Effect of Selected Agents on Immunomodulation in Chicken Immunocompromised by A Vaccine Strain of Infectious Bursal Disease Virus

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF POST-GRADUATE STUDIES)

PUSA, (SAMASTIPUR) BIHAR

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

VETERINARY MICROBIOLOGY

By

Mithilesh Kumar

Registration No. M/Vety. Micro./97/1996-97

Department of Veterinary Microbiology

BIHAR VETERINARY COLLEGE

PATNA, BIHAR (INDIA)

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2000

Dedicated

to

Almighty God

Dr. K.C.P. Singh

M. V. Sc., Ph. D.

Associate prof.-cum-Sr. Scientist.

Department of Veterinary Microbiology

Bihar Veterinary College, Patna – 800 014.

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CERTIFICATE – I

This is to certify that the thesis entitled **"EFFECT OF SELECTED AGENTS ON IMMUNOMODULATION IN CHICKEN IMMUNOCOMPROMISED BY A VACCINE STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS"** submitted in partial fulfillment of the requirements for the award of "Master of Veterinary Science (Veterinary Microbiology) in the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar is the record of bonafide research work carried out by **Dr. Mithilesh Kumar** (Admission No. VP/BVC/VMC/01/1996-97) son of **Shri Sidheshwar Prasad Sinha,** under my supervision and guidance. No part of the thesis has so far been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the course of this investigation and the sources of literature have been fully acknowledged.

(K.C.P. Singh)

CERTIFICATE – II

We, the undersigned, members of the Advisory Committee of **Dr. Mithilesh Kumar** 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 **"EFFECT OF SELECTED AGENTS ON IMMUNOMODULATION IN CHICKEN IMMUNOCOMPROMISED BY A VACCINE STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS"** may be submitted by **Dr. Mithilesh Kumar** in partial fulfilment of the requirements for the degree.

(K.C.P. Singh)

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Bihar Vety. College, Patna-14.
(Nominee, Dean, Post-Graduate Studies)

Dean, Post-Graduate Studies

CERTIFICATE – III

This is to certify that the thesis entitled **"EFFECT OF SELECTED AGENTS ON IMMUNOMODULATION IN CHICKEN IMMUNOCOMPROMISED BY A VACCINE STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS"** submitted by **Dr. Mithilesh Kumar** in partial fulfilment of the requirements for the degree of Master of Veterinary Science (Veterinary Microbiology) of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Pusa, Bihar was examined and approved on 16-01-2002. ~~2001~~.

16/01/2002

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Place: Patna
Date: 5/9/2000

Mithilesh kumar
Mithilesh Kumar

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Fig. No.

26. Microphotograph of bursa of fabricius of chicken sacrificed 96 hrs. post IBD vaccination showing necrosis and presence of eosinophilic mass in the follicle, in one of the follicle there is complete loss of lymphoid cells and only cystic space is visible (H & E X 100).
27. Microphotograph of bursa of fabricius of chicken sacrificed 96 hrs. post IBD vaccination showing marked depletion and vacuolar degeneration in the follicle (H & E X 200).
28. Microphotograph of bursa of fabricius of chicken sacrificed 96 hrs. post IBD vaccination showing marked degeneration of lymphoid cells in the follicle (H & E X 400).
29. Microphotograph of bursa of fabricius of chicken sacrificed 96 hrs. post IBD vaccination showing necrotic eosinophilic mass in the follicle (H & E X 200).
30. Microphotograph of bursa of fabricius of chicken sacrificed 96 hrs. post IBD vaccination showing marked necrosis and loss of normal structure of the follicle (H & E X 100) .
31. Microphotograph of bursa of fabricius of chicken sacrificed at termination of experiment showing repopulation of lymphoid cells in the follicle (H & E X 50).
32. Microphotograph of bursa of fabricius of chicken sacrificed at termination of experiment showing mild depletion of lymphoid cells (H & E X 50).

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LIST OF ABBREVIATIONS

Ab'	-	Antibody
AGPT	-	Agar gel precipitation test
ANOVA	-	Analysis of variance
BF	-	Bursa of fabricius
Carbo Veg	-	Carbo vegetabilis
^0C	-	Degree Celsius
Co.	-	Company
Dr.	-	Doctor
dpv	-	Days past IBD vaccination
d.w.	-	Drinking Water
ELISA	-	Enzyme Linked Immune Sorbent Assay
edn	-	Edition
fig	-	Figure
FCR	-	Feed Consumption Ratio
g	-	Gram
gr	-	Group
H & E	-	Haemotoxyline and Eosin
HA	-	Haemagglutination
HI	-	Haemagglutination Inhibition
Homeo dr.	-	Homeopathic drug
hr.	-	Hour
i.o.	-	Intraocular
IU	-	International Unit
IBDV	-	Infectious Bursal Disease Virus
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M

Ltd.	-	Limited
lb	-	Pound
MDA	-	Maternally Derived Antibody
Mab	-	Maternal Antibody
Micro	-	Microbiology
M.S.	-	Mean Sum of Squares
mg.	-	Miligram
ml.	-	Mililiter
No.	-	Number
NDV	-	Newcastle Disease virus
PBS	-	Phosphate Buffer saline
PI	-	Post Infection / Post Inoculation
Pvt.	-	Private
QAGPT	-	Quantitative Agar Gel Precipitation Test
RBC	-	Red Blood Cells
RDV	-	Ranikhet Disease virus
rpm	-	Revolution per minute
S.E.	-	Standard Error
Uv.	-	Unvaccinated
V.	-	vaccinated
vvIBD	-	very virulent Infections Bursal Disease
wt.	-	weight
w/v	-	weight by volume
%	-	percent

****

CHAPTER - I

INTRODUCTION

ineffective in controlling vvIBD, mostly because they can not work in the presence of high level of maternal antibody. On the other hand vvIBD virus can easily penetrate the maternal antibody of the young chicks between 2 to 4 weeks of age. It is in this backdrop that a new vaccine was introduced and is available in the market in the name of "hot strain", intermediate plus or IV95 vaccines. Whereas the vaccine strain has ability to penetrate the maternal antibody barriers of young chicks and incite immune response, it is relatively more invasive and has residual pathogenicity. The preliminary work undertaken in this department has sufficiently revealed that this vaccine strain possesses invasiveness and residual pathogenicity and also that it can induce immunosuppression. Therefore, it becomes necessary that some measures may be tried in order that the poultry birds receiving such vaccines remain unaffected from the effect of vaccine.

The role of levamisole in controlling immunosuppression due to IBD virus is being mentioned. The role of *Mycobacterium phlei* as well some indigenous products like immolyte (Vesper) zeetress (Indian Herbs) and stresroak (Dabur Ayurvet) in immunopotentiation of IBD infected birds have been reported. In addition several agents with proven antistress and adoptogenic effects have also shown encouraging effects in promotion of growth, improving feed conversion ratio, enhanced resistance to infectious diseases and lowered mortality rate. Further, in recent past probiotics are being increasingly used in poultry and they

INTRODUCTION

Infectious bursal disease, popularly known as Gumboro disease among poultry farmers, is an acute and highly contagious viral infection of young chicken which is characterised by destruction of B-cell in the bursa of fabricius. Clinically, the disease is marked by watery diarrhoea, ruffled feathers, in-coordination in gait, severe depression, hemorrhages in thigh and pectoral muscles with enlargements of bursa in early stage of infection.

The disease was first reported by Cosgrove in 1962 at Gumboro district, a place in Delaware in United States of America. In India, the disease was first recognized and reported by Mohanty *et al.* in 1971. The clinical pattern of IBD as it occurred before 1992 was characterised by low mortality and huge economic losses by way of immunosuppression which is marked by increased susceptibility to micro organisms pathogenic or otherwise, reduced body weight gain, poor feed conversion ratio as well as impaired immune response to vaccines. Since 1992 a new form of IBD, popularly known as very virulent IBD (vvIBD) are being reported from different parts of the country including the state of Bihar. This new form of IBD, besides other things, is largely characterised by very high mortality reaching as high as 80 % and above as well as relatively shorter course of disease. The conventional IBD vaccines such as mild strain (Lukert type), intermediate strain (Georgia strain) and inactivated IBD vaccines have proved rather

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cosgrove (1962) first recognised the outbreak of infectious bursal disease at Gumboro district, a place near south Delaware in United States of America. The clinical picture of disease was ruffled feathers, watery diarrhoea, severe depression, oedema and enlargement of bursa of fabricious. The disease was also named 'Gumboro disease" after the place of its first report.

In India, the disease was first reported from Uttar Pradesh by Mohanty *et al.* (1971) on the basis of histopathological studies. Since then severeal workers have reported the incidence of this disease in different part of country (Ajinkya *et al.*, 1980; Chauhan *et al.*, 1980; Singh *et al.*, 1994; 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 tissues in the bursa.

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.

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 bursal homogenate was treated with Areton 113. The quantitative agar gel precipitation test (QAGPT) was used to measure maternal antibody level in chicks from IBDV infected parents Wyeth and

Cullen, (1976) as well as Wood *et al;* (1979) standardized the QAGPT for determination IBDV antibodies level in chickens. They reported that the antigen concentration was of no significance within certain limits, but for clearity, high antigen concentration was recommended.

Winterfield and Thacker (1978) compared the usefulness of AGPT and VN test to study the immune responses of different strains of IBD applied as vaccines. They observed that even precipitin negative chickens were often protected, whereas AGPT positive chickens were always protected.

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

Edward *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 day old. Examination of bursal sections from chicks 7 day 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 8 of out of 10 bursa examined.

McFerran *et al.* (1982) conducted field studies with an inactivated vaccine against infectious bursal disease. Vaccination using an inactivated infectious bursal disease vaccine stimulated long lasting neutralizing antibodies. Highest titres were produced in the birds, which had previously been infected with a 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.

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 follicles. The lymphocytes were soon affected by three to four day PI. As the inflammatory reaction decreased, cystic cavity and fibroplasia of the inter follicular connective tissue developed.

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 SPF chickens were vaccinated with these vaccine. Two weeks after IBD vaccination they were vaccinated with Newcastle disease virus. The pathogenic and immunosuppressive effects of the IBD vaccines were evaluated by the antibody response to NDV vaccination, the bursal body weight index and histopathological lesions of the bursa. In chicks vaccinated at day old, bursa: body weight index varied from 0.23 to 1.0 whereas in case of chicks vaccinated at 3 weeks of age ratios ranged from 0.24 to 1.0. The HI antibody titre to NDV ranged from 3.7 to 61.7 and 13.0 to 59.2 respectively in chicks vaccinated at day old and those vaccinated at 3 weeks of age.

Khafagy *et al.* (1991) reported the isolation, identification and characteristics of 9 field isolates of very virulent infectious bursal disease virus prevalent on 9 chicken farms. The disease was characterized by sudden appearance with high morbidity usually nearly 100 percent and with mortality of up to 70 percent. The isolates were all identified as normal Gumboro strain (Faragher) with no variants.

Nakamura *et al.* (1992) compared the immunosuppressive effect of highly virulent infectious bursal disease virus on vaccination against Newcastle 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 diseases virus with high mortality in Japan. They isolated virulent infectious bursal disease virus 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 form 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 isolates from such outbreaks were investigated in SPF chickens. Infected birds developed diarrhoea within 24 hour of infection and showed depression, trembling, ruffled feathers and were prostate. The 5 isolates caused 30-70 percent mortality with Yamaguchi strains. Those that survived the disease, lost weight and 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 Mab ranged between 2^0 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 CS_{88} strains of IBD in 0.1 ml of inoculum and sacrificed 56 hours later and their bursae 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.

Singh *et al.* (1993) observed that levamisole treatment of IBDV infected chicks was able to restore their immune responses to sheep red blood cells to a level comparable to that of uninfected control. Immunomodulatory effect of levamisole was observed only in birds, which has undergone immunosuppression due to prior IBDV infection. This drug did not increase the immune response above the normal level in immunologically competent hosts. Thus the treatment of birds with levamisole may prevent the losses arising from immunosuppression as a result of sub clinical IBD.

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

El-Zanty-K (1994) studied the effect of virulent IBDV on 1week old broiler chicks fed diet containing different concentration of ascorbic acid (AA) (group 1, 110 mg AA/kg; group 2, 220 mg AA/kg; group 3, 330 mg AA/kg and group 4, 660 mg AA/kg diet respectively). At 4 weeks of age broiler chicks in different groups were infected intraocularly with 105.7 EID_{50}/0.1 ml virulent IBD virus. The severity of the clinical signs, bursal lesions and other pathological lesions were reduced in ascorbic acid fed

chicks. When bursal homogenate was tested by AGPT, titres were high in chicks receiving on AA but low in chicks from group 2 to 4. It was concluded that AA increases the resistance in broiler chicks to virulent IBD infection.

Kouwenhoven *et al.* (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 Netherland 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 (sterwin Laboratories, U.S.A), LZ228E (Mycoform Nederland B.V the Netherlands) and Bursa plus (Solvay Duphar, B.V. the Netherlands) 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 'hot' vaccines. They also observed that 'hot' vaccines were slightly more pathogenic than the Intermediate vaccines.

Pande *et al.* (1994) studied the immunomodulatory effect of zeetress in chicken vaccinated against ND (F strain) virus. Zeetress was administered at the rate of 5g/100 chicks through the drinking water for first 10 consecutive days and thereafter at the rate of 10g/100 birds form 24 to 35 days. On 35 day serum samples were collected for HI test. It was found that antibody titres, body weight gain and feed efficiency were significantly higher than untreated vaccinated control.

Singh *et al.* (1994) 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 from. The acute IBD was marked by high morbidity and high mortality ranging between 35-65 percent. Three virus isolates were recovered from the affected tissue. Majority of acute IBD outbreaks followed revaccination with RD vaccine.

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

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

chickens receiving zeetress.

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 strain 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 Bos (1996) conducted vaccination trail 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, feed conversion ratio and condemnation percentage.

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

Mahesh and Muniyappa (1996) studied the immunogenicity, pathogenicity and immunosuppressive potential of one less attenuated, three,

intermediate, one mild and combination of mild and inactivated IBD vaccines strain, and also monitored the maternally derived antibody response in both experimental and field conditions. The chicks were vaccinated against IBD according to manufactures 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 days 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.

Rao *et al.* (1996) concluded from the study that zeetress had a sparing effect on the damage to lymphoid follicles of bursa in IBD vaccinated group but of no consequence in the challenged/virulent IBD virus infected birds. It was concluded that zeetress had stimulated the rosette forming T-lymphocytes and enhanced the macrophage activity in the spleen. However it had no beneficial effects in virulent IBD virus infection.

Yamaguchi *et al.* (1996) studied the potency of a new vaccine in controlling highly virulent infections bursal disease virus (HV-IBDV) infection. They adapted some isolates of HV-IBDV through serial passage in embryonated eggs. The embryonated egg adapted HV-IBDV was adapted to grow in chicken embryo fibroblast (CEF). 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 test, the adapted strain immunized chickens showed good protection against the fatal infection of HV-IBDV. At 3 day after immunization the adapted strains showed effective immunogenicity against challenge infection.

Al- Mufarrej *et al.* (1997) studied the immunostimulating effects of Royal jelly (secretion of nurse bee) in chickens when immunized with sheep red blood cells and re immunized 10 days later of primary immunisation. Royal jelly treated and immunized group showed increased antibody production as compared with untreated immunized chickens. It is concluded that Royal jelly is an effective immunostimulant and that antibody production is more pronounced following subcutaneous administration compared with oral administration and during secondary immunization compared with primary immunization.

Bekhit (1997) reported highly virulent form of infectious bursal disease from Egypt in outbreak of IBD during 1988-1993. He observed severe outbreaks of IBD with usually high mortalities (56%). He suggested that the persent outbreaks of IBD were attributed to very virulent IBDV belonging to standard serotype I. 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 then antigenic variant such as proper timing of vaccination, handling and administration of vaccines, type of vaccine as well as vaccine doses.

Christopher *et al.* (1997) observed the influence of vvIBD on

immunity to 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 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 and the half life 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 vv IBDV could be the cause of this perceived difference in the RD-HI titre vbalues.

Panda *et al.* (1997) investigated the significance of Ashwagandha (*Withania somnifera*) root extract in the thyroid function of cockerel and they found that its root extract (20mg/day/bird for 30 days) increased serum thyroxins (T4) concentration significantly. The drug also increased the serum protein significantly. Interestingly liver and muscle protein concentration decreased following the drug administration. No significant change in body weight was observed between the treated and control groups.

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 percent protection, while IBDV strain 494 and strain 194 gave 88 percent and 76 percent protection respectively after challenge with 10^2 CID_{50} IBD virus. They opined that IBDV S394 may serve as prophylactic agent against IBD in poultry without any immunosuppressive effect and mortality in day old chicks.

Zorman-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 Slovenia. 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 days 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 days of age with mortality of 2.5 percent. It was concluded that neither vaccine can fully protect broiler against very virulent IBD virus strains.

Kumar *et al.* (1998) studied the influence of immunostimulation; with *Mycobacterium phlei* (ISMP) and bone marrow culture supernatant (BMCS) on decreased cytotoxic activity of day old white Leghorn chicks, and found the when the chickens were primed with ISMP a week before IBD infection the cytotoxic activity was approximately restored. However BMCS did not restore cytotoxic activity. It was concluded that immunositimulation may potentiate and restore the functional activity of in chicken infected with IBD virus.

Khaliel *et al.* (1998) observed the pathologic, immunocytochemical and immunologic studies on a new infections bursal disease vaccine "Intermediate Plus" in chickens. One-day old chicks vaccinated against Newcastle disease virus and challenged with a local virulent strain of IBD virus were examined for pathological and immunological effects of 2 types of IBD live vaccine (Intermediate and Intermediate plus). Grossly, moderate transient bursal atrophy was seen one week after immunization with the intermediate plus vaccine Microscopically, the severity of bursal lymphoid cell necrosis and the intensity of immonoperoxidase staining reaction

correlated with the degree of bursal atrophy. Ultrastraucturally, the necrotic lymphocytes appeared shrunken with nuclear fragmentation and chromatin condensation or margination. Immunologically, 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 against NDV vaccine. Protection against IBDV challenge was highest following immunization with Intermediate plus vaccine, particularly when given after the Intermediate vaccine. It was concluded that, despite the state of immunosuppression and the encountered bursal lesions following immunization with the Intermediate plus IBDV vaccine, it provided better protection against IBDV challenge. Both immunosuppressive and immunological effects of the vaccine were transient and within safe limit.

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

Szigeti (1998) evaluated a new type of immunostimulant to increase antibody production in response to viral and bacterial vaccines. An experimental product (IM-326) containing feed acidifiers, garlic and microbial cell extracts, was added to the drinking water of poultry at 1 ml /

litre 2-3 days before vaccination and for 17-20 days there after. It resulted in a 38-226 percent increased in GMT after parentral administration of inactivated vaccines against goose parvovirus, Newcastle disease and avian infectious bursitis and vaccines containing live egg drop syndrome aviadenovirus and killed *Salmonella enteritidis, Pasteurella multocida and Leptospira pomona.*

Barbar *et al.* (1998) studied the humoral and cell mediated immunopotentiation in vaccinated chicken layers by thymic hormones and zinc. The birds were vaccinated with trivalent killed vaccine (IBV, IBDV, NDV) and immunopotentiated by various combinations of thymic hormones and zinc group wise first group received thymopoietin and $ZnCl_2$, second group received thymopoietin and $ZnCl_2$, in the third group each bird received thymulin, thymopoietin and ZnCl2, while each bird of the fourth group received only $ZnCl_2$, Among all combination, the thymulin – $ZnCl_2$, resulted in birds with the highest humoral immunopotentiation to IBV, IBDV, and NDV antigens. The highest cell mediated delayed hypersensitivity reaction was obtained in chickens immunopotentiated by the thymulm thymopoietin $ZnCl_2$ combination.

Abdel-Fattah *et al.* (1999) studied the effects of crude thymus extracts on the immune response and protection against challenge with virulent IBDV in one-day old chicks. Oral administration of thymus extract (1ml/kg) markedly and significantly increased the total protein, albumin, globulin, tri-iodothyronine (T3), Thyroxine (T4) and the body weight gain in chickens. In addition, it increased the total lymphocytic count over four weeks after administration, Although vaccination also increased total protein, globulin, T4 and the total lymphocytic count but it significantly decreased the body

weight gain of the chicks and administration of thymus extracts, before, during or after vaccination markedly improved the vaccination effectiveness with significant elevation of the globulin level and body weight gain of the chicks. It also prevented the decrease in the relative weights of bursa, spleen and thyroid glands which commonly prevailed during vaccination. Chickens administered thymus extract and vaccinated with IBD vaccine showed 100 % protection against challenge with IBDV Meanwhile the vaccinated non thymus treated group exhibited 80 percent protection against IBDV challenge. These results indicate a potentiating effect of thymus extract on the immune system in baby chick. These findings are supported by ELISA results that showed a marked increase in antibody titres in thymus treated groups. Additionally microscopical examination of the bursa showed lymphoid hyperplasia in thymus treated group but not in vaccinated group supported these findings.

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 specific pathogen-free chickens were intraocularly inoculated with an intermediate vaccine strain (IBDV-vac) or 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. Intially, the virus caused extensive necrosis of bursal B lymphocytes. This lesion was 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

IBDB-vac than in the chickens exposed to IM-IBDV. By 7 week PI, 40 percent and 80 percent of bursal follicles in IM-IBDV and IBDV-vac inoculated chickens respectively were repopulated with IgM plus B lymphocyes. Both IBDV-vac and IM – IBDV caused suppression of the primary antibody response to antigens. However the antibody responses of the chickens exposed to either of the two IBDV strains used were compromised only during the first 6 weeks of virus exposure. Subsequently antibody response returned to normal level.

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

Saravanabava *et al.* (1999) planned an experiment to asses the effect of Tuftsin (a tetrapeptide) on immune response of birds immunosuppressed to IBD virus. The result indicated that the seroconversion to NDV vaccine as assessed by HI and ELISA were found to be higher in the birds vaccinated along with Tuftsin as compared to the birds vaccinated without Tuftsin in the

immunosuppressed and immunocompetant birds. The percentage of leucocyte migration inhibition was also found to be more in the tuftsin administered birds as compared to the birds without Tuftsion. Percentage of survivability was found to be more in birds vaccinated along with Tuftsin as compared to the birds vaccinated without tuftsin. All the unvaccinated birds succumbed to Newcastle disease. Administration of Tuftsin alone (without vaccine) did not produce any significant reversing effect in birds. It was concluded that Tuftsin produced significant reversing effect of immunosuppression caused by IBDV infection and significant immune enhancement in immunocompetant birds irrespective of the schedule of vaccination and type of vaccine virus used.

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CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS

Chicks:

3 weeks old apparently healthy broiler chicks and free from antibodies to IBD, were used for propagation of virus as well as production of antigen. The chicks were obtained from different commercial broiler farms located at Patna.

Antigen:

Poona strain of IBD virus being maintained in this department in the form of 50 percent bursal suspension was used as a reference antigen.

Antiserum:

The hyper immune serum against a vaccine strain of IBD virus (Georgia stain; Indovax Private Limited, Siswala, Hariyana) raised in this laboratory was used throughout this experiment as known positive antiserum. The serum was inactivated at 56^0C for 30 minutes and stored in the freezing chamber of the refrigerator.

Vaccines:

F strain RDV vaccine:

A commercially available F Strain of Ranikhet disease virus vaccine, manufactured by Indovax Private Limited, Siswala, Hissar Haryana, was used for vaccination of chicks after reconstitution.

LaSota strain RDV vaccine:

A commercially available LaSota strain of Ranikhet disease virus vaccine, manufactured by Indovax Private Limited, Siswala, Hissar, Haryana was used for vaccination of chicks after reconstitution.

IV 95 IBD vaccine:

A commercially available Invasive strain of Infectious bursal disease virus vaccine, manufactured by Indovax Private Limited, Siswala, Hissar, Haryana was used for vaccination after reconstitution.

Vitabland WM forte liquid:

The commercially available vitamin A (Agrivet farm care, Glaxo India Limited, Mumbai) was used.

Celin:

The commercially available vitamin C (Glaxo India Limited, Mumbai) was used.

Livol:

The commercially available herbal water soluble liquid (Indian Herbs research and supply Co. Pvt. Ltd. Saharanpur, U. P.) was used.

Lemasole– P:

The commercially available Levamisole hydrochloride (Ranbaxy Laboratories Limited) was used.

Carbo Vegetabilis:

Homeopathic drug manufactured by Hahnimann Laboratories, Culcutta was used.

Sporlac:

The commercially available probiotic (Unisankyo Limited, Banglore) was used.

METHODS

Production of hyperimmune serum:

Hyperimmune serum against IBDV was raised in 20 weeks old 6 apparently healthy chickens. Each bird was given Georgia strain of IBDV through occulo-nasal route at weekly intervals. Two weeks after the fourth

inoculation, the birds were test bled and the serum was tested for the presence of IBDV antibody by AGPT. This serum was stored at – 10^0C for further use.

Serum :-

2 to 3 ml blood was taken from the wing vein of birds with the help of 5 ml sterilized disposable syringe using 24-26 gauze needle (fig 1). It was kept in sterile test tube in slant position for 4 hours at room temperature for serum separation. The separated sera was collected in a sterilized vial of 2.0 ml capacity and were preserved by adding sodium azide (1:10000). The serum samples were stored at – 10^0C untill processed.

Red blood cell suspension:

Six adult chickens, age group 20 – 24 weeks, were used as donor of blood. One to 1.5 ml of blood was collected from each bird in Alsever's solution *(1:1)*. Supernatent fluid was removed after centrifugation at 1000 rpm for 10 minutes. The packed cells were washed three times with phosphate buffer saline. Finally 0.8 per cent RBC suspension was made in PBS and stored at refrigerator temperature. The RBC suspension was used only for 4 days after preparation and thereafter the fresh RBC suspension was prepared.

Preparation of antigen:

Poona strain of IBDV in the form of 10 percent bursal suspension in PBS, was inoculated into three weeks old broiler chicks at the rate of 0.2 ml of suspension per chick by intraocular route. The chicks were sacrificed 48 hours post inoculation (fig 2 & 3) and the bursa were collected aseptically and homogenized in a sterile mortar using glass wool as an abrasive.

The homogenate was diluted (1:1) in PBS (W/V), PH 7.4 (treated

with 10,000 units of Penicillin and 10mg streptomycin per ml of suspension). The suspension was 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 -10^0C and used as antigen. The normal bursal suspension (uninfected) prepared in the same manner served as negative antigen control.

BUFFERS

(1) For Agar gel Pricipitation test (Aziz, 1985)

(a) Solution A :

$Na_2 Hpo_4. 2H_2O$	1.4 g
Double distilled water	100 ml

(b) Solution B :

$Na\ H_2\ Po_4$	1.4 g
Double distilled water	100 ml

Composition of the agar gel :

Solution A	84.1 ml
Solution B	15.9 ml
Sodium chloride	8.0 g
Agarose	1.0 g
Sodium azide	0.01 g

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

(II) Composition of phosphate buffer saline (PBS) PH 7.2 to 7.4 (Aziz,

1985)

Nacl	2.0 g
Kcl	0.02 g
Na_2 H Po_4 2 H_2o	0.14 g
KH_2 Po_4	0.05 g
Double Distt. Water	250 ml.

The solution was sterilized by autoclaving at 15 lb pressure for 15 minutes and stored at refrigerator temperature.

(III) Alsever's Solution

Dextrose	2.05 g
Sodium chloride	0.42 g
Sodium citrate	0.90 g
Citric acid	0.50 g
Double Distt. Water	100 ml

Haemagglutination test (HA test) :

The HA test was carried in perspex plate according to Beard (1980). At first 0.2 ml of virus material was added in 0.8 ml of PBS and after that two fold dilutions were made. In the next step, 0.5 ml of 0.8 per cent RBC suspension was added to each well. A known positive and negative controls were included. The plate was stirred gently for mixing and uniform distribution of erythrocytes and inoculated at room temperature for 40 minutes. The RDV produced a diffused sheet of agglutinated RBC covering the bottom of the wells. Negative controls wells showed circumscribed compact buttons at the bottoms. Result of HA titre was recorded as reciprocal of the highest dilution showing HA.

Haemagglutination inhibition test (HI test):

The HI test was performed by microtitration techniques as suggested by Beard (1980). Four HA units of virus antigen (Charan *et al.*, 1981) and 0.8 percent chicken RBC suspension were used in the test. Using 0.25 ml of serum sample, two fold serial dilutions were made in PBS. Then in each serum dilution, 0.25 ml (4 HA units) of virus antigen was added. After a reaction time of 20 minutes at room temp. 0.5 ml of 0.8 percent 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 and inoculated at room temp. The result was recorded after 40 minutes. The reciprocal of highest serum dilution showing complete inhibition of haemagglutination was taken as the HI titres.

Agar gel Precipitation test (AGPT):

This test was carried out as per the procedure of Hirai *et at.* (1972) with some modifications. The glass microscopic slide was precoated by dipping them in 0.3 percent agar solution and dried in open air. The slides were placed on a horizontal level surface to obtain uniform gels. Approximately 4 ml of the gel was poured on each slide with the help of glass pipette and allowed to set. After setting the slides were kept at 4^0c overnight to facilitate punching of gels. With the help of a template, a hexagonal well pattern consisting of a central well surrounded by five peripheral wells were made, each well being 3.5mm in diameter and the centre to centre distance between the wells being 8mm.

The central well was charged with the antigen and one of the peripheral wells with the reference antiserum. The remaining four wells were used for test sera. The slides were kept in a moist chamber at room

temperature and observed daily for 3 – 4 days.

Quantitative Agar gel precipitation test (QAGPT):

The level of precipitating antibody was determind as per the method of cullen and wyeth (1975) with some modifications. A two fold serial dilutions 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 earried out simultaneously. The reciproeal of the highest dilution of serum which gave precipitating line was taken as the titre of the serum. The mean antibody titre of the positive samples were calculated according to villagas and purchase (1980).

Effect of selected agents on immunomodulation in chicken immuncompromised by a vaccine strain of infectious bursal disease virus:

The experiment was carried out as per the experimental design detailed below.

Experimental design:

A total of 400 day old chicks obtained through a local private poultry farm located at Ashiana Nagar Patna were procured and randomly divided into eight equal groups of 50 chicks each. All the chicks in the different groups were housed under identical managemental condition. Each group of chicks were given F strain Ranikhet disease vaccine intraocularly on the fourth day of age, and LaSota strain vaccine through drinking water on 19 day of age while IBD vaccine (IV95 strain) on 12 day of age intraocularly (group I to VII). The treatments that the birds received group wise are

shown in table 1.

Five birds of each group were sacrificed 96 hours post IBD vaccination as well as at termination of experiment (fig 4). Distribution of gross lesions were recorded (fig 5 & 6). A portion of bursa of fabricius, kidney, spleen and liver were collected in 10%, formalin saline for histopathological examination by H & E staining method. The bursa:body wt. ratio was determined on 96 hours post IBD vaccination as well as on 47 day of age.

Feed Conversion ratio:

The body weight gain of chickens were recorded by substrating the live weight of chicks at day of hatch from live weight of chicken at 47 day. Feed conversion ratio group wise at 47 day of age was determined as follows :

$$FCR = \frac{\text{Total feed consumed(g)}}{\text{Total wt. gain of chicken (g)}}$$

Histopathology:

The tissue samples for histopathalogical examination were processed in acetone benzene (Lillie and Fullmer 1976) and embedded in paraftin wax (melting point 62^0C). Five micron thick sections were cut and stained by haematoxylin 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 lesion 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 depletion, reticuloepithelial hyperplasia, vacuolar degeneration, follicular cyst, Interfollicular oedema, epithelial changes, interstitial fibrosis and cellular infiltration.

Statistical Analysis:

The mean and standard errors of the values obtained were determined. Analysis of variance (Anova) was performed whereever necessary as per Snedecor and Cochran (1967).

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TABLE 1 : PLAN OF EXPERIMENT

GROUP	No. OF CHICKS	TREATMENT AND AGE (DAY)				Observations
		F strain RD Vaccine day/route	IV 95 strain IBD Vaccine day/route	LaSota strain RD Vaccine day/route	DRUG SCHEDULE	
I	50	4 (i.o)	12 (i.o)	19 (d.w.)	Vitablend WM forte as source of vit A @ 4 ml/100 birds for 1st to 10th days later @ 6 ml/100 birds till 25th day of age in drinking water.	(i) Development of clinical signs and symptoms after IBD vaccinations if any. (ii) Collection of pre vaccinated blood samples on 11th day of age for determination of MDA level of IBD and HI titre to RD vaccine. (iii) Collection of blood samples at 7,14,21, 28 and 35 days post IBD vaccination for determination of (a) antibody titre to IBDV by QAGPT (b) antibody titre to RDV by HI test. (iv) Collection of bursa of fabricious, spleen, kidney and liver 96 hours post IBD vaccination as well as 47th day of age in 10% formalin saline for histopatho logical examination by H & E method. (v) Determination of body wt. gain and feed conversion ratio (FCR) for different groups of birds. (vi) Determination of bursa: body wt. ratio on 96 hour post IBD vaccination as well as on termination of experiment.
II	50	4 (i.o)	12 (i.o)	19 (d.w.)	Celin as source of vit C. @ 0.5 gm/lit of drinking water 1st day to 25th day of age.	
III	50	4 (i.o)	12 (i.o)	19 (d.w.)	Livol @ 4 ml/100 birds for 1st to 10th day later @ 6 ml/100 birds till 25th day of age in drinking water.	
IV	50	4 (i.o)	12 (i.o)	19 (d.w.)	Levamisole @ 15 mg/kg body wt.1st to 25th day of age in drinking water.	
V	50	4 (i.o)	12 (i.o)	19 (d.w.)	Carbo Vegetabilis 200 @ 20ml/100 birds twice weekly throughout the experiment.	
VI	50	4 (i.o)	12 (i.o)	19 (d.w.)	Sporlac @ 18g/100 kg of feed 1st day to 25th day of age via feed med.	
VII	50	4 (i.o)	12 (i.o)	19 (d.w.)	----------	
VIII (Control)	50	4 (i.o)	---	19 (d.w.)	----------	

Very virulent Infectious Bursal Disease

No Protection

High Protection

CHAPTER - IV

RESULTS

RESULTS

EFFECT OF SELECTED AGENTS/DRUGS ON IMMUNE RESPONSES TO IBDV (IV 95 STRAIN) IN BROILER CHICKENS.

The immune responses to IBD virus vaccine (IV 95 strain) in broiler chicken at different intervals post vaccination in different treatment groups are shown in table 2. The perusal of the table revealed that the mean QAGPT titres were highest in levamisole treated group overall intervals post vaccination when compared with the titres in the other treatment groups. The birds receiving a homeopathic drug, carbo vegetabilis demonstrated immune response to IBD vaccine which was lower than the corresponding values in levamisole treated group at all intervals of post vaccination except on 14 dpv when the precipitating antibody titres where almost similar in both the groups. Further the overall precipitating antibody titres of the carbo veg treated group (group V) was followed by vitamin A, sporlac, vitamin C and livol treated groups and the titre was lowest in the control group which was untreated (group. VII).

The overall pictures were suggestive of the fact that all the six drugs/agents employed in this study showed immune enhancing effect on responses to IBD virus vaccine in chickens when compared with the precipitating antibody titres of control birds (group VII) at all intervals post vaccination. Further the precipitating antibody titres in the different treatment groups (group I to VI) demonstrated increasing trend till the last day of observation (35 dpv). On the contrary, in untreated control group (group. VII) the QAGPT titres showed increasing trend only till 28 dpv (table 2).

TABLE 2: EFFECT OF DIFFERENT AGENTS ON IMMUNE RESPONSES TO IBD VIURS VACCINE (IV95 STRAIN) IN BROILER CHICKENS.

Group	Treatment	Age at IBD vaccination (Days)	Mean ± SE of QAGPT titre (log_2)* to IBD Virus Vaccine					
			Titre of Serum Samples at 11 days of age	Days Post IBD Vaccination				
				7	14	21	28	35
I	Vit A	12	- ve	$2.8^{ab}\pm0.314$**	$3.4^{b}\pm0.243$	$3.8^{bc}\pm0.374$	$4.0^{bc}\pm0.447$	$4.2^{bc}\pm0.311$
II	Vit C	12	- ve	$2.6^{ab}\pm0.400$	$3.4^{b}\pm0.245$	$3.6^{bc}\pm0.244$	$3.8^{bc}\pm0.319$	$4.0^{bc}\pm0.416$
III	Livol	12	- ve	$2.4^{ab}\pm0.214$	$3.2^{ab}\pm0.370$	$3.4^{ab}\pm0.219$	$3.6^{ab}\pm0.414$	$3.8^{b}\pm0.370$
IV	Levamisole	12	- ve	$3.2^{b}\pm0.371$	$3.6^{b}\pm0.248$	$4.4^{c}\pm0.241$	$4.6^{c}\pm0.281$	$4.8^{c}\pm0.245$
V	Carbo veg.	12	- ve	$2.8^{ab}\pm0.317$	$3.6^{b}\pm0.245$	$4.0^{bc}\pm0.411$	$4.2^{bc}\pm0.319$	$4.4^{bc}\pm0.374$
VI	Sporlac	12	- ve	$2.6^{ab}\pm0.421$	$3.4^{b}\pm0.240$	$3.6^{bc}\pm0.341$	$4.0^{bc}\pm0.447$	$4.2^{bc}\pm0.317$
VII	——	12	- ve	$2.2^{a}\pm0.291$	$2.4^{a}\pm0.245$	$2.6^{a}\pm0.432$	$2.8^{a}\pm0.371$	$2.8^{a}\pm0.379$
VIII (Control)	——	——	- ve	- ve	- ve	- ve	- ve	- ve

* Number of observations in each cell, n = 5

** Means with common superscript (a,b,c) in individual column did not differ significantly ($P<0.05$).

TABLE 3: IMMUNE RESPONSES TO RDV VACCINE (F & LaSota STRAIN) IN IBD VACCINATED BROILER CHICKEN AFTER ADMINISTRATION OF DIFFERENT AGENTS.

Group	Treatment	Vaccination (age in days)			Mean ± SE of HI titre (log_2)* to RD Vaccine					
		IBDV	RDV	RDV	7 days post F strain vaccination	Days Post IBD Vaccination				
		IV 95 strain	F strain	LaSota strain		7	14	21	28	35
I	Vit A	12	4	19	$3.4^{a}\pm0.219$**	$4.4^{ab}\pm0.241$	$6.0^{ab}\pm0.361$	$6.2^{abc}\pm0.374$	$6.4^{abc}\pm0.414$	$6.6^{abc}\pm0.417$
II	Vit C	12	4	19	$3.8^{a}\pm0.371$	$4.2^{ab}\pm0.274$	$6.0^{ab}\pm0.316$	$6.2^{abc}\pm0.319$	$6.4^{abc}\pm0.509$	$6.4^{ab}\pm0.487$
III	Livol	12	4	19	$3.4^{a}\pm0.214$	$4.4^{ab}\pm0.291$	$5.8^{ab}\pm0.311$	$6.0^{ab}\pm0.373$	$6.2^{ab}\pm0.315$	$6.4^{ab}\pm0.354$
IV	Levamisole	12	4	19	$3.8^{a}\pm0.388$	$4.8^{ab}\pm0.248$	$6.4^{ab}\pm0.509$	$6.8^{bc}\pm0.400$	$7.0^{bc}\pm0.583$	$7.2^{bc}\pm0.447$
V	Carbo veg.	12	4	19	$3.6^{a}\pm0.491$	4.6ab±0.244	$6.4^{ab}\pm0.431$	$6.6^{bc}\pm0.486$	$6.8^{bc}\pm0.317$	$7.0^{bc}\pm0.314$
VI	Sporlac	12	4	19	$3.8^{a}\pm0.345$	$4.4^{ab}\pm0.281$	$5.8^{ab}\pm0.447$	$6.2^{abc}\pm0.598$	$6.4^{abc}\pm0.360$	$6.6^{abc}\pm0.221$
VII	———	12	4	19	$3.6^{a}\pm0.411$	$3.8^{a}\pm0.317$	$5.2^{a}\pm0.352$	$5.4^{a}\pm0.241$	$5.6^{a}\pm0.509$	$5.6^{a}\pm0.341$
VIII (Control)	———	———	4	19	$3.4^{a}\pm0.219$	$5.0^{b}\pm0.447$	$6.8^{b}\pm0.583$	$7.0^{c}\pm0.477$	$7.4^{c}\pm0.441$	$7.6^{c}\pm0.244$

* Number of observations in each cell, n = 5

** Means with common superscript (a,b,c) in individual column did not differ significantly ($P<0.05$).

the groups (group I to VIII) overall the periods as evident from titres recorded 7 days post LaSota vaccination and afterwards. However, the booster responses due to LaSota vaccine were not marked on last day of observation (table 3).

EFFECT OF SELECTED AGENTS/DRUGS ON BURSA: BODY WEIGHT RATIO IN IBDV VACCINATED BROILER CHICKENS.

The bursa weight, body weight and bursa: body weight ratios are shown in table 4. The perusal of the table revealed bursa:body wt ratio which ranged between 1.883 ± 0.015 to 2.291 ± 0.010 in different groups of birds (group I to VIII). The b:b ratio recorded at the termination of the experiment on 47 day of age, ranged between 0.458 ± 0.0025 to 0.882 ± 0.0014 in different groups of birds (group I to VIII). Further bursa: body wt ratio at 96 hours post IBD vaccination in group VII and group VIII did not differ significantly ($P<0.01$) from each other whereas, both these values differ significantly from the values obtained in rest of the groups (table 4). The b:b ratio were invariably higher in different drug treated groups when compared with the values in case of group VII (vaccinated but untreated group) and group VIII (untreated unvaccinated). Similar trend in b:b ratios were observed in case of birds sacrificed at the termination of the experiment (table 5) except the values were markedly lower than the values recorded at 96 hours post IBD vaccination (as evident from the table 4 and table 5).

EFFECTS OF SELECTED AGENTS/ DRUGS ON GROSS AND HISTOPATHOLOGY IN IBDV VACCINATED BROILER CHICKENS.

The microscopic changes in the bursa of fabricious were typical of IBD and were mainly characterized by interfollicular oedema, lymphoid

TABLE 4: EFFECT OF DIFFERENT AGENTS ON BURSA WT., BODY WT. AND BURSA: BODY WT. RATIO OF IBD VACCINATED BROILER CHICKEN AT 96 HOURS POST IBD VACCINATION.

Group	Treatment	Vaccination (age in days)			Bursa wt. (g)*	Body wt. (g)*	Bursa: body wt. ratio
		IBDV IV95 strain	RDV F strain	RDV LaSota strain			
I	Vit A	12	4	19	$0.585^{d} \pm 0.0022$**	$264^{de} \pm 2.449$	$2.216^{d} \pm 0.013$
II	Vit C	12	4	19	$0.512^{b} \pm 0.0012$	$258^{c} \pm 1.532$	$2.107^{b} \pm 0.011$
III	Livol	12	4	19	$0.560^{c} \pm 0.0015$	$260^{cd} \pm 1.984$	$2.150^{c} \pm 0.015$
IV	Levamisole	12	4	19	$0.606^{e} \pm 0.0015$	$268^{e} \pm 1.603$	$2.258^{e} \pm 0.012$
V	Carbo veg	12	4	19	$0.644^{f} \pm 0.0018$	$281^{f} \pm 1.807$	$2.291^{e} \pm 0.010$
VI	Sporlac	12	4	19	$0.584^{d} \pm 0.0018$	$264^{de} \pm 1.714$	$2.212^{d} \pm 0.019$
VII	——	12	4	19	$0.506^{b} \pm 0.0016$	$240^{a} \pm 1.850$	$1.883^{a} \pm 0.015$
VIII (Control)	——	——	4	19	$0.475^{a} \pm 0.0010$	$252^{b} \pm 1.122$	$1.907^{a} \pm 0.011$

* No of observations in each cell, n = 5

** Means bearing common superscript (a,b,c,d,e,f) in individual column did not differ significantly ($P<.0.05$).

TABLE 5: EFFECT OF DIFFERENT AGENTS ON BURSA WT., BODY WT. AND BURSA: BODY WT. RATIO OF IBD VACCINATED BROILER CHICKEN ON TERMINATION OF EXPERIMENT (47 DAY OF AGE).

Group	Treatment	Vaccination (age in days)			Bursa wt. (g.)*	Body wt. (g.)*	Bursa: body wt. ratio
		IBDV IV95 strain	RDV F strain	RDV LaSota strain			
I	Vit A	12	4	19	$1.216^{e} \pm 0.0024$**	$1532^{e} \pm 3.741$**	$0.793^{c} \pm 0.0022$**
II	Vit C	12	4	19	$1.178^{d} \pm 0.0025$	$1516^{d} \pm 2.499$	$0.777^{c} \pm 0.0024$
III	Livol	12	4	19	1.110c ± 0.0031	1500c ± 3.162	$0.739^{c} \pm 0.0018$
IV	Levamisole	12	4	19	$1.280^{f} \pm 0.0060$	$1670^{f} \pm 3.161$	$0.766^{c} \pm 0.0033$
V	Carbo veg	12	4	19	$1.520^{g} \pm 0.0030$	$1718^{g} \pm 3.742$	$0.882^{d} \pm 0.0014$
VI	Sporlac	12	4	19	$1.158^{d} \pm 0.0037$	$1516^{d} \pm 4.000$	$0.763^{c} \pm 0.0038$
VII	——	12	4	19	$0.710^{b} \pm 0.0018$	$1340^{a} \pm 3.154$	$0.529^{b} \pm 0.0026$
VIII (Control)	——	——	4	19	$0.667^{a} \pm 0.0030$	$1451^{b} \pm 3.316$	$0.458^{a} \pm 0.0025$

* No of observation in each cell, n = 5

** Means bearing common superscript (a,b,c,d,e,f,g) in individual column did not differ significantly ($P<.0.05$).

depletion, lymphoid necrosis, interstitial fibrosis, cellular infiltration and epithelial changes including hyperplasia, epithelial invegination, vacuolation and cyst formation (fig 16 to 32). The mean lesion score was highest in birds which received only IBD vaccine but no medication (group VII). Interestingly the different drug given in group I to VI helped reduction of bursal lesion score (table 6 & 7). However, the ratios in treated groups better when compared with the values even in control. In the treatment group the lowest lesion score was detected in levamisole treated group followed by vit A, carbo veg, vit. C and livol. No changes were appreciable in the control (group VIII) which neither receive IBD vaccine nor any medication.

EFFECT OF SELECTED AGENTS/DRUGS ON BODY WEIGHT GAIN AND FEED CONVERSION RATIO IN IBD VACCINATED BROILER CHICKENS.

The body wt gains and feed conversion ratios in different treatment groups are shown in table 8. The body wt. gain was lowest in case of IBD vaccinated untreated group (group VII) followed by control group (group VIII) whereas, the values were invariably higher in case of different treatment groups (group I to VI) when compared with each of group VII and group VIII (fig 7a & b). Further the body wt. gain was highest in case of homeopathic drug treated group followed by levamisole, sporlac, vit A, vit C and livol group (fig 8a & b). Analysis of variance showed that the value of the body wt. gain in carbo Veg. treated group was significantly ($P<0.01$) higher than all other groups.

The feed conversion ratio was highest in group VII followed by control group (group VIII) and was lowest in homeopathic drug treated group of birds. The general trend suggested that whereas the ratios were poor

TABLE 6: MEAN ± S. E. OF BURSAL LESION SCORE IN DIFFERENT TREATMENT GROUPS OF BROILER CHICKENS SACRIFICED ON 96 HRS POST IBD VACCINATION (IV 95 STRAIN).

Group & Treatment	Inter-follicu-odema	Follicular changes					Epithelial changes				Intersti-tial fibrosis	cellular infitra-tion	Total lesion score
		Lymphoid necrosis	Lymphoid depletion	Reticulo-epithelial hyperplasia	Vacuolar degene-ration	Follicular cyst	Hyper-plasia	Epithelial invagi-nation	Cyst form-ation	vacuo-lation			
I Vit A	0.4^{a} ± 0.244*	1.8^{ab} ± 0.489	1.2^{a} ± 0.480	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.8^{a} ± 0.200	1.0^{a} ± 0.000	0.0 ± 0	0.0 ± 0	1.4^{d} ± 0.244	0.2^{a} ± 0.200	0.56^{ab} ± 0.187(60)
II Vit C	0.6^{ab} ± 0.241	2.0^{abc} ± 0.316	1.8^{bcd} ± 0.210	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.8^{a} ± 0.370	1.6^{bc} ± 0.400	0.8^{ab} +0.374	0.6^{a} ± 0.249	0.4^{a} ± 0.249	0.8^{bc} ± 0.211	0.78^{abc} ± 0.199(60)
III Livol	0.8^{ab} ± 0.200	2.2^{bc} ± 0.200	2.0^{bcd} ± 0.447	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.6^{a} ± 0.244	1.8^{bc} ± 0.440	1.0^{b} ± 0.316	0.8^{a} ± 0.211	0.6^{ab} ± 0.277	0.4^{ab} ± 0.244	0.85^{abc} ± 0.223(60)
IV Levamisole	0.4^{a} ± 0.240	1.6^{a} ± 0.410	1.4^{ab} ± 0.241	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.240	1.0^{a} ± 0.311	0.4^{a} ± 0.244	0.0 ± 0	1.0^{bcd} ± 0.315	0.4^{ab} ± 0.200	0.55^{a} ± 0.163(60)
V Carbo veg	0.4^{a} ± 0.244	1.8^{ab} ± 0.374	1.6^{abc} ± 0.240	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.6^{a} ± 0.241	1.4^{ab} ± 0.240	0.6^{ab} +0.241	0.0 ± 0	0.8^{abc} ± 0.211	0.6^{abc} ± 0.400	0.65^{abc} ± 0.186(60)
VI Sporlac	0.6^{ab} ± 0.400	2.0^{abc} ± 0.547	2.0^{bcd} ± 0.316	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.6a ± 0.200	1.6^{bc} ± 0.400	0.6^{ab} ± 0.240	0.4^{a} ± 0.244	0.6^{ab} ± 0.240	0.8^{bc} ± 0.374	0.78^{abc} ± 0.208(60)
VII No Med.	1.0^{b} ± 0.316	2.4^{c} ± 0.234	2.2^{d} ± 0.370	0.4^{a} ± 0.234	0.0 ± 0	0.0 ± 0	0.8^{a} ± 0.371	2.0^{a} ± 0.316	0.8^{ab} ± 0.200	0.6^{a} ± 0.400	0.6^{ab} ± 0.234	1.0^{c} ± 0.316	0.98^{c} ± 0.259(60)
VIII No Med./ No Vacc.	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	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0(60)

Number of observations in each cell, n = 5

* Means with common superscript (a,b,c,d) in individual column did not differ significantly ($P<0.05$)

TABLE 7: MEAN ± S. E. OF BURSAL LESION SCORE IN DIFFERENT TREATMENT GROUPS OF BROILER CHICKENS SACRIFICED AT TERMINATION OF EXPERIMENT (47 DAY OF AGE).

Group & Treatment	Inter-follicu-odema	Follicular changes					Epithelial changes				Intersti-tial fibrosis	cellular infitra-tion	Total lesion score
		Lymphoid necrosis	Lymphoid depletion	Reticulo-epithelial hyperplasia	Vacuolar degene-ration	Follicular cyst	Hyper-plasia	Epithelial invagi-nation	Cyst form-ation	vacuo-lation			
I Vit A	0.0 ± 0	1.0^{ab} ± 0.000	0.4^{a} ± 0.244	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.244	0.4^{ab} ± 0.244	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.244	0.0 ± 0	0.21^{ab} ± 0.090
II Vit C	0.2^{a} ± 0.200*	1.0^{ab} ± 0.316	0.6^{ab} ± 0.240	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.244	0.6^{b} ± 0.244	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{ab} ± 0.244	0.26^{ab} ± 0.096
III Livol	0.0 ± 0	1.2^{abc} ± 0.374	1.0cd ± 0.447	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.2^{a} ± 0.200	0.8^{bc} ± 0.374	0.0 ± 0	0.0 ± 0	0.2^{a} ± 0.200	0.0 ± 0	0.28^{ab} ± 0.129
IV Levamisole	0.0 ± 0	0.8^{a} ± 0.200	0.6^{ab} ± 0.400	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.2^{a} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.13^{a} ± 0.079
V Carbo veg	0.0 ± 0	1.0^{ab} ± 0.447	0.8^{bc} ± 0.200	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{ab} ± 0.244	0.0 ± 0	0.0 ± 0	0.2^{a} ± 0.200	0.2^{a} ± 0.200	0.21^{ab} ± 0.099
VI Sporlac	0.4^{ab} ± 0.244	1.2^{abc} ± 0.200	1.0^{cd} ± 0.316	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.2^{a} ± 0.200	0.6^{b} ± 0.241	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{ab} ± 0.244	0.33^{ab} ± 0.118
VII No Med.	0.6^{b} ± 0.240	1.4^{c} ± 0.244	1.2^{d} ± 0.440	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.4^{a} ± 0.244	1.0^{c} ± 0.310	0.4^{a} ± 0.244	0.0 ± 0	0.4^{a} ± 0.244	0.6^{b} ± 0.240	0.50^{b} ± 0.140
VIII No Med./ No Vacc.	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 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0

Number of observations in each cell, n = 5

* Means with common superscript (a,b,c,d) in individual column did not differ significantly ($P<0.05$)

TABLE 8: EFFECT OF DIFFERENT AGENTS ON GROWTH PARAMETERS IN IBD VACCINATED BROILER CHICKENS.

Group	Treatment	Vaccination (age in days)			Mean ± SE (g)**				Percent Mortality
		IBDV IV 95 Strain	RDV F Strain	RDV Lasota Strain	Initial body wt. on 0 day	Final body wt. on 47 day of age	Body wt. gain on 47 day of age	Feed conversion ratio	
I	Vit.A	12	4	19	49.90±0.317(50)	1534.28^{ab}±22.525 (35)*	1484.38^{a}±21.020 (35)	2.20	2.5
II	Vit.C	12	4	19	50.00±0.320(50)	1515.15^{ab}±23.862 (33)	1465.15^{a}±20.604 (33)	2.37	2.5
III	Livol	12	4	19	50.07±0.326(50)	1502.85^{ab}±35.677 (35)	1452.78^{a}±34.607 (35)	2.50	5.0
IV	Levamisole	12	4	19	50.02±0.385(50)	1680.88^{cd}±32.154 (34)	1630.80^{bc}±31.897 (34)	2.45	5.0
V	Carbo Veg.	12	4	19	50.02±0.327(50)	1713.23^{d}±29.350 (34)	1663.21^{c}±28.151 (34)	2.20	2.5
VI	Sporlac	12	4	19	50.15±0.296(50)	1551.56^{bc}±29.740 (32)	1501.41^{ab}±28.901 (32)	2.40	5.0
VII	——	12	4	19	49.90±0.300(50)	1425.75^{a}±28.959 (33)	1375.85^{a}±27.751 (33)	2.58	12.5
VIII (Control)	——	——	4	19	49.95±0.319(50)	1475.71^{ab}±29.015 (35)	1425.76^{a}±28.110 (35)	2.53	5.0

Figures in parentheses indicate number of observations.

* Means bearing common superscript (a,b,c,d,) in individual column did not differ significantly ($P<0.05$).

in IBD vaccinated but untreated group (group VII), it was better in birds receiving different treatments (group I to VI). Within the treatment groups lowest FCR was visible in homeopathic as well as vit A treated group and it was poorest in livol treated group. However, the ratios in different treatment groups were better when compared with the values even in control group (group VIII).

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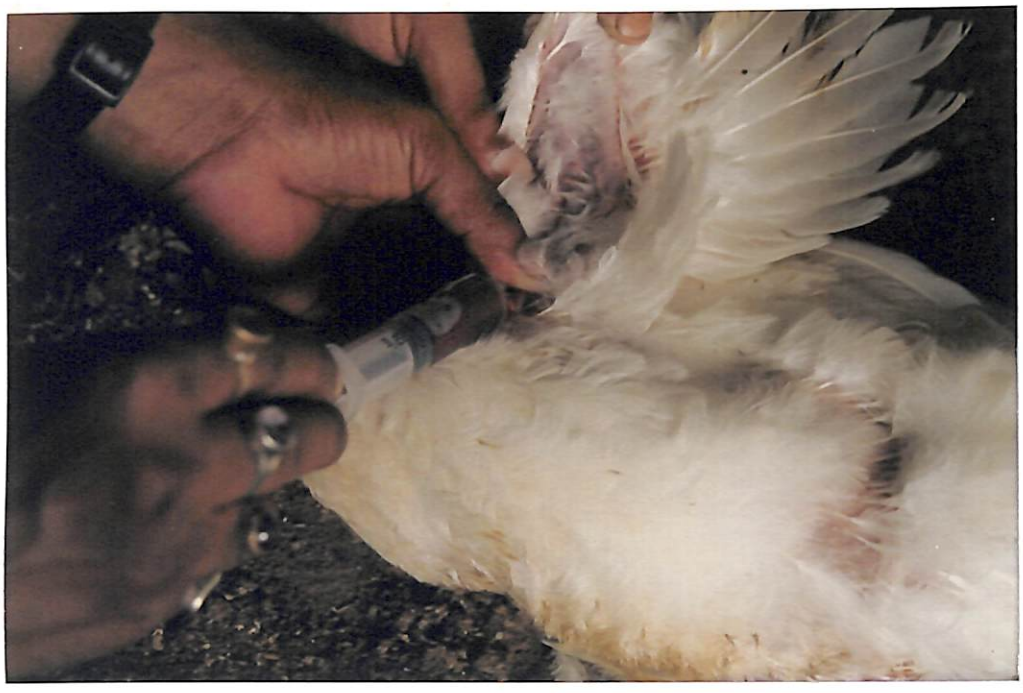
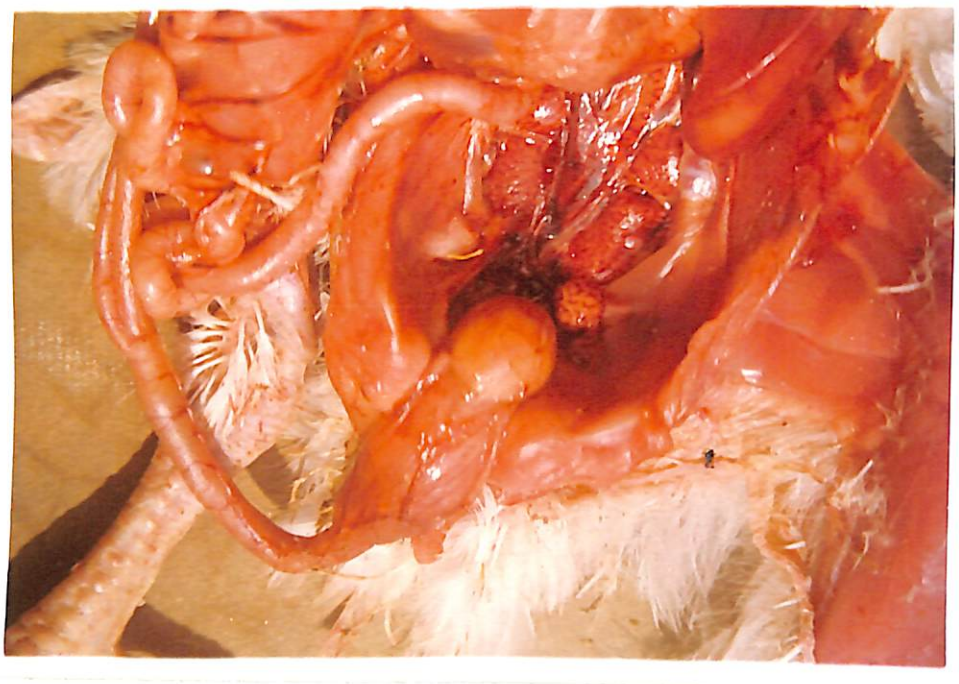




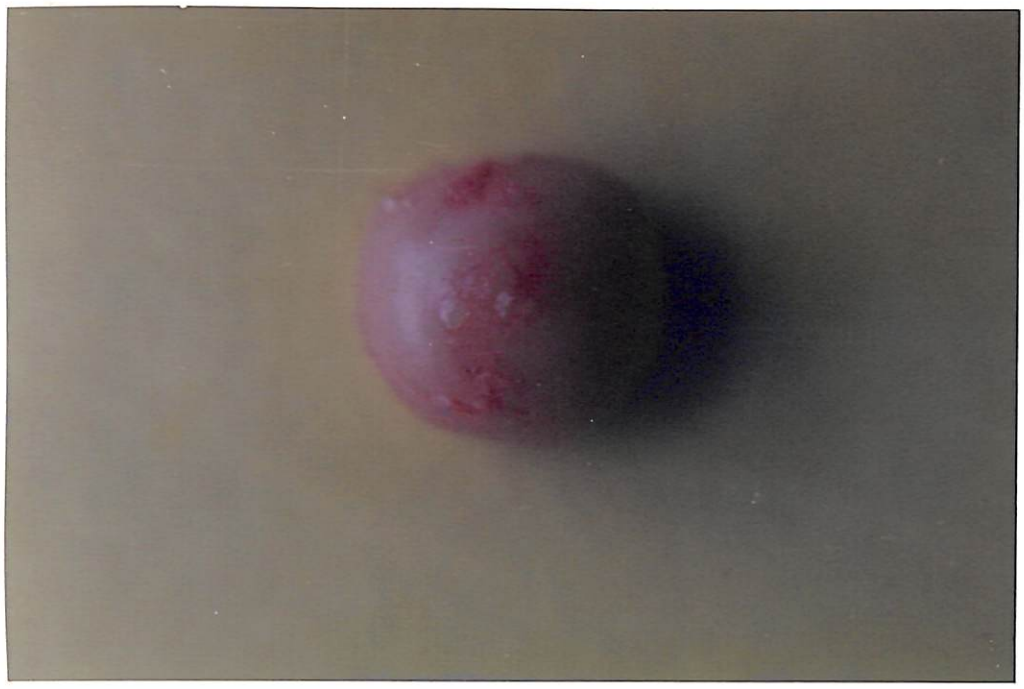


Figure 9 (a): Showing effect of different agents on antibody titre to IBD vaccine (IV 95) in broiler chickens.

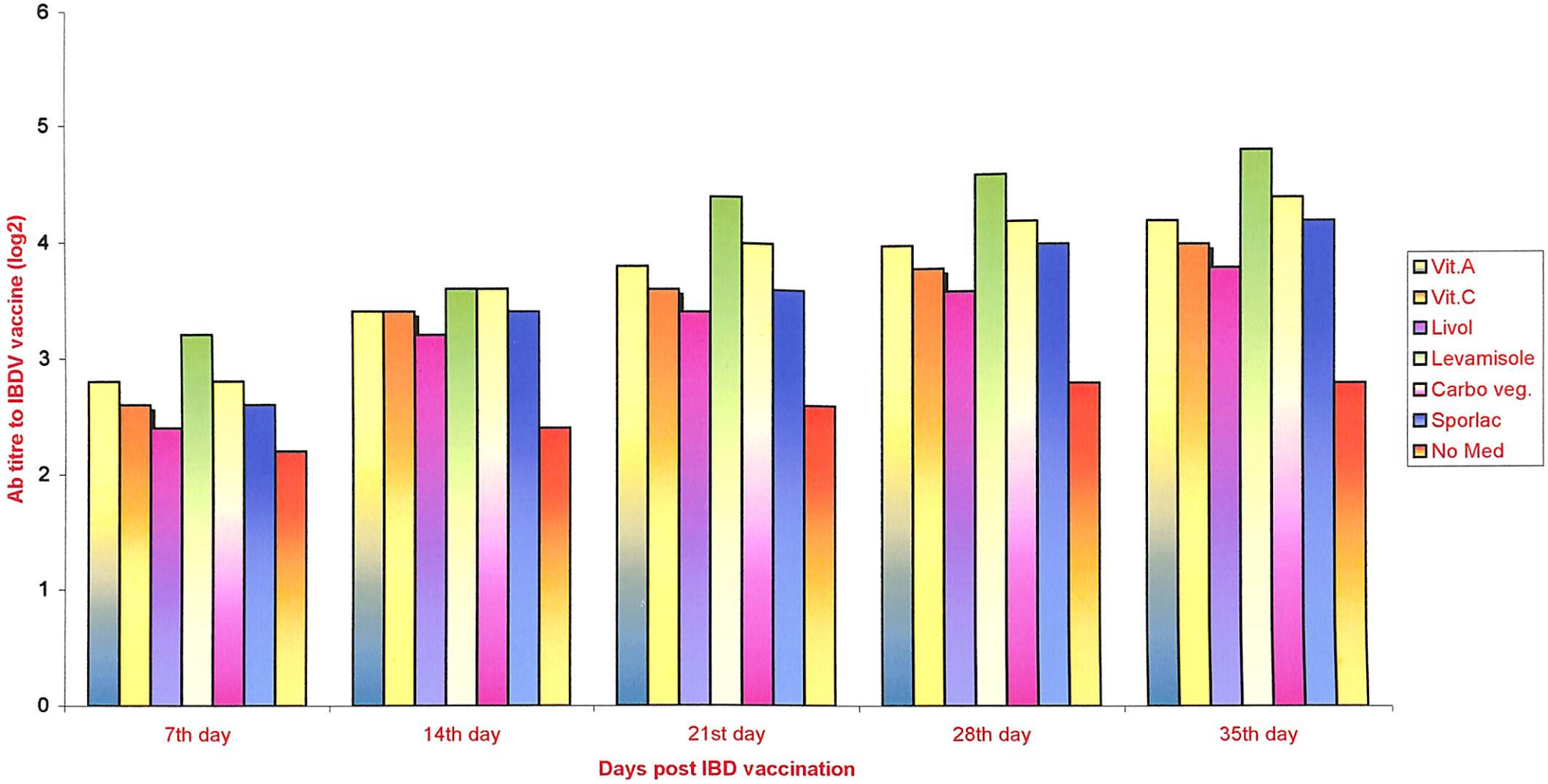
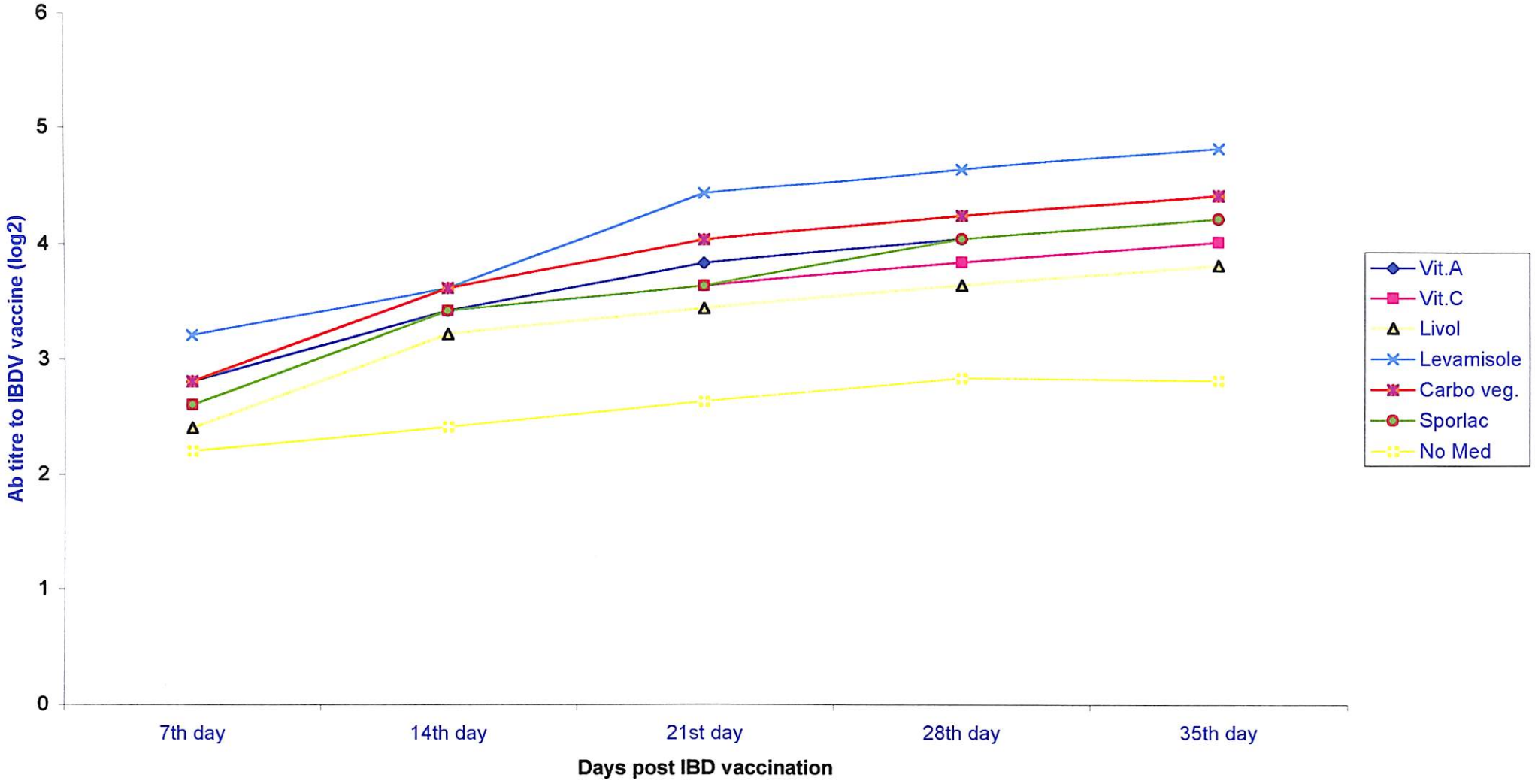


Figure 9 (b): Showing effect of different agents on antibody titre to IBD vaccine (IV 95) in broiler chickens.



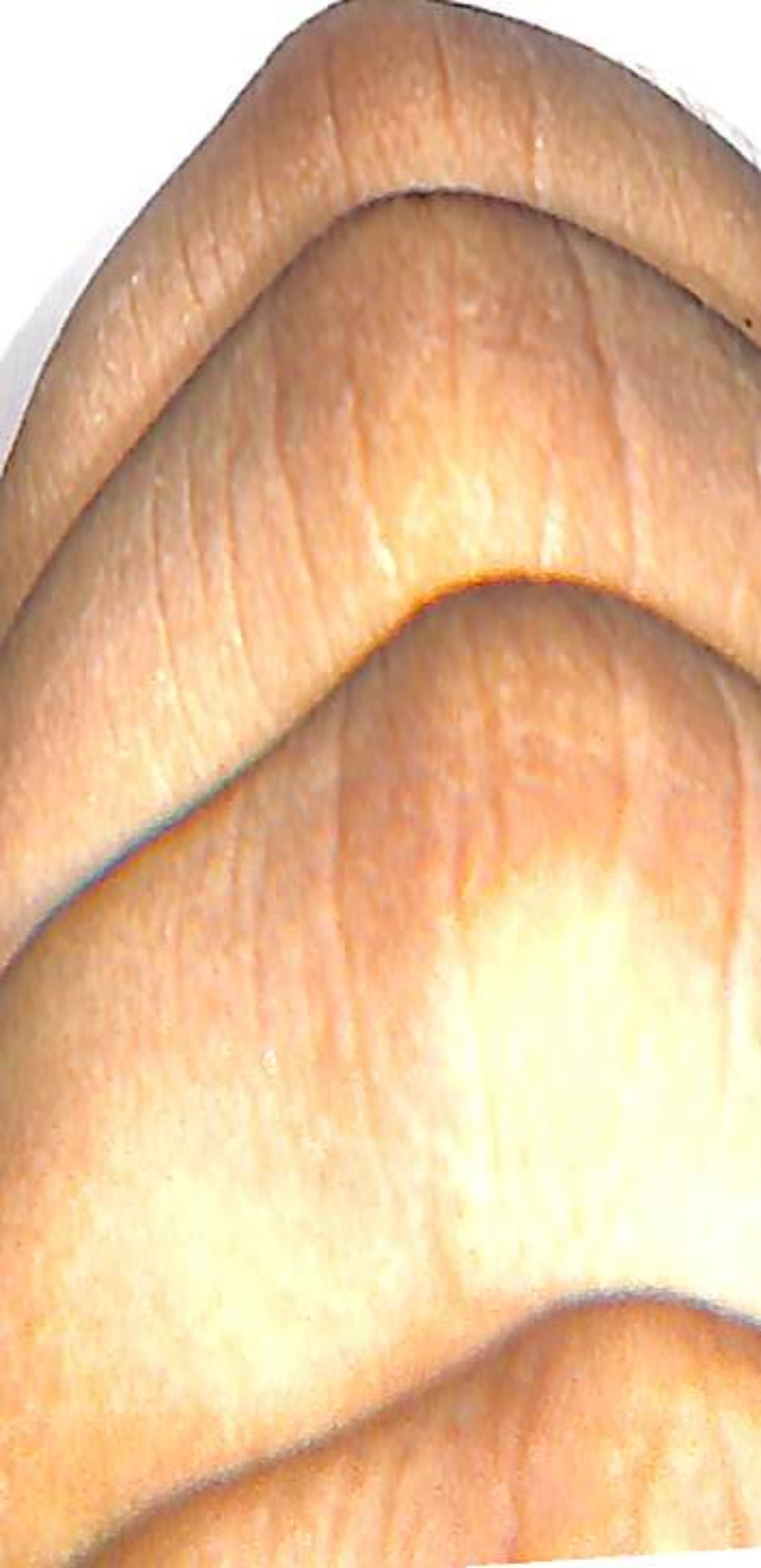


Figure 10 (a): Showing effect of different agents on antibody titre of RD vaccine in IBD vaccinated broiler chickens.

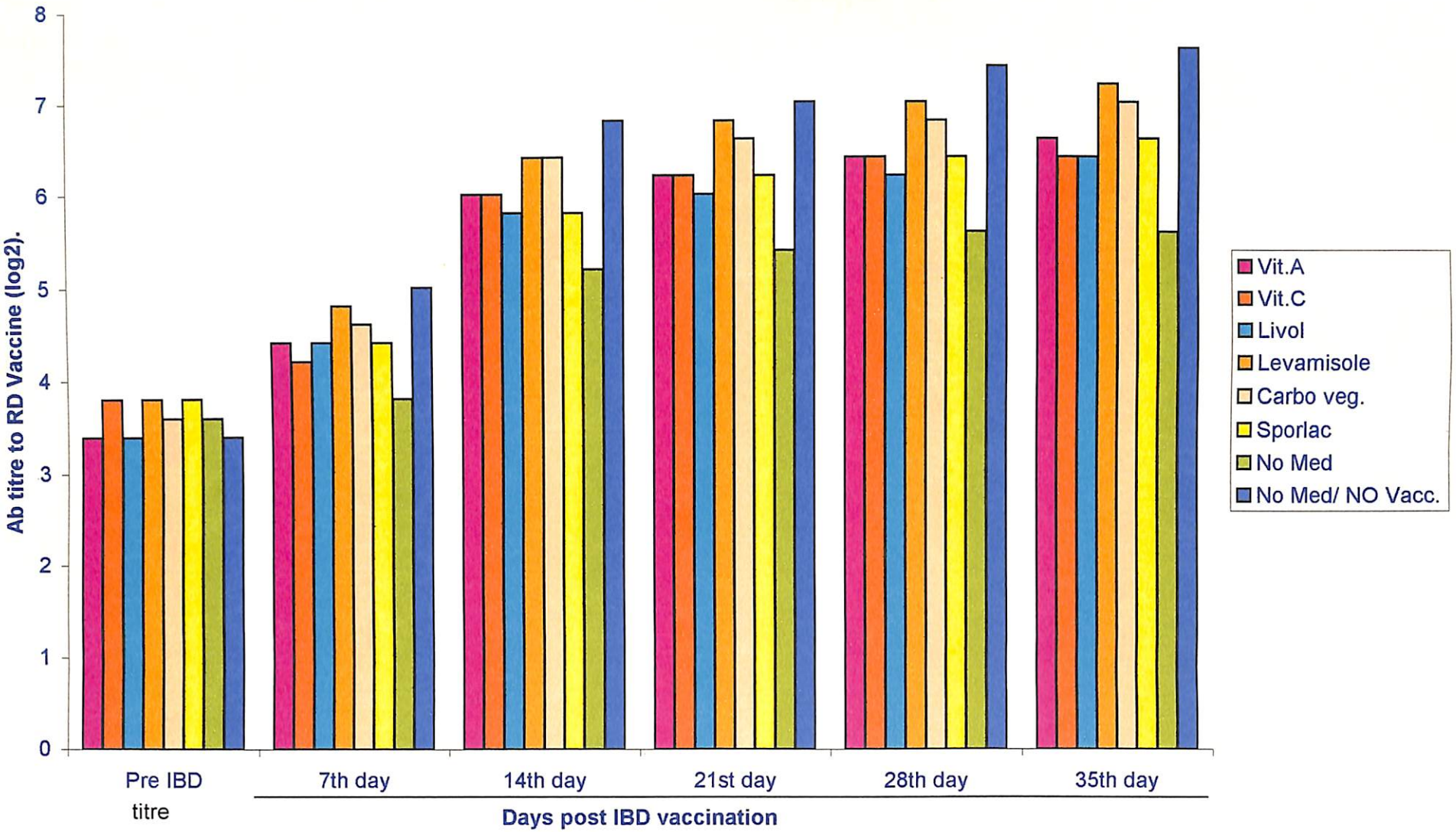


Figure 10 (b): Showing effect of different agents on antibody titre of RD vaccine in IBD vaccinated broiler chickens.

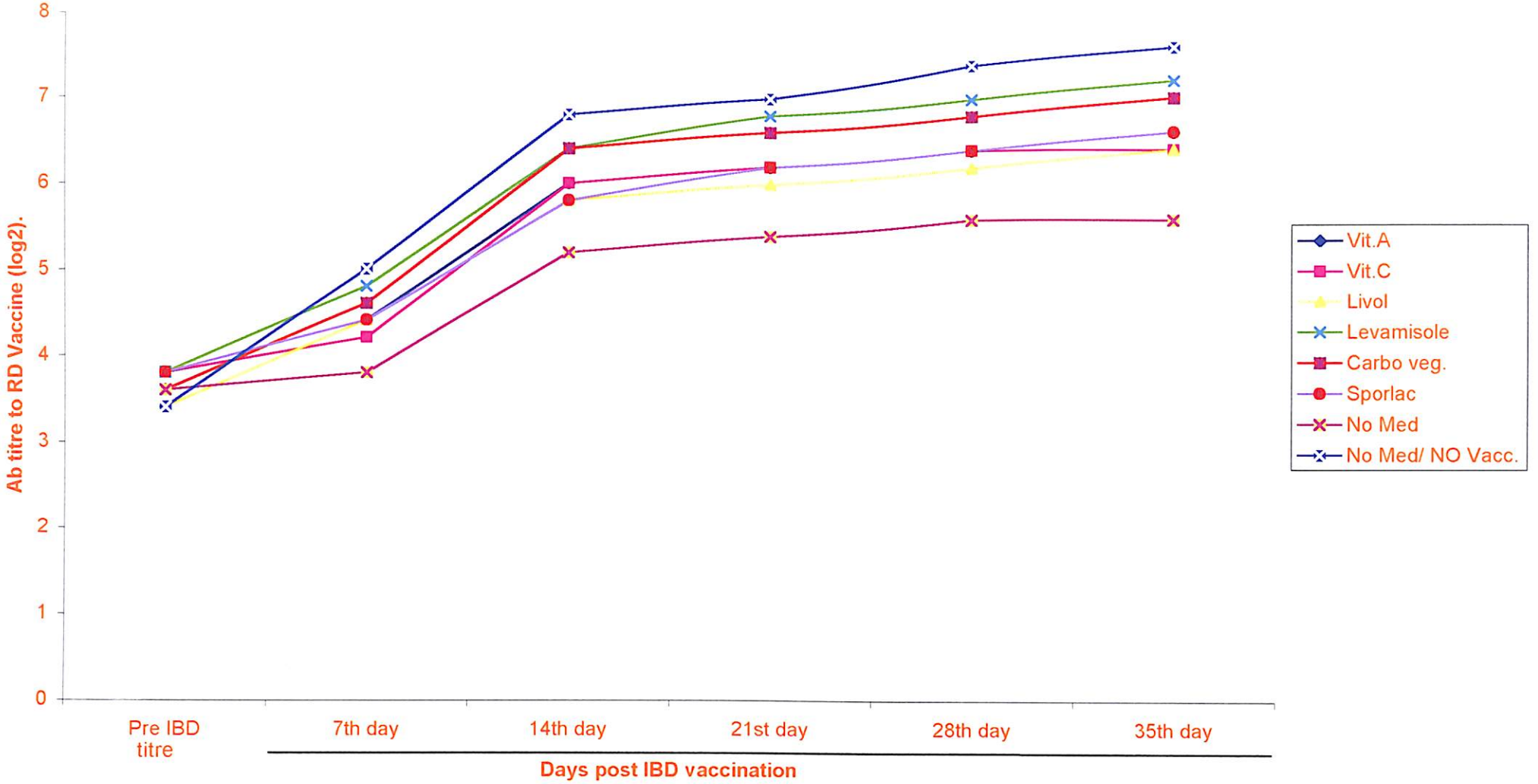


Figure 11: Showing effect of different agents on bursa: body wt. ratio at 96 hours post IBD vaccination in broiler chickens.

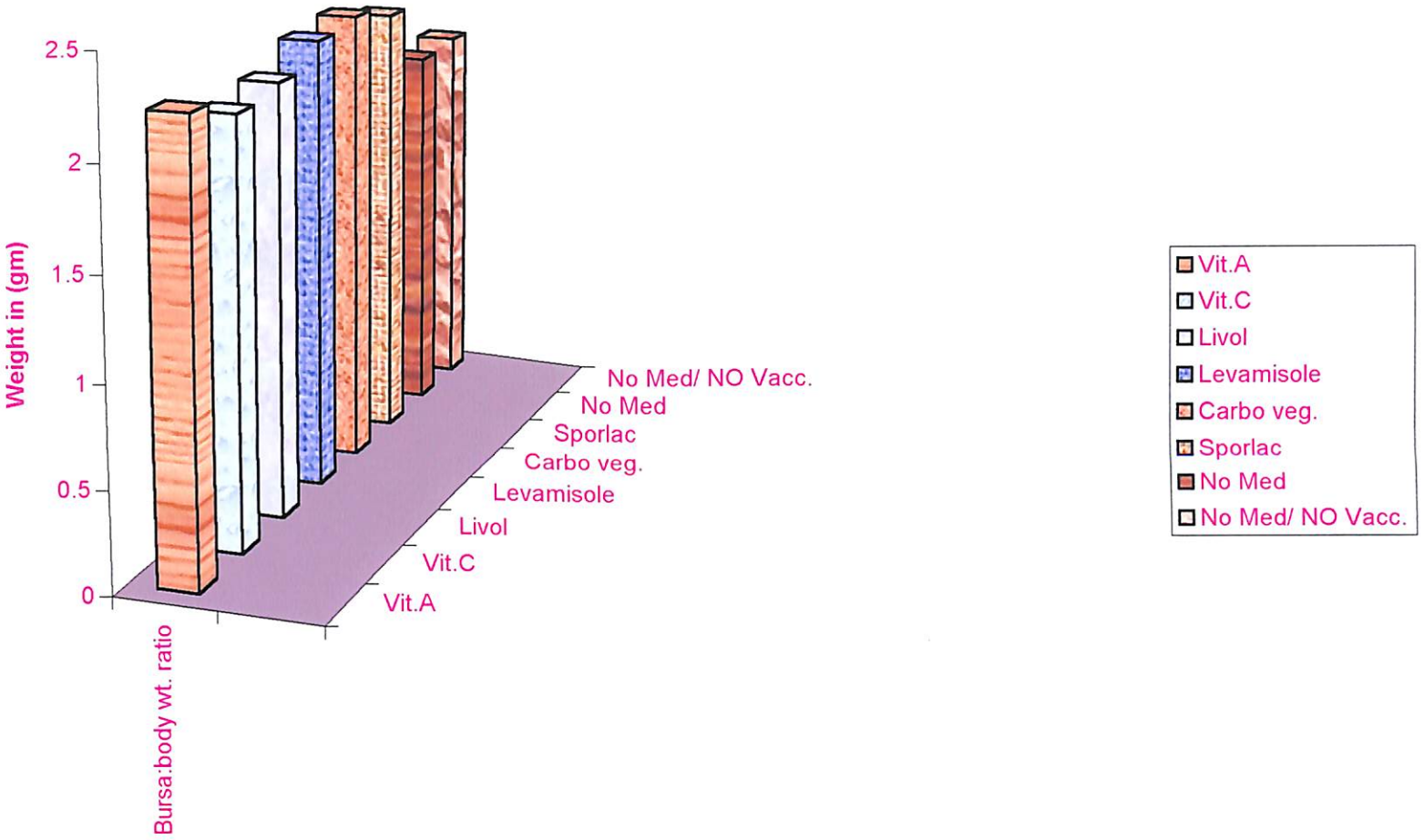


Figure 12: Showing effect of different agents on bursa: body wt. ratio on termination of experiment (47th day of age) in broiler chickens.

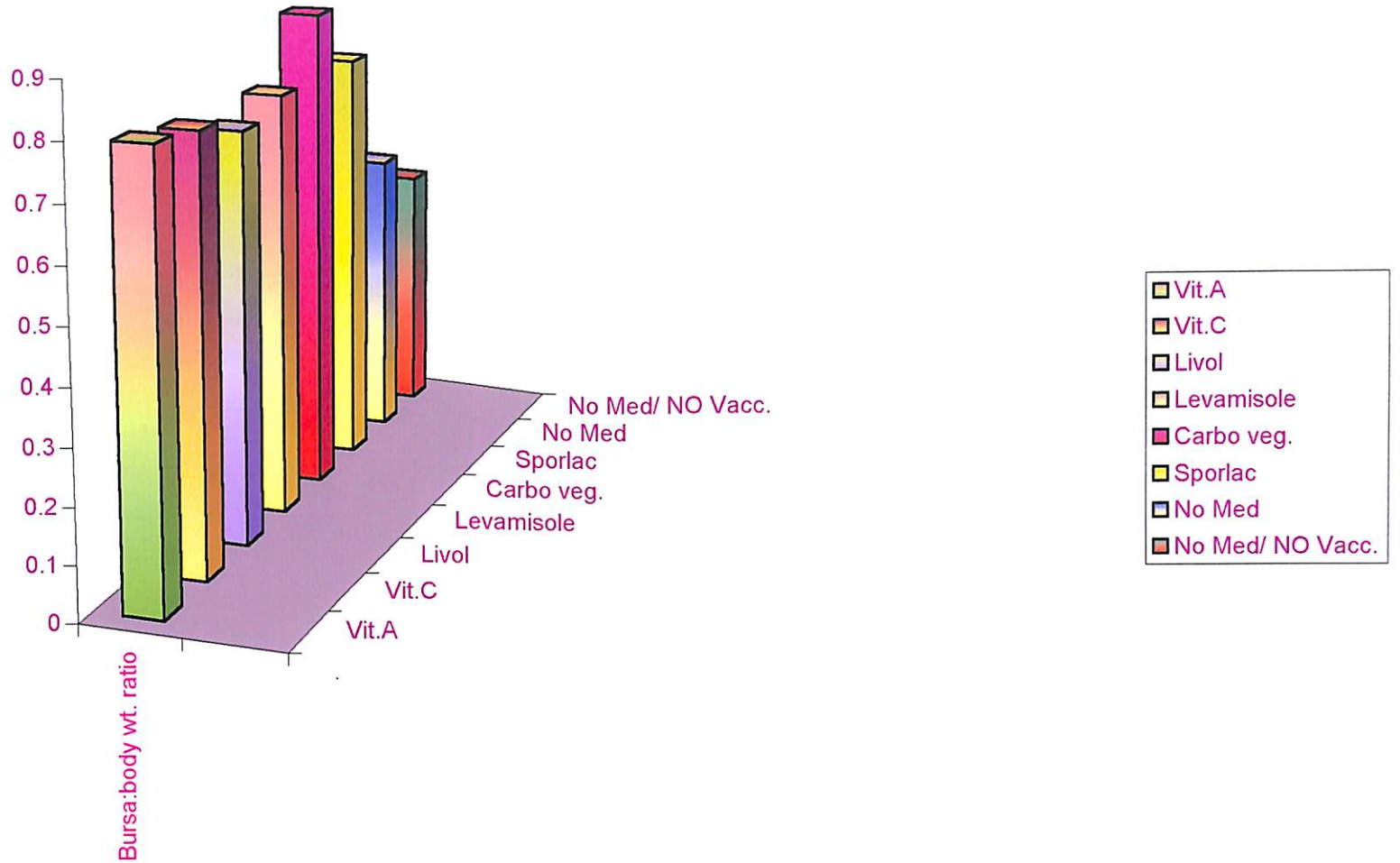


Figure 13: Showing body weight in different treatment groups of IBD vaccinated broiler chickens at termination of experiment (47 day of age).

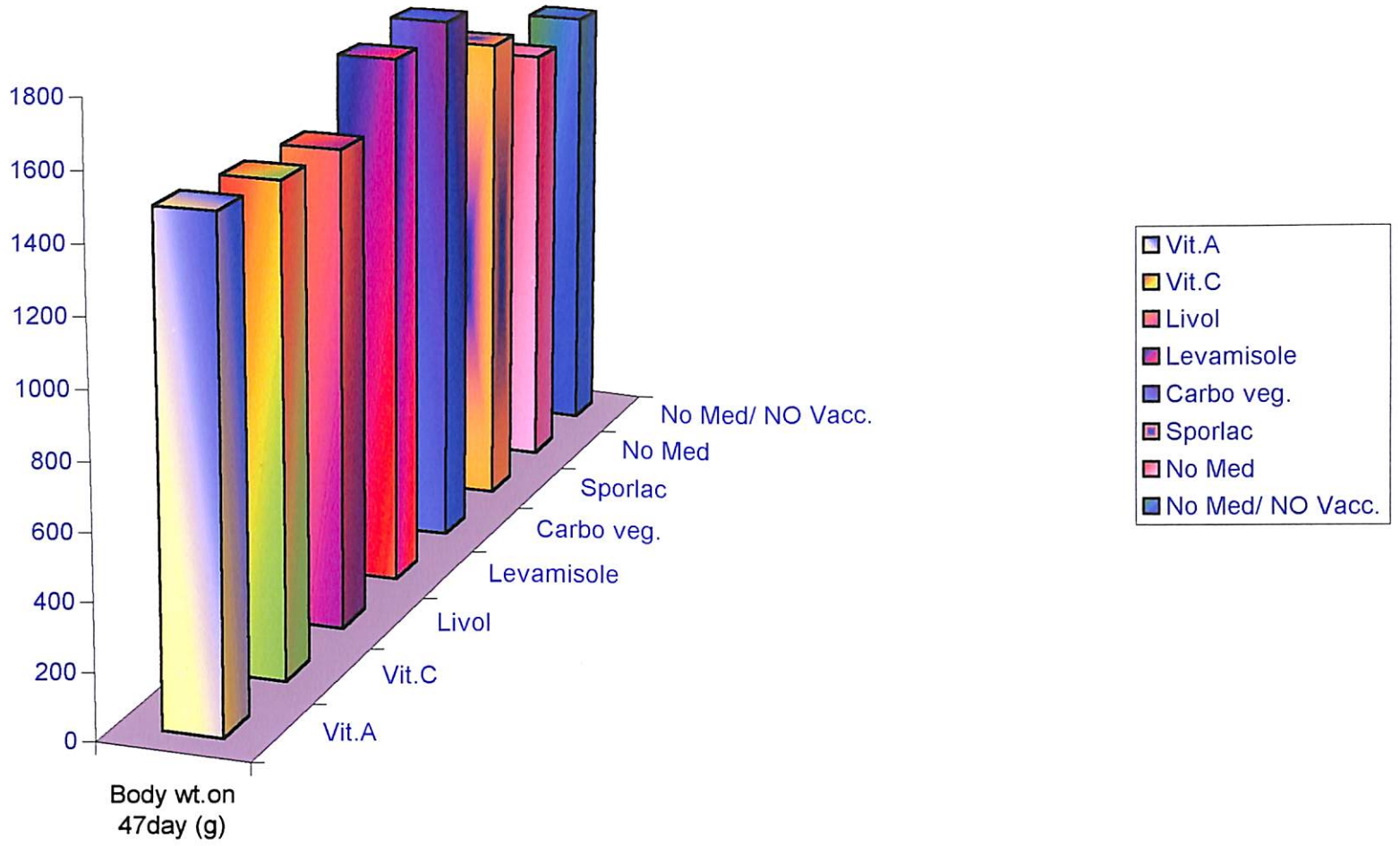


Figure 14: Percent mortality in different treatment groups of IBD vaccinated broiler chickens.

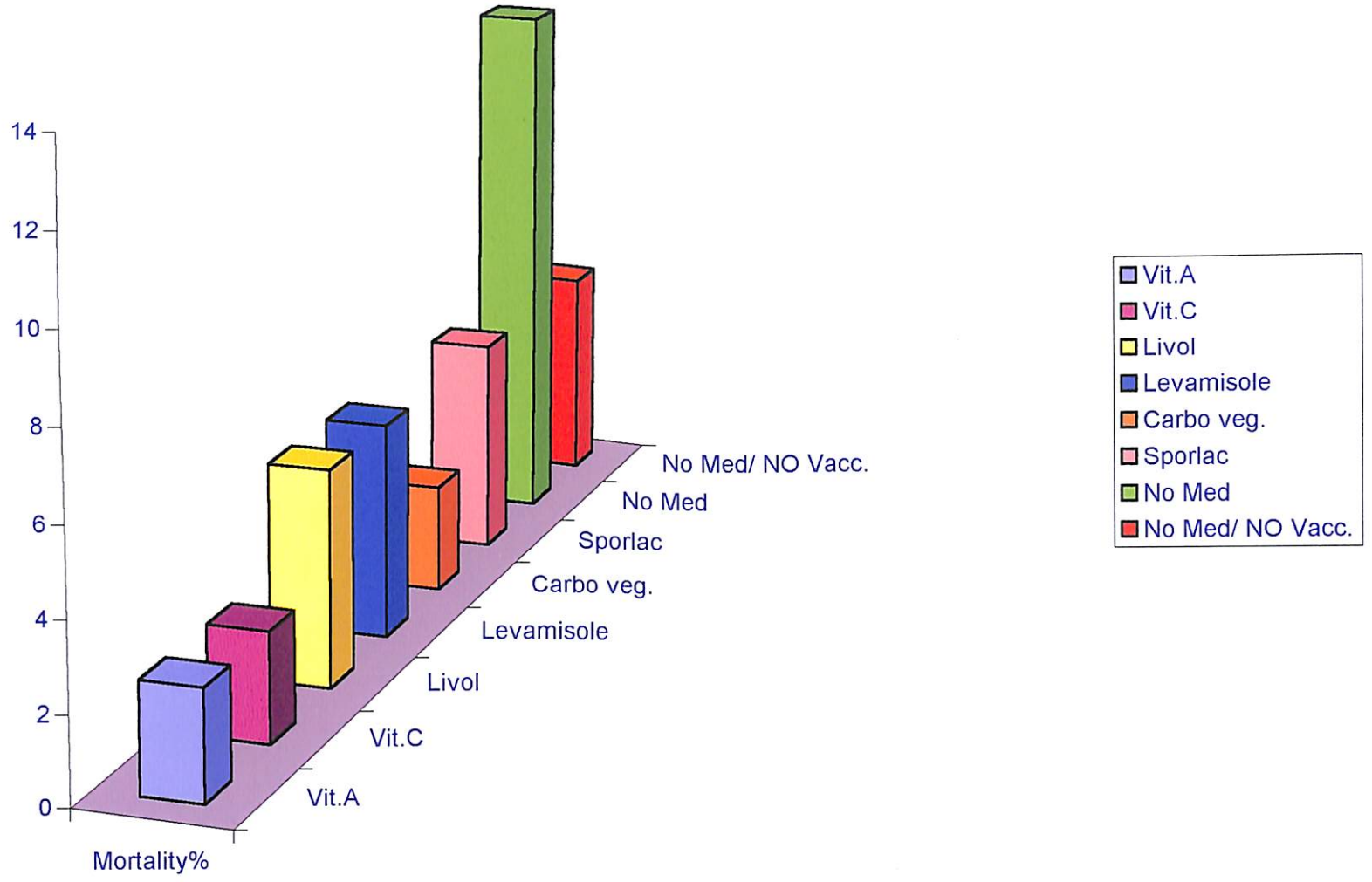
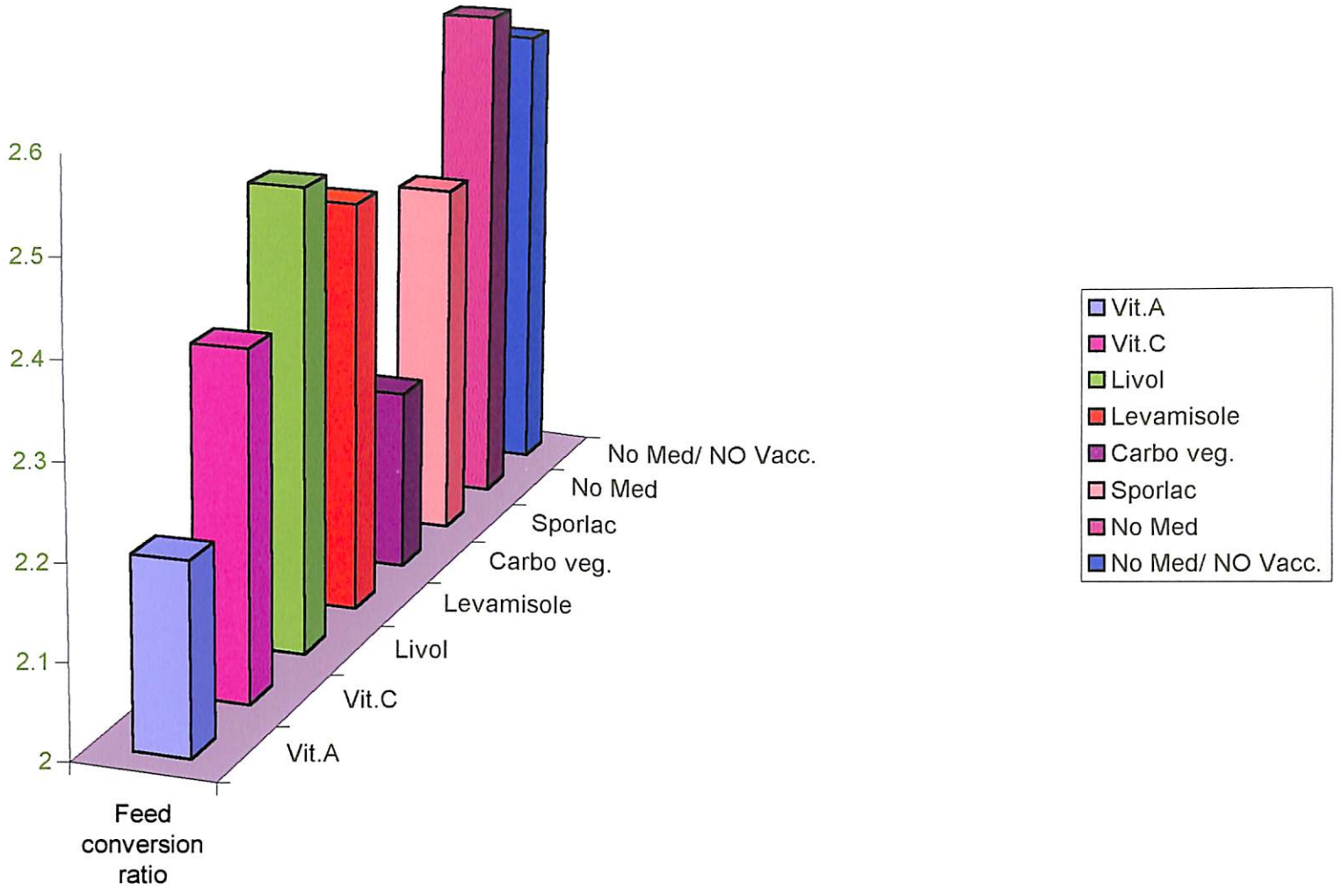
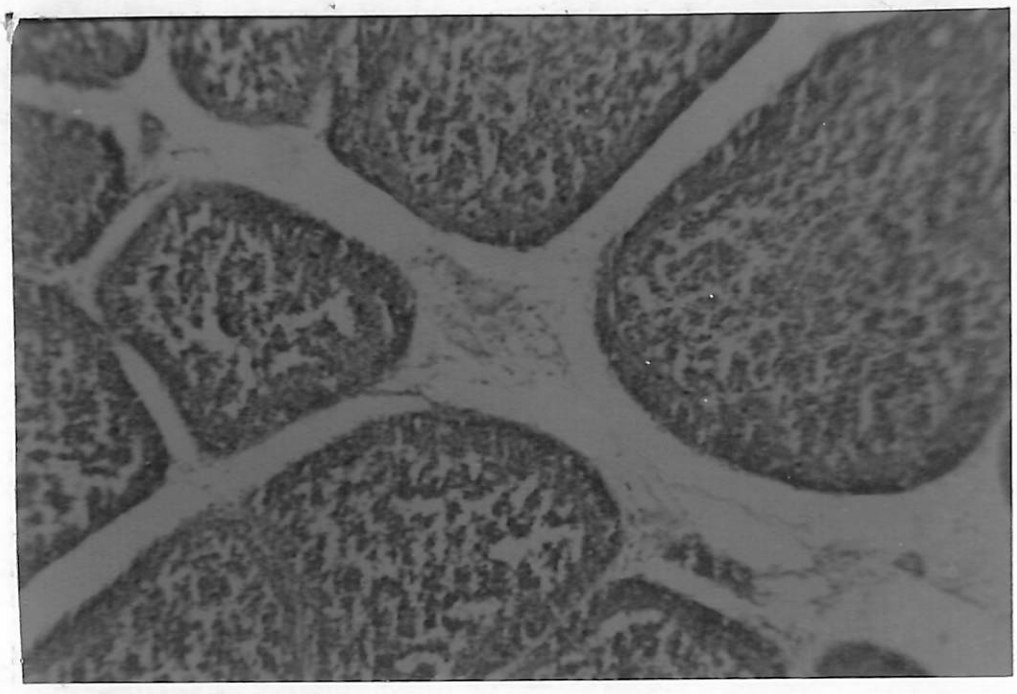
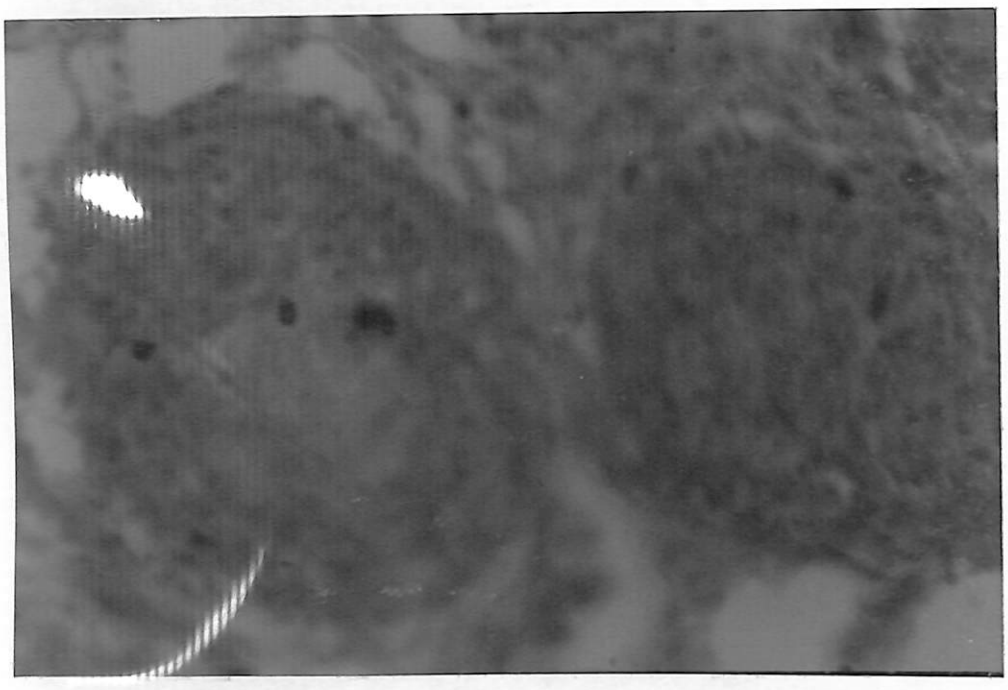
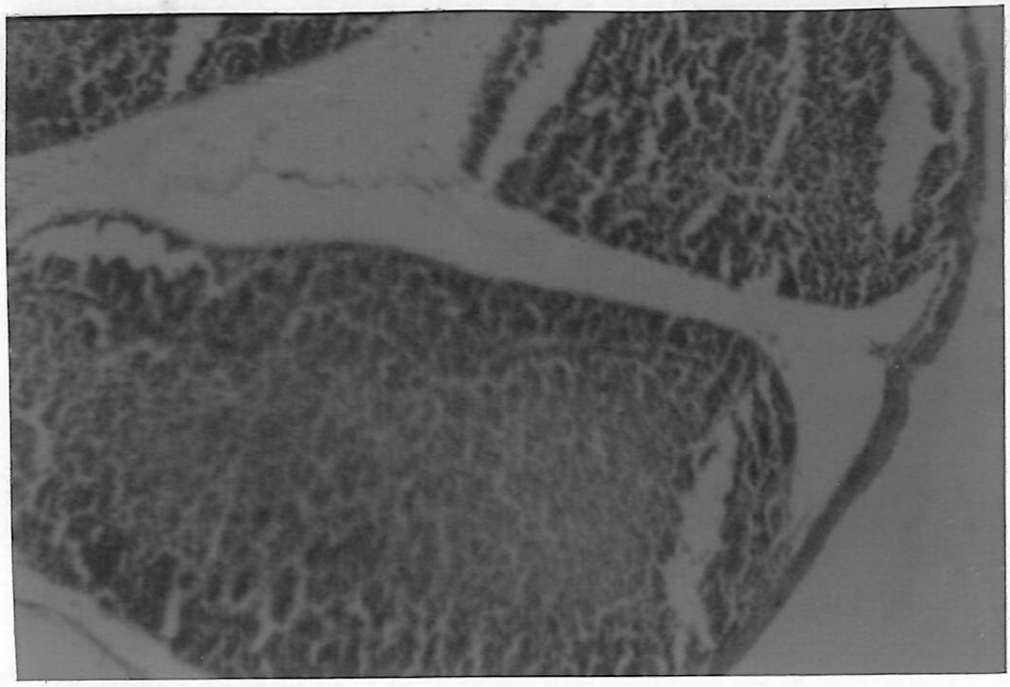


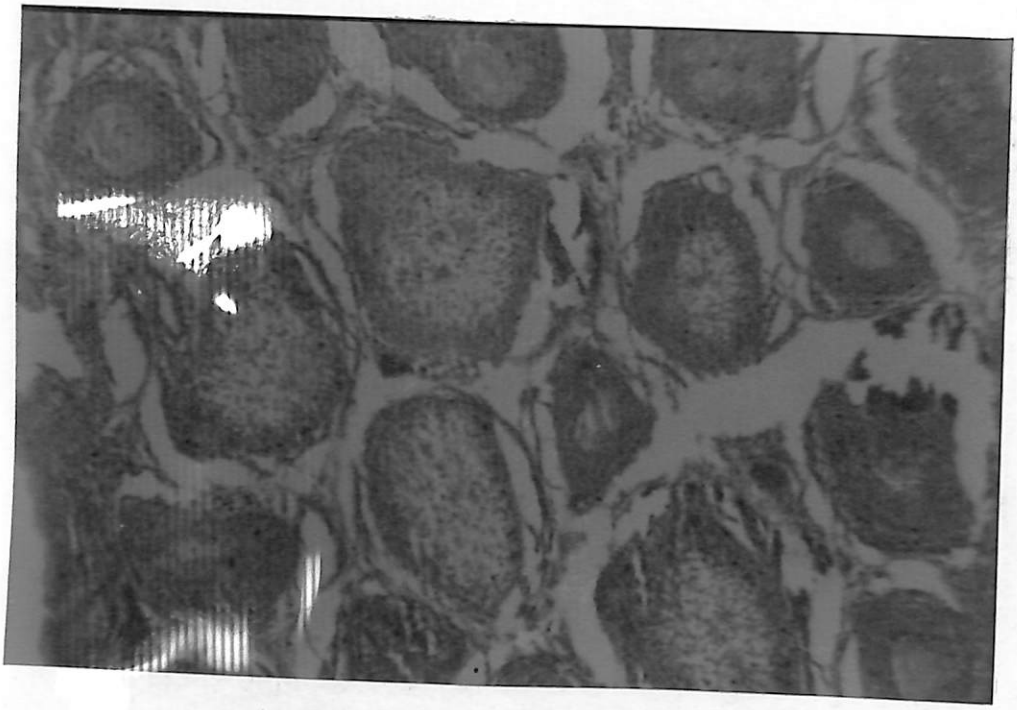
Figure 15:Showing feed conversion ratio in different treatment groups of IBD vaccinated broiler chickens.

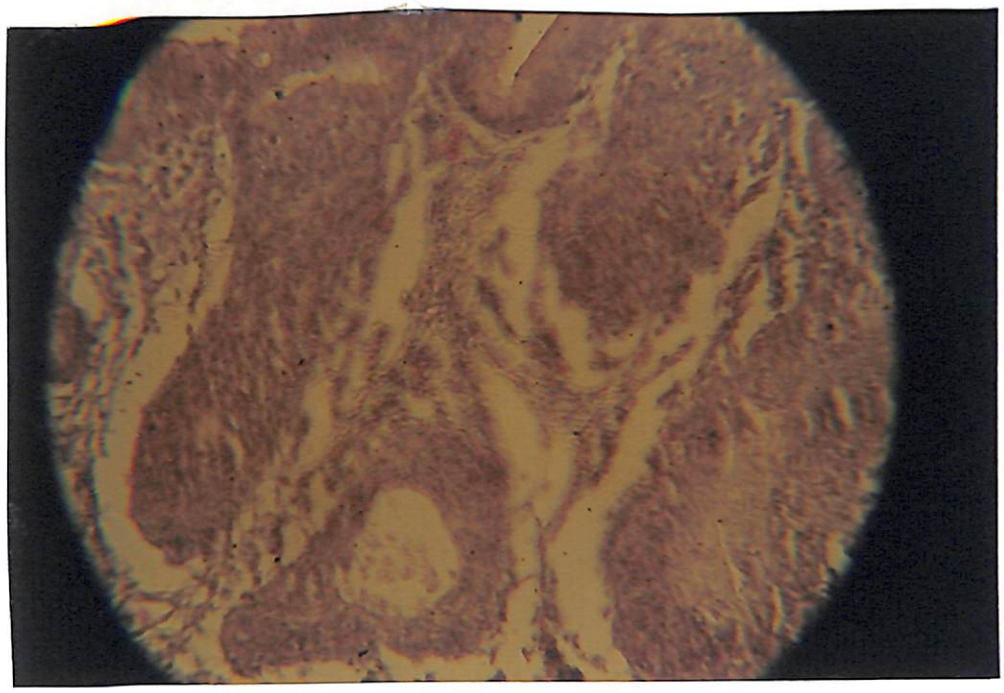


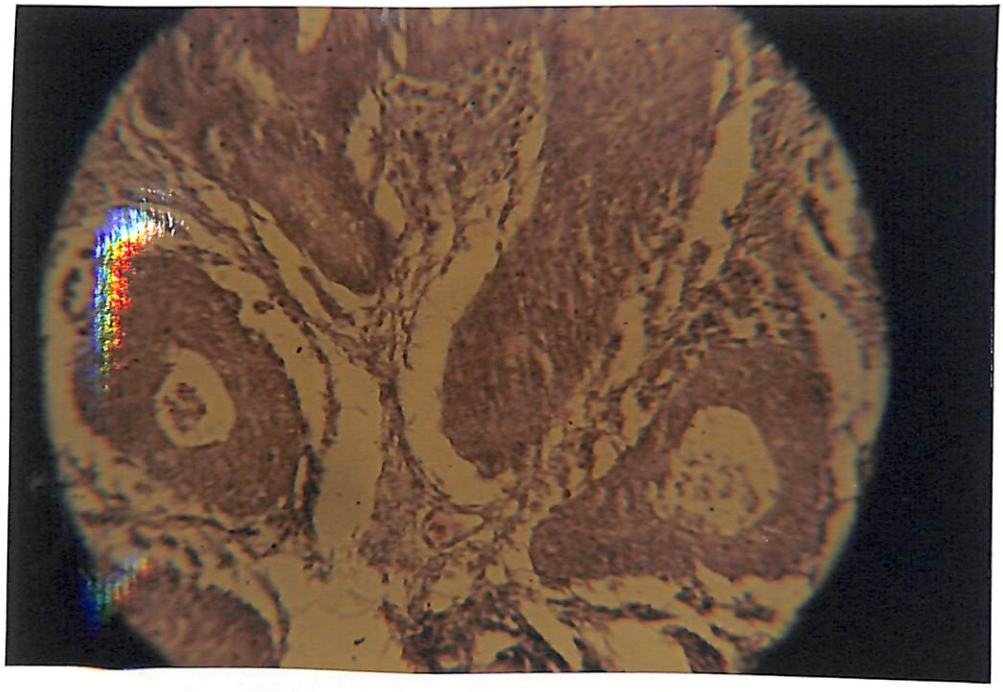


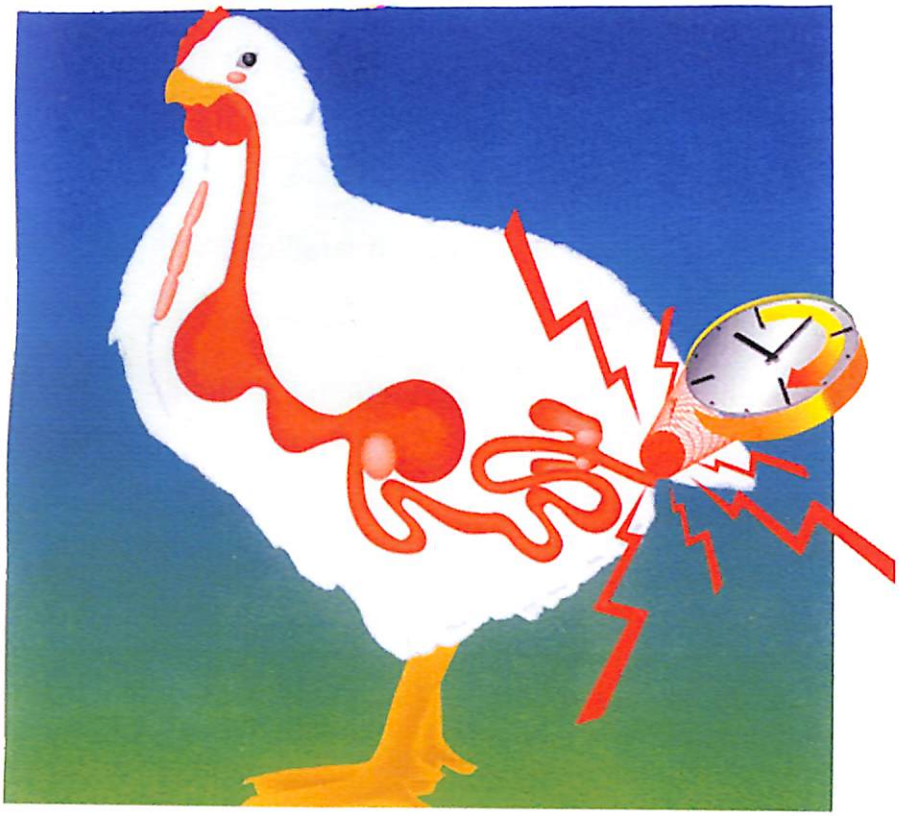












CHAPTER - V

DISCUSSION

DISCUSSION

Infectious bursal disease is a highly contagious viral disease of young ckens that results in immunosuppression and mortality in affected birds. e disease is caused by a double stranded, double-segmented RNA virus onging to the family Birnaviridae. Since the first report of this disease by sgrove (1962), it has been reported from almost all parts of the world. In dia, this disease was reported for the first time by Mohanty *et al.* (1971). e prevalence of a new form of the disease, also called very virulent ectious bursal disease (vvIBD) is being reported from the state of Bihar ce 1991 (Singh *et al.* 1994; Sinha, 1997). These outbreaks were racterized by high morbidity and mortality reaching up to 80 percent and ve. The disease mostly occurred in the age group of 3-8 weeks and in ne cases adult birds of 12 week of age were also affected. Surprisingly se outbreaks are also reported on such farms which had received ermediate strain IBD vaccine at two weeks of age. A number of measures re tried by the owners of different farms with a view to minimise the nomic losses as well as to stop the perpetuation of virus in the rroundings. These measures mainly included repeat vaccination with the ne intermediate strain IBD vaccine in the face of outbreaks as well as ministration of antibiotics, electrolytes, vitamin A, vitamin E, as well as me known immunomodulators like zeetress, levamisole etc. Though these eatments were not performed under controlled condition but there were dications of reduction in number of death and improvement in body weight in. However, the outbreaks due to vvIBD continued unawaited until such ne the new vaccine called invasive Intermediate strain or intermediate plus hot strain vaccine was introduced in this state by the end of the year 1996.

DISCUSSION

Infectious bursal disease is a highly contagious viral disease of young chickens that results in immunosuppression and mortality in affected birds. The disease is caused by a double stranded, double-segmented RNA virus belonging to the family Birnaviridae. Since the first report of this disease by Cosgrove (1962), it has been reported from almost all parts of the world. In India, this disease was reported for the first time by Mohanty *et al.* (1971). The prevalence of a new form of the disease, also called very virulent infectious bursal disease (vvIBD) is being reported from the state of Bihar since 1991 (Singh *et al.* 1994; Sinha, 1997). These outbreaks were characterized by high morbidity and mortality reaching up to 80 percent and above. The disease mostly occurred in the age group of 3-8 weeks and in some cases adult birds of 12 week of age were also affected. Surprisingly these outbreaks are also reported on such farms which had received intermediate strain IBD vaccine at two weeks of age. A number of measures were tried by the owners of different farms with a view to minimise the economic losses as well as to stop the perpetuation of virus in the surroundings. These measures mainly included repeat vaccination with the same intermediate strain IBD vaccine in the face of outbreaks as well as administration of antibiotics, electrolytes, vitamin A, vitamin E, as well as some known immunomodulators like zeetress, levamisole etc. Though these treatments were not performed under controlled condition but there were indications of reduction in number of death and improvement in body weight gain. However, the outbreaks due to vvIBD continued unawaited until such time the new vaccine called invasive Intermediate strain or intermediate plus or hot strain vaccine was introduced in this state by the end of the year 1996.

Though the new vaccine is being used strictly as per the manufacturer's direction, it is also not full proof because a number of complications are being reported on such farms which has used this vaccine. The occurrence of such complications has been correlated with the state of immunosuppressions which may be the consequence of administration of this new vaccine, which is reported to possess relatively more residual pathogenicity as well as invasiveness as compared to available conventional IBD vaccines. The study conducted in this department further confirm the residual pathogenicity and invasiveness inherent in this vaccine (Kumar, 1998). This study also limelighted the immunosuppressive effect of this new vaccine strain. It is in the light of the above study as well as taking into account the earlier reports of residual pathogenicity of the vaccine strain it was felt necessary to look for some alternative approach which could successfully counter the complications that follows this vaccination to make this vaccine more acceptable by the poultry farmers. It is in this backdrop that the present study has been planned to evaluate the efficacy of certain immunopotentiating agents/drugs in controlling the after effects of newly introduced vaccine.

The immune responses to IBD virus vaccine (IV95 strain) in broiler chicken at different intervals post vaccination in different treatment groups are shown in table 2. The perusal of this table revealed that the seroconversion occurred place by 7 day post IBD vaccination. A Number of workers have reported seroconversion between 3-7 days post infection/vaccination (Ley *et al.*, 1983; Das *et al.*, 1991; Thangavelu *et al.*, 1993; Kumar 1998). On the other hand several workers have reported seroconversion by 14 day post vaccination (dpv) when the birds were

carrying maternal antibody (Hirai *et al.*, 1972; Ley *et al.*, 1983; Zorman Rojs *et al.*, 1996; Kumar, 1998). However, in the present study the birds were negative for the presence of Mab at the time of vaccination. Besides, the vaccination in the present case was carried at 12 days of age. Both Mab level and age factor are considered to affect the immune response to IBD vaccine (Gordon and Jordan, 1982; Goddard *et al.*, 1994; Tsai *et al.*, 1995; Kumar *et al.*, 2000). The present finding of seroconversion to IBD vaccine by 7 dpv may be explained on above grounds as the vaccination in the present study was conducted on 12 day of age. Further, the seroconvension which was first detected at 7 dpv showed increasing trend till the last day of observation (47 day) in drug treated groups (group I to VI). But in case of vaccinated but untreated group (group VII) the increase in antibody titre continued only by 28 dpv. The result also demonstrated that the antibody levels in different drug treated groups were invariably higher at all the intervals post IBD vaccination than the titres recorded in group VII for the corresponding intervals. This clearly suggested that all the six agents/drugs employed in this study were effective in enhancing the immune response to IBD vaccine. A number of workers have reported applications of various agents/drugs in IBD infected/vaccinated birds as anti-stress, adoptogenic and immunopotentiator in order to combat the immunosuppressive effect of virus as well as to harness the potentials of vaccines to a maximum level. (Mcllory *et al.*, 1993; Singh *et al.*, 1993; El-Zanty K. 1994; Panda and Rao, 1994; Skalan *et al.*, 1994; Franchini *et al.*, 1995; Rao *et al.*, 1995; Szigeti *et al.*, 1998; Sadekar *et al.*, 1998a; Sadekar, 1998b; Kolte *et al.*, 1999; Saravanabava *et al.*, 1999). The present findings further support the earlier observations.

During the present investigation the AGPT was routinely employed for detection of antibodies and its titre against IBDV. Several workers have reported the application of AGPT for demonstration of antibodies and its level against IBDV (Schneider and Haass, 1969; Hirai *et al.*, 1974; Wyeth and Cullen, 1967; Wilke *et al.*, 1978; Wood *et al.*, 1979). Though a number of workers found merit in ELISA and SNT over AGPT (Nicholas *et al.*, 1985; Solano *et al.*, 1986; Dash *et al.*, 1991; Synder *et al.*, 1992; Desh Pande and Muniyappa, 1996; Mangla gowri *et al.*, 1996), there is general consensus that AGPT is a specific, easy to perform and highly reporducible (Wilke *et al.*, 1978; Dash *et al.* 1991; Thangavelu *et al.* 1993). In the present study also this test was found to be easily reproducible and easy to perform and hence used routinely. In general up to three precipitin lines were discernable when reference antigen and hyperimmune serum were employed. However, only one to two precipitin lines were seen between the central well containing reference antigen and peripheral well containing test serum samples. Whereas, the finest precipitation lines appeared between 16 to 30 hours depending on variation in room temperature and was closer to the antigen well, the second precipitation line generally appeared between 30-48 hours, and was midway between antigen and antiserum wells. The third precipitation line when detected appeared between 48 to 72 hours and was closer to antiserum wells. A number of workers reported similar pattern of precipitin lines in AGPT employing IBD anitigen and antibody (Faragher, 1971; Hirai *et al.*, 1974; Wilke *et al.*, 1978; Takase *et al.*, 1993). However, variations in pattern of precipitin lines with IBD antigen and antibody system have also been reported (Takase *et al.*, 1993). A number of factors determine the pattern of precipitation line including concentration of antigen

and antibody (Wood *et al.*, 1979; Mohanty *et al.*, 1981). Hence, some variations in pattern of precipitin lines are possible.

It may be mentioned that IBD virus has suppressive effect on immune responses to various vaccines whereas normally the response to IBD vaccine itself remain unaffected. Therefore, the use of such immunopotentiating agent for enhancing the response to IBD vaccine may not sound proper. However, in such a scenario where the birds are continuously exposed to several other immunosuppressive agents, infectious or otherwise including one most commonly encountered, the aflatoxin and also where the nutritional requirements of the birds are not provided to the optimum level, it would not be proper to expect getting normal immune responses to vaccines including IBD vaccines. Under such circumstances the relevance of immunopotentiating agent similar to one used in the present study cannot be denied. Besides there are also reports that factors like stress, climatic change and several other known or unknown factors can adversely affect the immune response to vaccines (Sharan and Sharma, 1996). Accordingly, it has become common practice to use some agents of proven immunopotentiating effect as an adjunct to vaccination programme, so that the immune response of the vaccine expresses to fullest level. The relatively better immune responses recorded in different treatment groups (group I - VI) over all the intervals may be due to antistress, adoptogenic and restorative effects of the agents/drugs used.

The role of vitamin A in immunomodulation, growth promotion and enhancement to resistance to diseases has been well documented (Tengerdy, 1975; Tengerdy *et al.*, 1990; Kurtoglu *et al.*, 1996). The above action of this vitamin has been attributed to enhancement of humoral immune response,

and antibody (Wood *et al.*, 1979; Mohanty *et al.*, 1981). Hence, some variations in pattern of precipitin lines are possible.

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phagocytosis and some other mechanism which are hitherto unillustrated. In the present study this vitamin was used mainly to examine its role in encountering the immunosuppressive effects of IV95 IBD virus vaccine by way of enhancement of immune response to RD virus vaccine and improvement in certain growth parameters to be discussed after wards. In the process it was also felt necessary to see that how does this vitamin affects the immune response to IBD vaccine itself. The result amply demonstrated that it has potentiating effect on humoral immune response to IBD vaccines as evident from antibody titres in group I (table 2) overall the periods post IBD vaccination when compared with the antibody titre to group VII for the corresponding intervals.

The application of vitamin C is widely advocated in poultry rearing to counteract the adverse effect of stress in poultry-both broilers and layers (Seokand and Singh, 1996; Daghir, 1996). It is also considered to be a general antioxidant (Murray *et al.*, 1996). The usefulness of this vitamin has also been established in enhancement of immune response to IBD vaccine (Shadaksharappa *et al.*, 1998). Several workers have also reported supplementation of vitamin C in poultry feed or through drinking water to replace the severe loss of this vitamin that is likely to occur during stress. Since, any vaccination exerts some sorts of stress it would be logical to expect higher immune response when vitamin C supplementation is done. It was with this intention that the effect of vitamin C on immure response to IBD vaccine was studied and the result revealed its enhancing effect on immune response to IBD vaccine as evident from antibody titres recorded at different intervals post IBD vaccination when compare with the titres for the corresponding intervals of the control group (table 2, fig 9 a & b). The

present observation is in agreement with the findings observed by (Shadaksharappa *et al.*, 1998). However, the titres observed in vitamin C treated groups were invariably lower than the QAGPT titres recorded in levamisole and carbo veg treated groups. Hence, it would be desirable to ascertain the utility of vitamin C after considering its effect on various parameters to be described later in this text.

There is increasing interest in the use of herbal preparations which may have role in immune enhancement, increasing body resistance and bringing improvement in growth. This is also because the herbal preparations are easily available, cost effective and by and large free from side effects. Livol is an important and widely used herbal preparation given to livestock and poultry to tone up the liver to optimise hepatic functions. It also helps to enhance bile secretion and thereby helps in digestion and assimilation of fat. Now a days this drug is widely used for improving feed conversion efficiency for more weight gain in broilers and higher egg production in layers. The product information bulletin also advocates its utility in counteracting the damaging effects of aflatoxins. The drug has further been suggested for use to increase body resistance as well as to counter debility and general weakness. In the present study efforts were made to study the influence of this drug on immune response to IBD vaccine (IV95 strain). The results indicated higher precipitating antibody titre in livol treated group than the titres recorded in group VII which received IBD vaccine but not any drug treatment over all the periods post vaccination. The comparison of antibody titres between the different drug treated groups (group I to VI) showed lowest antibody titre in livol treated group (table 2, fig 9 a & b). But the very fact that the drug has been helpful in bringing

improvement in precipitating antibody titre to IBD vaccine over all the intervals post vaccination when compared with antibody titres in group VII for the corresponding periods and also that it is easily available, cost effective, almost free from any side effects, provides sufficient evidence to suggest the application of this drug in IBD infected / vaccinated birds. It also proves satisfactory in improving feed efficiency, growth rate as well as response to RD vaccine. It would be also advisable to consider this drug to control IBD scenario after comparing its effect along with other important herbal preparations which are proven immunopotentiator such as – zeetress (Kumar, 1998) stressroak (Anjali, 1997) and like.

Levamisole is a broad spectrum anthelmintic commonly used in veterinary and human medicine. It has been largely used immunopotentiating agent to enhance the immune response of man, livestock and poultry to different vaccines (Panigraphy *et al.*, 1979; Hogarth-Scott *et al.*, 1980; Panda and Rao, 1994; Shadaksharappa *et al.*, 1997; Shadaksharappa *et al.*, 1998). In the present study levamisole treated birds (group IV) exhibited highest precipitating antibody titres to IBD vaccine at all the intervals post vaccination when compared with the titres of other groups (group I to III & V to VII) for the corresponding periods (table 2, fig 9 a & b). Several workers have reported the application of levamisole in IBD vaccinated chickens and have also reported enhancement of immune response in treated groups (Shadaksharappa *et al.*, 1997; Shadaksharappa *et al.*, 1998) which support the present finding. It may be mentioned that in most of the cases levamisole is used in livestock and poultry as an immunomodulator to enhance immune responses to different vaccines in immunocompromised animals. IBD virus is a proven immunosuppressant which

immunocompromises the birds to immune response to several vaccines including RD vaccine and the drug like levamisole is being used in such cases to enhance the antibody level (Singh and Dhawedkar 1994). However, application of this drug for enhancement of antibody titre to IBD vaccine itself is a new approach and needs consideration whether it is at all necessary. This is so because there appears to be dearth of information on failure of immune response to IBD vaccines to an unprotective level. However, such a situation cannot be denied when the birds are immunosuppressed prior to receiving IBD vaccine. Immunosuppression due to aflatoxin and other mycotoxins is widespread and well documented (Allan *et al.*, 1972; Thaxton *et al.*, 1974). There are also reports that immune response to IBD vaccine may be adversely affected in birds which are already immunocompromised due to aflatoxin. (Giambrone *et al.*, 1978). Beside, there are several other immunosuppressive agents to which the birds may be exposed leading to immunosuppression. In all such conditions, the response to IBD vaccine may not be proper when the use of levamisole or similar immunomodulatory agents may have to be considered along with this vaccine. In any case the purpose to use levamisole in the present investigation was to counter the after effects of IV95 strain vaccine which is known to have relatively more residual pathogenicity and immunosuppressive effect. It is in this process that its effects on immune response to IBD was also examined and the drug was found to be immunostimulatory in this regard too. The result obtained is suggestive of the additional benefits of levamisole to which the poultry farmers, who may be using have to be apprised for better acceptability of this drug. The present finding may also be considered encouraging even in a situation when IBD

vaccine could have produced optimum antibody level by itself but after administration of levamisole the titre is enhanced further. It is hoped that such enhancement of antibody level due to levamisole may be helpful in providing better resistance in vaccinated birds so much so that such birds can withstand exposure to even high multiplicity of infection when there are outbreaks of the disease in the surrounding localities. Further, any enhancement in the antibody level over and above the optimal vaccinal response will certainly allow the persistence of protective antibody level for longer period and thus immunity of longer duration which is always welcomed. Accordingly, this finding may have far reaching consequences and hence needs to be highlighted.

Homeopathic medicines have occupied important position in the treatment of a various alignments. The application of this group of medicine have been advocated to cure various conditions in poultry (Patra, 1983; Bera, 1983; Jagtap *et al.*, 1993). Presently some homeopathic drugs have also been employed to combat the adverse effect of vaccines, improve growth condition and counter stresses (Patra, 1983; Jagtap *et al.*, 1993). Though the exact mechanism of action of most of the homeopathic drugs are yet to established it was logical to believe that the homeopathic drugs with indications of improving general vitality, countering stresses and ability to promote growth should work through a mechanism which may also involve immunological component and defence system of the body. It was in this background that homeopathic medicines were considered for use along with IV95 IBD vaccine. The homeopathic drug, carbo vegetabilis used in this study has clearly indicated role in improving general vitality in the body and controlling side effects of various bacterial and viral vaccines

(cowpaerthwaite, 1960; Allen, 1967). The perusal of the result demonstrated the immunopotentiating effect of this drug response to presently used IBD vaccine (table 2, fig 9 a & b). Earlier a homeopathic drug combination, consisting of carbo vegetabilis, carbo anaemalis and thuja occidentalis was employed to study its effect on immune response to interplus IBD vaccine (Hoechst Roussel Pvt. Ltd.) and it was found that this homeopathic drug combination had quite encouraging effect on enhancement of immune response to this vaccine in broiler ohicken (Hindustani, 2000). After this study it was felt necessary to ascertain the effect of individual component of the drug combination and it was in this process this time only carbo vegetabilis component was used for the purpose. Interestingly it was revealed that carbo vegetabilis alone also produced enhancing effect on immune response to invasive intermediate strain IBD vaccine which was even better than the immune response exhibited when this drug had been used in combination with the two other drugs namely thuja occidentalis and carbo anaemalis. Futher, carbo vegetabilis alone produced increasing trends in QAGPT titre till the last day of the observation (35 dpv) whereas the immune response after administration of three drug combination had been studied only upto 28 dpv (Hindustani, 2000). Therefore, the present finding may be considered important because only carbo vegetabilis component of the drug combination employed by the earlier worker (Hindustani loc cit) can be taken to produce the same or even better effect which could be possible after administration of the drug combination. This is so because carbo veg has produced quite encouraging result with respect to other parameters also (to be described later in this text).

Strains of *Lactobacillus* and *Streptococcus* that alter the microbial

species present in the gastro intestinal system to the benefit of the treated animal are selected and included in the probiotic feed additives. In general, the probiotics act through the stimulation of development of desirable bacteria, prevention of development of coli bacteria and inhibition of enterotoxins in the digestive system, which reduces the breakdown of proteins to nitrogen (Fuller, 1977; Mikulec *et al.* 1999). This leads to better utilization of proteins, improved body wt. gain and feed conversion ratio. A number of workers have also reported increase in body resistance, enhancement of immune response of chickens to Newcastle disease vaccine (Ramdan *et al.*, 1999: Gerendai, 1993) and lower mortality rate due to the climination of some pathogens from the intestinal tract (Ramadane *et al.*, 1991: Gerendai, 1993; Mikulli *et al.*, 1999).Khajararen and Ratansethakul(1998) also reported enhancement of both HI titres of Newcastle disease vaccines and ELISA titres to IBD vaccines. They also reported that oral administration of *Lactobacillus casei, Lactobacillus acidophillus* and *Lactobacillus delbrucckii* subsp. *Bulgaricus* have enhanced both the cellular and humoral immune response in mice. In the present study sporlac, which contains *Lactobacillus sporogens* was incorporated in the diet to evaluate its influence on different production parameters in broiler chickens (table 8) as well as its effects on immunological response to IBD vaccine and RD vaccine. The result indicated higher QAGPT titres to IBD vaccine in the group which received sporlac (group VI) when compared with the titres in the IBD vaccinated but untreated group (group VII) for the corresponding intervals (table 2,fig 9 a & b). Though there are no report on application of probiotic for enhancement of immune response to IBD vaccine and it is also fact that IBD virus leads to lower immune response to

several vaccines but for IBD vaccine itself. In such a scenario the application of probiotic for enhancement of immune response to IBD vaccine may be questionable. But the very fact that any type of vaccination produces some sorts of stress, production of stress after IBD vaccination may not be ruled out. Further, stress has adverse effect in optimum manifestation of vaccinal response. A number of workers have reported that every stress, regardless of its cause can result in the increase of a number of undesirable micro-organisms *(Staphylococcus, Streptococcus, Corynebacterium, Enterobacteriaceae)* in digestive system of poultry to the determinant of *Lactobacillus* which ultimately results in disrupted digestion, reduced feed utilization, decline in production parameters, health, lowered resistance and poor immune responses to vaccines. (Suzuki *et al.* 1989, Ramadan *et al.* 1991; Gerendai, 1993). Therefore, the enhancement of immune response to IBD vaccination in sporlac treated group (group VI) may be explained on the above ground. The present finding appears to be interesting on two accounts – (a). there is scope for enhancement of immune response to IBD vaccine and (b). sporlac used as probiotic has immune enhancing effect on response to IBD vaccine. Further, both the dose of probiotic and composition of poultry feed provided to the birds have important bearing on different production parameters as well as immune response to vaccine. Therefore, it is desirable to undertake further study in order that a suitable dose schedule of probiotic as well as poultry ration which are cost effective as well as eco friendly may be determined for on farm application for bringing improvements in immune response to this vaccine.

Immunosuppressive effect of IBD virus in well documented (Faragher *et al.*, 1974; Ajinkya *et al.*, 1980; Nakamura *et al.*, 1992; Prakeen *et al.*,

1995). However, variation in degree of immunosuppression have been reported by a number of workers from time to time (Giambrone and clay, 1986a; Mazariegos *et al.*, 1990). A number of factors such as strain of virus, age of birds at infection as well as size of inoculum have been reported to be responsible for variation in degree of immunosuppression (Ezeokoli *et al.*, 1990; Mazariegos *et al.*, 1990; Mahesh and Muniyappa 1996). Control of IBD by vaccination is in practice worldwide. However, there are a number of reports that even IBD vaccine produce some degree of immunosuppression (Ezeokoli *et al.*, 1990; Mazariegos *et al.*, 1990; Dash *et al.*, 1996). Kumar (2000) also reported that IBD vaccine had lowering effect on HI titre to RDV. In the present study IV95 strain IBD vaccine showed lowering effect on HI titre to RDV (F & LaSota strain) vaccine when compared with the corresponding value of the control group (group VIII) over all the intervals till the termination of experiment (table 3, fig 10 a & b). The recently introduced hot strain IBD vaccine marketed in the name of invasive intermediate strain (IV95), Inter plus, Bursine plus etc are known to have relatively more residual pathogenicity and invasiveness and consequently such strains are more prone for production of immunosuppressive effect of higher magnitude than the conventional vaccines (Kowenhoven and Bos, 1994; Coletti *et al.*, 1994; Survashe, 1996, Khaliel *et al.*, 1998; Kumar 2000). In a situation where even the vaccine strain can have marked immunosuppressive effect as apparent from significantly lower level of antibody titres to RD vaccines in IBD vaccinated birds of group VII when compared with the titres in group VIII (table 3), it would not be out of way to suggest the use of agents/drugs in conjugation with IBD vaccine in order that the after effect of vaccine is prevented or at least minimized without in

any way affecting the response to IBD vaccine. In the present study all the six drugs/agents employed showed immune enhancing effect on antibody response to RD vaccine in IBD vaccinated chickens (table 3, fig 10 a & b). Interestingly, these agents also showed potentiating effect on response to IBD vaccine itself which have been discussed above in this text. The comparison of antibody titres to RD vaccine in the treatment groups (I through VI) revealed that the antibody levels did not differ significantly ($P<0.01$) among themselves. By and large the titres were higher than that recorded in vaccinated group which did not receive any drug (group VII). Further, the comparison of antibody titres of treatment groups with the titres observed in non IBD vaccinated untreated control group (group VIII) showed that whereas all the six drugs had different degree of immunopotentiating effect on response to RD vaccination, none of the treatment group exhibited antibody titres which could have been comparable to the titres in group VIII at any interval post IBD vaccination. In other words none of the immunopotentiating agents was able to bring improvement in antibody level to RD vaccine at par with the levels of antibody shown by non IBD vaccinated control birds (group VIII). A number of workers have reported that in general the immunomodulators have enhancing effect on immune response to vaccines in immunocompromised animals but in most of the cases they failed to bring the antibody level comparable to one observed in immunocompetent animals (Singh *et al.*, 1993; Saravanbava *et al.*, 1999) which support the present findings.

In the present study all together six drugs were evaluated for their immunopotentiating effect on response to RD vaccine in birds receiving hot strain IBD vaccine (IV95 strain). The levamisole showed the best immune

enhancing effect on HI antibody titre to RD vaccine followed by carbo veg vit A as well as sporlac, vit C and livol. As the titres in the different treatment groups did not differ significantly ($P<0.01$) among them selves and also that the titres were always higher than the protective level (2^4). All the six drugs should be acceptable subject to the cost effectiveness, duration of treatment and case of availability. It may also be mentioned that the investigation conducted so far with the respect to the mechanism of action of these have largely thrown light on mode of action of levamisole, vit A, sporlac, vit C and livol. However, the use of homeopathic drugs to control immunosuppressive effect of IBD virus is rarely reported and therefore, it would be advisable to undertake further study to suggest its mechanism of action. In the mean time the present study is clearly indicative of the fact that the drugs used have immune enhancing effects and therefore, may be advised to poultry farmers for use in chicks to be vaccinated with IBD vaccine especially intermediate plus strain of IBD vaccine or IV95 strain.

Pathological changes and lesion score proved a very sound criteria for determining the virulence of IBD virus (Naqi *et al.*, 1980; Mazariegos *et al.*, 1990). The same criteria have also been extensively used to assess the residual pathogenicity of vaccine strain of IBD virus (Mazariegos *et al.*, 1990; Khaliel *et al.*, 1998). The microscopic changes noted in the bursa of vaccinated chickens were characteristic of IBD virus (Winterfield *et al.*, 1980; Ezeokoli *et al.*, 1990). In the present study lymphoid necrosis and depletion in the bursa of fabricius constituted the predominant lesions. Several workers have recorded both necrosis and depletion in the bursa of fabricius as the main lesions in IBD infected/vaccinated birds which corroborates the present findings (Ajinkya *et al.*, 1980; Ley *et al.*, 1983;

Lukert and Hitchner, 1984, Jhala *et al.*, 1990; Khafagy *et al.*, 1991). The other changes recorded during this study such as interfollicular oedema, epithelial invegination, vacuolation in plical cells, hyperplasia, cellular infiltration (table 6 & 7, Fig 11&12) have also been reported by one or other workers from time to time (Panigraphy *et al.*, 1986; Del Bono *et al.*, 1968; Lukert and Hitchner, 1984). Whereas the histological changes recorded in the bursa of fabricius were clearly suggestive of the possession of residual pathogenicity in the IV95 strain IBD vaccine virus (Kouwenhoven and Bos, 1994; Khaliel *et al.*, 1998; Kumar, 2000), in none of the groups the clinical signs and symptoms typical of IBD virus could be observed. Thronton and Pattison (1975) also studied nine IBD vaccines obtained from seven different sources and found that each of the vaccines invariably produced bursal damage of varying degree as evidenced by histopathological changes but none of them produced clinical disease. Histopathological examination of section of liver, kidney and spleen did not reveal any changes. A number of workers reported mild changes in non bursal organs in IBD infected birds (Aziz, 1985; Singh, 1987; Sah *et al.*, 1995). However, Ley *et al.*, (1983) reported that the changes observed in non bursal organs were non specific. In the present study liver, kidney, spleen did not reveal any significant ($P<0.01$) changes which supported the earlier observations by a number of workers (Bos, 1994; Khaliel *et al.*, 1998; Kumar 2000).

Several workers have studied the body weight gain and feed conversion ratio in order to find out the over all response of IBD vaccine in chicken. (McIlroy *et al.*, 1993; Tengerdy, 1975; Rao *et al.*, 1995). In the present study also the birds which have received only IBD vaccine but no any drug (group VII) exhibited lower body wt. gain and higher FCR

findings may be explained on the basis of the ground reality that in a situation where there is continuos risk of exposure of animals to immunosuppressants like aflatoxin, different types of stress, nutritionally deficient feed formulations as well as different types of infections, the normal expression in terms of body weight gain, FCR and so on may not be possible on every occasions.

The present study finally suggested that there is scope of further improvement in immune response to IBD vaccine. The study also revealed that the vaccine virus has residual pathogenicity and immunosuppressive effect as evidence by immune response to RD vaccine, bursal lesion score, body weight gain, FCR and percent mortality. Further, it was also observed that by application of different drugs/agents it is possible to bring improvement in respect of above parameters. Further all the six drugs used in this study have immunopotentiating effect but levamisole appeared to be the most suitable in terms of immune response to IBD vaccine and RD vaccine in IBD vaccinated chicks followed by the homeopathic medicine, carbo veg vit A and sporlac, vit C and livol. Further, when the effects of levamisole and carbo veg were compared in terms of body weight gain, FCR and percent mortality, carbo veg was found to be better than even levamisole.On over all consideration the homeopathic drug, carbo veg may be considered to be most acceptable and it may be recommended for use by poultry farmers as measure to control the after effect of IBD vaccine especially the moderate "hot" vaccine like one used in the present study. This is so because in terms of immune response to IBD and RD vaccine it was found next to levamisole but in terms of its effect on economic parameters like body weight gain, FCR, the results obtained in carbo veg

treated group was most encouraging. Further, it may also be advisable to keep this comment reserved till such time similar study with levamisole is conducted using different dose rates and regimen.

On the other hand, since all the six drugs employed in this study proved to be quite effective in reducing the immunosuppressive effect of IBD vaccine in terms of immune response to RD vaccine, body weight gain, FCR and percent mortality, bursal lesion score, any of the drugs may be recommended for use by poultry farmers depending on local availability, cost effectiveness and its eco-friendly potentials. However, as body weight gain and FCR are two very vital components of poultry farming which determine the profitability and carbo veg having a profound effect on these two parameters, the preference for recommending this drugs in controlling the after effect of IBD vaccine is but natural.

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