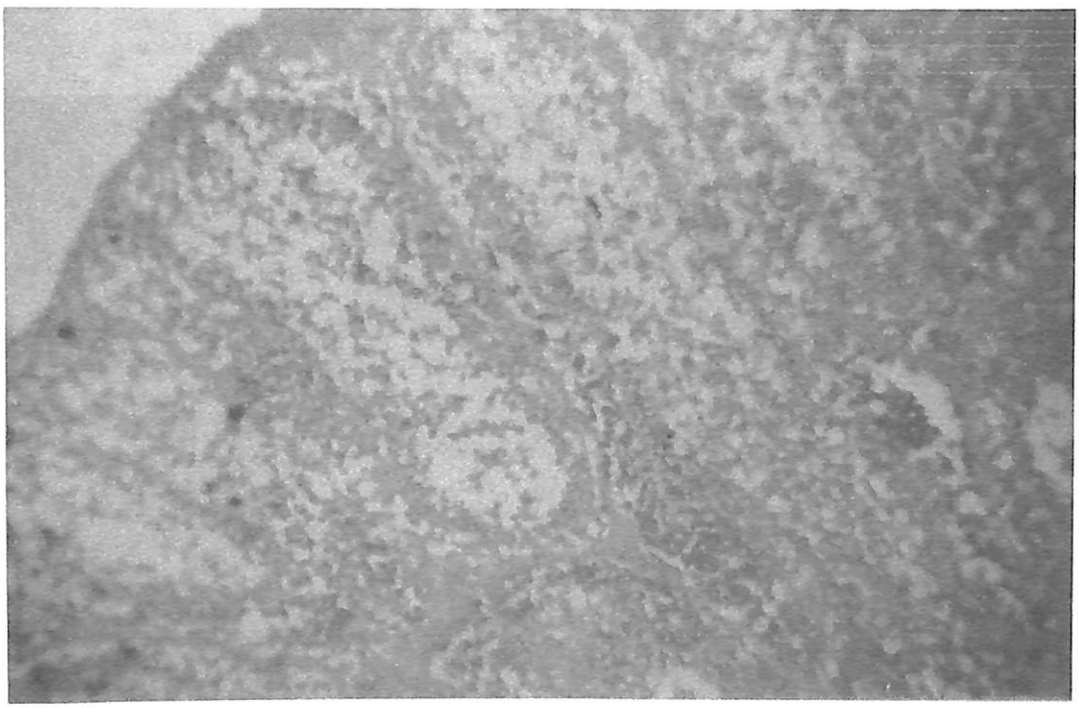


Fig 22 : Microphotograph of bursa of Fabricius of chick of group III sacrificed on 11th day post IBD vaccination showing total dageneration in medullary region of follicle only vacuolar network left, interfollicular fibrosis.

(H&Ex400)

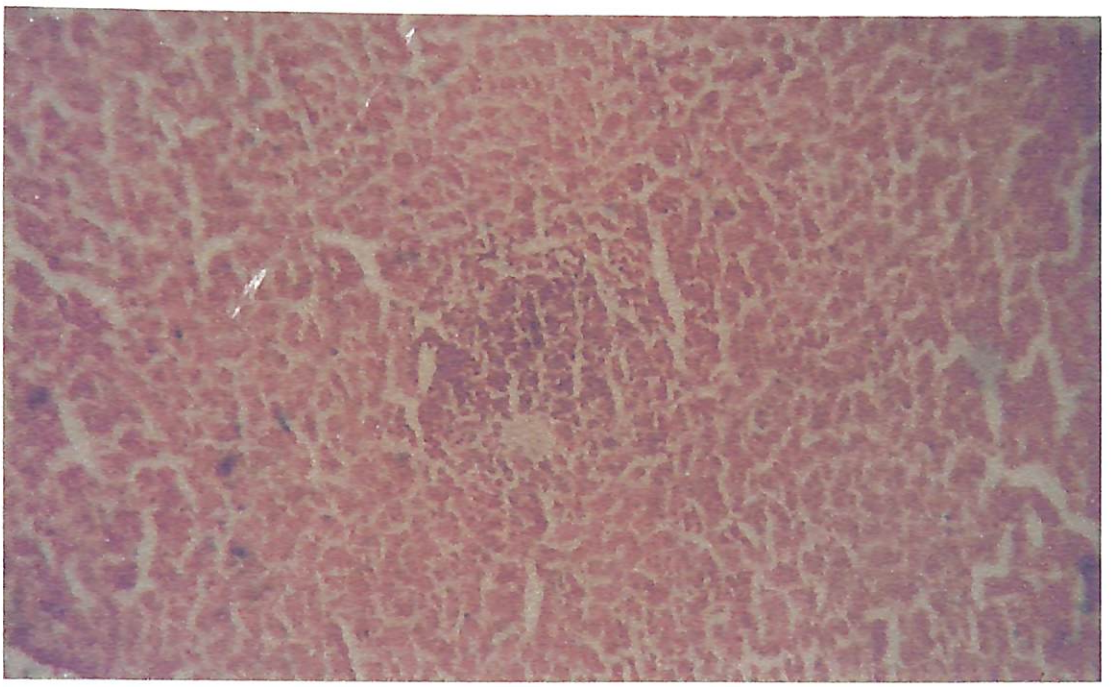


Fig 23 : Microphotograph of liver of chick of group III sacrificed on 11th day post IBD vaccination showing focal infiltration of heterophilic cells and degeneration of hepatic cells. (H&Ex400)

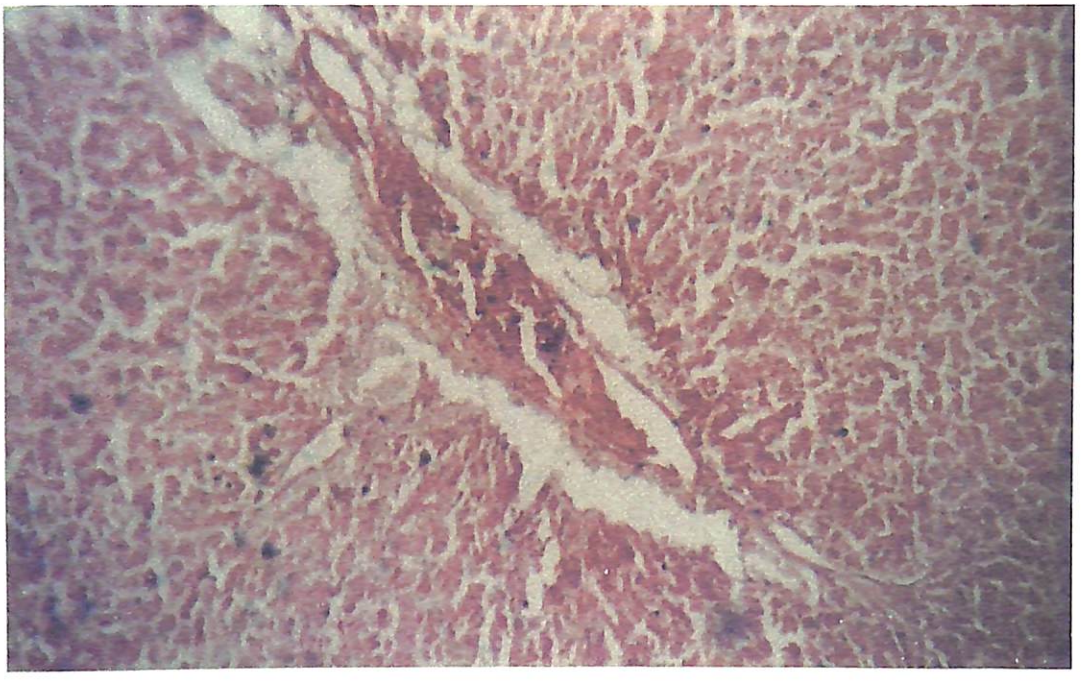


Fig 24 : Microphotograph of liver of chick of group III sacrificed on 11th day post IBD vaccination showing dialatation of central vein and congestion in portal area (H&Ex400)

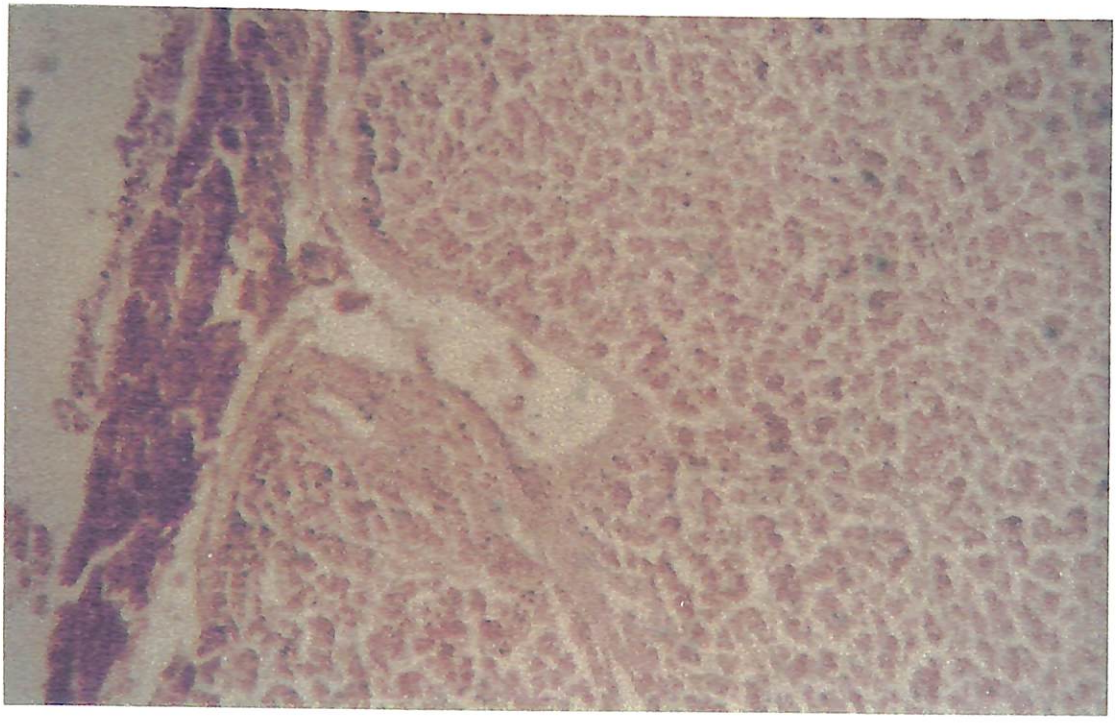


Fig 25 : Microphotograph of liver of chick of group III sacrificed on 11th day post IBD vaccination showing proliferation of connective tissue in the hepatic cells, degeneration with pyknotic nuclei. (H&Ex400)

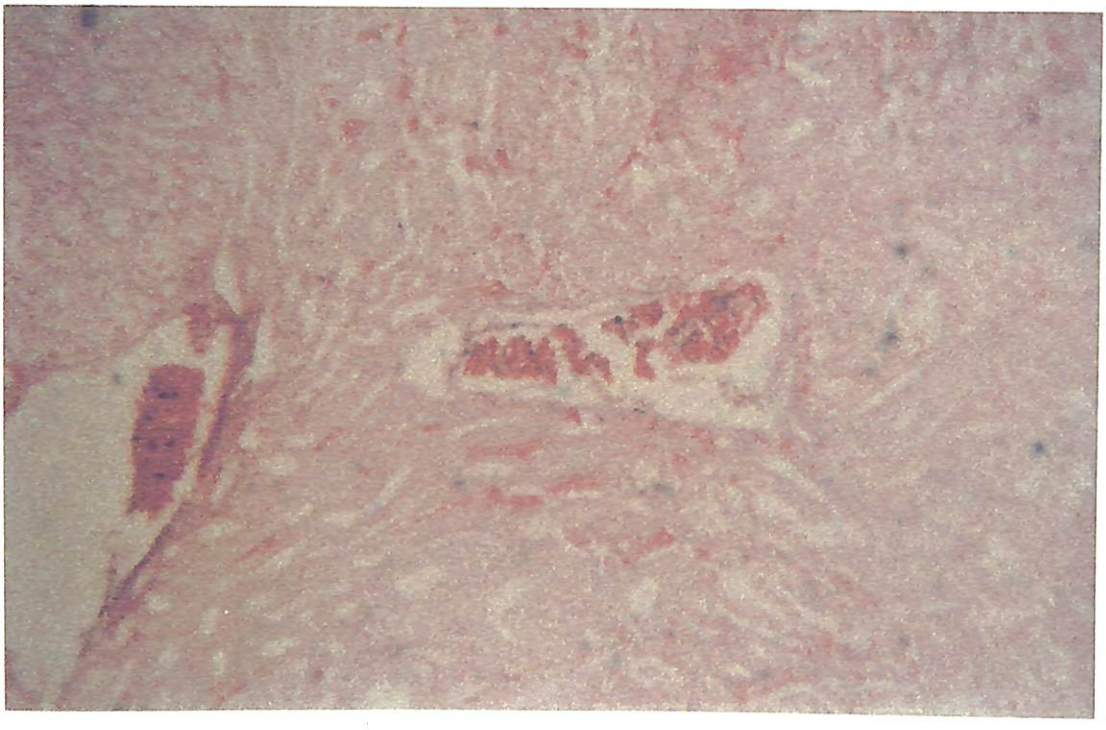


Fig 26 : Microphotograph of kidney of chick of group III sacrificed on 11th day post IBD vaccination showing congestion of blood vessels. (H&Ex400)

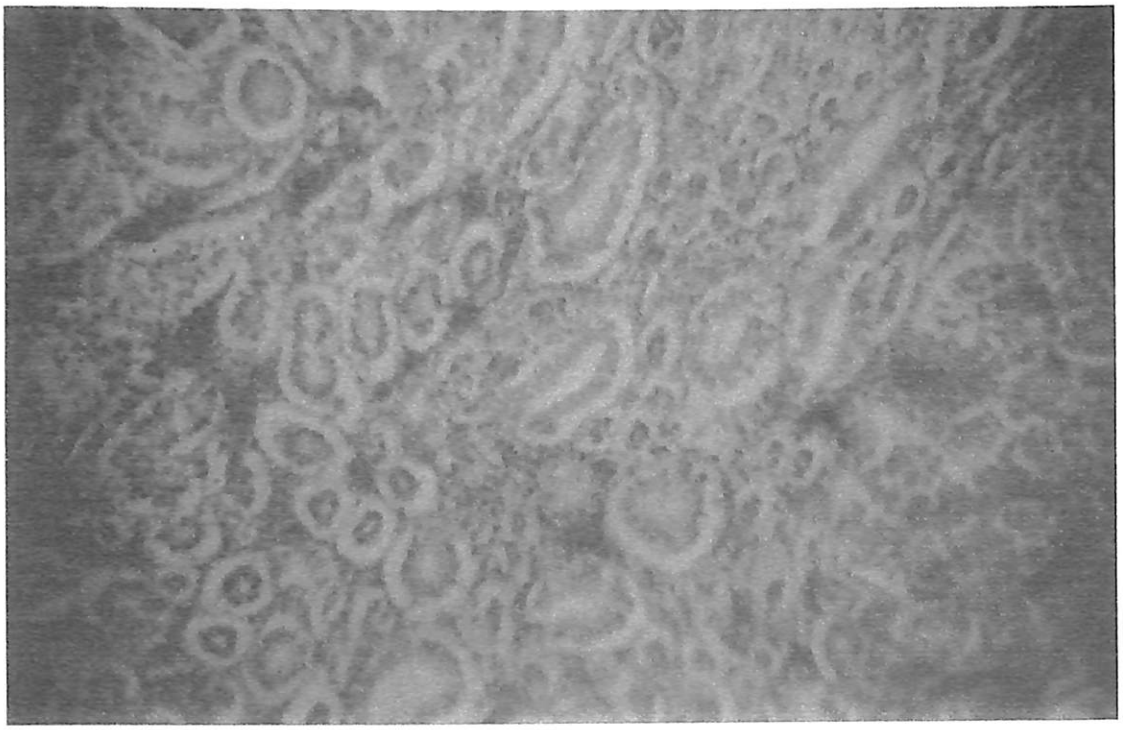


Fig 27 : Microphotograph of kidney of chick of group III sacrificed on 11th day post IBD vaccination showing hyperaemic changes in blood vessels, haemorrhages in interstitial tissue, degenerative and necrotic changes in the lining epithelium of renal tubules. (H&Ex100)

CHAPTER

DISCUSSION

Discussion

Studies conducted so for have clearly established the occurrence of aflatoxin in poultry feeds and feeding ingredients (Bryden *et al*, 1980; Buckle, 1983; Jelinek *et al.*, 1989) . The adverse effect of the aflatoxin in terms of its toxicity on liver (hepatotoxin) and on kidney (nephrotoxin) as well as by way of carcinogenic and immunosuppressive effects have also been revealed on a number of occasions (Campbell *et al.*, 1988; Mohiuddin, 1993; Gabal & Azzam, 1997). Attempts to ascertain the reason for the toxicity of the aflatoxin have yielded information in respect of the factors such as nutritional status, age (younger birds are more susceptible than the adult birds), sex, genetic make-up, challenges due to pathogenic organisms and certain climatic stressers like heat or cold (Smith and Hamilton,1970). The poultry farmers are specially concerned with the immunosuppressive effects of the aflatoxin which leads to a population of birds which are immuno-compromised and therefore, demonstrate impaired immune responses to various microbial vaccines and thereby throwing challenges to the whole gamut of poultry health programme. The present scenario in respect of the effect of the aflatoxin needs rethinking given the fact that the poultry population is already exposed to the risk of serious immuno suppressing agents like infectious bursal disease (IBD) virus. The earlier studies undertaken to know the status of IBD in poultry population in this part of the country have established the occurrence of this disease both in broiler and layer chickens, specially in the age group of 2-8 weeks. The clinical picture in respect of IBD has suddenly changed after 1992 due to occurrence of new form of IBD, very commonly known as vvIBD (very virulent IBD). These days it is only this new form of IBD which is taking toll of poultry birds. To control this dreaded form

of IBD a new type of vaccine is being used and one such vaccine widely used in this area of the country is invasive strain commonly called IV95 strain of IBD virus. The works conducted in this laboratory (Kumar, 1998) and else where also have shown the possession of residual pathogenecity and immunosuppressive effect in such vaccines strain. The residual immunosuppressive effect of such vaccine strains of IBD is acceptable to a larger extent in the given circumstances, specially where there is no other option. However, in our conditions where it is practically not possible to provide poultry feed free of aflatoxin and also given the fact that the levels of aflatoxin in the feed samples during the survey works have revealed relatively higher level over and above the maximum limit which can be tolerated by the birds (less than 20 ppb) even such IBD vaccine strains with minimum residual immunosuppressive effect may demonstrate precipitating effect in combination with varying level of aflatoxin to which the birds are exposed almost in a routine manner.

It is in this context that it has been planned to study the effect of different level of aflatoxin on immune response to IBD vaccines as also the asceleration of the residual immunosuppressive effect contained in this vaccine strain in terms of its effect on immune response to RD vaccine and certain other parameters.

At present the vaccination programme against IBD is undertaken employing three conventional IBD vaccines namely mild (Lukert type), intermediate (Georgia type) strains as well inactivated IBD vaccines. The immune responses to the above named vaccines have been conducted and reported on number of occasions (McFerram *et al.*1982; Giambrone and Clay, 1986a; Mahesh and Muniyappa, 1996; Khaliel and El-Manakhly, 1998; Thangavelu *et al*, 1998). Both intermediate and moderate hot strain vaccines

which include IV95 strains also have been shown to possess residual immuno suppressive effect (Nagi *et al.* 1980; Khaliel and El-Manakhly, 1998; Kumar, 1998). Paradoxically the IBD virus is known to impair immune response to several microbial agents/ vaccine but without impairing its own immune response. (Giambrone *et al.*, 1979; Jhala *et al.*, 1990; Rao & Rao., 1992; Prabhakaran.*et al.*, 1997).

However, there are reports that aflatoxin impairs the immune response to IBD virus as well (Giambrone *et al*, 1978; Gabal & Azzam, 1998) .In the present study the precipitating antibody titre to IBD vaccine in aflatoxin treated groups (gr I-III) were lower than corresponding value in the control group (gr IV) over all the periods post IBD vaccination (Table-2) Further the immune suppressive effect got apparent from the precipitating Ab titre getting gradually lowered with increase in dose level. The present findings are in agreement with the observation of Ghosh and Chauhan, (1991) and Mani *et al*, (2000).

However, as in the present study only three dose levels of aflatoxin were employed and therefore it could not be exactly ascertained the dose level of the aflatoxin which could be none immunosuppressive in terms of Ab titre to IBD vaccines.

The immunosuppressive effect of aflatoxin in young chickens is well documented (pier *et al* .,1971; Giambrone *et al.*, 1978; Jassar & Singh., 1989; Bakshi *et al.*, 1998; Gabal & Azzam, 1998;). Further the immune suppression 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. Since, immunosuppressive effect of IBD virus is mainly by way of destruction/ necrosis of B dependent lymphocytes in the bursa of Fabricius, there are reasons to believe that due to the exposure of the birds the duo (IBD virus and aflatoxin simultaneously) should produce immune suppression of higher degree, because while the IBDV acts mainly on B-cells, aflatoxin may not allow proper synthesis of immunoglobulins by the immuno - competent cells (mainly B-cells) which have escaped the destructive effect of IBD virus. Besides, by causing enhanced degradation of pre-formed Ab by lysosomal enzymes in the liver cells as well as by causing enhanced degradation of antigen, the aflatoxin may lead to further immunosuppression over and above what is already produced by IBD virus. Therefore, the higher degree of immunosuppression as recorded in terms of lowered Ab titre to IBD vaccine strain virus in aflatoxin treated groups (gr I-III) when compared with the values in the control group (gr IV) may be explained in the light of the reason cited above.

The suppresive effect of both IBD virus and aflatoxin on immune response to RD vaccine are widely accepted (Faragher *et al* ., 1974; Giambrone *et al* ., 1978 ; Hirai *et al.,* 1979; Higashihar *et al.*, 1991; Bakshi *et al* ., 1998). In the present study, it was demonstrated that combined effect of both the agents (ie IBD virus and aflatoxin) was more immunosuppressive as measured by lowered level of HI titre in aflatoxin treated groups than values recorded in non-aflatoxin control groups (gr.IV). Therefore, the present findings are in consonance with the observation recorded by Giambrone *et al* . (1978). Mangat *et al.* (1989). Jassar and Singh . (1993). Mani *et al* . (2000). Further it was also recorded that the immune suppression was more marked when the dose of aflatoxin was higher (Table –3). These findings are in agreement with the observation of Mani *et al.* (2000) and Bakshi *et al.* (2000). It also demonstrated that the degree of inhibition caused by aflatoxin is dose related - higher the

dose of aflatoxin poorer the HI titre to RD vaccine which also supports the present findings. The findings in terms of HI titter to RD vaccine as noted in the birds under group III receiving the highest level of aflatoxin (200 ppb) in this experiment, specially at 35 and 42 days post IBD vaccination are alarming because the interaction of the two (virus and aflatoxion) has lead to lowering of the HI titre below the protective level of 2^3 . The present study warrants the regular monitoring of feed and their ingredients independently in respect of the level of aflatoxin, specially in such area where IBD is endemic and also when the vaccine such as one employed in the present study (IV95 strain/ moderate hot strain) is used to control the vvIBD scenario.

The another interesting findings was the decline in HI titre to RD vaccine by 21 days post IBD vaccination as clearly depicted in group II and group III which received 100 ppb and 200 ppb of aflatoxin respectively. The present finding is suggestive of the fact that the aflatoxin in combination with even non-pathogenic/avirulent strain of IBD like the one used in the present study (IV95 strain) can produce immunosuppression of higher degree as evidenced by the decline in HI titre in group II and III. Further, in case of group I, IV and V, the decline in the titre to RD vaccine was first revealed by 28 days post IBD vaccination which suggested as the dose of aflatoxin is reduced the HI titre remains maintained and the trend is almost equivalent in the group without aflatoxin but which received IBD strain vaccine (gr IV) as well as in the group which neither received aflatoxin nor received IBD vaccine (gr V). It is also to be noted that the administration of 2^{nd} dose of RD vaccine (Lasota strain) has been able to keep the HI titre increasing and interestingly the titre remained higher than the protective level of 2^3 in all the groups except the group which received 200 ppb of aflatoxin (gr III) (graph-3).

The present study is indicative of the desirable effect of the 2nd RD vaccination with Lasota strain in terms of encouraging secondary immune response. This study also points to the fact that the repeated vaccination with Lasota strain may be necessary in a situation where continuous exposure to aflatoxin is likely and also when Gumboro is endemic in such area. The poultry farmers need to be cautioned about the likely risk involved in vaccinating their stock with moderate hot strain IBD vaccine to control the vvIBD scenario in such areas.

There are numbers of reports that even the vaccines strains of IBD virus produces some degree of pathogenecity and immunosuppression in vaccinated chickens. (Winterfield and Thacker ., 1978; Mazariegos *et al.*, 1990; Khaliel and El-Manakhly., 1998; Jeurissen *et al.*, 1998). Further the degree of the changes produced in the bursa of Fabricius were relataively more marked when intermediate plus strain of vaccine was used than intermediate strain of IBD virus vaccine (Kouwenhoven and Vanden Bos., 1994; Tsukamoto *et al.*, 1995) . In the present study also changes typical of IBD virus were recorded when IV95 strain of virus was used. The present findings is in consonance with the earlier work conducted in this laboratory (kumar 1998) as well as reports of the several workers from different part of globe. (Kouwenhoven and vanden Bos., 1994). It was also observed that such changes were more marked in groups of birds which also received aflatoxin (gr I-III) (Table- 4,5 and 6). The present findings are similar to the reports received earlier that aflatoxin and IBD virus together produce additive effects (Giambrone *et al.*, 1978; Chang and Hamitton., 1982; Somvanshi and Mohanty ., 1991). Further it was noticed that the severity of the bursal lesion got enhanced as the dose level of aflatoxin was increased (group II and III) as shown in table – 4,5and 6.

In the present study histopathological examination of section of liver, Kidney and spleen showed some changes like extensive vacuolar changes in hepatocytes, necrosis of liver cells (Fig –23, 24 & 25), proliferation of connective tissue around bile duct, congestion of blood vessels and vacuolar degeneration in tubular epthelium of kidney (Fig –26 & 27). These findings are in agreement with the observation of somvanshi & Mohanty . (1991); Giambrone *et al.* (1978). A number of worker reported mild changes in bursal organs in IBD infected birds (Aziz., 1985; Sah *et al.*, 1995). However, Ley *et al* (1993) reported that the changes observed in non bursal organs were non specific. In the present study, it is suggested that severe and certain gross and microscopic lesions may have been due to additive effect of IBDV and aflatoxin.

Total serum protein concentration is convenient indicator of hydration (Ley *et al.*, 1983). A number of workers have reported decrease is total serum protein in IBD infected chickens (El. Batrawi and Awad., 1993; Singh *et al.*, 1994; Al – Afaleq ., 1998). In the present study, also reduction in total serum protein value was noticeable in IBD vaccinated group (gr IV) when compared with the values in the unvaccinated control group (gr V). Cheville (1967) and panigraphy *et al* (1986) observed that the decline in serum total protein values in IBD infected chickens may be due to inflammatory exudation of serum albumin into the bursa of Fabricius and its subsequent excretion in the faces which will finally lead to reduction in total serum protein values. However, this needs further confirmation since some workers have also reported no change in serum total protein after IBD infection (Ley *et al.*, 1983).

Further it was also observed that there was sharp reduction in total serum protein values in IBD vaccinated groups of birds which has simultaneously received graded doses of aflatoxin (gr I to III); (Table –7). The relatively greater decline of total serum protein value levels in aflatoxin treated

groups as noted above may be due to the inhibitory effect of aflatoxin on protein synthesis in hepatic cells, inactivation of amino acids for protein synthesis in liver and blocking of RNA synthesis in the nucleolus. It is also important to mention that the decline in total serum protein value got deepened as the dose level of aflatoxin was enhanced (Table –7) . The present findings is suggestive of the gravity of the situation being confronted by the poultry farmers in a complex scenario where the chances of aggravation of the adverse effect of IV95 type IBD vaccine are likely to occur because of the simultaneous exposure of birds to aflatoxin.

There was lowering of albumin values when compared with the unvaccinated control (Table-8). in overall the periods post IBD vaccination. On the other hand the picture in respect of serum albumin values in group I,II and III demonstrated albumin values which were higher than the values recorded in vaccinated control group (gr IV). Further, the globulin values were also noted to be on decline side when compared with the IBD vaccinated control group (gr IV).

There are several reports in IBD vaccinated chickens where the globulin values were higher. (Chevillle, 1967; Ley *et al.*, 1983; Panigraphy *et al.*, 1986). However, in the present case whereas the trend remained the same in group of birds which received only IBD vaccine, the reduction in both albumin and globulin values were noticeable in birds of group I-III (Table 8 & 9). Since there was reduction in total serum protein value, the lowering of both albumin and globulin value in group of birds which received both aflatoxin and IBD is understandable. But the simultaneous reduction in both albumin and globulin value is alarming because the globulin contributes to the production of the antibody. In this respect the simultaneous administration of aflatoxin and IBD is not advisable. Therefore, there is urgent need that the poultry feed being given

to the birds should be monitored regularly in order that the birds which are likely to be exposed to IBD may not get access to aflatoxin through feed.

In the present study there was decline in serum calcium level as the doses of aflatoxin increases.(gr I-III, Table –10) These findings support earlier works by Chang & Hamilton (1982). It is also observed that the calcium level of IBD vaccinated alone group (gr IV) was also less than the unvaccinated control group (gr V) at all interval of time, though all these values were within the physiological range. These observation revealed that dietary aflatoxin decreased plasma calcium (Table –10) while the IBD infection alone did not but the factors combinedly interacted to produce a much more severe hypocalcemia, (Chang and Hamilton 1982).Number of workers suggested that the aflatoxin inhibits calcium absorption from the intestine. Poor mineral absorption invariably results in poor bone strength (Dimri *et al.*, 1994). The same mechanism might probably explain the low level of serum inorganic phosphorus as well. Several workers reported earlier that increased levels of aflatoxin resulted in a linearly decreasing effect on serum calcium and phosphorus (Fazal *et al.*, 1980; Shukla & Pachauri, 1995) . In the present study the serum inorganic phosphorus level were decreased with increased dose level of aflatoxin (Table-11)

A number of workers have reported the alteration in haematological values due to IBD virus in chickens, (Panigraphy *et al*, 1986; Kumar & Rao 1991; Singh and Dhawedkar 1994a) . In a number of cases such reports are conflicting. Both hemoglobin and packed cell volume value have been reported to decrease in IBD infected chicken (Kumar and Rao., 1991; Cho & Edger., 1972). On the contrary in the present study both the Hb and pcv values did not differ significantly in vaccinated control group (gr IV) when compared with the unvaccinated control group gr (V) . (Table – 12 & 13) In the present study, the

values of Hb and pcv value decreased when doses of aflatoxin increased. (Table 12 & 13) A number of workers have reported that the Hb and pcv values were inversely proportional to the dietary aflatoxin levels. (Mani *et al.*, 1993; Oguz *et al.* 2000). Further Chang and Hamilton (1982) also observed that reduction in packed cell volume and Hb value were due to aflatoxin but not due to IBD infection.

In present study there was decline in total WBC count in the birds which received IBD vaccine alone (gr IV) when compared with unvaccinated control group (gr. V) (Table –14). Hudson *et al.*, (1975); Kumar & Rao (1991) and Singh *et al.*, (1994) also reported decline in total WBC count in IBDV infection. It may be mentioned that lymphocytes constitute approximately 80 percent of WBC (Lucas and Jamroz 1961) and also majority of the circulating lymphocytes have been found to be T cells (Hudson *et al.*, 1975; Tizzard 1996). Therefore, it is likely that decrease in total WBC count in IBDV infected chickens may be related to the decrease in circulating T cell numbers. However, another group of workers failed to detect any significant difference in total WBC count (Montogomery *et al.*, 1986) of IBDV infected chickens from that of uninfected control. In the present study, slight reduction of WBC count was observed (Table –14). Further it was observed that the WBC count decreased with increase dose level of aflatoxin. These findings are in consonance with the earlier findings reported by Yaman *et al.*, (1990) Misri (1994) and Mishra (1995).

In the present study a significant reduction in a percent lymphocyte count was observed in IBD vaccinated birds (gr IV) as compared to unvaccinated group (gr V) (Table –15). There are a number of reports on lowering of percent lymphocyte count in IBD infected birds (Cheville., 1967 ; Kumar and Rao., 1991; Singh & Dhawedkar 1994a). The reduced percent lymphocyte count as observed in the present study may be attributed to nuclear

vacuolation and chromatolysis associated with cytoplasmolysis in blood lymphocyte (Bayoumi *et al.*, 1984) necrosis and depletion of thymic lymphocyte (Cheville ., 1967; Ley *et al.*, 1983) as well as increased level of plasma α 1-acid glycoprotein (AGP) (Inoue *et al.*, 1997). Further it was also observed that the percent lymphocyte count decreased with the increased dose level of aflatoxin (gr I – III) (Table-15). Misri (1994) and Mishra (1995) had reported progressive reduction in lymphocyte count in the aflatoxin treated birds. They had stated that reduction in lymphocyte count might be attributable to damage in lymphoid tissues leading to depletion of lymphoid cells.

Kumar and Rao (1991) reported heterophilia and monocytosis in IBD infected birds. In the present study, also similar results were obtained (Table 16 & 17) in the group which received IBD vaccine alone (gr IV) at all intervals post IBD vaccination. Further it was also observed that there was marked increased in heterophil count at the different dose levels of aflatoxin Mohiuddin *et al* (1986) observed considerable increase is heterophils in chickens fed with aflatoxin . It is postulated that during aflatoxicosis in chickens, occurrence of heterophilia may be due to the fact that aflatoxin acts as foreign particles in the body leading to different inflammatory changes (Mishra 1995). In present study, also monocytosis was observed in IBD vaccinated birds (gr I -IV) (Table –17). The percent monocyte count increased as the dose level of aflatoxin increased. These findings were also reported by Benjamin (1985). He reported that monocytosis might occur during stress stimulating conditions and haemarrhages into the tissue, Misri (1994) and Mishra (1995) also observed mild form of monocytosis in aflatoxin treated groups.

Mishra had reported a mild form of eosinophilia in aflatoxin fed chickens. In the present investigation also mild form of eosinpohilia was observed (Table - 18) . Montogomery *et al.*, (1986) and Singh & Dhawedker (1994a) did not find

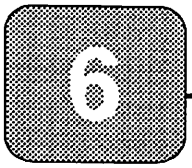
any significant difference in eosinophil count in IBD vaccinated chickens. So in the present study, increased number of eosinophil may be due to aflatoxin treatment.No significant difference was observed in blood basophil count in different groups of chickens in the present study. A number of workers could not find any significant change in basopholic count during IBD (Montogomery *et al.*, 1986 and Singh & Dhawedkar., 1994a;) and aflatoxicosis (Mishra 1995).

The bursa : body wt ratio provides one of the important criteria for determining the residual pathogenecity and immunosuppressive effect of IBD virus by a number of workers (Giambrone and clay., 1986a; Ezeokoli., 1990; Mazariegos *et al.*, 1990). In the present study, the B:B ratio were higher in groups which received IBD vaccines (gr I- IV) when compared with the value in group V which did not receive IBD vaccine. In the present study, reduction of bursa wt was dose dependent. (Table –20,21 and 22) A number of workers reported earlier that aflatoxin feeding had resulted in a significant regression in bursa of Fabricius weight and regression was directly proportional to the dietary aflatoxin level, (Smith & Hamilton.,1970 ; Pier *et al.*, 1971 ; Thaxton *et al.*, 1974; Mani *et al.*, 2000;). The reduction in the size of the bursa of Fabricius could be due to depletion of lymphocytes from these organs (Ghosh *and Chauhan,.* 1991)

In the present study, marked increase in weight of spleen in aflatoxin treated group (gr I – III) than non-aflatoxin treated group (gr IV & V). (Table 23,24, and 25) Similar increase in the weight of the spleen resulting from aflatoxin was reported by Reddy *et al* (1982) and Smith and Hamilton (1970) in the chicken. The increase in weight of the spleen could be due to hemolytic anaemia. The spleen was enlarged in an additive fashion by both IBD infection and aflatoxin. These findings support the earlier study carried out by Chang & Hamiltion(1982).

Body weight gain and feed conversion ratio are two important factors being considered to measure the degree of immunosuppression (Thornton and Pattison., 1975; Henry and Williams, 1980; Wyeth *et al.*, 1981). In the present study both body wt gain and FCR were poor in IBD vaccinated group without aflatoxin (group IV). Both the factors were still poorer in birds which received both aflatoxin and IBD (gr I-III,Table – 26). This clearly depicts the additive effects of aflatoxin and IBD virus. The present study warrants that all measures should be taken to ensure that the aflatoxin are not made available to the birds wherever the threat of IBD is there and also when the vaccination against IBD is likely to be planned.

CHAPTER



SUMMARY

Summary

A total of 125 newly hatched chicks were obtained from private poultry farm, Patna and divided into five equal groups of 25 chicks each. Birds in all the five groups were given F-strain RDV vaccine intraocularly at zero day of age, while birds from group I–IV received IBD vaccine (IV95 strain) intraocularly at 14 days of age. Further, the birds of group I – III were given 50 ppb, 100 ppb and 200 ppb of aflatoxin respectively. The birds of group IV and V were given non–aflatoxin supplemented feed. The group IV and V served as vaccinated and unvaccinated control. At 28 days of age all groups (gr. I–V) of birds were given RD (Lasota strain) vaccine. Post IBD vaccinated blood and serum samples were collected at weekly intervals from each group of chickens. Blood samples were evaluated for haematological and serum biochemical profiles. Serum sample were evaluated for determination of Ab titre to IBD vaccine by QAGPT titre and RD (F-strain) vaccine by HI test. Five birds from each group were sacrificed on 3rd, 7th and 11th day Post IBD vaccination for histopathological examination of bursa of Fabricius, kidney, liver and spleen and for bursa : body weight ratio and spleen : body weight ratio were determined on 3rd, 7th and 11th day Post IBD vaccination. Body wt. gain and FCR were determined at the end of experiment (i.e. on 56 days of age).

All the three doses of aflatoxin employed in this study produced lower QAGPT titre ranged between 2.40 ± 0.374 to 1.00 ± 0.00 than the titre (3.20 ± 0.374) recorded in the IBD vaccinated but without aflatoxin fed group of birds (gr. V). However, the lowest level of precipitating Ab was recorded in group III followed by group II and I. The result clearly depicted that all the three doses of

aflatoxin employed in this study had lowering effect on immune response to IBD vaccine.

The IBD vaccine used in this study showed immunosuppressive effect on immune response to RD vaccine (F-strain) as evident from lowered HI Ab titre recorded in IBD vaccinated group of birds (gr. IV) when compared with the titre shown by none IBD vaccinated control birds (gr. V). The result clearly depicted that the aflatoxin in combination with IBD vaccine (IV95 strain) produces immunosuppression of higher degree as evidenced by the decline in HI titre in group I-III. It is also to be noted that the administration of 2^{nd} dose of RD vaccine (Lasota strain) has been able to keep the titre increasing and the titre remained higher than the protective level of 2^3 in all the groups except the group which received 200 ppb of aflatoxin (group III).

Further characterization of IV95 strain vaccine virus revealed the possession of residual pathogenecity as characterized by production of typical lesion in the bursa of Fabricius which included lymphoid depletion and necrosis, interfollicular oedema, epithelial invagination, interstitial fibrosis, cellular infiltration and vacuolar degeneration. The application of different doses of aflatoxin (50, 100 and 200 ppb) increased the lesion scores as evident from the values of the lesion scores in IBD vaccinated alone group (gr. IV) (table 4, 5 & 6).

The study on serum total protein of IBD vaccinated birds in general demonstrated decrease in total serum protein and serum albumin but increase in globulin value in comparison to the corresponding value recorded in non-IBD vaccinated control bird (gr. V). Again aflatoxin treatment had lowering effect on serum total protein, albumin and globulin (gr. I-III). The marked decrease in serum total protein observed as in group III.

The serum calcium and phosphorus value of IBD vaccinated (gr. I-IV) birds were lower as compared to the unvaccinated control group (gr. V). Further, the values were decreased as the doses of aflatoxin increased. The reduction was most marked at the dose level of 200 ppb of aflatoxin.

The haematological profile showed that the haemoglobin and Packed Cell Volume (PCV) value of aflatoxin fed group (gr. I-III) differed significantly with vaccinated control (gr. IV). The Hb and PCV value were lowest in group III followed by II and I. Further, the study also revealed that the TLC value in the unvaccinated control group was highest when compared with IBD vaccinated birds (gr. I-IV). The TLC value decreased as the doses of aflatoxin increased.

The differential leucocytic count showed that the lymphocyte percent value in the vaccinated control group and unvaccinated control were higher than the corresponding value in first three groups (gr. I-III) till termination of experiment. The percent lymphocyte counts in birds receiving different level of aflatoxin (gr. I-III) were significantly lowered than gr. IV and V. It is clearly depicted that the value decreases as the aflatoxin level increased. Marked heterophilia were observed in all IBD vaccinated birds when compared with the unvaccinated control group. The heterophil percentage was highest in group III which received 200 ppb of aflatoxin followed by group II and I which received 100 ppb and 50 ppb of aflatoxin respectively. Monocytosis were observed in aflatoxin fed groups (gr. I-III) when compared with the non- aflatoxin fed group (gr. IV & V). The eosinophil percentage were found to be higher in aflatoxin fed groups (gr. I-III) as compared to vaccinated and unvaccinated control group (gr. IV and V). No significant differences was observed in basophil count at the termination of experiment in any groups.

The B : BW ratio was lowest in group III followed by gr. II, I, V and IV. The vaccinated alone group (gr. IV) demonstrated lower Spleen : Body wt. ratio

than the value of the ratio in group of birds which neither received IBD vaccine nor aflatoxin. However, the administration of aflatoxin increases the Spleen : Body wt. ratio (gr. I – III).

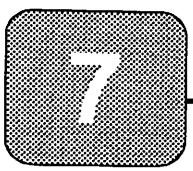
Further study revealed the lowering effect of vaccine strain of IBD virus on body weight gain and poor FCR values. The administration of aflatoxin had further lowering effect on body wt. gain. The FCR value were poorer in group III followed by group II and I.

Finally the present study suggested the presence of residual pathogenecity and immunosuppressive effect of the vaccine strain of IBD virus (IV95) as employed in this study. Further, the different doses of aflatoxin included in this study proved to have lowering effect on immune response to IBD vaccine as well as on response to RD vaccine in birds immunocompromised by moderate hot strain vaccine (IV95) which is widely used these days to control vvIBD scenario in this state. Comparative evaluation of the three level of aflatoxin in respect of immunosuppressive effect on response to IBD vaccine as well as RD vaccine in IBD vaccinated chickens revealed that the immunosuppressive effect is most marked at the dose level of 200 ppb of aflatoxin. Therefore, on overall consideration it may be concluded that together both aflatoxin and infectious bursal disease virus have potentiating effect on severity of the disease. The present observation points to the fact that poultry farmers should keep their finger crossed and remain alert to keep the poultry stocks free from aflatoxin as far as possible, more so, in an areas where the IBD is endemic. Aflatoxin contamination of poultry feed stock should be avoided or kept at minimum level in such area where vvIBD is widely prevalent. In such a situation where continuous and regular monitoring of aflatoxin at the farm premises is not feasible, the farmer should be advised to use some

immunopotentiating agents/immuno modulators alongwith moderate hot strain of IBD vaccine in consultation with the competent authority/agency.

Alternatively study may be planned to evaluate the efficacy of the commonly used immunomodulaters in controlling the combined immunosuppressive effect of moderate hot strain IBD vaccine like one used in this study as well as aflatoxin in varying dose levels.

CHAPTER



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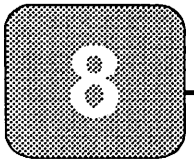
CHAPTER



APPENDIX

Appendix

APPENDIX - I

I. Analysis of variance showing effect of aflatoxin and days on the Ab titre against IBD in chickens.

Sources of variation	D.F	M.S.	F
Between treatment group	3	2.383	34.38**
Between days (pre - post IBD vaccination)	5	0.706	10.18**
Error	15	0.0693	
Total	23		

** Indicate significance at P<0.01.

II. Analysis of variance showing effect of aflatoxin and days (pre & dpv) on Ab titre against RDV(F- strain) in chickens.

Sources of variation	D.F	M.S.	F
Between treatment group	4	5.25	24.52**
Between days (pre-post IBD vaccination)	6	7.01	32.74**
Error	24	0.2141	
Total	34		

** Indicate significance at P<0.01.

APPENDIX – II

III. Analysis of variance showing effect of different level of aflatoxin on Total bursal lesion score in chicken sacrificed on 3rd, 7th and 11th day post IBD vaccination.

Sources of variation	On 3rd dpv			On7th dpv		On 11th dpv	
	DF	M.S.	F	M.S.	F	M.S.	F
Between treatment	4	6.826	10.86**	7.329	9.66**	5.804	8.08**
Within treatment	295	0.628		0.788		0.718	
Total	299						

** Indicate significance at P< 0.01.

APPENDIX - III

IV. Analysis of variance showing effect of aflatoxin treated groups and days (pre and dpv) on the serum profile in chickens.

Source of variation	D.F	Total serum protein		Serum albumin		Serum globulin	
		M.S.	F	M.S.	F	M.S.	F
Between treatment groups	4	0.235	5.04**	0.057	1.44^{NS}	0.107	9.72**
Between days (pre & dpv)	6	0.478	10.25**	0.161	4.075**	0.126	11.45**
Error	24	0.0466		0.0395		0.011	
Total	34						

^{NS} indicate non - significance $P < 0.05$
** indicate significance at $P< 0.01$.

APPENDIX - IV

V. Analysis of variance showing effect of aflatoxin treatment groups and days (pre & dpv) on serum calcium and serum inorganic phosphors level in chickens.

Sources of variation		Serum calcium		Serum inorganic phosphors	
	DF	M.S.	F	M.S.	F
Between treatment groups	4	3.97	20.55**	0.287	2.81^{NS}
Between days (pre & dpv)	6	0.628	10.33**	2.10	20.58**
Error	24	0.189		0.102	
Total	34				

^{NS} indicate nm significance.
** indicates significance at P<0.01

APPENDIX – V

VI. Analysis of variance showing effect of aflatoxin treatment groups and days (pre & dpv) on Hb, pcv in chickens.

Sources of variation	DF	Haemoglobin		Packed cell volume	
		M.S.	F	M.S.	F
Between treatment groups	4	0.585	2.03^{NS}	9.99	10.43**
Between days (pre & dpv)	6	4.73	16.42**	8.74	9.13**
Error	24	0.288		0.957	
Total	34				

^{NS} indicates non significance.
** indicates significance at P < 0.01.

VII. Analysis of variance showing effect of aflatoxin treatment groups and days (pre & dpv) on TLC in broiler chickens.

Sources of variation	D.F	M.S	F
Between treatment days groups	4	4.89	8.92**
Between days (pre & dpv)	6	1.96	3.57*
Error	24	0.548	
Total	34		

* indicates significance at P < 0.05.
** indicates significance at P< 0.01.

APPENDIX – VI

VIII. Analysis of variance showing effect of aflatoxin treatment groups and days (Pre & dpv) on DLC in chickens.

Sources of variation	D.F	Lymphocyte		Heterophil		Monocyte precent		Basophil percent		Eosinophil percent	
		M.S	F	M.S	F	M.S	F	M.S	F	M.S	F
Between treatment groups	4	17.77	5.05**	9.59	7.32**	0.682	9.09**	15.83	32.97**	1.91	9.25**
Between days (pre & dpv)	6	34.85	9.92**	20.00	15.26**	2.67	35.6**	6.17	12.61**	0.511	2.68*
Error	24	3.512		1.31		0.075		0.489		0.190	
Total	34										

* indicates significance at (P<0.05).
** indicates significance at (P<0.01).

IX. Analysis of variance showing effect of aflatoxin treatment groups on Bursa : Body wt. ratio on 3rd, 7th and 11th days post IBD vaccination.

Sources of variation	On 3rd dpv		On7th dpv		On 11th dpv		
	DF	M.S.	F	M.S.	F	M.S.	F
Between treatment groups	4	0.02	3.33*	0.01	1.00NS	0.052	8.00**
Within treatment	20	0.006		0.01		0.006	
Total	24						

NS indicates non – significance.
* indicates significance at (P < 0.05).
** indicates significance at (P < 0.01).

APPENDIX – VII

X. Analysis of variance showing effect of aflatoxin treatment groups on spleen : Body wt. ratio on 3rd , 7th and 11th day post IBD vaccination .

Sources of variation	D.F	Spleen : Body wt. ratio					
		3rd dpv		7th dpv		11th dpv	
		M.S.	F	M.S.	F	M.S.	F
Between treatment groups	4	0.141	35.2**	0.65	81.0**	0.1575	39.37**
Within treatment	20	0.004		0.05		0.004	
Total	24						

** indicates significance at (P < 0.01).

XI. Analysis of variance showing effect of aflatoxin treatment groups on initial body wt., Final body wt., weight gain and feed conversion ratio.

Sources of variation	D.F	Initial weight		D.F	Body weight on 56 day		D.F	Body wt. gain on 56 days		D.F	Feed conversion ratio	
		M.S	F		M.S	F		M.S	F		M.S	F
Between treatment groups	4	40.185	2.01NS	4	12746.22	29.10**	4	11096.7	28.05**	4	0.0475	2.04NS
Within treatment	45	19.96		37	437.936		37	395.6		37	0.0232	
Total	49			41			41			41		

NS indicates non significance.
** indicates significance at (P < 0.01).