EFFECT OF SELENIUM ON IMMUNE RESPONSEAND SUSCEPTIBILITY OF VACCINATED COCKERELS TO INFECTIOUS BURSAL DISEASE

BY

Audu, SHEKARO

DEPARTMENT OF VETERINARY PATHOLOGY,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

AUGUST, 2012

EFFECT OF SELENIUM ON IMMUNE RESPONSE AND SUSCEPTIBILITY OF VACCINATED COCKERELS TO INFECTIOUS BURSAL DISEASE

BY

Audu, SHEKARO
DVM ABU (1994)
MSc/VET-MED/14754/2007-2008

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN VETERINARY PATHOLOGY

**DEPARTMENT OF VETERINARY PATHOLOGY,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA**

AUGUST, 2012

DECLARATION

I, Audu SHEKARO, hereby declare that this thesis titled "**EFFECT OF SELENIUM ON IMMUNE RESPONSE AND SUSCEPTIBILITY OF VACCINATED COCKERELS TO INFECTIOUS BURSAL DISEAS**" was carried out by me in the Department of Veterinary Pathology, Ahmadu Bello University Zaria under the supervision of Prof. S.B. Oladele, Prof. P.A. Abdu and Prof. N.D.G. Ibrahim.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at any University.

Audu SHEKARO _____ _____
(Name of student) Signature Date

CERTIFICATION

This thesis titled "**Effect of selenium on immune response and susceptibility of vaccinated cockerels to infectious bursal disease**" by Audu SHEKARO, meets the regulations governing the award of the degree of Master of Science (Veterinary Pathology) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Prof. S.B. Oladele, D.V.M, M.Sc., PhD (A.B.U) _____ _____
Chairman, Supervisory Committee Signature Date

Prof. P.A. Abdu, D.V.M, M.Sc., PhD (A.B.U) _____ _____
Member, Supervisory Committee Signature Date

Prof. N.D.G. Ibrahim, D.V.M, M.Sc., PhD (A.B.U) _____ _____
Member, Supervisory Committee Signature Date

Dr. B. Mohammed, DVM, M.Sc., PhD (A.B.U) _____ _____
Head, Department of Veterinary Pathology, Signature Date
Ahmadu Bello University, Zaria

Prof. A, A, Joshua, BA, MA., PhD` (ABU) _____ _____

Dean, School of Postgraduate Studies Signature Date
Ahmadu Bello University, Zaria

DEDICATION

This research work is dedicated to the Omnipotent God and my family.

ACKNOWLEDGMENTS

My profound gratitude goes to God Almighty whose mercy saw me through this research work, to Him be the glory.

I wish to express my sincere and profound gratitude to my major supervisor, Prof. S.B. Oladele, who gave me not only the professional but also brotherly advice and guidance throughout the duration of this work. Although I was a little bit slow for him yet I never exhausted his patience in guiding me through. I thank him for his accessibility and patience.

I am also very grateful to Profs. P.A. Abdu and N.D.G. Ibrahim who as members of my supervisory team were always available and willing to assist me in every way possible despite their tight schedule and other commitments. I appreciate all my postgraduate lecturers for preparing me for this work through the knowledge they imparted on me during lectures periods.

My appreciation also goes to Mr. Bitrus Bako of the Department of Pathology and Microbiology and Mr. Adetiba Bamidele of the Department of Human Anatomy; they processed all my histopathological slides. Worth mentioning is Mrs Deborah M. Anueyiagu of the Clinical Pathology Laboratory, National Veterinary Research Institute, Vom, who assisted me in the haematological analysis. Thanks also to all the staff of Viral Research Department, National Veterinary Research Institute, Vom for their technical assistance and cooperation.

I wish to also extend my gratitude to Prof. U.A. Lar of the Department of Geology and mining, University of Jos for allowing me access to the Departmental Laboratory and Machine to analyse the trace element of interest. Thanks also to the technical staff of that Department, Mr. Wudima I. Marcus and Mrs Makplang Daret for their cooperation and assistance.

I appreciate my nephew, Mr. James B. Ndock who took care of the experimental birds. He worked round the clock just to make sure that the experiment was successful. I am grateful to my employer, the National Veterinary Research Institute Vom, for affording me an opportunity to further my education which gave birth to this work. Thanks to all my colleagues in Kaduna Outstation, for absorbing some of my responsibilities while I was away for this work.

I appreciate my dear wife, Mrs Hwonghei C.D. Shekaro, who stood by me and allowed me to use the family meagre resources to sponsor this research. Thanks also to my three children, Master Jinom A.D. Shekaro, Master Nomsi A.D. Shekaro and Miss Fendok A.D. Shekaro for sacrificing some little comfort they would have enjoyed, just for this work.

Thanks to my younger brothers, Mr. Monday Shekaro and Mr. Ishaya Shekaro for all their moral support, love and encouragement.

Last but not the least, I am grateful to the Postgraduate Fellowship of the Fellowship of Christian Students, Ahmadu Bello University Zaria, for their spiritual and moral support.

To God be the glory for His faithfulness, I owe Him my love and allegiance.

ABSTRACT

Selenium (Se) is a micronutrient that has been used to stimulate the immune system in animals. Infectious bursal disease (IBD) outbreaks in spite of vaccination are a common occurrence in poultry production in Nigeria. This study was conducted to determine the effect of Se supplementation on immune response and resistance of cockerels to IBD. In the experiment, 300 Dominant black marshal cockerels (DBMC) were divided into 12 groups of 25 birds each and fed graded levels of dietary Se. Groups A, B, C received 0.1 ppm, 0.5 ppm and 1.0 ppm of Se in feed, respectively. They were vaccinated with IBD vaccine (M.B strain) at 1 and 3 weeks old and challenged with IBD virus (IBDV) at 6 weeks old. Groups D, E, F received 0.1, 0.5 and 1.0 ppm of Se in feed, respectively; they did not received IBD vaccine, but were challenged at 6 weeks old with IBDV. Groups G, H, I received 0.1, 0.5 and 1.0 ppm of Se respectively, received IBD vaccine at 1 and 3 weeks old, but were not challenged with IBDV. Group J was not supplemented with Se but vaccinated with IBD vaccine at 1 and 3 weeks old but not challenged with IBDV. Group K was not supplemented with Se, not vaccinated with IBD vaccine and not challenged with IBDV. Group L did not receive Se supplementation, not vaccinated against IBD, but challenged with IBDV at six weeks old. Selenium concentration in the feed and tissues sample was determined using inductively coupled plasma optical spectroscopy. Antibody titre was determined using a commercial ELISA kit. Mortality and Protective rates in the challenged birds were calculated. Gross and microscopic lesions were scored. Haematological parameters were determined using auto haematology analyzer. Biuret method was used to determine total protein. After graded levels of Se supplementation for 10 weeks, there was no correlation between the dose of Se in feed and its concentration in the liver, muscles, blood and serum, but there was a positive correlation ($r = 0.94$) in the kidneys. Increase in dietary Se concentration led

to increase in antibody titres. There was a negative correlation (r = - 0.93) in the vaccinated and (r = -0.95) in unvaccinated chicks between the concentration of Se in feed and mortality rate in chicks challenged with IBDV. There was a positive correlation between Se concentration in feed and haematological parameters. Lesions were severe in birds that received lower doses of Se than those that received higher doses. It was concluded that Se supplementation in feed enhances the immune response of DBMC to IBD and it also reduces the severity of the disease. Selenium supplementation in poultry feed was therefore recommended for industrial trial.

.

TABLE OF CONTENTS

Cover page i

Title page..... ii

Declaration..... iii

Certification..... iv

Dedication.....v

Acknowledgements..... vi

Abstract.....viii

Table of Contents..... x

List of Figures.....xv

List of Tables..... xvii

List of Plates..... xviii

List of Abbreviations..... xix

List of Appendices..... xxiii

CHAPTER 1

INTRODUCTION

1.0 Background..... 1

1.1 Statement of research problem.....3

1.2 Justification of the study.....3

1.3 Aim of the study.....4

1.4 Objectives of the study.....4

1.5 Hypothesis.....5

CHAPTER 2

LITERATURE REVIEW

2.1 Poultry sub-sector: an overview.....6

2.2 Selenium.....6

2.2.1 Historical Background of Selenium.....6

2.2.2 Forms of Selenium and their uses in Poultry.....9

2.2.3 Biological roles of Selenium.....9

2.2.4 Selenium Metabolism and Bioavailability.....10

2.2.5 Tissue Distribution and Concentration of Selenium in the Body.....11

2.2.6 Selenium Excretion from the Body.....13

2.2.7 Selenium Deficiency.....13

2.2.8 Selenium Toxicity.....21

2.2.9 Selenium and the Immune System.....22

2.3 Infectious Bursal Disease.....24

2.3.1 Historical Background of Infectious Bursal Disease.....25

2.3.2 Prevalence of Infectious Bursal Disease.....28

2.3.3 Aetiology of Infectious Bursal Disease.....28

2.3.3.1 Types and Subtypes of Infectious Bursal Disease Virus.....29

2.3.3.2 Biological Properties of Infectious Bursal Disease Virus.....30

2.3.3.3 Physiochemical Properties of Infectious Bursal Disease Virus.....31

2.3.3.4 Antigenic Properties of Infectious Bursal Disease Virus.....33

2.3.3.5 Replication of Infectious Bursal Disease Virus.....37

2.3.4 Hosts of Infectious Bursal Disease Virus.....40

2.3.5 Pathogenesis of Infectious Bursal Disease Virus.....40

2.3.6 Infectious Bursal Disease Virus and Immunity.....44

2.3.6.1 Effect of Infectious Bursal Disease Virus on Innate Immunity.....44

2.3.6.2 Effects of Infectious Bursal Disease Virus on Humoral Immunity.....44

2.3.6.3 Effect of Infectious Bursal Disease Virus on Cellular Immunity.....45

2.3.7 Immunology of Infectious Bursal Disease Virus.....46

2.3.8 Immunosuppression during Infectious Bursal Disease Virus Infections.....49

2.3.9 Apoptosis in Infectious Bursal Disease Virus Infection.....51

2.4 Clinical Signs of Infectious Bursal Disease.....53

2.5 Pathology of Infectious Bursal Disease.....54

2.5.1 Gross Lesions.....54

2.5.2 Microscopic Lesions of Infectious Bursa Disease Infection.....55

2.6 Diagnosis of Infectious Bursal Disease.....57

2.6.1 Clinical Signs.....57

2.6.1 Virus Isolation.....58

2.6.2 Serology.....59

2.6.2.1 Enzyme Link Immune Sorbent Assay.....59

2.6.2.2 Viral Neutralization Test.....59

2.6.2.3 Agar Gel Precipitating Test.....60

2.7 Differential Diagnosis of Infectious Bursal Disease.....60

2.8 Management of Infectious Bursal Disease.....61

2.8.1 Prevention and Control of Infectious Bursal Disease.....61

2.8.1.1 Vaccination against Infectious Bursal Disease.....61

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Chickens and Rearing.....69

3.2 Vaccines.....70

3.3 Feeds.....70

3.4 Infectious Bursal Disease ELISA Kits.....70

3.5 Sample Collection.....70

3.6 Selenium Analysis in Tissues, Feed and Blood.....71

3.7 Infective Bursal Disease Virus.....72

3.8 Measurement of Antibody Titre to Infectious Bursal Disease Vaccine.....72

3.9 Determination of Mortality and Protective Rates.....73

3.10 Pathology and Lesion Score.....73

3.10.1 Gross Lesion Score.....73

3.10.2 Histopathology Lesion Score.....74

3.11 Determination of Haematological Parameters.....75

3.11.1 Red Blood Cell Count, Packed Cell Volume and Haemoglobin Concentration.....75

3.11.2 Differential Leukocyte Count.....75

3.11.3 Determination of Total Protein.....76

3.12 Statistical Analysis.....76

CHAPTER 4

RESULTS

4.1 Selenium Concentration in Feed and Tissues..... 77

4.2 Serum Antibody Titre to Infectious Bursal Disease Vaccine.....81

4.3 Selenium Supplementation and Mortality Rates.....81

4.4 Graded Levels of Selenium Supplementation and Protective rate of Infectious Bursal Disease Vaccine.....86

4.5 Gross Pathology.....86

4.5.1 Bursa of Fabricius.....86

4.5.2 Kidneys.....86

4.5.3 Liver.....92

4.5.4 Leg Muscles.....92

4.5.5 Pectoral Muscles.....92

4.5.6 Proventriculus.....99

4.6 Gross Lesion Score.....103

4. 7 Histopathology.....105

4.8 Haematology.....111

CHAPTER 5

DISCUSSION

Discussion.....119

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary.....127

6.2 Conclusions.....127

6.3 Recommendations.....127

REFERENCES.....128

LIST OF FIGURES

FIGURE	TITLE	PAGE
Figure 4.1	Mean concentration of selenium (ppm) in the tissues of day old Dominant black marshal cockerels	79
Figure 4.2	Mean selenium concentration in the tissues of Dominant black marshal cockerels after dietary supplementation for ten weeks	80
Figure 4.3	Infectious bursal disease antibody titres in selenium supplemented (vaccinated and unvaccinated) Dominant black marshal cockerels challenged at 6 weeks old	83
Figure 4.4	Selenium supplementation and cumulative mean antibody titre to infectious bursal disease vaccine on Dominant black marshal cockerels vaccinated at 1st and 3rd week of age	84
Figure 4.5	Graded levels of selenium supplementation and mortality rate of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus	85
Figure 4.6	Graded levels of selenium supplementation and the protective rate of infectious bursal disease vaccine on Dominant black marshal cockerels infected with infectious bursal disease virus	87

Figure 4.7 Selenium supplementation and the gross pathology of bursa of Fabricius of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and infected with infectious bursal disease virus.....88

Figure 4.8 Selenium supplementation and gross pathology of kidneys of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and infected with infectious bursal disease virus.....91

Figure 4.9 Selenium supplementation and gross pathology of liver of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and infected with infectious bursal disease virus.....93

Figure 4.10 Selenium supplementation and gross pathology of leg muscles of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and infected with infectious bursal disease virus.....94

Figure 4.11 Selenium supplementation and gross pathology of the pectoral muscle of Dominant black marshal cockerels vaccinated with infectious bursal diseases vaccine and infected with infectious bursal disease virus.....95

Figure 4.12 Selenium supplementation and gross pathology of proventriculus of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus.....100

Figure 4.13 Gross pathological lesions scores in vaccinated and unvaccinated Dominant black marshal cockerels with and without selenium supplementation, infected with infectious bursal diseas virus.....104

Figure 4.14 Graded levels of selenium supplementation and red blood cell count of Dominant black marshal cockerels.....113

Figure 4.15 Graded levels of selenium supplementation and haemoglobin concentration of Dominant black marshal cockerels.....114

Figure 4.16 Graded levels of selenium supplementation and packed cell volume of Dominant black marshal cockerels.....115

Figure 4.17 Graded levels of selenium supplementation and percentage lymphocyte Of Dominant black marshal cockerels.....116

Figure 4.18 Graded levels of selenium supplementation and percentage heterophil of Dominant black marshal cockerels.....117

x

Figure 4.19 Graded levels of selenium supplementation and total blood protein of Dominant black marshal cockerels.....118

LIST OF TABLES

TABLE	TITLE	PAGE
Table 3.1	Treatment groups as it relates to dose of selenium supplemented, age at which infectious bursal disease vaccine was given and age at which the birds were challenged	74
Table 4.1	Levels of selenium concentration in four commonly used commercial chick	78
Table 4.2	Antibody titres to infectious bursal disease in day old Dominant black Marshal cockerels before selenium supplementation	82
Table: 4.3	Haematological parameters and total blood protein of day old Dominant Black marshal cockerels	112

LIST OF PLATES

PLATE	TITLE	PAGE
Plate I	Bursa of Fabricius and kidneys of a chick from group E (selenium supplemented at dose of 0.5 no vaccination, but challenged with infectious bursal disease virus at 6 weeks).	89
Plate II	Incised bursa of Fabricius from a chicken from group L (no Se supplementation but challenged with infectious bursal disease virus at six week of age).	90
Plate III	Thigh and leg muscle of chick from group B (Birds received 0.5 ppm of Se, they were vaccinated and later challenged with infectious bursal disease virus at 6 weeks)	96
Plate IV	Leg and pectoral muscles of a chicken from group E (Se supplemented at a dose of 0.5 ppm, not vaccinated with infectious bursal disease vaccine, but challenged with infectious bursal disease virus at 6 weeks)	97
Plate V	Leg and pectoral muscle of chick from group (No Se supplementation, no	

vaccination but challenged with infectious bursal disease virus at 6 weeks).....98

Plate VI Proventriculus of a chick from group C (birds received 1 ppm of Se, they were vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus at 6 weeks of age). ……101

Plate VII Proventriculus of a chick from group F (birds recieved 1 ppm of Se, they were not vaccinated with infectious bursal disease vaccine, but challenged with infectious bursal disease virus at 6 weeks). 102

.

Plate VIII Photomicrograph of the normal bursa of Fabricius from uninfected Group.....106

Plate IX Photomicrograph of bursa of Fabricius from group B, vaccinated and Supplemented with 0.5 ppm of Se and challenged. 107

Plate X Photomicrograph of bursa of Fabricius from group A (vaccinated and supplemented with 0.1 ppm 0f Se).....108

Plate XI Photomicrograph of bursa of Fabricius from group E (not vaccinated but supplemented with 0.5 ppm 0f Se).....109

Plate XII Photomicrograph of the bursa of Fabricius from group L, (not vaccinated, not supplemented but challenged) …..……..……...110

LIST OF ABBREVIATIONS

AGPT	Agar gel precipitating test
AIDS	Acquire immune deficiency syndrome
ATSDR	Agency for toxic substance and drug registry
BF	Bursa of Fabricius
BSV	Blotched snakehead virus
C	Congestion
CE	Chicken embryo
CEB	Chicken embryo bursa
CEF	Chicken embryo fibroblast
CEK	Chicken embryo kidney
CMI	Cell mediated immunity

CPE	Cytopathic effect
Cscl	Celsium chloride
CY	Cyclophosphomide
DBMC	Dominant black marshal cockerel
ED	Exudative diathesis
EDTA	Ethylene diamine tetra acetic acid
EH	Ecchymotic haemorrhage
EID	Embryo infective dose
ELD	Embryo lethal dose
ELISA	Enzyme link immune sorbent assay
FAO	Food and Agriculture Organization
FDA	Food and drug administration
GDP	Gross domestic product
GMT	Geometric mean titre
GI	Gastrointestinal tract
GSH-Px	Glutathione peroxidase
H	Hour
HET	Heterophil
HGB	Haemoglobin
HIV	Human immunodeficiency virus
IB	Infectious bronchitis
IBA	Infectious bursal agent
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
IBV	Infectious bronchitis virus
ICPOES	Inductively couple plasma optical emission spectroscopy
ID	Iodothyronine deiodinase

IF	Immuno florescence
IM	Intramuscular
IP	Intraperitoneal
IPNV	Infectious pancreatic necrotic virus
IV	Intravenous
KD	Kidney
LM	Leg muscle
LV	Liver
LYM	Lymphocyte
M	Minute
MAP	Mitogen activated protein
MD	Marek's disease
MDA	Maternally derived antibody
MDV	Marek's disease virus
Ml	Millilitres
mRNA	Messenger RNA
N	Normal
ND	Newcastle disease
NDV	Newcastle disease virus
NFkB	Nuclear factor-koppa B
NO	Nitric oxide
NPA	Nutritional pancreatic atrophy
NRC	National research council
PBL	Peripheral blood lymphocyte
PCR	Polymerase chain reaction
PCV	Packed cell volume
PH	Petechial haemorrhage

PI	Post infection
PM	Pectoral muscle
PPM	Parts per million
PR	Proventriculus
PVJ	Proventriculus- ventriculus junction
RBC	Red blood cell
RDA	Recommended daily allowance
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT PCR	Real time polymerase chain reaction
Se	Selenium
SEC	Selenocystein
SeMet	Selenomethione
SPF	Specific pathogen free
TP	Total protein
tRNA	Transfer RNA
UGA	Uracil guanine adenosine
USA	United State of America
UV	Ultraviolet
VN	Viral neutralization
VP	Viral protein
vvIBDV	Very virulent infectious bursal disease virus
WBC	White blood cells

LIST OF APPENDICES

Appendix 1 Concentration of selenium (in ppm) in the tissues of day old Dominant black marshal cockerels before selenium supplementation.....156

Appendix 2 Concentration of selenium in the tissues of Dominant black marshal cockerels after graded levels of supplementation with selenium for ten

weeks.....157

Appendix 3 Graded level of Selenium supplementation and mean antibody titre to Infectious bursa disease vaccine in Dominant black marshal cockerels vaccinated with intermediate infectious bursal disease vaccine at 1st and 3rd week and challenged with IBDV at 6th week.....158

Appendix 4 Graded level of selenium supplementation and mortality rate of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus.....159

Appendix 5 Selenium supplementation and protective rate of infectious bursa disease vaccine.....160

Appendix 6 Selenium supplementation and gross pathology of the bursa of Fabricius (a), kidneys (b), liver (c), leg muscles (d), pectoral muscles (e) and proventriculus (f).....161

Appendix 7 Selenium supplementation and gross mean lesion score of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus.....162

Appendix 8 Haematological parameters of day old Dominant black marshal cockerels.....163

Appendix 9 Graded levels of selenium supplementation and mean haematological values of Dominant black marshal cockerels challenged with infectious bursal disease virus.....164

CHAPTER 1

1.0 INTRODUCTION

The poultry industry is one of the fastest growing segments of the agricultural sub-sector in Nigeria. It accounts for 9-10% of the agricultural gross domestic product (GDP) with the net worth value of over 250 million US dollars (Maina, 2008). The poultry population in Nigeria is estimated to be about 140 million and is subdivided into commercial (25%), semi commercial (15%) and rural /backyard (60%) (Maina, 2008).This poultry population in Nigeria is facing serious challenges from infectious disease such as Infectious bursal disease (IBD; Gumboro disease) which is second only to Newcastle disease in prevalence (Obi *et al*., 2008)

Infectious bursal disease is an acute and highly contagious viral disease of immature chickens caused by a member of the virus *Birnaviridae* family, whose genome consists of two segments of double stranded RNA (van de Berg *et al.,* 2000; Gary and Richard, 2003). Infectious bursal disease was first recognized in the United States of America (USA) in 1962 and has since been reported worldwide, including Nigeria (Cosgrove, 1962; Ojo *et al*., 1973; Onunkwo, 1975; Okoye and Uzoukwu, 1981., Abdu, 1988).

The main target cells for viral replication and lyses during IBD infections are actively dividing IgM-bearing B lymphocyte in the bursa of Fabricius (Sharma *et al*., 2000; Arshard *et al.,* 2005). In spite of vaccination programme in the field, IBD continues to cause economic losses due to the presence of more virulent strains, causing a higher incidence of acute form of the disease with high mortality (Chen *et al*., 1998; Ture *et al*., 1998; van de Berg, 2000). Abdu (1986) reported outbreaks of IBD in vaccinated flocks in Zaria. Vaccination failures due to improper storage, transportation and administration of vaccines have been reported and presence of

maternal antibody has been strongly suspected to contribute to some vaccination failures (Abdu, 1986).

Selenium (Se) as a trace element was first discovered in 1817 by the Swedish Chemist, Berzelius and later Se became known as an essential mineral for human health (Rayman, 2000). It is established that selenium displays antioxidant activity, anti inflammatory, chemopreventive and antiviral characteristics (Rayman, 2000). Its effect on human health is due to its presence within some proteins (Kryukov *et al.*, 2003). Unlike other metal elements that interact with proteins in the form of cofactors, Se becomes cotranslationally incorporated into polypeptide chain as part of the amino acid selenocysteine (Sec), hence it is also described as the 21st amino acid (Gromer *et al.,* 2005).

Selenium supplementation in animal diet is known to enhance the immune status and ability of the immune system to respond to disease challenges (Maldron and Vrzgulova, 1998). The parenteral administration of selenium has been reported to enhance humoral immune response in steers (Broke and Loerch, 1989). Selenium strengthens the immune system and increases the natural resistance of steers and broiler chicks by increasing response of animals to antigenic stimuli (Calnago *et al.,* 1984; Maldron and Vrzgulova, 1998). If the above information is true for other animal's species, selenium supplementation may improve immune response of chickens to infectious diseases, such as infectious bursal disease. This will presumably reduce the incidence of vaccination failures and hence outbreaks of the disease.

1.1 STATEMENT OF RESEARCH PROBLEM

Infectious bursal disease remains a problem in the poultry industry in Nigeria. Affected chickens have reduced antibody response to vaccinations and increased susceptibility to concurrent or

secondary infections (Gary and Richard, 2003). The disease was rated as one of the most prevalent disease of commercial poultry in Nigeria (Ojo *et al*., 1973; Abdu, 1986).

Studies have shown that severe deficiencies and excess of essential trace elements impair the ability of the mammalian hosts to combat infectious pathogens. Se deficiency increases the incidence, duration and severity of viral infections (Mark, 2003). Also, in another study Se deficiency in growing chicken was reported to cause diathesis with early signs, which include unthriftiness and ruffled feathers usually occurring at 5-11 weeks of age. The birds bruise easily with large scabs often forming on an old bruise (Richard, 1986).

1.2 JUSTIFICATION OF THE STUDY

Selenium has attracted great interest as an essential element, particularly as certain diseases have been controlled by its dietary supplementation on account of its ability to improve humoral and cellular immune responses of animals such as steers, pigs and chicken (Richard, 1986; Calnago *et al.,* 1994; Maldron and Vrzgulova, 1998; Mark 2003). As a component of antioxidant enzyme glutathione peroxidase, Se is essential for normal mammalian development and it plays a major role in protecting the cells from free radicals (Bou-resil *et al.*, 2002). In addition, a decrease in Se level or concentration leads to various pathological changes in humans and in various laboratory animals (Bou-resil *et al*., 2002).

There is increase report of IBD vaccine breaks and failures by practitioners and farmers. Despite that various vaccination programmes and route of administration have been developed the outbreak has continued to be recorded in both vaccinated and non vaccinated flocks (Abdu, 1997; Ezeokonkwo, 1997; Oyekunle and Adeniji, 2008).

Since Se supplementation in the diet was reported to result in enhanced immune response of animals to infectious agents, determination of the levels of humoral and cellular responses of poultry vaccinated against IBD and those challenged with the virus, following feed supplementation with varying levels of Se (Ayo and Oladele, 1996) is imperative.

It is expected that the results from this study would be an additional consideration in the control of IBD.

1.3 AIM OF THE STUDY

This study was aimed at investigating the effect of dietary Se supplementation on the immune response and resistance of cockerels vaccinated against Gumboro disease.

1.4 OBJECTIVES OF THE STUDY

The objectives of the study were to:

1. Determine the level of Se in the liver, kidneys, muscles, serum and whole blood before and after feeding cockerels with commercial feed supplemented with Se for ten weeks.

2. Determine the effect of Se supplementation on humoral immune response against IBDV in cockerels.

3. Assess the effect of Se supplementation on mortality rate and protective rate following challenge of birds with IBD virus.

4. To study the pathological changes of the bursa of Fabricius, thymus, liver, spleen, kidneys, of birds fed selenium supplemented feeds that were challenged with IBD virus.

5. Determine the effect of Se supplementation on the haematological parameters of cockerels challenged with IBDV.

1.5 HYPOTHESES

NULL

There is no effect of Se supplementation in feed on:

1. Liver, kidneys, muscles, serum and whole blood levels.

2. Humoral immune response against IBDV in cockerels.

3. Mortality and protective rates of cockerels challenged with IBDV.

4. Pathological changes on the bursa of Fabricius, thymus, liver, spleen, kidneys of cockerels challenge with IBDV.

5. Haematological parameters on cockerels challenged with IBDV.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 POULTRY SUB-SECTOR: AN OVERVIEW

Livestock contribute about 3% to Nigeria's gross domestic product (GDP) and about 38 million people are in the agricultural sector in Nigeria (FAO, 2005). Poultry meat and eggs offer considerable potential for meeting human needs for dietary animal protein supply (Folorunsho and Onibi, 2005). Poultry production in the past was not counted as an important occupation, in some communities, the fowl was used as a means of knowing the time but of recent poultry production has developed and occupies a place of pride among the livestock enterprises due to its rapid monetary turnover (Laseinde, 1994). This singular reason, among others has made the enterprise attractive and popular among small, medium, as well as large scale poultry farmers. The poultry industry has become a diverse industry with a variety of business interests such as egg production, broiler production, hatchery and poultry equipment business (Oluyemi and Roberts, 1979).

2.2 SELENIUM

2.2.1 Historical Background of Selenium

The element Se was discovered in Sweden in 1817 by Jons Jacob Berzelius, when he found a residual slime forming during the oxidation of sulfur dioxide from copper pyrites (Rayman, 2000). Selenium toxicity did receive considerable attention before its essentiality was established. Marco Polo had the first recorded observation of Se toxicity in 1295 when he described a disease he called "hoof rot" (Spallholz, 1994).

It was not until 1842 that Se was first proved toxic but was not associated with general livestock poisoning until 1931 when it was named "alkali disease" (Moxon and Rhian, 1943). In 1928, Dr. Kurt W. Franke was the first to observe the signs of poisoning in farm animals and took many samples from regions where poisonings were rarer. In the first half of the twentieth century, "blind staggers" was reported in several papers (Moxon and Rhian, 1943). Selenium toxicity was confirmed in 1933 to occur in livestock that consumed plants of the genus *Astragalus, Xylorrhiza, Oonopsis* and *Stanleya,* which were Se accumulator plants (Moxon and Rhian, 1943).

It was in the 1950s that Se interest focused on nutritional importance when it was discovered that it played a significant role in preventing liver necrosis in vitamin E-deficient rats and chicks (Patterson *et al.,* 1957). Since the discovery of gluthathione peroxidises (GSH-px), there have been several other selenium-containing proteins discovered which include, iodothyronine deidinases, thioredoxin reductase and selenoprotein synthetase (Behne and Kyriakopoulos, 2001).

In 1974 the United States Food and Drug Administration (USFDA) permitted the use of Se as a feed supplement for pigs and turkeys at the level of 0.1 ppm and 0.2 ppm, respectively. Eight years later the USFDA increased the levels of Se in poultry feeds to 0.3 ppm (Surai, 2002). In 1989 the recommended daily allowance (RDA) of Se, was set by the United States National Research Council at 70 μg/day for adult men and 55 μg/day for adult women (NRC, 1989).

Selenium is an ultramicro trace mineral which exists in both the inorganic and organic forms. The inorganic form can be found in soils and both inorganic and organic forms can be found in plant and animal tissues (Johnson *et al*., 1999). When Schwarz and Foltz established selenium's

essentiality in rats, they ignited research into its chemical properties (Schwarz and Foltz, 1957). Selenium is a structural component of several synthetic as well as naturally occurring compounds. Some of these compounds have antioxidant properties (Gladyshev *et al*., 1998).

In chickens, Se is associated with several selenoproteins including glutathione peroxidase and thioredoxin reductase. Both are ubiquitous enzymes that function to quench oxygen and nitrogen free radicals that can be damaging to proteins, lipids and nucleic acids (Brigelius-Flohe, 1999; Arner and Holmgren, 2000; Rayman, 2000). Generated free radicals in the body that are not removed can affect many different tissue functions, which can lead to organ malfunctions and physiological disruptions (Beckett *et al*., 1992).

Selenium can be toxic in large amounts, but trace amounts are needed for proper cellular function. There are four natural oxidiation states of Se and it occurs naturally in a number of inorganic forms. These forms include: -2 (hydrogen selenide, sodium selenide, dimethyl Se, trimethyl Se, and selenoamino acids, such as selenomethionine), (elemental Se), +4 (Se dioxide, selenous acid, and sodium selenite), and +6 (selenic acid and sodium selenate). The valance state can affect its water solubility and the gastrointestinal absorption rate. Selenium usually occurs in soils in soluble forms, such as in a selenate form, and these can be leached into rivers and surface waters by runoff. The organic Se compounds are the major Se forms in cereal and vegetables (Barceloux *et al*., 1999).

Animals receive Se mainly in the form of selenomethionine in plant-based feed ingredients. Selenium has a biological role and is found in organic compounds, such as dimethyl selenide, selenomethionine and selenocysteine. In these compounds, Se plays an analogous role to sulfur (Johnson *et al*., 1999).

Selenium can be toxic in valence states –2, +4 and +6, but trace amounts of Se these states, have essential roles in the maintenance of homeostasis (Rayman, 2000; Ursini, 2000). The primary function of Se in animals' systems is as a component of the antioxidant enzymatic cascade. This is responsible for the protection of cells from damaging lipid peroxides as well as oxygen and nitrogen free radicals (Rayman, 2000; Ursini, 2000). Selenium is also involved in the regulation of energy metabolism, spermatozoa function, thyroid hormone activation, immunity, and is recognized for having anticarcinogenic as well as antiviral properties (Rayman 2000; Ursini, 2000).

2.2.2 Forms of Selenium and Their Use in Poultry

There are two forms of Se which are organic and inorganic. The later can be found as selenite, selenite or selenide and in the metallic form where as an organic compound, it is found incorporated within the amino acids methionine and cysteine in forages, grains and oil seed meals (Surai, 2002). In 1974, when the USFDA approved Se as a feed supplement, inorganic Se, primarily as sodium selenite, became the traditional source for dietary supplemental Se for poultry and livestock (Leeson and Summers, 1991). The use of inorganic Se supplements in feeds has improved the general health and performance of all classes of commercial poultry (Snyder, 2009).

2.2.3 Biological Roles of Selenium

The importance of Se as microelement for the nutrition of mammals and birds was discovered over 50 years ago (Schwarz and Foltz, 1957). Currently, it is well established that the biological function of Se in the body is mediated via specific selenoproteins/selenoenzymes, hydrogen selenide and methylated Se compounds, respectively (Behne and Kyriakopoulos, 2001). About

30 specific selenoproteins have been identified but only half of them were functionally described (Behne and Kyriakopoulos, 2001).

2.2.4 Selenium Metabolism and Bioavailability

The bioavailability of Se can be defined as the part of absorbed Se from the intestine which is metabolically available for the maintenance of the normal structures and physiological processes of an organism under normal conditions (Wolffram, 1999). Selenium can be absorbed in three ways which are: inhalation, through the gastrointestinal tract and skin (Barceloux and Barceloux, 1999). Data are limited on the inhalation of Se. Animal studies have supported the theory that the lungs readily absorb Se compounds. However, the rate of absorption depends on the chemical form of Se. Barceloux showed that in a study with animals, after 4 h the absorption of elemental Se form the lungs was 57% which is less than with selenious acid of 97% (Barceloux and Barceloux, 1999).

Studies on the gastrointestinal tract absorption are also limited but like the lungs the gastrointestinal absorption depends on the source and forms of Se (Snyder, (2009). As for the dermal absorption data, it is also limited. A study using rat skin showed that approximately 10% of a 0.1 M solution of sodium selenite that was applied to the skin was absorbed within 1 h (Barceloux and Barceloux, 1999).

Organic Se compounds, such as selenomethionine or Se yeast tend to be more bioavailable than inorganic Se sources such as selenite and selenite, organic Se primarily in the form of selenomethionine is metabolized in the same way as methionine (Wolfram, 1999). This is transported through the intestinal membranes during absorption and is actively accumulated in tissues such as the liver and muscles. Selenomethionine is not synthesized in humans or animals,

and it must be derived from feed sources (Schrauzer, 2000). Inorganic Se, on the other hand, is absorbed as a mineral and little is retained in the body's tissue's (Wolfram, 1999). Absorption of Se appears to be less efficient in ruminants than it is in monogastric animals (Wolfram, 1999).

Apsite *et al*. (1994) found selenite to be passively absorbed in the chicken's intestine. The highest concentration of selenite was located in the duodenum, liver and kidneys of the chicken. Pesti and Combs (1976) found the absorption of Se to be the greatest in the duodenum and anterior ileum of the chicken. In a study with rats fed selenite-supplemented diets at 0.2 ppm of Se compared to those of a Se deficient diet (0.009 ppm of selenium), the absorption of Se from selenite, selenate, and selenomethionine (SeMet) was determined. Vendeland, *et al*., (1992) used ligated loops from the duodenum, jejunum and ileum of two categories of rats. They found that Se deficiency had no effect on absorption of any selenocompound in any intestinal segment. Selenomethionine was absorbed from all segments but selenate and selenite were more efficiently absorbed from the ileum (Vendeland *et al*., 1992).

Bioavailability of Se is quite variable and depends on the form of Se (Barceloux and Barceloux, 1999). The absorption of Se is certainly dependant on the form of Se. The absorption of organic Se occurs through an active transport mechanism, which is like that of methionine. The absorption of Se from amino acids tends to be greater than that of inorganic Se, the absorption of inorganic Se occurs via passive diffusion (Barceloux and Barceloux, 1999).

2.2.5 Tissue Distribution and Concentration of Selenium in the Body

Selenium binds mostly to a plasma protein in the blood, after an intravenous injection Se from selenite has been shown to move to the liver and kidneys (Barceloux and Barceloux, 1999). After primates ingested 600 μg of selenomethionine per kilogram of body weight, Se was found to

move into the liver, kidneys, spleen, pancreas, cardiac muscles, brain, and skeletal muscles, respectively. When a daily dose of 200 μg Se, was given as selenomethionine, the Se content in haemoglobin increased to a higher amount than when the equivalent dose of Se selenate was given (Barceloux and Barceloux, 1999). In the body of primates, the total amount of Se averages approximately 14 mg with a range of 3-20 mg, which depends on the intake of Se. Of the total body pool of Se 40-50% is incorporated in skeletal muscles as selenomethionine (Barceloux and Barceloux, 1999).

Tissue concentration of Se depends on several factors including the particular tissue, the amount of Se available in the diet, the length of time the diet is fed, the species the diet is fed to, the age of the animal, and the form of Se fed (Wolfram, 1999). Typically, the Se status of the mother affects the tissue concentration of Se in her offspring. The organic form of Se is usually retained in tissues, but the inorganic forms are absorbed as minerals with little retention in the tissues (Wolfram, 1999).

The tissue concentration of Se usually follows the following order in most species: kidneys, liver, pancreas, heart and skeletal muscles in decending order (Arnold *et al.*, 1973). However, in chickens, the concentration generally follows the following order: feathers, liver, kidneys, muscles and plasma (Arnold *et al*., 1973; Echevarria *et al.*, 1988). In a study by Apsite and Atlavin (1994), Se was injected into the bird and observations were made 4 h later. Selenium concentration was more in the duodenum followed by liver, kidneys, feathers, jejunum and ileum, spleen, pancreas, blood, breast muscles in decending order (Apsite *et al*., 1994). Muscles concentration of Se is relatively low, but muscle is a large mass containing nearly 40% of the total body Se followed by the liver with 30% and all other organs and tissues with less than 10% (Behne and Wolters, 1983).

2.2.6 Selenium Excretion from the Body

Selenium excretion is dependent on the amount of available dietary Se and the form of the Se, which are: the lungs, the urinary and the intestinal tract (ATSDR, 2003). Most of the Se which enters the body is excreted within 24 h. Selenium in the urine increases as the amount of Se concentration in the body increases, it can also build up in the human body; but the amount that builds up depends on the chemical form of the Se. Normally, Se builds up in the liver and kidneys but it can also build up in the blood, lungs, heart and testes. Selenium can also build up in the nails and hair (ATSDR, 2003).

2.2.7 Selenium Deficiency

Dietary Se deficiency has long been associated with cell-mediated and humoral immune dysfunction, opportunistic infections, cancers and ultimately death (Baum *et al*., 2000). Surai (2002) added that in chickens, a deficiency in Se is responsible for exudative diathesis, nutritional encephalomalacia and nutritional pancreatic atrophy (Surai, 2002).

Selenium deficiency has been associated with impaired immunocompetence, reduced egg production and increased embryonic mortality (Combs and Combs, 1984). Selenium deficiency is relatively rare in healthy well-nourished individuals, but it can occur in those who have compromised intestinal functions, or undergoing total parenteral nutrition, or in those who grow their food on Se deficient soils (Barceloux and Barceloux, 1999).

Selenium was detected in normal human tissue samples as early as 1916. In 1957, Schwartz and Foltz found that liver necrosis induced in rats by feeding a purified vitamin E diet could be prevented by the addition of Se to the diet. Many other studies have shown that Se is essential for

adequate growth and fertility, and that a Se-deficient diet or a combined low Se and vitamin E status may lead to various disorders (Combs and Combs, 1984).

A Se-deficiency has been known to cause a disorder in humans known as Keshan Disease. Keshan disease was named after an epidemic outbreak in 1935 in Keshan County, China. This disease occurred in the Se-deficient soil areas (Cheng and Qian, 1990). Keshan disease is an endemic cardiomyopathy that occurs with signs of congestive heart failure or stroke from diffuse cardiac thromboses (Aro *et al.*, 1994).

The myocarditic Coxsackievirus has been associated with the pathogenesis of this disease. The virus will become virulent in Se-deficient or vitamin E-deficient animals (Beck, 1994). A change in virulence has been found to be due to specific mutations in the virus itself (Beck *et al*., 1995). Se-enriched salt supplementation of the diets has reduced significantly the incidence of Keshan disease in Se deficient areas (Li, 2000).

Another disease found in humans as a result of Se-deficiency is Kashin-Beck disease. Like Keshan disease, Kashin-Beck is most prevalent in areas of the world with Se-deficient soils. A range of bone and joint deformations that develop during childhood and puberty characterizes the disease. The hands and feet are usually affected and the bones of the wrist and ankles may be smaller in size or sometimes completely absent (Yang, 1993).

Radiographs of these affected individuals have shown that the disease causes a deformation and fragmentation of the epiphyseal plate, uneven mineralization of the extracellular matrix and an irregular bone surface. One diagnostic feature of Kashin-Beck disease is the broken, blocky or conical shape of the growth apparatus (Yang, 1993).

Histological observations attribute these signs to the necrosis of chondrocytes during bone growth and necrosis of the growth plate. Although, the importance of Se for bone metabolism is still unknown, the consequences of the disease demonstrate the requirement for Se in bone metabolism (Moreno-Reyes *et al*., 2001).

Selenium deficiency can actually be a consequence of some diseases such as liver necrosis in rat, poultry and mouse, muscular dystrophy in poultry, sheep and cattle, exudative diathesis in chicken and turkeys and cancer in humans (Marcus, 1993; Ramaekers *et al*., 1994). Many illnesses cause an altered nutritional status, poor nutrient absorption from the gastrointestinal (GI) tract, and decreased immunity. Se-deficiency has been associated with muscle weakness and tenderness, nail bed changes and cardiomyopathy with decreased cardiac function (Marcus, 1993). Selenium deficiency is also characteristic of patients with a reduced protein intake due to phenylketanuria, gastrointestinal disorders such as Crohn's disease and renal failure (Abrams *et al.*, 1992; Darling *et al.*, 1992).

Other possible health effects that have been reported in numerous studies indicate that Se deficient patients are more prone to seizures, rheumatoid disease, arteriosclerosis, miscarriages, neurological disorders, depression and even cancer (Ramaekers *et al*., 1994; Rayman, 2000).

Humans are not the only species that can be affected by Se-deficiency, Se deficiency in poultry, especially when combined with vitamin E deficiency, can be responsible for a range of avian diseases, including exudative diathesis (ED), nutritional encephalomalacia and nutritional pancreatic atrophy (NPA) (Century and Hurwitt, 1964; Thompson and Scott, 1969; Combs and Hady, 1991; Bartholomew *et al*., 1998).

Nutrional pancreatic atrophy is the only disease that is caused by Se deficiency alone (Combs, 1994). Se-deficiency in chickens is associated with impaired immunocompetence and increased embryonic mortality (Combs and Combs, 1984). In a study carried out by Combs and Scott (1974), birds were fed a low Se diet and peroxidized fat. This resulted in a significant decrease in the hatchability of eggs, which was restored by Se and vitamin E supplementation.

Selenium deficiency in poultry affects fertility and hatchability. Combs and Scott (1974) reported low fertility and hatchability when birds were fed a basal (low Se) diet, but this reproductive disorder could be corrected partly by vitamin E supplementation and completely by Se-supplementation. Eggs from hens fed a very low level of Se were more likely to be infertile, more likely to have high embryo mortality and lower rate of hatchability of fertile eggs (Latshaw *et al*., 1977).

Hatching weights of chicks from hens supplemented with 0.05 and 0.1 ppm of Se were significantly heavier than those of hens with no supplemental Se (Latshaw *et al*., 1977). Exudative diathesis in hatchlings from hens with no Se supplementation was also elevated (Hassan *et al*., 1990). Exudative diathesis is a common problem in chickens deficient in Se and vitamin E. Exudative diathesis is attributed to increased capillary permeability due to endothelial cell failure in skeletal muscles and reduced levels of blood proteins (Combs and Scott, 1974).

The sign of ED arises from leakage of body fluids through the capillaries and from small haemorrhages in the muscle tissues and can occur at any age but most frequently in young chicks or turkey poults (Whitehead and Portsmouth, 1989; Hassan *et al*., 1990). Hassan *et al.* (1990) studied ED in chicks from laying hens fed a Se and vitamin E deficient diets. Exudative diathesis was observed at hatching whereas signs of ED were not present in the chicks from Se and

vitamin E supplemented hens indicating that signs of ED can develop during the development of embryos from Se and vitamin E deficient dams Hassan *et al.* (1990).

Exudative diathesis has been associated with low levels of muscle Se, liver GSH-px, and an increase in liver non-Se-dependent GSH-px (Hassan *et al*., 1990). Bartholomew *et al.* (1998) hypothesized that ED may be an inflammatory response associated with a Se/vitamin E deficiency. Pro-inflammatory cytokines are recruited because of a lack of antioxidant enzymes, might be responsible for the fluid accumulation and haemorrhage Bartholomew *et al.* (1998). Supplementing the diet with Se rather than vitamin E is more effective in preventing the occurrence of ED Snyder, (2009). Vitamin E supplementation at 15 ppm was not enough to prevent ED while levels as low as 0.15 ppm of Se was sufficient (Hassan *et al*., 1990). Therefore, ED is considered to be a Se-deficiency syndrome in chickens (Machlin *et al*., 1962).

Selenium deficiency can lead to weakened membranous structures of spermatozoa that are characterized by a significant increase in midpiece abnormalities and decreased motility (Edens, 1996). Watanabe and Endo (1991) studied mice fed Se-supplemented or Se-deficient feed. In the Se-deficient group, the proportion of abnormal sperm was significantly higher than in the Se-supplemented group. The same results were shown in boars under the same conditions and it was observed that fewer sperms were able to penetrate oocytes in the Se-deficient group (Marin-Guzman *et al*., 1997).

In chickens, a low Se diet increased the amount of spermatozoa with bent midpieces to 18.7%, while diets supplemented with either sodium selenite or SeMet decreased the amount of deformed midpieces to 6.2% and 0.7%, respectively (Edens, 2002). These studies have shown

that adequate Se is required to maintain sperm membrane integrity, motility and fertilizing capability. The combination of both vitamin E and Se further improves semen quality.

Health problems in different species of animals might possibly be associated with impaired selenoprotein functions associated with antioxidant systems that are caused by frank and marginal Se deficiency (Moustafa, 1998). All selenoproteins contain Se in the form of selenocysteine, which is encoded by a Uracil Guanine Adenosine (UGA) triplet in the messenger RNA (mRNA) of the selenoprotein (Moustafa, 1998).The amount of available Se limits the synthesis of selenocysteine for the synthesis of selenoproteins (Moustafa, 1998). There are actually two forms of transfer RNA (tRNA) [Ser] Sec, which are required for the synthesis of selenoproteins. Both tRNA forms are isoforms, but differ by the methylation of a ribose portion of the wobble nucleotide of the anticodon (Moustafa, 1998). The methylation of tRNA [Ser] Sec has been thought to be relevant to the translation of glutathione peroxidase and possibly other selenoproteins (Moustafa, 1998).

Selenium deficiency might reduce the amount of methlylated tRNA [Ser] Sec, which would account for the decrease in selenoprotein mRNA concentrations Snyder, (2009). An example of this hypothesis was seen in a severely Se deficient rat model in which almost all transcription of cGSH-px in the liver and heart were suppressed and Type I iodothyronine deiodinase (ID) was decreased by 50% (Saedi, 1988).

Selenium deficiency affects the total antioxidant system because the mineral in the inorganic forms can be a pro-oxidant and in the organic form (SeMet) it can be an anti-oxidant as well (Edens, 2002). This lack of antioxidant protection can lead to an increase in oxidative stress and alterations in redox signaling (Gladyshev *et al.*, 1999). Kayanoki *et al.* (1996) demonstrated that

Se-deficient bovine renal epithelial cells were more susceptible to hydrogen peroxide induced apoptosis than the cells supplemented with Se. Several signaling pathways are also disrupted by reactive oxygen species (ROS). Selenium has been shown to have influence on signaling molecules such as nuclear factor-*kappa* B (NFκB) and caspase-3 (Nomura *et al*., 1999). Both molecules are important in apoptosis signaling.

Selenium has a direct effect on mitogen-activated protein (MAP) kinases, which are upstream molecules for the activation of NFκB (Adler *et al*., 1996). All of this information supports the hypothesis that Se deficiency can be detrimental because of increased cellular damage and apoptosis Snyder, (2009). In vertebrate animal species, Se is the pivotal element in an enzyme, iodothyronine deiodinase (types I, II, and III), which is needed for the conversion of the thyroid hormone, thyroxine (T4) to triiodothyronine (T3) (Edens and Gowdy, 2005).

If T4 is not converted, deficiencies can arise causing hypothyroidism, which leads to numerous metabolic disorders that have numerous signs such as extreme fatigue, goiter, miscarriages, mental slowing, and cretinism (Edens and Gowdy, 2005). Shamberger and Frost (1969) were the first to indicate that Se helped to reduce cancer mortality rates. Since that time, there have been numerous experimental and epidemiologic studies to investigate this hypothesis Snyder, (2009). Several scientific studies suggest that an increased risk of cancer occurs as a result of low concentrations of Se in the diet (Schrauzer *et al*., 1977; Combs and Gray, 1998; Rayman and Clark, 2000).

Evidence has been amassed to suggest strongly that Se should be taken to aid in the prevention of many types of cancer. One study involved 1312 patients who daily took 200 ug of Se yeast or low-selenium yeast placebo for 4.5 years. Selenium supplementation was shown to reduce the

total mortality and mortality from cancers as well as the incidence of lung, colorectal and prostate cancer by 46%, 58% and 64%, respectively when the subjects were seen 6.4 years after the ingestion of the supplements (Clark *et al*., 1996). Nevertheless, the lack of appropriate apoptosis in Se deficient animals might exacerbate the growth of neoplastic tumours (Edens *et al*., 2007).

Research has shown a link between HIV/AIDS and Se. Selenium supplements offer a low-cost, simple and safe treatment for people with HIV (Rayman, 2000). Hurwitz *et al*. (2007), included 174 subjects using both genders. The subjects consumed 200 μg/day of Se from high- Se yeast or a placebo for 9 months. Selenium supplementation decreased HIV-1 viral load.

The effect of Se supplementation on the immune function in healthy adults has been investigated. Twenty-two adults, who had relatively low plasma concentrations of Se, were randomly assigned to consume either 50 or 100 μg/day of Se from either sodium selenite or a placebo for 15 weeks. Sodium selenite supplements increased the plasma concentration of Se, the exchangeable Se pool and glutathione peroxidase activity in both lymphocyte phospholipids and cytosol (Broome *et al*., 2004).

In a study on the impact of Se type II diabetes, 56 people with type II diabetes were randomized to receive either 960 μg/day Se or a placebo for three months. Ten non diabetics also participated in the study as the controls. Selenium supplements significantly increased plasma Se concentrations and red-cell Se glutathione peroxidase activity. Selenium supplements reduced nuclear factor-*kappa* B (NF-*k*B) activity in people with type II diabetes to a level near that of the non diabetic controls proving that Se helps protect against type II diabetes (Faure *et al*., 2004).

2.2.8 Selenium Toxicity

Although Se is an essential trace mineral, it is also toxic at high levels of dietary supplementation (Franke, 1934). Selenium toxicity was observed in the Western United States in the 1930's ranch animals that consumed plants with high levels of accumulated Se (Spallholz, 1997).

Selenosis or Se toxicity in any species can be classified into two categories depending on the amount of Se ingested and the duration of exposure (Kim and Mahan, 2001). Acute selenosis is a result of a high dietary intake (>20 mg/kg) of Se for a short period of time (Mahan and Moxon, 1984). Signs of acute selenosis are respiratory distress, ataxia, diarrhoea, and even death (Mahan and Moxon, 1984). Chronic selenosis is a result of a dietary intake of 5 to 20 mg/kg over a long period of time (Goehring *et al*., 1984).

Chronic selenosis produces reduced feed intake and growth rate in livestock followed by hair loss, shedding of hooves, liver cirrhosis and anaemia (Ekermans and Schneider, 1982). There are several mechanisms through which Se can produce toxic effects. The most common and important mechanism is the production of the superoxide radical (Stohs and Bagchi, 1995). The superoxide radical has the ability to interact with thiols in mammals and in birds (Klaassen, 1985; Hoffman *et al*., 1991). Reactions with thiols can alter the activity of many antioxidant sulfhydryl-containing enzymes as well as structural proteins in the body (Spallholz and Hoffman, 2002).

When superoxides react with thiols they can generate destructive free radicals, which can cause irreversible cellular damage (Spallholz, 1994). Another way that Se can exert its toxic effects is through generation of excess hydrogen selenide as an intermediate in inorganic and organic Se

metabolism. Hydrogen selenide accumulates in animals receiving excess selenocysteine as a result of Se methylation metabolism being inhibited (Sayato *et al*., 1997).

Excess hydrogen selenide can cause hepatoxicity and can result in other Se related injuries (Spallholz and Hoffman, 2002). Since Se can substitute sulfur in the formation of selenoproteins, Spallholz and Hoffman (2002) hypothesized that excess dietary SeMet might result to massive incorporation into sulfur-containing enzymes and structural proteins that might cause embryo malformations and toxicity in aquatic birds. However, Spallholz and Hoffman (2002) pointed out that pure SeMet injected into the embryo was far more toxic than Se incorporated into the embryo's proteins.

Toxicity of Se is not only related to the amount and duration of exposure but also the form that is ingested. Most toxicity studies have examined the effects of sodium selenite. In a study done by Latshaw *et al.* (1977) female chickens were fed graded levels of sodium selenite to determine at what level toxicity occurred, toxic effects were observed at 5 ppm. In vitro studies with human lymphocytes have demonstrated toxic responses to the addition of sodium selenite at 5 μM/l but not with the addition of selenomethionine (Kelly and Powers, 1995).

2.2.9 Selenium and the Immune System

Dietary Se is necessary for optimum functioning of the immune system. Selenium has beneficial influences at several points in both the cell-mediated and antibody-mediated branches of the immune system (Jianhua *et al*., 2000). Not only does the immune system rely on the antioxidant properties of Se to control oxidative bursts by phagocytic cells it also has some endocrine effects that benefit antibody-mediated immunity (Jianhua *et al*., 2000). The role of Se in the immune system of chickens is of growing interest to many commercial production companies Snyder,

(2009). March *et al.* (1986) fed low levels of sodium selenite to chickens and observed an increase in the relative weights of lymphoid organs. The increase in lymphoid organ weights was an indication of a more proliferative immune system. The authurs saw a degeneration of the bursal epithelium in Se-deficient birds.

Chang *et al.* (1994) studied the influence of sodium selenite and vitamin E on T and B cell markers and lymphocyte proliferation. Lymphocyte proliferation was impaired with Se and vitamin E deficiency, and the amount of CD4+ CD8+ T cells increased significantly when chickens were fed the Se-deficient diet. These results indicate that vitamin E and Se deficiencies affect the maturation of T cells and the proliferative responses of peripheral lymphocytes (Chang *et al.,* 1994). Leng *et al.* (2003) compared the influence of either sodium selenite or organic Se sources on the immune system of layer chickens. They determined that organic Se sources increased CD3+, CD4+ and CD8+ surface markers on T cells located in several lymphoid structures in young chickens. All markers are involved in associating the T cells with antigen presenting cells (APCs). Leng *et al.* (2003) concluded that organic Se supplements improved the status of the avian immune response to disease agent by increasing the ability of immunocompetent cells to respond to an antigen challenge.

Though the relationship between the immune system and modern-day poultry performance is complex. The general opinion is that modern poultry have been selected to have a less responsive immune system in order to provide energy for the increased growth rate and body weight in a confined environment. The decreased immune functions are due to energy demand and nutrient requirements that are no longer being met by the nutrition of the broiler chicken. Supplementation of the appropriate form of Se in modern-day poultry diets is one factor that can enhance the immune functions of poultry (Snyder, 2009).

2.3 INFECTIOUS BURSAL DISEASE

Infectious bursal disease is an acute and highly contagious viral disease of immature chickens, 3-6 weeks of age caused by a member of the virus *Birnaviridae* family whose genome consists of two segments of double stranded RNA (van de Berg *et al.*, 2000; Gary and Richard, 2003). It is characterized by destruction of lymphoid cells in the bursa of Fabricius (Arshad *et al*., 2005).

After its first outbreak in 1962 in Gumboro area of Southern Delaware, USA the disease was recorded in poultry from all over the world (Cosgrove, 1962). In most parts of the world before 1987, the disease was subclinical with lower morbidity and mortality rates. But after 1987, increased morbidity rate up to 100% and mortality rate up to 25% in broilers were reported from different parts of Europe and Asia (Sah *et al.,* 1995; Cao *et al.*, 1998). During the 63rd General Session of the Office International des Epizooties, IBD was considered as a disease of socio-economic importance at international level, as the disease was present in more than 95% of the member countries (Eterradossi *et al*., 1997).

Since the disease was recognized over 50 years ago, it continues to pose a threat to the commercial poultry industry (Eterradossi *et al.,* 1997). The economic impacts of the disease are manifold, including losses due to morbidity and mortality, immunosuppression in the surviving chickens since IBDV infection exacerbates infections with other disease agents, reduction in the chicken's ability to respond to vaccination and risk of introduction to exotic places from importing infected poultry products (Eterradossi *et al*., 1997). The economic impact of the disease is influenced by pathogenicity of the strain of virus, susceptibility and breed of flock, other prevalent pathogens and environmental and management practices (Eterradossi *et al*., 1997). Infectious bursal disease virus infection also lowers egg production, leads to deterioration of egg shell and internal egg quality (Muller *et al*., 2003). Infectious bursal disease virus

infection results in immunosuppression which is marked by higher viral respiratory infections and increased mortality due to airsacculitis and colisepticemia during the end of the growing period in broilers (Muller *et al*., 2003).

2.3.1 Historical Background of Infectious Bursal Disease

In 1957, Albert S. Cosgrove recognized a syndrome, on a broiler farm near Gumboro, Delaware USA. It was named "Gumboro disease" after the geographic location of the first recorded outbreak. The disease was characterized by ruffled feathers, watery diarrhoea, trembling, severe prostration, dehydration, haemorrhages in the leg and thigh muscles, increased mucous in the intestine and enlargement of the bursa of Fabricius. The syndrome was characterized by 10% flock morbidity and mortality ranging from 1- 10% occurring throughout the Delmarva region (Lasher and Davis, 1997).

Initially, variant infectious bronchitis virus (Gray strain) was suspected to be the causative agent since the kidney lesions induced by the Gray virus and those seen in avian nephrosis were similar; hence the syndrome was given the name avian nephrosis nephritis (Winterfield *et al*., 1962). They observed that concurrent occurrence of two diseases, lack of SPF eggs and limited ability of the diagnostic tools to isolate the virus, led to this conclusion. Subsequent studies indicated that birds immune to Gray virus could still be infected with the IBD virus and would develop changes in the cloacal bursa like IBD (Lukert and Saif, 2003).

None of the conventional treatments like antibiotics, vitamin supplements, molasses or different management practices altered the course of the disease and different management practices had variable or insignificant effect on the severity of the disease (Parkhurst, 1964; Lasher and Davis,

1997). It was also noticed that the disease was transmitted from farm to farm through leftover feed from the affected farms (Edgar and Cho, 1965; Edgar and Cho, 1976).

Winterfield *et al*. (1962) isolated the agent in embryonating eggs and noticed that it was difficult to maintain on serial passage. The isolate was called infectious bursal agent (IBA) and regarded as the true cause of IBD Winterfield *et al*. (1962). Edgar and Cho, (1965) named the syndrome "infectious bursal disease" (IBD) instead of Gumboro disease while Gray virus was identified as an isolate of infectious bronchitis virus with nephrotoxic tendencies (Lukert and Saif, 2003).

Planned infection with the disease agent was proposed as a control measure on the premises where IBD was already established (Hitchner, 1963). Hitchner studied the clinical manifestations of the infectious bursal agent (IBA) and differentiated them from infectious bronchitis virus (IBV) (Hitchner, 1963). Cho and Edgar (1969) characterized IBA in experimentally infected chickens and observed gross changes in the bursa and population shifts in circulating blood cells over the course of infection.

It was concluded that the aetiologic agent of IBA was a virus, relatively resistant to extreme temperature and pH and a wide range of chemical treatments (Benton *et al*., 1967; Cho and Edgar, 1969). Moulthrop and Wills (1967) were able to adapt an isolate to chicken embryos (Snedeker *et al*., 1967). Cho (1970) suggested that IBD infection was equivalent to "biological bursectomy". Allan *et al.* (1972) reported that IBDV infection at an early age had immunosuppressive effects. Several studies reported significant immunosuppression following exposure to IBDV and Newcastle disease virus (NDV), Marek's disease, *E. coli* or *S. typhimurium* (Cho, 1970; Allan *et al*., 1972; Faragher *et al.*, 1972; Faragher *et al*., 1974; Wyeth., 1975). These observations greatly emphasized the need for the development of control measures.

By 1976 the structural and growth characteristics of IBDV became available in detail and it was concluded that the virus could not be placed into any previously recognized group and should be placed in a new taxanomic category (Nick *et al.*, 1976). Classic strains like Edgar 2512 and Irwin Moulthrop (IM) was isolated from the USA in the early 60's and caused 30-60% mortality in chickens causing haemorrhagic inflamation and B cell depletion (Liu and Vacharia, 2004).

The vaccine strain, Winterfield 2512, was isolated by Winterfield in 1965 and was modified for vaccine production (Winterfield, 1969). A mild strain adapted to chicken embryos was used to produce the first licensed vaccine "Bursa Vac" (Snedeker *et al.*, 1967). The existences of the second IBDV serotype were reported in 1980 and were found to be non-pathogenic to chickens (McFerran *et al.*, 1980). Variant viruses like Delaware and GLS were isolated in the 80's from vaccinated farms in Delmarva poultry producing area. These viruses did not cause mortality or trigger inflammation. These strains were able to break through the maternal immunity against standard or classic IBDV strains (Saif, 1984).

At the beginning of the 1980's most commercial broiler farms were not vaccinated against IBD in many European countries, since it was believed that maternal immunity from the vaccinated chickens would protect against IBD infection (Shamaila, 2005). The very virulent IBDV (vvIBDV) first appeared in the Netherlands in the 80's and rapidly spread all over the world including Central Europe, South Eastern Europe, Japan and Russia (Nunoya *et al.*, 1992; Lin *et al.*, 1993; Savic *et al.*, 1997; Zorman-Rojs *et al.*, 2003). Australia, New Zealand, Canada and United States were free from the vvIBDVs (Shamaila, 2005). These viruses were able to breakthrough the immunity provided by the MDAs and cause more than 70% mortality and in addition to the bursa of Fabricius, they caused lesions in the thymus and bone marrow (Brown *et al.*, 1994).

2.3.2 Prevalence of Infectious Bursal Disease

Classical IBDV have traditionally affected poultry worldwide ever since the first outbreak of the disease was reported from Delaware, Maryland and Virginia (Delmarva) region (Jackhwood and Saif, 1983). Cosgrove was the first to report this disease in 1962 (Cosgrove, 1962). By 1970, the disease had been reported from Canada, Mexico, Europe, the Middle East, Africa and Asia, (Mohantey *et al.*, 1971; Langraf *et al*., 1972; Lucio *et al*., 1972; Ide and Stevenson, 1973; El-Zein *et al*., 1974; Onunkwo, 1975; Gukelberrger *et al*., 1977).

The first reported outbreak of IBD in Nigeria was in 1969 (Ojo *et al.*, 1973). Epizootics are said to occur in the poultry-rich southern states of Nigeria, where between 90 to140 outbreaks involving about 0.5 million chicks are recorded annually (Nawathe and Lamorde, 1982). Abdu, (1986) reported a prevalence of 13.4% in vaccinated flocks in Zaria, also Abdu, (1987) reported a concurrent infection of IBD and spirachetosis in pullet chicks in Zaria with 39% mortality. Serological studies that were carried out in Osun, Oyo, Ogun, Ekiti and Lagos states showed 34% prevalence in 2000 local chickens that were examined (Oyedotun and Durojaiye, 1999). A similar study in the Jos area of the Plateau state reported 61.9% prevalence in local chickens, 33.3% in guinea fowls and 56.6% in local ducks (Okwori *et al.*, 2006). Mboku *et al*, (2010) reported a prevalence of 7.26% in retrospective study of IBD diagnosed at Poultry Unit of Ahmadu Bello University, Nigeria. Infectioos bursal disease ranks second to Newcastle disease (ND) among poultry diseases of highest prevalence in Nigeria (Obi *et al*., 2008).

2.3.3 Aetiology of Infectious Bursal Disease

The causative agent of IBD is a bisegmented, double stranded RNA virus that belongs to the family *Birnavirideae*. Two distinct serotypes have been recognized. Pathogenic strains are grouped in serotype 1 viruses while serotype 2 strains are non-pathogenic (Muller *et al*., 2003).

Initially recognized strains were named classical strains. Mutations in the RNA genome of the virus have resulted in the appearance of variant strains in the US and very virulent strains all over the world (Muller *et al.*, 2003).

2.3.3.1 Types and Subtypes of Infectious Bursal Disease Virus

Two distinct serotypes of the IBDV have been recognized. Serotype 1 viruses are pathogenic to chickens while serotype 2 viruses are nonpathogenic to chickens and have been isolated from chickens and turkeys (Lim *et al*., 1999). Serotype 1 viruses can be further categorized into 4 groups on the basis of their pathogenicity: classical, variants, attenuated and very virulent strains (Lim *et al*., 1999).

Variant IBDV strains appeared in the US in 1983. These strains were antigenically different from classic strains and caused a rapid and severe bursal atrophy and in contrast to classical strains produced no clinical signs of illness (Vakharia *et al*., 1994). Antigenic variants have been recognized by their ability to escape cross-neutralization by antiserum against the classical strains, while attenuated strains have been generated by adapting the classical and variants strains to chicken embryo fibroblasts (CEF) or other cell lines (Lim *et al*., 1999). Very virulent strains have been characterized by severe clinical signs and high mortality ranging from 60-100% (van de Berg, 2000). Very virulent strains can breakthrough the immunity provided by maternal antibodies (van de Berg, 2000). The vvIBDVs produce similar signs as the classical strains and the same incubation period of 4 days but the acute phase is more severe and more generalized in affected flocks (van de Berg, 2000). Serotype 2 strains do not produce any clinical disease in the chickens (Abdel-Alim and Saif, 2001). Both serotypes 1 and 2 co-exist in chicken and turkey flocks in the USA and antibodies to both serotypes are prevalent in the field (Jackwood and Saif, 1983).

2.3.3.2 Biological Properties of Infectious Bursal Disease Virus

The IBDV is a non-enveloped virus with a diameter of 60 nm and a density of 1.336 g/ml in CsCl (Kibenge *et al*., 1988). Capsid of the virion consists of a single layer of 32 capsomers arranged in a 5:3:2 symmetry (Hirai *et al.*, 1974). Density equilibrium in CsCl is the common method of purifying IBDV except for the Australian 002/73 and IM strain which are unstable in CsCl gradient (Azad *et al*., 1985).

The IBDV particle has sedimentation co-efficient of 460S in sucrose gradient (Dobos *et al*., 1979). Various densities reported for the IBDV particle range from 1.29 g/ml to 1.33 g/ml (Hirai *et al.*, 1974; Nick *et al*., 1976; Bottcher *et al*., 1997). A single virus preparation has been reported to have as many as 6 bands (Muller *et al*., 1986).

Usually the buoyant density of 1.33 g/ml corresponds to the typical IBDV particle in cesium chloride (CsCl). Bands obtained at 1.32 g/ml had double stranded RNA and represent incomplete virus particles and those at 1.31 g/ml had incomplete virus particles without RNA while 1.29 g/ml had irregular shape, poor assembly and an unusual amount of dsRNA. The bands showed similar SDS-PAGE profile except the band obtained at 1.29 g/ml. Particles of 1.29 g/ml were obtained when the virus was grown in CEF as opposed to bursa of Fabricius. Incomplete virus particles are not normally obtained from bursa. Formation of incomplete virus particles can be one of the reasons for the loss of pathogenicity for chickens when IBDV is passaged repeatedly in CEF cells (Muller and Becht, 1982., Kibenge *et al*., 1988).

The three dimensional structure of IBDV virion has been determined by electron cryomicroscopy. The outer and inner surfaces of the capsid are made of trimeric subunits (Bottcher *et al*., 1997). Virus architecture is based on a T=13 lattice (Ozel and Gelderblom,

1985). Capsid is 9 nm thick and nonspherical in shape since the subunits close to the 5 fold symmetry axes are at a larger radius than those close to 2-3 fold axes. The viral protein 2 (VP2) forms the external trimeric subunits and protrude out of the shell forming a honeycomb surface. The VP3 forms the inner Y shaped trimers that are packed closely to form a continuous shell and are connected to VP1. Viral protein 4 formed the rim around each 5 fold axis on the inner surface of the capsid (Bottcher *et al.*, 1997). This model suggests 780 copies of VP2, 600 copies of VP3, 60 copies of VP4 and is in accordance with the observed composition of 51% VP2, 40% of VP3, 6% VP 4 and 3% VP1 (Dobos *et al*., 1979).

2.3.3.3 Physiochemical Properties of Infectious Bursal Disease Virus

The IBDV is non-enveloped and quite resistant to physical and chemical agents. Due to the stability and hardiness of the virus, it persists in poultry premises even after thorough cleaning and disinfection. The virus is inactivated at a pH of 12.0 but not at pH 2.0, the virus is resistant to chemical agents and hard to eradicate from poultry houses (Benton *et al*., 1967).

Benton *et al*. (1967) reported that IBDV survived a temperature of 37^{o}C for 90 min and 56 ^{0}C for 5 h. A marked reduction in infectivity of the virus was observed after treatment with 0.5% formalin for 6 h. The virus remained unaffected by ether, choloroform, phenol, thiomaesal, Staphene and Hyamine 2389 treatments. The virus survived treatments with various concentrations of three disinfectants (an iodine complex, a phenolic derivative and a quaternary ammonium compound) for a period of 2 minutes at 23^{o}C, only the iodine complex had any deleterious effects (Benton *et al*., 1967).

Cho and Edgar (1969) reported that the virus was inactivated by exposure for 1 h to 1% formalin, 1% cresol and 1% phenol. It remained stable at 60^{o}C for 90 minutes and was still infectious at

room temperature for approximately 25°C for 21 days. Petek *et al.* (1973) observed that IBDV was more resistant than Reovirus to heat, ultraviolet irradiation and photodynamic inactivation.

The hardiness of the IBDV makes eradication difficult from poultry houses after an outbreak (Alexander and Chettle, 1998). Heat resistance of IBDV is an important factor to be considered in trade of poultry due to extensive international trade of processed and partially processed poultry meat. Alexander and Chettle (1998) constructed the heat inactivation curves of classical IBDV at 70^{o}C, 75^{o}C and 80^{o}C. These biphasic multiple kinetic curves showed an initial rapid drop in infectivity followed by a more gradual decline. In the second phase it took 18.8 min at 70^{o}C, 11.4 min at 75^{o}C and 3.0 min at 80^{o}C for reducing the infectivity of IBDV by 1 log $_{10}$. Landgraff *et al*., (1972) showed that IBDV survived at 60^{o}C but did not survive at 70^{o}C for 30 min and 0.5% chloramines killed the virus after 10 min. Invert soaps with 0.05% sodium hydroxide either inactivate or had a strong inhibitory effect on the virus (Schnitzler *et al.*, 1993).

To investigate heat liability of the virus, it was subjected to 37, 65, 71, 74, 77, 82 or 100 ^{0}C for 1 min. Virus titre declined greatly at temperatures above 65^{0}C, 71^{0}C and 100 ^{0}C at rates of > 90, 99 and 99.99% respectively (Lukert and Davis, 1974). Similar viral reduction curves were obtained after heating for 6 min at 71 ^{o}C or 74 ^{o}C (Lukert and Davis, 1974). Minimum heating patterns for 1 log 10 reduction in titre ranged from 1 min at 65 ^{0}C to 18.8 min at 70 ^{o}C (Alexander and Chettle, 1998).

The IBDV is unusually resistant to inactivation by cooking so there is a risk of introduction to the backyard flocks through uncooked chicken meat products since viable virus might be present in meat from apparently healthy chickens (Lukert and Davis, 1974). Drumsticks and chicken patties experimentally injected with 10^{7}-10^{9} $TCID_{50}$ of virus and cooked to internal temperature

of 71^{o}_{C} and 75^{o}C, respectively in hot oil or steam in a flame grill still contained the infectious virus (Lukert and Davis, 1974). Electron microscopy showed that virus morphology is changed after pressure treatment. Elevating pressure also destroyed the infectivity of the virus while retaining its immunogenicity (Tian *et al*., 2000).

2.3.3.4 Antigenic Properties of Infectious Bursal Disease Virus

Infectious bursal disease virus is endemic throughout the world but several different antigenic and pathogenic types exist in specific geographic locations (Macreadie *et al*., 1990). Two serotypes of IBDV occur in Europe and USA as recognized by the virus neutralization test. These two serotypes are antigenically distinct (Macreadie *et al*., 1990). Serotype 1 viruses are pathogenic to chickens and differ in their virulence (van den Berg, 2000). They cause lesions in the bursa of Fabricius by lymphocytic depletion (Rosenberger *et al*., 1975). Whereas serotype 2 viruses are avirulent to chickens and isolated mainly from turkeys and chickens (Ismail and Saif, 1991; Kibenge and Dhama, 1997).

Serotype 1 viruses can be broadly divided into classic, variant and very virulent IBDVs. Until 1987, the strains of virus were of low virulence and were controlled by vaccination. Emergence of variant viruses was first reported in USA in 1987, these viruses were reported to undergo an antigenic drift against which the classical IBD vaccines were not protective against it (Ivanyi and Morris, 1976; Shwed *et al*., 2002).

Six antigenic subtypes of IBDV serotype 1 viruses have been identified by the virus neutralization test (Ivanyi and Morris, 1976). Variant viruses occur in the USA and Australia and are different from the classic viruses in terms of pathogenicity and immunogenicity. They

overcome the immunity induced by classic serotype 1 viruses and cause rapid bursal atrophy with minimal or no inflammatory response (Macreadie *et al*., 1990; Hassan *et al*., 1996).

Vaccination with serotype 1 subtype cannot insure protection from challenge with another subtype suggesting that variant viruses are antigenically different from classical viruses (Ismail and Saif, 1990; Macreadie *et al*., 1990). Variant viruses present in the USA and Australia are not closely related to each other (Savic *et al*., 1997). Significant antigenic differences exist among serotype 1 strains as detected by virus neutralization and led to the grouping of the serotype 1 viruses into 6 subtypes (Ivanyi and Morris, 1976). Virus neutralization test proved to be serotype specific and could distinguish between the two serotypes (Ivanyi and Morris, 1976; Macreadie *et al*., 1990; Jackwood *et al*., 1996; Jackwood *et al*., 2001).

Serotype 1 induced protection against challenge with variant viruses (Ismail *et al*., 1987). Serotype 2 viruses are immunologically distinct from serotype 1 viruses since vaccination with serotype 2 (OH) viruses did not confer protection against serotype 1. Cross protection studies indicated that the variant viruses were different from other subtypes of serotype 1 IBDVs. Both serotype 1 and 2 viruses share common group antigens which could be detected by AGPT, flourescent antibody test and ELISA (Chettle *et al*., 1985; Jackwood *et al*., 2001). Capsid proteins VP2 and VP3 contain epitopes that are responsible for group antigenicity (Becht *et al*., 1988).

The VP2 carries the serotype specific antigens responsible for the induction of neutralizing protective antibodies (Azad *et al*., 1987; Becht *et al*., 1988). Variation in IBDV antigenicity depends on amino acid changes in peak A and B. Serotype 2 strains 23/82, North American antigenic variants A, E, GLS-5 and DS 326 neutralization resistance escape mutants all exhibit

amino acid changes in the hydrophilic peaks, only serotype 1 strains show changes in hydrophobic domain. Two smaller hydrophilic areas of VP2 variable domain also influence IBDV antigenicity (Eterradossi *et al*., 1998).

Deletion mutagenesis studies with Australian strain 002/73 showed that virus neutralizing monoclonal antibody recognized a discontinuous epitope on VP2 (Azad *et al*., 1987). The antigenic region responsible for the production of neutralizing antibody is highly conformation dependent since the antibodies immunoprecipitate VP2 but did not react with the denatured protein in the immunoblot (Becht *et al*., 1988; Fahey *et al*., 1989; Oei *et al*., 2004). The VP2 carries the epitopes which elicit neutralizing antibodies and distinguish the two strains as well as those which elicit nonneutralizing antibodies and are common to both strains (Cruz-coy *et al*., 1993).

Since protective immunity against IBDV is dependent on the presence of neutralizing antibodies in susceptible birds, precise knowledge of antigenic sites responsible for the induction of neutralizing antibodies is of fundamental importance (Azad *et al*., 1987; Becht *et al*., 1988). At least three distinct conformation dependent serotype 1 specific virus neutralizing antigenic sites have been identified on VP2 and one linear antigenic site on VP3 (Azad *et al*., 1987; Becht *et al*., 1988).

Two conformational virus neutralizing antigenic sites were localized in the central region of VP2 consisting of 156 amino acids residues while linear epitope was mapped to C terminal 105 amino acid residues of VP3. Sequence specific epitope on VP2 are common to both serotypes (Becht *et al*., 1988). Of the two non-neutralizing monoclonal antibodies defined epitopes, one epitope is common to both serotypes whereas the second is distinct for serotype 1 and 2.

With the help of the monoclonal antibodies against the variant strain DEL-E and GLS, showed that the presence of six distinct but closely related neutralization epitopes clustered in two or three sets on the VP2 (Snyder, 2009). Very virulent strains are not antigenically different from the classic strains unlike variant strains from USA and Australia (Roessler and Rosenberger, 1989). It is not clear whether vvIBDV evolved from classical strains or pre-existed in nature with other avian species (Inoue *et al*., 1992).

Very limited antigenic variation has been reported in the French vvIBDVs (Fahey *et al*., 1991; Eterradossi *et al*., 1997). Fahey *et al*, (1991) reported two non overlapping epitopes recognized by the virus neutralizing monoclonal antibodies on VP2. Four conformation dependent neutralization epitopes were recognized on VP2. The VP2 sequencing results confirmed that neutralizing epitopes are clustered in the variable domain which is highly hydrophobic (Tanimura and Sharma, 1998).

In the vvIBDV strain there is no evidence of antigenic variation from the classical strains (Tanimura *et al*., 1995). A modified epitope has been described with the help of neutralizing monoclonal antibodies corresponding to a mutation at amino acid position 222 in the first hydrophilic peak of VP2. No antigenic drift has been demonstrated by cross neutralization tests. Hydrophilic peak B in the VP2 protein is involved in the formation of one or more neutralizing epitopes (Jackwood and Saif, 1983). Several mutations have been observed in the area between amino acid positions 317-323 by the nucleotide sequencing of the region (Heine *et al*., 1991; Dormitorio *et al;* 1997; Eterradossi *et al.*, 1998).

In France, some field isolates have shown atypical antigenicity due to the critical amino acid changes in the second hydrophilic peak but these have not replaced the more typical prevalent

vvIBDVs (Eterradossi *et al.,* 1997; Eterradossi *et al.,* 1998). A neutralization epitope has been possibly modified in European pathogenic IBDV strains because two monoclonal antibodies bind to the Faragher 52/70 strain, but not the atypical 89 163 isolate in an antigen capture ELISA (Eterradossi *et al.,* 1997). No genetic or antigenic absolute marker for virulence has been described. So the only way to demonstrate virulence is still by the *in vivo* inoculation (Eterradossi, 2001).

2.3.3.5 Replication of Infectious Bursal Disease Virus

A single replication cycle takes 6-8 h for IBDV and the virus binding protein has been found at various types of chicken cells for IBDV (Regenmortel, 2003). The cell receptor recognition site on the virus is not known (Lukert and Saif, 2003). Replication of IBDV is not restricted by the presence of specific receptor sites. Strains of both IBDV serotypes bind to lymphoid cells isolated from bursa, thymus or spleen indicating that all of these cells are susceptible and have the specific receptor site for the entry of the virus (Nieper and Muller, 1996). The specificity of binding was determined by saturation and competition experiments (Nieper and Muller, 1996). The CEF has receptors common to both serotypes and specific ones for each serotype. Receptor sites common to both serotypes were also present on lymphoid cells; however, additional serotype specific sites were demonstrated for apathogenic serotype 2 strain (Nieper and Muller, 1996).

The biochemical steps involved in replication of IBDV or other birnaviruses have not been completely characterized (Kibenge *et al.,* 1988). Two factors restricting the study of replication process are that only a low number of cells support virus replication *in vitro* and sensitivity of virus towards Actinomycin D (a selective inhibitor of cellular RNA synthesis) (Spies *et al.,* 1987).

It has been suggested that cell tropism of the virus is determined by receptor-mediated entry step (Boot *et al*., 2000; Brandt *et al*., 2001). After entry into the host cell, the virion RNA dependent RNA polymerase becomes activated and produces two genome length (24 S) mRNA molecules from each of the 14S dsRNA genome segments (Regenmortel, 2003). These mRNA lack a cap and 3' poly A tail and have a VPg attached to their 5′ end (Regenmortel, 2003). Genome –linked proteins have been described indicating that the virus replicates by a strand displacement mechanism (Spies *et al*., 1987). The separate strands of the genomic ds RNA can be translated *in vitro* (Azad *et al*., 1985).

Replicative intermediates have been described in the infected cells however, there are no reports on the minus strand synthesis (Regenmortel, 2003). The two mRNAs are synthesized in the same relative proportions throughout the replicative cycle (twice as many A as B mRNA molecules) (Regenmortel, 2003). On a plus-strand IBDV segment A cRNA template, minus-strand synthesis occurred in such a way that a covalently linked double-stranded RNA product was generated by a 'copy-back' mechanism (Von Einem *et al*., 2004). Importantly, enzyme activity was observed only with templates that comprised the 3' non-coding region of plus-strand RNAs transcribed from IBDV segments A and B, indicating template specificity (Von Einem *et al*., 2004).

There are two initiation codons upstream of the polyprotein start codon of segment A. The first at postion 66 at the start of 12 amino acid polyprotein and second at position 98, potentially initiating the trasnslation of 17K polypeptide. According to Kozak's rules the codon at 66 and initiation codon for polyprotein are good candidates but the one at 98 is a poor candidate for the polyprotein synthesis (Bayliss *et al*., 1990). N-terminus position of VP4 is completely conserved and might be required for the correct activity of the protease. Ribonucleic acid polymerase activity can be demonstrated without any pretreatment of the virus particle showing that

transcription and replication occur after the virus had entered the host cells without uncoating (Spies *et al*., 1987). The viral nucleic acid replicates by strand displacement reaction. Both ends are circularized by 90 Kd VP1 (Spies *et al*., 1987).

The activity of viral RNA dependent RNA polymerase requires removal of Ca^{++} from the reaction mixture. Optimal polymerase activity was found to be at pH 8.5 at 40 ^{0}C in the presence of Mg^{++} ions. Monovalent cations Na^+ and K^+ significantly increase the enzyme activity. The RNA products synthesized *in vitro* are 24S single stranded RNA (the newly transcribed mRNA), 14 S double stranded RNA (the two genomic double stranded RNA) and large intermediate transcription products containing complexes of RNA (Kibenge *et al.,* 1988).

It has been reported that the synthesis of the host proteins is not shut off in CEF infected with IBDV (Becht, 1980). Restriction of IBDV to lymphoid B cells might not be determined by the presence of a specific receptor, but cis–acting elements might play a role in restriction of virus replication in B lymphocytes (Schroder *et al*., 2001). Virus particles assemble and accumulate in the cytoplasm. Auto-proteolytic cleavage of polyprotein is the first step governing the IBDV capsid assembly. Although the primary cleavage sites are known, the proteolytic cascade involved in the processing is still not understood. Residues Ser-652 and Lys-692 are important for the protease activity of VP4. The VP4 proteases of birnaviruses are species specific, since they do not cleave heterologous substrates (Lejal *et al*., 2000).

The VP4 accumulates in the nucleus and the cytoplasm, the mechanism of viral release is unknown (Regenmortel, 2003). The VP5 and N-terminus of VP2 upto 647 nucleotides were not responsible for the different pathotypes of IBDV serotypes 1 and 2 as shown by the production of chimeric viruses. Birnaviruses are cytolytic viruses but the exact mechanism of viral release is

not known (Lombardo *et al*., 2000). Viral protein expression or the formation and accumulation of virus particles are thought to induce changes in membrane permeability eventually leading to cell lysis. Expression of single viral proteins have been demonstrated in several viruses e.g. 2B protein of poliovirus, NSP4 of rotavirus and E3-11.6 K of adenovirus to induce lysis of the cell (Flint *et al*., 2000). The VP5 caused severe cytotoxic effects resulting in the cell lysis and it was proposed that VP5 acts as a death protein and helps in viral release (Lombardo *et al*., 2000).

2.3.4 Hosts of Infectious Bursal Disease Virus

Infectious bursal disease virus is host specific and has been reported in Ostriches, in Baltic ducks and herring Gulls, various raptors and passerine species in Japan (Hitchner, 1970; Nieper and Muller, 1996; Gouch *et al*. 1998). Infectious bursal disease antibodies have been reported from Antarctic penguins (Gardner *et al*., 1997). Experimental inoculation of pheasants, partridges, guinea fowls and quails showed no signs of disease (Tham and Moon, 1996). Japanese quails are refractory to IBDV infection (Greenfield *et al*., 1986). They tested negative for virus replication, but precipitating or virus neutralizing antibodies as well as gross and microscopic changes in the bursa of Fabricius associated with infection (Tsukamato *et al*., 1995). However, quail-chicken hybrids can be infected with IBDV (Greenfield *et al*., 1986). In one study dogs were evaluated as potential carriers of the virus since viable virus persisted in the faeces two days after initial ingestion of IBDV infected-chicks and maintained its original characteristics (Torrents, *et al*., 2004).

2.3.5 Pathogenesis of Infectious Bursal Disease Virus

Pathogenesis is defined as the mechanism used by the virus to cause injury to the host with mortality, disease or immunosuppression as a consequence, the injuries can be evaluated at the level of host, the organ and the cell (Tanimura and Sharma, 1997). Infectious bursal disease virus

usually infects young chickens between 3-6 weeks of age and causes a clinical disease, while subclinically infecting older birds (Moody *et al*., 2000). The outcome of IBDV infection is dependent on the strain and amount of the infecting virus, the age and breed of the birds, route of inoculation and presence or absence of neutralizing antibodies (Moody *et al.*, 2000).

Sequential studies of tissues from orally infected chickens using immunoflorescence detected the viral antigen in macrophages and lymphoid cells in the caecum at 4 h PI and in the lymphoid cells of duodenum and jejunum at 5 h PI (Muller *et al*., 2003). The virus reaches the liver at 5 h PI and enters the bloodstream from where it is distributed to other organs; bursal infection was followed by second viremia. The virus persists in the bursa of experimentally inoculated SPF chickens till 3 weeks of age but the presence of maternal antibodies in the commercial chicken decreases the duration of its existence in the bursa (Abdel-Alim and Saif, 2001). Appearance of viral antigen in the bursa is accompanied by an infiltration of T cells while IgM + cells undergo a precipitous decrease and the immunoglobulin level remains the same (Kibenge *et al*., 1999). The ratio of CD4 and CD8 cells were the same during the 1st seven days PI but CD8 cells became predominant afterwards (Kibenge *et al*., 1999).

Infectious bursal disease virus induced bursal T cells have increased surface expression of MHC-II and IL-2 receptors, elevated expression of cytokine genes like IFN-γ and IL-6 like factor (Saif, 1991). Thymic dependant cells from the bursa of the IBD recovered birds proliferate when exposed *in vitro* to purified IBDV. While the spleen cells from IBDV exposed chickens produced nitric oxide (NO) stimulating factor when stimulated *in vitro* with purified IBDV. Bursal T cells also suppressed the mitogenic proliferation of the spleen from normal, virus free chickens (Saif, 1984).

The target organ for pathogenic serotype 1 is the bursa of Fabricius (BF). The BF reaches the maximum development between 3-6 weeks of age and at this time chickens are most susceptible to the disease and infection results in high mortality during the acute course of the disease or in B cell deficiency after recovery from infection (Becht, 1980; Jagadish *et al*., 1988). Chickens infected with IBDV immediately after hatching develop a chronic infection with atrophy of BF and B cell depletion (Winterfield *et al*., 1972; Hudson *et al*., 1975). Bursectomized chickens survive the IBDV infections lethal for normal chicken (Kaufer and Weiss, 1980). High concentrations of antigens and high infectivity titres were found in the BF of infected chickens, whereas only traces of antigen and low virus titres were detected in the thymus, spleen and peripheral blood (Kaufer and Weiss, 1980; Burkhardt and Muller, 1987). *In vitro* infection studies have shown that IBDV replicates in the population of proliferating B cells but not in very immature lymphobalsts or competent B cells (Becht, 1980; Beug *et al*., 1981; Muller, 1986).

Treatment of chicken with cyclophosphamide (CY) or surgical bursectomy at 4 weeks of age is known to prevent IBDV infection; bursectomized chickens did not show the disease and had transient lesions in the lymphatic tissues. However, these chickens contained virus and produced antibodies against it (Jackwood *et al*., 1984). The virus concentrations in the bursectomized chickens were about 1,000 times lower than the non bursectomized chickens (Jackwood *et al*., 1984). It appeared that the availability of a large number of bursal cells is an essential factor in the development of IBD (Ismail *et al*., 1987).

The bursa and spleen were reported to have substantially higher concentration of the virus as compared to any other tissue. The bursa harvested from chickens at 72 h PI yielded high virus titres followed by spleen and kidneys. No virus was detected beyond 10 days PI in the pancreas (Mackenzie and Spradbrow, 1981). Infectious bursal disease virus was reported to persist in the

chicken for a few days but the lesions could be seen for at least 10 weeks, the longest interval evaluated in that study (Winterfield *et al*., 1972). The IBDV was re-isolated most consistently from the bursa and less frequently from thymus, liver, kidneys, lungs and spleen. No virus was isolated from the pancreas (Mackenzie and Spradbrow, 1981). The virus was not detected beyond 11 days PI in commercial chickens when inoculated at 1, 7 or 14 days of age. In birds inoculated at 21 days of age, IBDV was re-isolated till 8 days only. The precipitating antigen was detected only in the bursa and only at 3^{rd}, 4^{th} and 5^{th} day PI but was not detected in any organ of chicken infected at 21 days of age (Mackenzie and Spradbrow, 1981).

Skeeles and Lukert (1979) reported that after chickens were inoculated with an attenuated cell culture adapted virus at one day of age, the virus was detected in cell culture inoculated with homogenate of BF, spleen, thymus, liver, kidneys and lungs for up to 14 days PI, in one experiment and 10 days PI in another. No virus was detected after the levels of neutralizing antibodies became significantly high. Also, the virus was not detected in tissues from birds given the virus at three weeks and low virus neutralizing antibody titres were detected, indicating an age resistance to CEF virus or insufficient dose.

When SPF chickens were inoculated with IBDV at 3 week of age, the viral RNA was detected from the BF by real time polymerase chain reaction (RT PCR) till 21 days PI, but attempts to isolate infectious virus from bursal homogenates failed (Abdel-Alim and Saif, 2001). Infectious virus was detected by embryo inoculation up to 7 days PI in the bursa of SPF chickens inoculated at 2 or 3 weeks of age, whereas the viral RNA was detected by RT PCR for up to 28 days PI. In SPF chickens inoculated at 1 day of age, the bursa-derived virus or its RNA was detected at 7 and 14 days PI when inoculated at a high dose 10^4 EID_{50}/bird or at a low dose $10^{2.5}$ EID_{50}/bird. In commercial 1-day-old broiler chickens, the bursa-derived virus was detected at 7

and 14 days PI when inoculated at a high dose 10^4 EID_{50}/bird, whereas the virus was detected only at 14 days PI when inoculated at a low dose $10^{2.5}$ EID_{50}/bird. In SPF and commercial chickens, vaccinated with a modified live IBDV vaccine, the virus persisted in the bursa of SPF chickens till 3 weeks but maternal antibodies in the commercial chickens rapidly eliminate it from the bursa since no live vaccine virus nor its RNA was detected in commercial broilers vaccinated at 1 day or 2 weeks of age (Abdel-Alim and Saif, 2001).

2.3.6 Infectious Bursal Disease Virus and Immunity

2.3.6.1 Effect of Infectious Bursal Disease Virus on Innate Immunity

Infectious bursal disease virus has been shown to modulate the macrophage function by altering the *in vitro* phagocytic activity, macrophages from infected chickens have upregulated cytokine gene expression and produce increased levels of NO (Kim *et al*., 2000).

2.3.6.2 Effects of Infectious Bursal Disease Virus on Humoral Immunity

Infectious bursal disease virus has a predilection for the immature actively dividing B lymphocytes and causes lytic infection of IgM bearing B cells resulting in decrease in circulating IgM+ cells. Infected chicken produce less level of antibodies against the IBDV antigen (Sivanandan and Maheswaran, 1980; Kibenge *et al*., 1999). Only primary antibody responses are affected. Secondary responses remain unaltered (Sharma *et al*., 1989; Rodenberg *et al*., 1994). Infectious bursal disease virus induced humoral deficiency is reversible and overlaps with the restoration of bursal morphology (Sharma *et al*., 2000).

Chickens infected with IBDV at 1 day of age were found to be completely deficient in serum immunoglobulin G and produced only a monomeric immunoglobulin M (IgM) (Ivanyi, 1975; Ismail and Saif, 1991). IgG levels varied depending on the age at the time of infection (Hirai *et*

al., 1979). The number of B cells in peripheral blood was reduced after infection with IBDV but T cells were not appreciably affected (Sivanandan and Maheswaran, 1980). The adverse effect on antibody responses is due to the damage to the B cells in the bursa and the blood. Since the virus has a predilection for actively dividing B cells as compared to the mature B cells (Sivanandan and Maheswaran, 1980).

2.3.6.3 Effect of Infectious Bursal Disease Virus on Cellular Immunity

The extent to which the cellular immune response is affected is not well understood. However, it is known that the effect of IBDV on cell mediated immunity (CMI) is transient and less pronounced than the effect on humoral response. Infected chickens show a poor cellular response to certain antigens and show increased susceptibility to disease that are under the control of cellular immune defense (Anderson *et al*., 1977). The thymic lesions were transient and appeared within the first week of infection, peaked at 3-4 days PI and then subsided. The presence of thymic lesions were not associated with active viral replication of the virus in the thymic cells as shown by the immunoflorescence (IF) and antigen capture ELISA. In addition, T cells from infected chickens during the early stages of virus infection fail to respond optimally to mitogens *in vitro* (Confer *et al*., 1981).

Maximum depression in the cellular immunity was shown to occur at 6 weeks PI by using the lymphoblast transformation assay (Sivanandan and Maheswaran, 1981). The reason for the delay in this response is not clear considering that the virus persists in the host for approximately 3 weeks, it was speculated that this depression is the overall depression of T-cell function during the virus infection (Sivanandan and Maheswaran, 1981). The effect of IBDV on two CMI functions i.e. natural killer cell cytotoxicity and mitogenic response had been studied. It was reported that IBDV had an inconsistent effect on the natural killer cell cytotoxicity but caused a

transient early depression of the blastogenic response of spleenic cells to phytohaemagglutinin (Sharma and Lee, 1983). *In vitro* mitogen hypo-responsiveness of T cells is mediated by the suppressor cells in the spleens of the infected chicken; the mechanisms of reduced *in vivo* cellular immunocompetence are not known (Lam, 1991).

Infectious bursal disease virus infected chickens were shown to have a normal natural killer cell levels, mononuclear phagocytic activity and delayed type hyper sensitivity reaction (Giambrone *et al*., 1977; Howie and Thorsen, 1981). Neither did virus infection alter the normal proportions of CD4 and CD8 subsets of T cells in the circulation and spleen, it was reported that variant A strain of IBDV had a significantly higher effect on CMI as compared to the standard Edgar strain when given to day old chickens which lingered until 5 weeks (Sharma *et al*., 1993). A similar effect was reported in chickens infected at 3 weeks of age (Craft *et al*., 1990).

Harderian glands are another component of immune system associated with the local immune system of the respiratory tract (Dohms *et al*., 1981). Infectious bursal disease infection of 1-5 day-old chickens produced a dramatic decrease in plasma cell content of the harderian gland that lasted till 7 weeks (Dohms *et al*., 1981).

2.3.7 Immunology of Infectious Bursal Disease Virus

Immunoglobulin M (IgM) producing cells are the target cells for the IBDV. During the acute phase of the disease the bursa undergoes atrophy as the bursal follicles get depleted of B cells. Virus replication causes extensive damage to lymphoid cells in medullary and cortical regions of the follicle. Apotosis of the neighbouring B cells augments the destruction of the bursal morphology. By this time an ample amount of viral antigen can be detected in other lymphoid organs like caecal tonsils and spleen (Snedeker *et al*., 1967; Saif, 1991). During the acute phase

of the IBD, there is a reduction in the circulating IgM + cells but circulating IgG level remains the same (Giambrone *et al*., 1977; Hirai *et al*., 1981; Kibenge *et al*., 1988; Proffitt *et al*., 1999). Maternally derived antibodies (MDA) protect chickens against subclinical IBD and immunosuppression (Giambrone and Clay., 1986). A maternally derived antibody is known to protect the chickens against IBD for up to 3 weeks of age (Lasher and Shanes, 1994).

Thymus cells are resistant to infection by IBDV, during the acute phase of the disease lesions appear in the thymus which is quickly overcome within a few days, a dramatic influx of T cells is reported in and around the site of virus replication (Hirai *et al*., 1979). The infiltrated T cells could be detected from 1^{st} to 12^{th} weeks PI although the viral antigen disappears by the 3^{rd} week (Hirai *et al*., 1979). The IBDV induced cytotoxic T cell, limit the spread of the virus by destroying the cells expressing the viral antigen and thus can initiate the recovery process. At the same time IBDV induced T cells might enhance the viral lesions by producing inflammatory cytokines. T helper cells produce inflammatory cytokines like IFN-γ which activates the macrophages to produce NO (Sharma *et al*., 2000).

Both humoral and cellular arms of the immune system are compromised during the IBDV infection due to lysis of the B cells and altered antigen-presenting cells. Infectious bursal disease virus causes a transient inhibition of *in vitro* proliferative activity of T cells to mitogens. The virus stimulates the macrophages to produce T cell cytokine like IFN-γ to produce nitric oxide (NO) and other cytokines with anti-proliferative activity (Sharma *et al*., 2000). Infectious bursal disease virus did not affect natural killer cells levels in chickens (Sharma *et al*., 2000).

The NO production after IBDV infection exerts antiviral effect since the immune-suppressed chickens that failed to induce NO had more severe disease and higher degree of virus replication,

but does not seem to correlate with the haemorrhagic lesions which result from the reaction of host factors and the determinants responsible for virus virulence and virus clearance (Poonia and Charan, 2005).

The IBDV induced damage to humoral immunity is reversible and antibody production correlates with the morphologic restoration of the bursal follicles (Shamaila, 2005). Mitogenic response of T cells returned to the normal levels. During the course of mitogenic inhibition, T cells of infected chicken also failed to secrete IL-2 upon *in vitro* stimulation (Knoblich *et al*., 2000).

The T cells play a significant role in the pathogenesis of IBDV. Intra bursal T cells and T-cell-mediated responses play significant role in viral clearance and promoting recovery from infection, they defend the host cell by reducing the viral burden but at the same time produce inflammatory cytokines and NO inducing factor that enhance tissue destruction and also delay the recovery process (Sharma *et al*., 2001).

Chickens that survive the IBD clear the virus and recover from its pathologic effects (Sharma *et al*., 2000). It has been shown that the more virulent the virus the stronger is the suppression of the humoral and cell mediated immunity (Shamaila, 2005). Virulent virus also produced a detectable NO production in serum. Humoral immunity is the primary mechanism of the protective immune response (Shamaila, 2005). Infection with IBDV results in the formation of antibodies to the group and serotype specific antigens (Jackwood *et al*., 1985).

Field exposure or vaccination results in VN titre higher than 1:1000, but weak responses are obtained in chickens immunized with purified viral polypeptides since viral protein conformation is important in eliciting a high antibody response (Fahey *et al*., 1985; Azad *et al*., 1987).

Antibody production is stimulated at the primary site of viral replication in gut associated tissue and they can be detected as soon as 3 days PI. These antibodies prevent the spread of the virus to other tissues. Due to the rapid onset of antibodies, the necrotic foci that form in the bursa of Fabricius stop expanding and are completely eliminated (Becht, 1980).

2.3.8 Immunosuppression during Infectious Bursal Disease Virus Infections

Immunosuppression caused by IBDV has a significant economic impact due to widespread nature of the disease in commercial chickens (Shamaila, 2005). Infectious bursal disease virus infection at an early age compromises the humoral and local immune responses of chickens (Shamaila, 2005). Allan *et al,* (1972) made the earliest observation about the immunosuppressive potential of IBDV. The extent of the immunosuppressive effect is related to the age at infection. The most pronounced damage results if the infection occurs within the first 2-3 weeks of hatch (Allan *et al*., 1972). Birds less than three weeks of age do not exhibit clinical signs but are immunosuppressed (Rosenberger *et al*., 1994). Following the ingestion of the virus, lymphoid cells and macrophages in the intestine are infected and carry the virus to the BF (Muller *et al.*, 2003). Clinical signs and lesions of IBDV appear shortly thereafter (Muller *et al*., 2003). Chickens infected with IBDV are more susceptible to various other infections. Chickens exposed to IBDV at 1 day of age had lower antibody responses and were more prone to infection with Newcastle disease (ND) virus (Faragher *et al*., 1972). The infected chicken had a decreased humoral response to vaccines e.g ND, fowl pox and fowl typhoid (Hirai *et al*., 1974).

Immunosuppression resulted in lower flock performance, more secondary infections, poor feed conversion, less protective response to vaccines and higher rate of carcass condemnation at the processing level (Sharma *et al*., 2000). The immunosuppressive effects of IBDV are dependent on the strain of the IBDV (Craft *et al*., 1990; Mazariegos *et al*., 1990; Higashihara *et al*., 1991;

Sharma *et al*., 2000). Chickens infected with IBDV at an earlier age succumbed to other infections like inclusion body hepatitis reovirus, coccidiosis, Marek's disease, haemorrhagic-aplastic anemia, gangrenous dermatitis infectious laryngotracheitis, chicken infectious anemia, salmonellosis, colibacillosis and mycoplasmosis (Wyeth, 1975; Fadly *et al*., 1976; Anderson *et al*., 1977; Giambrone *et al*., 1977; Rosenberger and Gelb, 1978; Schnitzler *et al.,* 1993).

An enigma surrounding IBDV infections was that although there was immunosuppression against many antigens, the response to IBDV itself remained normal even in day old susceptible chicken (Snedeker *et al*., 1967). Selective stimulation of the proliferative B cells committed to anti-IBDV antibody production seems to occur. Peripheral blood lymphocytes (PBL) from chickens infected with IBDV have depressed proliferative responses to stimulation with the mitogens concanavalin A or phorbol myristate acetate (McNeilly *et al*., 1999; Sharma *et al*., 2000). Reduction in the number of B cells in the BF due to viral infection is the major cause of immunosuppression. Suppression of B cell function might be caused by damage to helper T cells or other cells involved in generating the immune responses (Sharma *et al*., 1993). Chickens infected with IBDV have suppressor cells in the spleen, which cause *in vitro* mitogenic hypo responsiveness to concanavalin A. These cells prevent normal spleen cells from responding to the mitogen. The impairment of T cells and development of suppressor cells was demonstrated *in vitro* by using proliferation tests or by measuring the cytokine release after mitogen activation of T cells (Confer and MacWilliams, 1982; Saif, 1984; Lam, 1988).

Besides lymphocyte lysis, apoptosis also plays a role in immunosuppression (Vasconcelos and Lam, 1995; Ojeda *et al*., 1997; Tanimura and Sharma, 1998). Apoptosis could occur in a variety of organs like thymus, BF and spleen (Allan *et al*., 1972; Inoue *et al*., 1994; Vasconcelos and Lam, 1995; Lam, 1997). Chicken embryo fibroblasts and Vero cells infected with IBDV show

the biochemical features of apoptosis (Tham and Moon, 1996). In addition to causing necrosis, IBDV can also induce apoptosis in avian lymphocytes *in vitro*, a correlation exists between virus replication and apotosis in cells of BF (Nieper *et al*. 1999; Vasconcelos and Lam, 1995). Viral proteins like VP2 and VP5 have been implicated in the induction of apoptosis (Fermandez-Arias *et al*., 1997; Yao *et al*., 1998). Expression of VP2 polyprotein in transiently transfected DT40 B lymphocyte culture suppresses cell growth and proliferative responses to mitogen stimulation indicating that IBDV polyprotein is a mediator of immunosuppression (Peter *et al*., 2004). Apoptotic cells have also been observed in antigen negative bursal cells indicating that immunological mediators like cytokines might be involved in the process (Tanimura and Sharma, 1998)

2.3.9 Apoptosis in Infectious Bursal Disease Virus Infection

Apoptosis or the programmed cell death plays a major role in the immuno-pathology of IBDV characterized by destruction of cells of BF (Jackwood *et al*., 1984; Burkhardt and Muller., 1987; Becht and Muller, 1991). Only 20% of the lymphoid cells in the BF contain replicating IBDV, the severe damage to the bursa can be ascribed to apoptosis (Muller, 1986; Burkhardt and Muller, 1987). In addition to necrosis, marked atrophy of the BF occurs without eliciting an inflammatory response that is a characteristic sign of the apoptotic process.

Replication of the virus in the BF results in secondary viremia thus spreading the virus to other tissues (Tanimura and Sharma, 1997). It has been suggested that early after infection, the cells containing the viral antigen are protected from apoptosis to ensure viral replication. Anti- viral mechanisms kick in and destroy the neighbouring cells to prevent the spread of the virus (Lam, 1988). During the late infection, the infected cells undergo apoptosis thus seeding the virus to other cells. The IBDV infection of a susceptible chicken has been shown to induce apoptosis in

the bursa as well as thymus (Lam, 1988; Inoue *et al*., 1994; Ojeda *et al*., 1997; Tanimura and Sharma, 1997).

Morphological and biochemical features of apoptosis were also observed after *in vitro* infection of chicken peripheral blood lymphocytes and CEF with IBDV (Vasconcelos and Lam, 1995; Tanimura *et al*., 1995). Apoptosis occurs in lymphocytes of various organs like thymus, bursa of Fabricius and spleen (Lam, 1988; Inoue *et al*., 1994). Some researchers believed that apoptosis induced by IBDV in cell cultures following *in vitro* infection was an early genetic response of the host cells and was independent of virus replication while others showed that appearance of cytopathic effect (CPE) coincided with virus replication (Tanimura *et al*., 1995).

Jungmann *et al*., (2001) however, showed that proportion of apoptotic cells increased from 5.8 % at 4 hrs PI to 64.5% at 48 h PI. in chicken embryo (CE) cells after infection with IBDV strain Cu-1 however, treatment of CE cell cultures with ultraviolet light (UV) inactivated IBDV did not induce apotosis (Jungman *et al*., 2001). Whether apoptosis is triggered via virus receptor activation is not yet known. Double labeling technique revealed that during the course of early infection maximum number of antigen expressing cells were not apoptotic. It was only later in the infection that the double-labeled cells appeared (Jungman *et al*., 2001).

Double labeling technique determined the distribution of both apoptotic cells and cells containing viral antigen in the same section of BF (Tanimura and Sharma, 1998). Double labeling studies for apoptotic or antigen positive cells revealed that apotosis in the bursa occurs both in IBDV positive and IBDV negative cells whereas apoptosis in the thymus occurs in the antigen negative cells only (Tanimura and Sharma, 1998). It was concluded that IBDV induced apoptosis indirectly in non-bursal organs. It has been postulated that IBDV impairs the

withdrawal of apoptotic cells and therefore results in the increased number of the apoptotic cells. Apoptotic cells were located mostly in an area between the cortex and medulla whereas majority of cells positive for viral antigens were found in medulla of the BF (Ojeda *et al*., 1997).

Indirect mechanisms might also be involved in the induction of apoptosis and could have induced apotosis in vivo resulting in rapid depletion of cells in BF. In the infected follicles large numbers of cells were apoptotic but very few contained the viral antigen (Tanimura and Sharma, 1998; Jungman *et al*., 2001). Interferon production occurs after IBDV infection and is thought to be the major apoptosis–inducing factor in the neighboring cells along with TNF-α (Jungman *et al*., 2001).).

2.4 CLINICAL SIGNS OF INFECTIOUS BURSAL DISEASE

The disease is characterized by ruffled feathers, watery diarrhoea, trembling, severe prostration, dehydration, haemorrhages in the leg and thigh muscles, increased mucous in the intestine and enlargement of the bursa of Fabricius Cosgrove, (1962). Losses due to the classical IBDV could reach up to 50% morbidity and less than 3% mortality in broilers and up to 20% mortality in commercial Leghorn pullets (Muller *et al*., 2003). Losses due to very virulent strains of the virus in Europe have reached approximately 30-40% mortality in broilers and 50-70% in commercial layers (Chettle *et al*., 1989; van de Berg *et al*., 1991; Contreras *et al*., 2000). Okoye *et al.* (1999) reported a mortality rate of 85%, 66.7%, 30% and 20% for the pullets, cockerels, local chickens and broilers, respectively in Nigeria.

2.5 PATHOLOGY OF INFECTIOUS BURSAL DISEASE

2.5.1 Gross Lesions

The tissue distribution and severity of lesions is dependent on the subtype and pathogenicity of the IBDV (Rosenberger and Cloud, 1986; Tanimura *et al*., 1995). Infected birds are dehydrated and have darkened discoloration of pectoral muscles. Haemorrhages occur in the thigh and pectoral muscles and are also reported from the mucosa at the proventriculus-ventriculus junction and on the serosal surface and plica of the bursa (Rosenberger and Cloud, 1986; Tanimura *et al*., 1995). There is increased mucus in the intestine and renal changes are observed in diseased birds that are due to dehydration (Lukert and Saif, 2003). The kidneys, tubules and ureters are so distended and filled with urates that they appear white (Cosgrove, 1962).

The bursa is the target organ for the replication of IBDV and hence the most severely affected. Characteristic pattern of bursal changes observed during the course of infection differ for the classic and variant viruses (Rosenberger and Cloud, 1986). During infection with classic viruses, the bursa increases transiently in size accompanied by inflammation. After the inflammation subsides, rapid bursal atrophy occurs. On the other hand, infections with some variant viruses like VA isolate are not accompanied by inflammation while other variants like IN produce inflammation (Sharma *et al*., 1989; Hassan *et al*., 1996). Variant viruses result in a rapid atrophy of the bursa (Rosenberger and Cloud, 1986).

In a detailed study using the Edgar strain of the IBDV Cheville (1967) recorded the bursal weights for 12 days PI. The bursa began to increase in size and weight due to oedema and hyperemia on 3 days PI and by day 4 it doubled in size. By day 5, the bursa returned to its normal weight and from day 8 it atrophied further and became one third its original weight. By day 2 or 3 post-infection, the bursa had a gelatinous yellowish transudate covering the serosal

surface. Longitudinal striations became prominent and the colour changed from white to creamy. The transudate disappeared as the bursa returned to its normal size and the bursae turned gray during the period of atrophy (Lukert and Saif, 2003).

Extensive haemorrhages could be seen on the entire bursa. Pathologic changes in the spleen and thymus were less prominent than those of the bursa (Cosgrove, 1962; Inoue *et al*., 1994). The spleen might be slightly enlarged and usually had small gray foci uniformly dispersed on the surface (Lukert and Saif, 2003). Lesions in the spleen and thymus were noticed at the same time as the changes occurred in the bursa and resolved within 1 or 2 days of appearance (Helmboldt and Garner, 1964).

The very virulent infections are characterized by severe clinical signs, high mortality, and a sharp death curve followed by rapid recovery. The vvIBDV strains have the same clinical signs and incubation period of 4 days as classical viruses but the acute phase is exacerbated (Van de Berg, 2000). The vvIBDV strains cause more severe lesions in the caecal tonsils, thymus, spleen and bone marrow and a greater decrease in thymic weight index as compared to the moderately pathogenic strains, but bursal lesions are similar (Tanimura *et al*., 1995). It has been shown that the pathogenicity of field strains of IBDV correlate with lesion production in non-bursal lymphoid organs. The results also suggest that the pathogenicity of IBDV may be associated with virus antigen distribution in non-bursal lymphoid organs (Tanimura *et al*., 1995).

2.5.2 Microscopic Lesions of Infectious Bursa Disease Infection

Histopathologic lesions occur in the bursa of Fabricius, spleen, thymus, harderian glands and caecal tonsils, the first signs of infection occur in the bursa and it is the most severely affected organ. Degeneration and necrosis of individual lymphocytes in the medullary region of the bursa

occur as early as 1 day post infection. Lymphocyte degeneration is accompanied by nuclear pyknosis and formation of lipid droplets in the cytoplasm (Cheville, 1967). Other lesions in the bursa of Fabricius include: congestion of blood vessels, interfollicular edema or fibroplasias, hypertrophy of follicular cortico-medullary epithelium, atrophy of lymphoid follicles, reticuloendothelial hyperplasia, cystic formations and epithelial erosion (Abdu, 1986).

Degenerating lymphocytes are surrounded by macrophages; lymphocytes are soon replaced by heterophils, pyknotic debris, and hyperplastic reticuloendothelial cells. By 3 or 4 days post infection, all lymphocytes have been affected. At this point the bursal weight increases due to edema, hyperemia, and accumulation of heterophils. As the inflammatory reaction subsides, cystic cavities appear in the medullary region of the bursal follicles. Necrosis and phagocytosis of the heterophils take place and fibroplasia occurs in the inter-follicular connective tissue (Helmboldt and Garner, 1964; Cheville, 1967; Lukert and Saif, 2003).

Proliferation of the bursal epithelial layer occurs producing glandular structures of columnar epithelial cells containing globules of mucin. Follicular regeneration and repopulation of follicles with lymphocytes occur but healthy follicles are not formed during the observed time span of 18 days (Helmboldt and Garner, 1964). Variant A isolate of IBDV cause extensive lesions in the bursa but lacks an inflammatory response (Sharma *et al.*, 1989).

The spleen shows hyperplasia of the reticuloendothelial cells around the adenoid sheath arteries during the early stages of infection. Lymphoid necrosis occurs in the periarteriolar lymphoid sheath by three days post infection. The spleen recovers shortly without any sustainable damage to the germinal follicles (Cheville, 1967; Lukert and Saif, 2003).

Changes in thymus and caecal tonsils appear shortly after infection and include areas of lymphoid necrosis and hyperplasia of the reticular and epithelial components in the medullary region of thymic follicles (Cheville, 1967). The damage is less extensive than in the bursa and is quickly repaired by 12 days post infection (Cheville, 1967). Variant virus A is reported to cause milder lesions in the thymus than a standard (IM) strain (Sharma *et al*., 1989).

The Harderian glands are reported to be severely affected by the IBDV in 1- day old chickens (Survashe *et al*., 1979). Normally, the glands are populated with plasma cells as the chicken ages but the infection prevents this infiltration. A Harderian gland of the chickens infected at 1 day of age has 5-10 folds fewer plasma cells than those of uninfected chickens from 1-7 weeks of age (Dohms *et al*., 1981). However, lymphoid follicles and heterophil populations in the Harderian gland are not affected by IBDV infection, nor could necrotic or degenerative changes be found in the acini or excretory ducts. In contrast, the broilers infected at three weeks of age have a 51 % reduction in plasma cell content at 5-14 days post infection, plasma cell numbers reduction was temporary and levels became normal after 14 days in the Harderian glands (Dohms *et al*., 1988). Histologic lesions appearing in the kidneys were nonspecific and resulted from dehydration (Helmboldt and Garner, 1964). The liver had some slight perivascular infiltration of monocytes (Peters, 1967).

2.6 DIAGNOSIS OF INFECTIOUS BURSAL DISEASE

2.6.1 Clinical Signs

Acute clinical outbreaks of classical IBDV in susceptible chicken flocks 3-6 weeks old broiler and replacement pullet flocks are characterized by a sudden onset, high morbidity, spiking mortality curves and a rapid recovery time of about 5-7 days from clinical signs. Grossly visible changes are observed in the bursa that includes changes in colour and size of the bursa during the

course of infection Cosgrove, (1962). Infections of young chickens or of those having maternal antibodies are diagnosed by histopathological examination of the bursa (Lukert and Saif, 2003). Variant viruses differ from the standard viruses in that they cause less mortality and produce splenomegaly and liver necrosis of the embryos (Lukert and Saif, 2003).

2.6.1 Virus Isolation

The bursa and spleen are used for the isolation of the IBDV (Hitchner, 1970; Lukert and Saif, 2003). The virus can be found in other organs such as the thymus, liver and bone marrow but in significantly low quantities than in the bursa (Cheville, 1967; Tanimura and Sharma, 1997). The inoculum for virus isolation is prepared by homogenizing the tissue sample in antibiotic containing buffer that is centrifuged to remove larger tissue particles and is used for inoculating embryonated eggs and tissue culture (Lukert and Saif, 2003).

The chorioallantoic route of inoculation in 9-11 days old embryos is the most sensitive route for the isolation of the virus. Classic viruses usually kill the embryos in 3-5 days and produce lesions of vascular congestion and subcutaneous haemorrhages in the embryos (Hitchner, 1970; Lasher and Shane, 1994). Variant viruses however, do not kill the embryos but cause embryo stunting, discoloration, splenomegaly and hepatic necrosis (Lukert and Saif, 2003).

Primary cell cultures of CEF, bursa (CEB) and kidney (CEK) have been used to propagate the virus (Shamaila, 2005). McFerran (1980) reported that three out of seven chicken isolates failed to grow in CEF cells but propagated well in embryonating eggs. Some strains grow well in embryos but are not readily adapted to grow in CEF or CEK (McFerran *et al*., 1980). Cell cultures containing 50% bursal lymphocytes and 50% CEF have been used to isolate and serotype IBD virus successfully (McFerran *et al*., 1980). The fibroblasts served as a matrix for

lymphocytes and infected lymphocytes are detected by immunoflorescence (Lukert and Saif, 2003).

2.6.2 Serology

2.6.2.1 Enzyme Link Immune Sorbent Assay

Serological tests generally used for the detection of IBDV are enzyme link immune sorbent assay (ELISA), viral neutralisation (VN) test and agar gel precipitating (AGP) test. The ELISA is the most commonly used test for the detection of antibodies to IBDV. It is economical, simple, quick and tests a large number of samples at the same time, it is also adaptive to automation to computer software (Lukert and Saif, 2003).

The ELISA allows the quantification of antibodies to IBDV and is therefore used for monitoring the immune status of the chicken flocks, to check response to vaccination, natural field exposure and decay of maternal antibody titre (Marquardt *et al*., 1980; Lasher and Shane, 1994; Lukert and Saif, 2003). However, ELISA cannot differentiate between the antibodies specific to the two IBDV serotypes (Ismail and Saif, 1990; Lukert and Saif, 2003). Therefore, while using ELISA for monitoring the chicken flocks for antibodies to IBDV, careful consideration should be given to the fact that serotype 2 viruses are widespread in commercial chickens and could result in erroneous impression of antibody levels of the flock (Abdel-Alim and Saif, 2001).

2.6.2.2 Viral Neutralization Test

Constant virus - diluting serum VN test was the most common procedure before the use of ELISA (Skeeles *et al*., 1979). The VN is the only serologic test to distinguish the serotypes 1 and 2 of IBDV and also to differentiate the antibodies to different subtypes of IBDV (Jackwood and Saif, 1987; Lukert and Saif, 2003).

At least six different antigenic subtypes of IBDV serotype 1 viruses have been identified by the *in vitro* cross-neutralization test; the VN titres accurately reflect the relative protection of chickens to IBDV (McFerran *et al*., 1980; Jackwood *et al*., 1985; Jackwood and Saif, 1983; Ismail and Saif, 1990). It is essential to use appropriate indicator viruses in VN tests to avoid artificially low titres due to the existence of several antigenic variants (Ismail and Saif, 1990).

Most chicken sera have high levels of neutralizing antibodies to a broad spectrum of antigenically diverse viruses due to vaccine and field exposure. However, VN is laborious and time consuming and therefore its use is limited to research applications. Although *in vitro* VN tests can be used for detection of antigenic differences between the virus strains, *in vivo* cross protection studies are essential for determining immunogenicity of the virus and complete evaluation of host response (Jackwood and Saif, 1983).

2.6.2.3 Agar Gel Precipitating Test

Another method used to detect antibodies to IBDV is the AGP test. This test has been adapted to the quantitative format (Cullen and Wyeth, 1975). It is rapid but insensitive. It does not detect serotypic differences and measures primarily group-specific soluble antigens (Lukert and Saif, 2003).

2.7 DIFFERENTIAL DIAGNOSIS OF INFECTIOUS BURSAL DISEASE

The lesions and symptoms of coccidiosis are similar to IBD, both diseases are common and severe in young chicks, symptoms such as ruffled feathers, dirrhoae and high mortality in chicks are common to both diseases. However, muscular haemorrhages, oedema of the bursa and nephrosis differentiate IBD from coccodiosis. Also, caecal and intestinal haemorrhages seen in coccidiosis differentite it from IBDV (Lukert and Saif, 2003).

Another disease that resembles IBD is infectious bronchitis (IB), both IB and IBD are highly infectious, chickens are the only birds that are naturally infected by these diseases, gross lesions common to both diseases include enlargement of the kidneys with urates deposition and distention of the ureters. Unlike in IBD, IB affects all ages and is characterised by respiratory rales, catarrhal exudates in nasal cavity, caseous plugs in bronchi in chicks and egg peritonitis in layers. On the contrary the characteristic muscular haemorrhages, bursal enlargement seen in IBD distinguish it from IB (Lukert and Saif, 2003).

Marek's disease (MD), can also be confused with IBD because MD like IBD is an immunosuppressive disease and bursal enlargement is common to both diseases. However, the presence of visceral tumours, unilateral or bioccular involvement of tumours, featured in MD is absent in IBD. The characteristic high mortality, shorter course of the disease and muscular haemorrhagic lesions noticeable in IBD distinguish it from MD (Lukert and Saif, 2003).

2.8 MANAGEMENT OF INFECTIOUS BURSA DISEASE

2.8.1 Prevention and Control of Infectious Bursal Disease

The epidemiology of infection is not extensively studied but it is known that the IBDV is contagious so contact with the infected birds and contaminated fomites could result in the spread of infection (Shamaila, 2005). Rigorous biosecurity measures have to be implemented in order to stop the spread of virus from one flock to the next (Shamaila, 2005). The virus is environmentally stable and resistant to many physical and chemical agents (Alexander and Chettle, 1998). Integrated nature of commercial poultry operations and vectors like lesser mealworm, mosquitoes and rats pose extra problems for the control of this infection (Shamaila, 2005).

No therapeutic treatment has been found to have an effect on the course of the viral infection (Cosgrove, 1962; Lukert and Saif, 2003). There are no reports of the use of the antiviral compounds and interferon inducers for the treatment of IBD (Lukert and Saif, 2003).

2.8.1.1 Vaccination against Infectious Bursal Disease

Before the immunosuppressive effects of IBDV were known, planned infection of birds at an early age was used as a measure to control IBD. Young chickens less than 2 weeks of age did not exhibit clinical signs of IBD. Planned infection was usually done on a farm where disease was already established and the chickens would have maternal antibodies. These infections were accomplished by exposing the chickens to the contaminated litter or by mixing infected chickens with susceptible ones (Lasher and Davis, 1997). This technique lowered IBD mortality but often resulted in immunosuppression and further dissemination of field virus. Later when the severe immunosuppressive effects of IBDV were discovered, the practice of intentional exposure of chickens became less desirable on many farms, due to lack of thorough cleaning the virus persists and provides an early exposure naturally (Lukert and Saif, 2003).

Due to the highly infectious nature of the virus and its hardiness, vaccination is inevitable under high infection pressure and it is mandatory to protect chickens against infection during the first weeks of age. Thus, immunization of the chickens is the principal method of prevention of IBD in chickens. A live attenuated vaccine based on a mild strain passaged in eggs was developed, early vaccines included the virus isolated by Edgar, Mouthrop-Snedecker and Winterfield (1968). Edgar isolate was a moderately pathogenic bursa-origin virus strain used as a live vaccine (Mazariegos *et al*., 1990).

The other two isolates, Mouthrop-Snedecker and Winterfield were attenuated by serial passage in eggs and became some of the first commercially available vaccines, Bursa Vac® (Lasher and Davis, 1997) and IBD BlenTM respectively (Winterfield *et al*., 1981). These vaccines reduced clinical signs but caused significant bursal pathology (Winterfield *et al*., 1981).

Once the immunosuppressive potential of the disease and role played by maternal immunity became clear, focus shifted to the effective vaccination of the breeder flock in order to provide maternal antibodies to the progeny. Such parental immunization protects the chickens from earlier infections. Maternal immunity protects the chicken for 1-3 weeks of age and passive immunity might be extended for 4-5 weeks by immunizing the breeder flocks with oil-adjuvanted vaccines (Lucio and Hitchner, 1979). Vaccination of breeders at 8-12 weeks of age with live attenuated virus followed by a booster of inactivated oil-emulsion prior to the onset of lay at 20-21 weeks resulted in greater transfer of maternal antibodies to the progeny (Wyeth and Cullen, 1979; Eidson *et al*., 1980).

In the sensitized hens, the inactivated vaccine induces a long lasting high titre serum antibody response, which is then transferred to the progeny (Lukert and Saif, 2003). The major problem with active immunization of maternally immune chickens is to determine the proper time of vaccination that allows adequate replication of the vaccine virus and at the same time protects young chicken from disease (Lukert and Saif, 2003). The time of vaccination varies with the level of maternal antibodies, route of vaccination and virulence of the vaccine virus, for a successful vaccination program, factors like environmental stresses, management and flock profiling for the presence of maternal antibodies should be taken into account (Lukert and Saif, 2003).

The decay of maternal antibodies was reported to be linear, in white leghorn chickens, the half-life of IBDV antibodies was reported to be from 3-8 days (Cosgrove, 1962; Skeeles *et al*., 1979). Chickens having medium levels of maternal antibodies were shown to be refractory to infection till 2 weeks, while those having high levels of maternal antibodies were resistant to infection up to 4 weeks of age. Chickens having high levels of maternal antibodies (GMT of VN = 1:3275) at hatch were shown to be protected from IBDV infection up to 5.5 weeks of age (Al-Natotur *et al*., 2004).

Live vaccines available in the US are classified according to their virulence as mild, mild intermediate, intermediate, intermediate plus or hot (Shamaila, 2005). Vaccines containing the Delaware variants, either in combination with classic strains or alone, are also available (Shamaila, 2005). Live attenuated vaccines are recommended for use at 1 day-of age. An improvement in the total performance of broilers after one–day of age vaccination has been observed (Knoblick *et al*., 2000). Combined vaccination with IBDV and Marek's disease vaccine at one-day of age results in less rapid decline in IBDV-specific neutralizing maternal antibodies as compared to vaccination with IBDV and MDV alone. In addition, IBDV vaccination at 1 day of age slowed down the accelerated rate of IBDV neutralizing antibody decline caused by vaccination with MDV (Knoblick *et al*., 2000).

Highly virulent (hot), intermediate and avirulent strains break through maternal VN antibody titres of 1:500, 1:250 and less than 1:100 respectively (Lucio and Hitchner, 1979; Skeeles and Lukert, 1979). Intermediate strains vary in their virulence and induce bursal atrophy and immunosuppression in 1 day old and 3 week old SPF chickens (Lukert and Mazariegos, 1985). Chickens might be vaccinated with the avirulent strains of the virus if the maternal antibody titre

is less than 1:1000 (Shamaila, 2005). The vaccine virus replicates in the thymus, spleen and bursa where it persists for 2 weeks (Lukert and Rifuliadi, 1982).

After the maternal antibody level wanes out, there is a primary response to the persisting vaccine virus (Lukert and Rifuliadi, 1982). A complex vaccine made by mixing an intermediate strain and a vaccine strain with a measured amount of IBDV antibody before injection has been used to immunize day-old chickens in the presence of maternal antibodies. This vaccine induced active immunity and provided some protection against challenge with the standard IBDV challenge (Shamaila, 2005).

Killed virus vaccines in oil-adjuvant are usually used to boost and prolong immunity in breeder flocks but are not used for inducing a primary response in young chickens while oil adjuvant vaccines are most effective in chickens that have been primed with live virus either in the form of a vaccine or field exposure to the virus (Wyeth and Cullen., 1978; Lukert and Saif, 2003). Oil-adjuvanted vaccine might contain both standard and variant strains of the virus. Maternal antibody profiling of the breeder flock should be done to assess the effectiveness of vaccination and persistence of antibody (Lukert and Saif, 2003).

Antigen content of inactivated IBD vaccines was shown to be a reliable indicator of the protective serological response after vaccination and could be used as a measure of vaccine potency. Neutralizing antibody titte correlated well with the VP2 content of the vaccine. A significant correlation was also observed with the VP3 content and antibody response (Oei *et al*., 2004).

A novel concept is *in ovo* vaccination of chickens against multiple viral agents at 18 days of incubation (Negash *et al.*, 2004). The immune system in birds develops early during

embryogenesis and various immune reactions are induced in the late stage of embryonic development. Compared with post-hatch vaccination, *in ovo* vaccination stimulates both the innate and adaptive immune responses. Due to prenatal immunization, *in ovo* vaccinated chickens have good protection by the time of hatch (Negash *et al*., 2004). A single *in ovo* injection of a vaccine containing serotype 1, 2 and 3 of Marek's disease virus (MDV), a vaccine strain of serotype 1 of IBD and recombinant fowl pox vaccine with HN and F genes of NDV (rFP-NDV) induced protection against virulent MDV, IBDV, NDV and fowl poxvirus. This vaccine induced specific antibodies against the agents present in the mixture and did not adversely affect the hatchability of the chicken. This technique is labour saving and has a potential to circumvent the effects of maternal antibodies and initiating a primary immune response (Gagic *et al*., 1999).

A universal vaccination program for IBDV could not be offered due to variability in maternal immunity and due to different management and control practices (Lukert and Saif, 2003). Vaccination of broilers might not be needed if high levels of maternal antibodies are achieved and the field challenge is reduced. Chickens have been vaccinated with attenuated and intermediate vaccines from as early as 7 days to 2 or 3 weeks of age. Infectious bursal disease vaccines could be given to broiler at 1 day of age along-with MD vaccine. Breeder replacement chickens are vaccinated with a live vaccine at 10-14 weeks of age. Killed oil-adjuvant vaccines are given at 16-18 weeks of age. If antibody profiling undergoes a major drop then re-vaccination of breeders is required (Lukert and Saif, 2003).

Recombinant vaccines have been described but none is currently available commercially (Fahey *et al*., 1989; Macreadie *et al*., 1990; Baylis *et al*., 1991; Heine and Boyle, 1993; Vakharia *et al*., 1993; Snyder *et al*., 1994; Darteil *et al*., 1995). Attempts to produce VP2 subunit vaccines in *E.*

coli were unsuccessful due to improper folding and post–translational modification of the protein (Fahey *et al*., 1989). Immunization with these expressed proteins resulted in antibodies low titre (Fahey *et al*., 1991). It also indicates that the VP2 by itself might be insufficient to mount a good immune response. Recently, the genome segment A of the highly IBDV was cloned and amplified in *E. coli* and vaccinations with the resultant empty viral particles were shown to confer protection to challenge (Rogel *et al*., 2003).

Yeast expression system produced more immunogenic recombinant proteins that induced VN antibodies that were passed on to the progeny and were shown to be protective against challenge with virulent IBDV (Macreadie *et al*., 1990; Fahey *et al*., 1991). A number of different recombinant viral expression vectors have been described as potential IBDV vaccines (Baylis *et al*., 1991; Heine and Boyle, 1993; Vakharia *et al*., 1994; Dybing and Jackwood, 1998; Fernandez-Arias *et al*., 1998). A fowl pox recombinant virus expressing VP2 under the control of fowl pox early/late promoter induced VN antibodies that protected significantly against challenge but the protection was still less than what complete virus offers (Heine and Boyle, 1993).

A fowl pox vector containing VP2 from the virulent virus strain 52/70 as a β-galactosidase fusion protein provided protection but this vaccine caused bursal pathology (Bayliss *et al*., 1991). A fowl adenovirus carrying VP2 from the Australian strain 002/73 protected SPF chicken against an intermediate virulent classic strain via IV, IM, IP or subcutaneously but not via conjunctival sac (Sheppard *et al*., 1998). Immunogenic IBDV protein expression using baculovirus and recombinant turkey herpes virus vectors have also been described (Vakharia *et al*., 1994; Dybing and Jackwood, 1998).

A chimeric virus between classic and variant strains was produced (D78-Chim). This vaccine induced high VN antibodies that were comparable to both classic and variant strains by themselves. D-78 and D78-Chim induced comparable bursal damage in the presence of maternally derived antibodies (MDA) and were able to break through a similar level of MDA (Mundt *et al*., 2003).

Deoxyribonucleic acid vaccines encoding the polyprotein from segment A have been described. Vaccination with a DNA vaccine expressing variant E polyprotein provided 90% protection against the homologous strain, while that expressing a classic standard challenged (STC) strain provided 100% protection against the homologous strain (Chang *et al*., 2001). None of the afore mention vaccines are currently available in the Nigerian market for use. The molecularly cloned vaccines produced by site directed mutagenesis have a great risk of reversion to the wild type that has greatly limited their practical application as potential vaccines in the field (Raue *et al*., 2004).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL CHICKENS AND REARING.

Three hundred day-old Dominant black Marshal Cockerels were purchased from a commercial hatchery in Ota, Nigeria. The chickens on arrival were randomly divided into twelve groups (A-L) of twenty five chicks per group and housed under deep litter system with a floor space of 0.14 square meters per bird. Water and feed (chick mash) containing varying levels of Se were given to them *ad libitum* throughout the period of the experiment (Table 3.1).

Table 3.1 **EXPERIMENTAL DESIGN**

Group	Dose of selenium in ppm	Age of vaccination of birds with IBD vaccine (weeks)	Age of challenge with IBD virus (weeks)
A	0.1	1 and 3	6
B	0.5	1 and 3	6
C	1.0	1 and 3	6
D	0.1	No vaccination	6
E	0.5	No vaccination	6
F	1.0	No vaccination	6
G	0.1	1 and 3	No challenge
H	0.5	1 and 3	No challenge
I	1.0	1 and 3	No challenge
J	0.0	1 and 3	No challenge
K	0.0	No vaccination	No challenge
L	0.0	No vaccination	6

3.2 VACCINES

The chickens were vaccinated against Newcastle disease, using Newcastle disease vaccine intraocular (NDVi/o) at day one, at the dose of 0.05 ml into each open eye, NDV La Sota in drinking water at the dose of 10 ml per bird at two weeks of age, NDV Komarov intramuscularly at week six at the dose of 0.2 ml and fowl pox vaccine via the wing web at a dose of 0.02 ml per bird at six weeks of age. Infectious bursal disease vaccine (batch number 20621062E, expiry date August 2012, ABIC, Israel) purchased from a commercial sales outlet in Kaduna, were used in this experiment.

3.3 FEEDS

The following feed, TF, VF, LF and EF were obtained from commercial distributors of the products in Jos; Plateau State. The feed given to the birds had the following composition in gm per 100 gm of feed: Crude protein 19, fats 5, crude fibre 5.00, available phosphorus 0.45, calcium 1.00, lysine 1.00, methionine 0.40, salt 0.3 and metabolizable energy 2650 kcal.

3.4 INFECTIOUS BURSAL DISEASE ELISA KIT

Immuno Comb diffusion test kit was purchased from Biogal Galed laboratories in Israel. The Immunocomb kit which is based on solid dot ELISA technology has antigen applied to test spots on the solid phase which is a comb-shaped plastic card. The kit was stored at 4^{o}C until used.

3.5 SAMPLE COLLECTION

At the beginning of the experiment, 10 grams of chick mash each from LF, TF, EF and VF were collected and package in clean polythene bags to determine the level of selenium. Ten chickens were randomly selected from the 300 cockerels and bled terminally. Two millilitres (ml) of blood was collected from each chick using 23G sterile hypodermic needles and syringes

intracardially into two set of bottles, one containing EDTA and the other without EDTA, the later was allowed to clot, serum harvested into sterile sample bottles and kept at -20^{o}C until analysed. Five out of the ten blood samples clotted and so not suitable for haematological test, the remainin five were used for the determination of haematological parameters, while the ten sera sample were used to test for IBDV antibody titre.

Sections of liver, kidneys and muscles were collected from the dead chicks using tissue forceps and surgical blade into clean polythene bags. These tissues were refrigerated at 4^{o}C until used for Se analysis as described by Cappello *et al*. (1990). On a weekly basis (for seven weeks) 2 ml of blood was aseptically collected from five randomly selected birds in each group as described above and used to determined haematological parameters and antibody titres.

3.6 SELENIUM ANALYSIS IN TISSUES, FEED AND BLOOD

The tissues samples were taken to the Biochemistry Department of the National Veterinary Research Institute, Vom. The samples were placed in Petri dishes inside a laboratory oven at 60^{o}C for 4 days to dry; they were then removed and grinded using Agate pestle and mortar. The grinded tissue samples were thereafter transferred to the Geomining Department of the University of Jos where the Se concentrations in various tissues were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES), model optima 2000DV, produced by Perkinelmer.

One gram of the grinded tissue sample was weighed into a Teflon crucible and 6 ml of nitric and percloric acid was added. The sample was then placed on the sand bath for 3-4 hours to dry, 2 ml of nitric acid was then added and heated for 5 minutes and then 10 ml of deionised water was added and heated for 15 minutes.

The tissue sample was then transferred into a 100 ml volumetric flask by filtering through a funnel and rinsed with deionised water. It was made up to the 100 ml mark with deionised water. The ICPOES machine was calibrated using Se standard. The sample in the volumetric flask was then presented to the analyser probe tip of the machine which was connected to a computer. The aspirate key was pressed to start the analysis. The result was printed out in a hard copy from the computer printer, and the result recorded. The same procedure as described above was used to analyse Se in feed and blood except that there was no need of drying and grinding.

3.7 INFECTIVE BURSAL DISEASE VIRUS

The infective IBDV was provided by the Viral Research Department of the National Veterinary Research Institute Vom, Plateau State. The IBDV inoculums were obtained from a confirmed outbreak of IBD in Jos Nigeria, in which the mortality was more than 70%. A 20% homogenate of the infected bursae was prepared in a phosphate buffered saline (PBS), PH 7.2 and centrifuge at 112 g for 10 minutes and passed through a 450 nm filter. The supernatant was stored at -20^{O}C until used for the inoculation of the chicks (Okoye and Uzoukwu, 1981). Each chick was inoculated with 0.04 ml of virus inoculum equivalent to 16 x $10^{4.6}$ ELD_{50} via conjuctival instillation at 6 weeks of age.

3.8 MEASUREMENT OF SERUM ANTIBODY TITRE TO INFECTIOUS BURSAL DISEASE VIRUS

The multi chamber developing plate of the ELISA kit was removed from the pack and incubated at 37^{o} C for 20 minutes. Row A of the developing plate was unsealed and 0.5 microlitre of the test sample serum was dispensed into wells A1 to A10, then positive control and negative control were dispensed into A11 and A12, respectively which were mixed by aspirating and releasing the contents with pipette several times.

One comb was removed from its protective cover and inserted into row A with print side facing the researcher, and the comb was gently moved up and down to mix the reagent and then left for10 minutes, after which the comb was withdrawn and shaken to dry.

Row B was unsealed and the comb was inserted for 2 minutes, and removed then transferred to row C for 10 minutes. The same process was done for row D for 2 minutes and row E for 2 minutes, while row F was for 10 minutes (to allow colour reaction to take place). The comb was again removed from row F and taken back to row E for 2 minutes to fixed colour. Finally the comb was removed from row E and shaken to remove excess water. The control was read to set the comb scale. The most suitable colour from the comb scale that matches with the colour on the control was selected and the titre read in the yellow windows.

3.9 DETERMINATION OF MORTALITY AND PROTECTIVE RATES

Mortality recorded in the different groups after challenge with IBD virus at six weeks of age was calculated at the end of the experiment as a percentage of the initial number of the birds (Babiker *et al.* 2008). Also the protective rate was calculated using the formula;

$$PR = \frac{MRUV-MRV}{MRV}$$

Where PR = is the protective rate, MRUV = mortality rate in unvaccinated, MRV= mortality rate in the vaccinated

3.10 PATHOLOGY AND LESION SCORE

3.10.1 Gross Lesion Score

Dead chickens were examined for gross pathology and the proportion of dead chickens showing specific gross lesions was noted. Also lesions scores were subjectively graded as normal (0), mild (1); mild to moderate (2); moderate (3) moderate to severe (4); severe (5), based on the severity of the lesions on the bursa of Fabricious, body skeletal muscles surfaces, kidneys,

erosions and hemorrhages of the mucosa of proventriculus (Hair-Bejo, 2000; OIE, 2004 and Babiker, *et al*. 2008).

3.10.2 Histopathology Lesion Score.

Tissues were collected from the bursae of Fabricious and fixed immediately by immersion in 10% buffered neutral formalin solution. The tissues were cut into blocks and identified and were then dehydrated through a series of graded alcohols (70%, 80%, 90%, 95% and 100%). The blocks were cleared in xylene and then infiltrated with molten paraffin wax. Sections of 5 microns (μm) were cut from embedded tissue using Jung Rotary Microtome (model 42339). The tissues were mounted on grease free clean glass slides and kept at room temperature then stained alternatively with Hematoxylin and Eosin (H & E). The prepared slides were studied using light microscope (Olympus binocular microscope) and examined at x 40 and x 100 magnifications. Photomicrographs of the tissues were taken using a digital microscopic objective camera. The pictures were transferred to a computer and detail studies carried out to acertain the Histopathological lesions (Babiker and Tawfeeg, 2008).

Bursal lesions scores were subjectively graded as follows: (Krishnamoorthy *et al.* 2007 and Babiker *et al*. 2008).

0- Normal follicles.
1- Follicles showing scattered lymphoid depletion in the cortex and medulla.
2- Follicles showing lymphoid depletion and necrosis in less than 50% of cells and mild interfollicular fibrosis.
3- Follicles showing lymphoid depletion and necrosis in more than 50% of cells and moderate interfollicular fibrosis.

4- Follicles showing glandular transformation of epithelelium, cystic changes and marked interfollicuar fibrosis.
5- Atrophy of follicles with fibroplasias.

3.11 DETERMINATION OF HAEMATOLOGICAL PARAMETERS

3.11.1 Red Blood Cell Count, Packed Cell Volume and Haemoglobin Concentration

Red blood cell count, packed cell volume and haemoglobin concentration were determine using Mindray auto haemo analyser, model BC-3000 PLUS, produced by Shenzhen Mindray Biomedical Electronics Ltd, China. The haemo analyser machine was switched on and it automatically flushed the fluidic lines, checked the background and entered the count screen. The menu key was pressed to select the sample mode, and whole blood was selected. The blood in the sample tubes were well mixed using Stuart roller mixer machine model SRT9D, after bringing it to room temperature. It was then presented to the analyser probe tip making sure it enters very well. The aspirate key was pressed to start the count. The result was printed out in a hard copy from the machine, and the result recorded (Mohammed *et al.*, 2012).

3.11.2 Differential Leukocyte Count

Samples were well mixed using Stuart roller mixer machine model SRT9D. A drop of the blood was placed one centimetre away from the edge of a clean greased free slide. A spreader was placed in front of the blood to allow the blood to spread across its edge. The spreader was moved forward quickly and smoothly. The smear was allowed to air dry, and the slide labelled. The air dried smear was then placed on a staining rack and flooded with filtered Leishman's stain and fixed for two minutes. The smeared slides were then flooded with equal volume of buffered distilled water (pH 6.8) and allowed to stain for 8 minutes. Excess stain was washed off and differentiated until salmon pink, and was allowed to drain. The slides were air dried and viewed

using oil immersion objective, starting from the thin end a systematic meander system of slow, careful and detailed leucocytes count was made, and the result recorded (Anderson and Stephen, 1970).

3.11.3 Determination of Total Protein

Biuret method was used to determine total protein, using the method described by Benjamin (1978). A blank test tube, standard and the test sample tubes were labelled. 1,000 microlitre of biuret reagent was pipetted into each tube, and 200 microlitres of distilled water, standard and test samples were pipetted into the respective tubes. They were thoroughly mixed and incubated for 30 minutes at 25^0 C. The spectrophotometer (model 6400/6405 made by Jenway, Barloworld Scientific, England) was set at 546 nm and zero using blank test tube, the absorbance was read, and the total protein calculated using the formula:

$$\frac{A1 \times C}{A2}$$

Where A_1 = Absorbance of the test sample, A_2 = Absorbance of the standard, C= Standard concentration

3.12 STATISTICAL ANALYSIS

Data collected were analyzed using SPSS version 16.0. Pearson correlation was used to determine the effect of Se supplementation on antibody titre, mortality rate and haematological parameters in infected birds. T- test was used to compare the means of antibody titre in Se supplemented and unsupplemented group, value of $p < 0.05$ was considered significant

CHAPTER 4

4.0 RESULTS

4.1 SELENIUM CONCENTRATIONS IN FEEDS AND TISSUES

Selenium analysis in four different commercial feeds showed that only one commercial feed contained Se with a concentration of 0.32 ppm (Table 4.1).

Mean concentrations of Se in serum, blood, muscles, liver and kidneys of day old DBMC were 0.08 ± 0.01 ppm, 2.77 ± 0.45 pmm, 57.34 ± 1.53 ppm, 47.99 ± 5.05 ppm, and 52.72 ± 10.85 ppm respectively, (Figure 4.1).

After supplementation of Se for ten weeks at graded levels of 0.1, 0.5 and 1.0 ppm, the mean concentrations of Se in serum was 2.8 ±0.27 ppm, 4.1 ± 0.28 ppm and 4.6 ± 0.31 ppm respectively, the mean concentrations of Se in the blood was 3.1 ± 0.38 ppm, 8.5 ± 0.50 ppm and 9.0 ± 0.87 ppm respectively, mean concentrations of Se in muscles was 76.9 ± 3.46 ppm, 96.2 ± 2.81 ppm, 96.1 ± 4.48 ppm respectively, means concentrations of Se in liver was 94. 0 ± 2.05 ppm, 96.6 ± 5.58 ppm and 97.0 ± 6.74 ppm respectively, while mean concentrations of Se in the kidneys was 82.3 ± 7.23 ppm, 91.1 ± 7.93 ppm and 96.9 ± 17.89 ppm respectively (Figure 4. 2).

Table 4.1: Levels of selenium concentration in four commonly used commercial chick mash.

Chick feed	Selenium concentration (ppm)
T.F	0.32
E.F	0.00
L.F	0.00
V.F	0.00

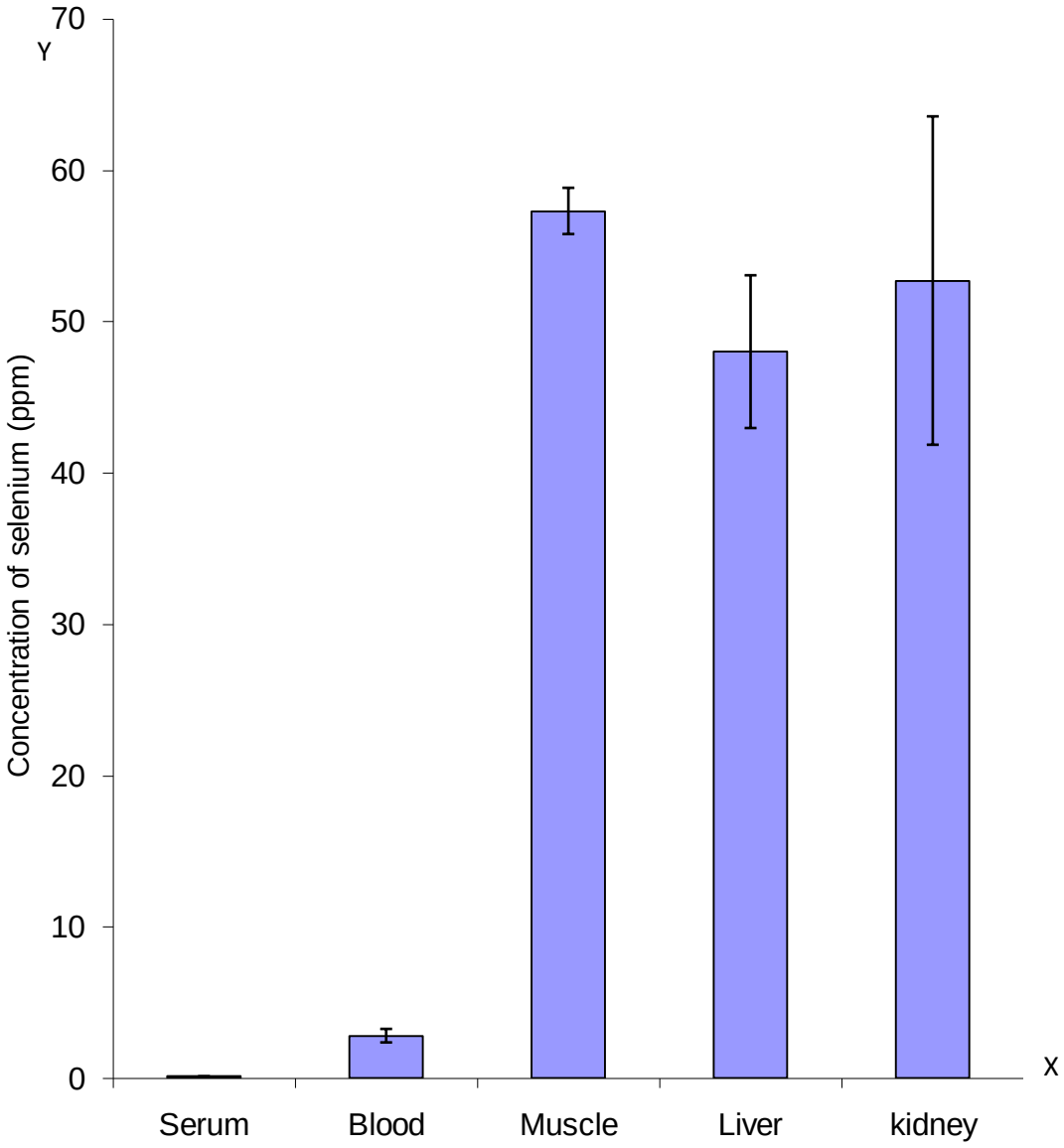


Figure 4.1: Mean concentration of selenium (ppm) in the tissues of day old Dominant black marshal cockerels.

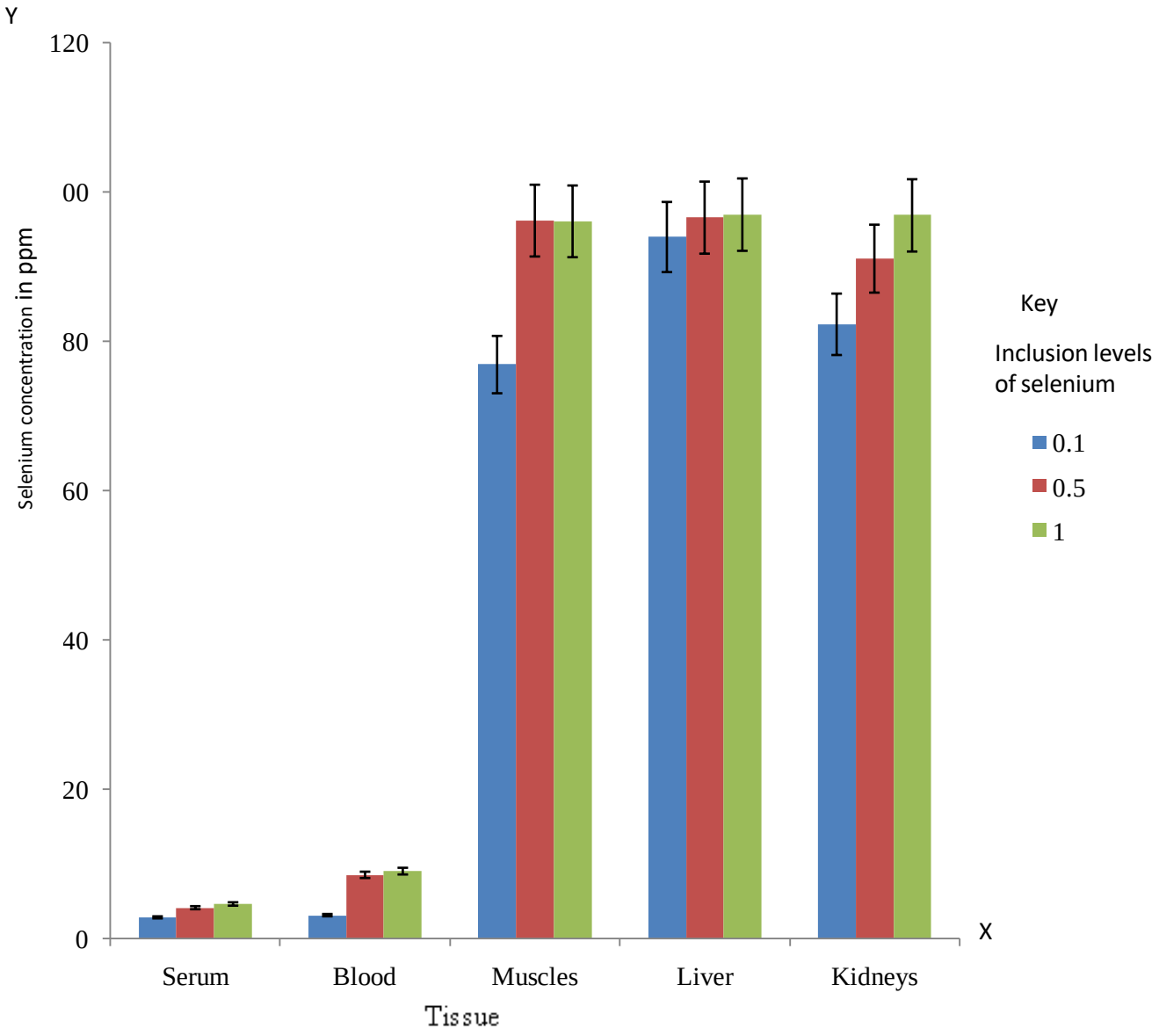


Figure 4.2: Mean selenium concentration in the tissues of Dominant black marshal cockerels after dietary supplentation for ten weeks.

4.2 SERUM ANTIBODY TITRE TO INFECTIOUS BURSAL DISEASE VACCINE

The results of screening for antibody titre to IBDV, in the chicks at day old revealed the mean ComScore (antibody titres) to IBD to be 1.4 (Table 4.2).

Result presented in Figure 3 showed the mean antibody titres to IBDV ranges from 1 in week one to 5.2 in week six; in group B the mean titres ranges from 1 in week one to 5.6 in week six; in group C the mean titre ranges from 1.1 in week one to 5.8 in week six; for group D, E, F and K, the mean titres ranges from 0 in week six to 1 in week one; in groups G, H, I and J the mean titre ranges from 1.2 in week one to 5.4 in week six; 1.2 in week one to 5.8 in week six; 1.2 in week one to 6.2 in week six and 1.3 in week one to 5.1 in week six, respectively. In group L the mean titre ranges from 0 in week six to 1.2 in week one. The cumulative mean antibody titre ranges from 1 in group D to 4.7 in group I (Figure 4.4).

4.3 SELENIUM SUPPLEMENTATION AND MORTALITY RATES

Clinical signs were observed in all the groups (A, B, C, D, E, F and L) challenged with IBDV. These signs include diarrhoea, anorexia, ruffle feathers, huddling, poor water intake, trembling, prostration and death. Figure 4.5, showed mortality rate of 64% in group L, which was not vaccinated and not supplemented with selenium but infected at six weeks of age. Also, mortality rates of 36%, 18%, 12%, 56%, 52% and 32%, were observed in groups A, B, C, D, E and F respectively. There was a negative correlation ($r = -0.93$ in the vaccinated and $r = -0.95$ in the unvaccinated chicks) between the concentration of Se in feed and mortality rate of infected chicks.

Table 4.2: Antibody titres to infectious bursal disease in day old Dorminant black marshal cockerels before selenium supplementation.

Serial number	ComScore to IBD
1	2
2	1
3	1
4	2
5	0
6	1
7	2
8	1
9	1
10	3
	Mean 1.4 ± 0.2667

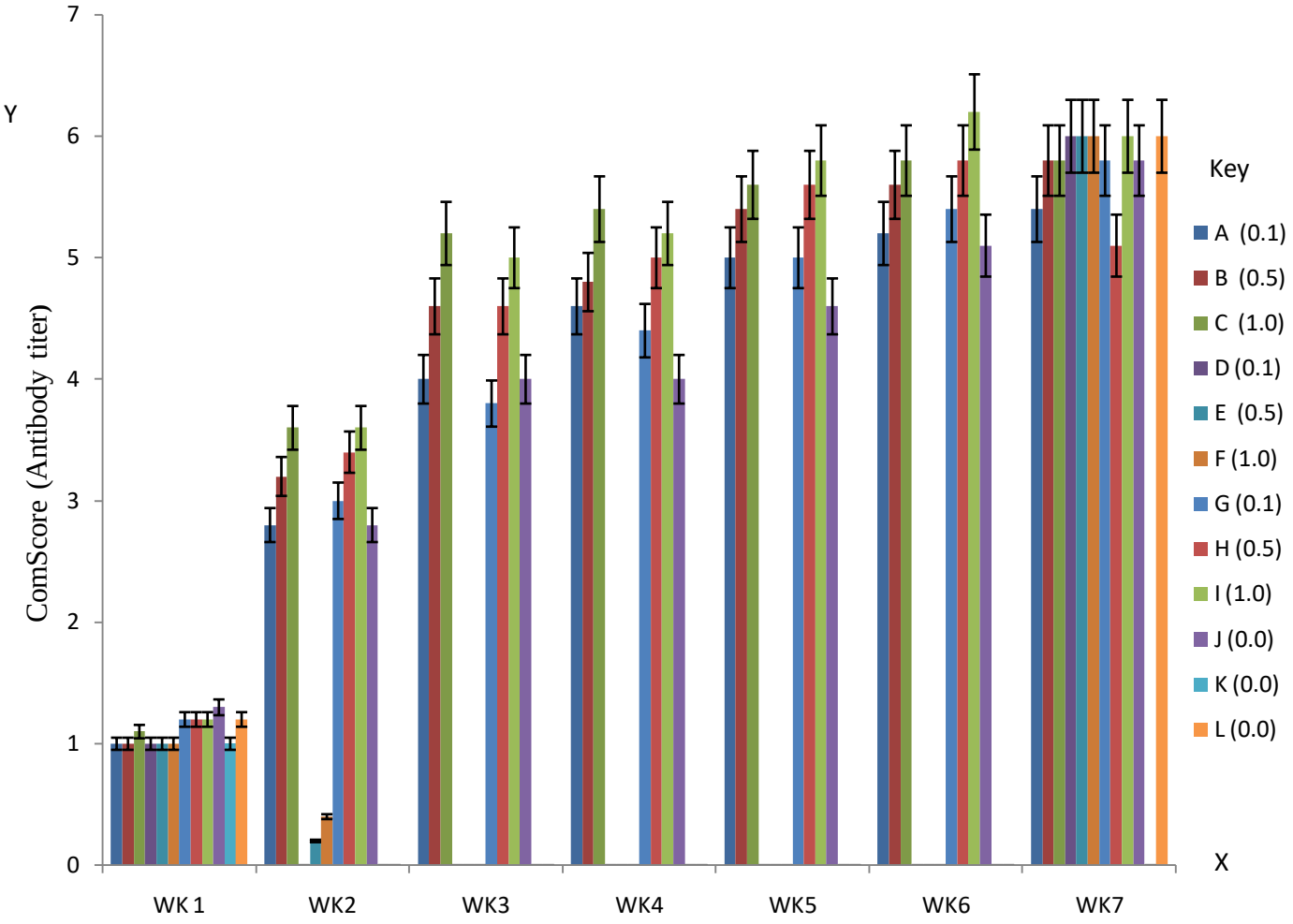


Figure 4.3: Infectious bursal disease virus antibody titers in selenium supplemented (vaccinated and unvaccinated) Dominant Black Marshal Cockerels challenged at 6 week old

Groups A, B, C= were vaccinated with IBD vaccine at week 1 and week 3 of age, they were challenged at 6 weeks of age with IBDV.
Groups D, E, F = were not vaccinated with IBD vaacine but challenged at 6 weeks of age with IBDV.
Group J = vaccinated with IBD vaccine at 1 and 3 weeks of age but not challenged with IBDV.
Group K = not vaccinated with IBD vaccine and not challenged with IBDV.
Group L = not vaccinated with IBD vaccine but challenged with IBDV at 6 weeks of age.

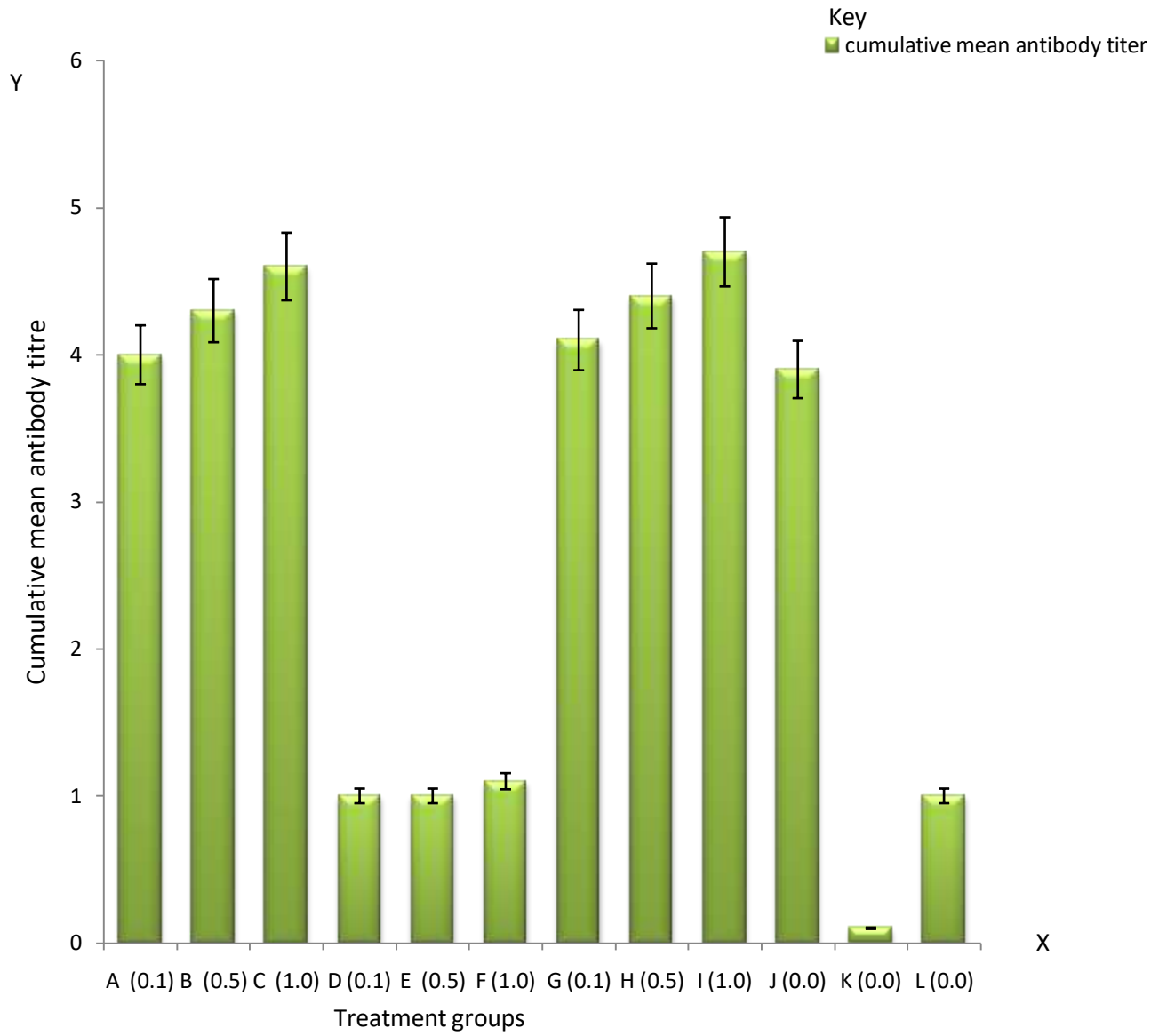


Figure 4.4: Selenium supplementation and cumulative mean antibody titre to infectious bursa disease vaccine in Dorminant black marshal cockerels vaccinated at 1st and 3rd week of age.

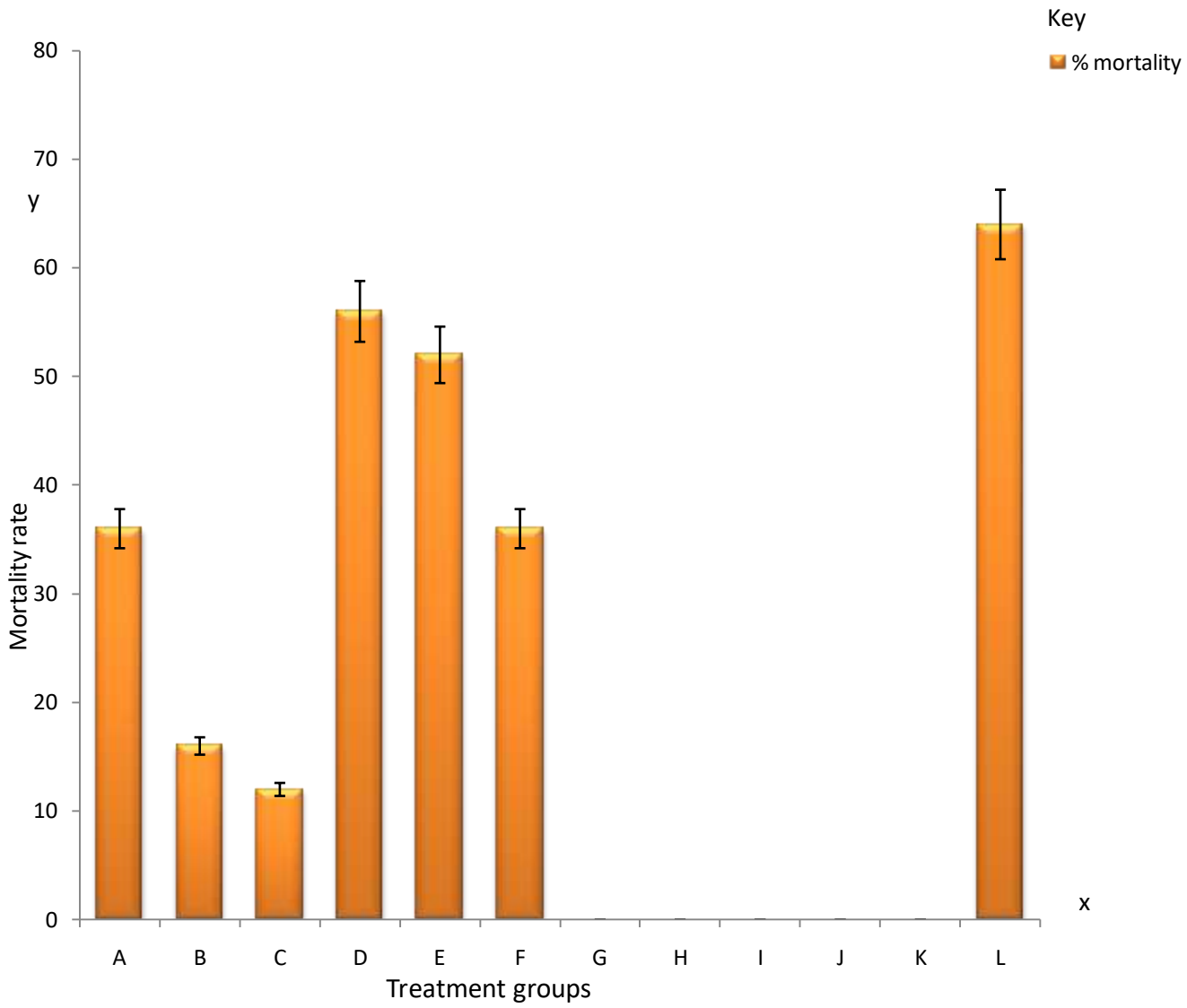


Figure 4.5: Graded levels of selenium supplementation on the mortality rate of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus.

Groups A, B, C= were vaccinated with IBD vaccine at week 1 and week 3 of age, they were challenged at 6 weeks of age with IBDV.

Groups D, E, F = were not vaccinated with IBD vaacine but challenged at 6 weeks of age with IBDV.

Group J = vaccinated with IBD vaccine at 1 and 3 weeks of age but not challenged with IBDV.

Group K = not vaccinated with IBD vaccine and not challenged with IBDV.

Group L = not vaccinated with IBD vaccine but challenged with IBDV at 6 weeks of age.

4.4 GRADED LEVELS OF SELENIUM SUPPLEMENTATION AND

PROTECTIVE RATE OF INFECTIOUS BURSAL DISEASE VACCINE

The effect of graded levels of Se supplementation on the protective rate of IBD vaccine is presented in Figure 4.6. At 0.1 ppm, 0.5 ppm and 1 ppm, the protective rate was 35.7%, 69.2% and 67.7% respectively.

4.5 GROSS PATHOLOGY

4.5.1 Bursa of Fabricius: As presented in Figure 4.7, a total of 9, 4, 3, 14, 13, 9 and 16 carcasses were examined for gross pathological lesion in groups A, B, C, D, E, F and L, respectively. In group A, 28.6% of the bursae were haemorrhagic, 71.4% enlarged and 11.1% normal. In group B, 75% of the bursae were enlarged and 25% normal. In group C, 66.7% of the bursae were enlarged and 33.3% were normal, while in group D, 33.3% were haemorrhagic, 88.9% enlarged and 7.1% normal. In group E, 15.4% of the bursae were haemorrhagic, 84.6% enlarged and 7.7% were normal. In group F, 11.1% of the bursae were haemorrhagic, 66.7% enlarged and 22.2% normal. In group L, 50% of the bursae were haemorrhagic, 81.3% enlarged and 6.3% normal. Plates I and II shows varying degrees of bursal enlargement.

4.5.2 Kidneys: It was observed that 14.3% of the kidneys in group A were swollen, 28.6% pale and 71.4% normal. All the 4 kidneys in group B and 3 kidneys in group C were normal. In group D, 22.2% of the kidneys was swollen, 28.6% pale and 71.4% were normal. In group E, out of the 13 kidneys examined 15.4% were swollen, 23.1% pale and 69.2% normal. For group F, 11.1% of the kidneys were swollen, 33.3% pale and 68.8% normal. Out of the 16 kidneys examined in group L, 31.3% of them was swollen, 22.5% pale and 55.6% normal (Figure 4. 8). Plate I shows the enlargement of the kidneys.

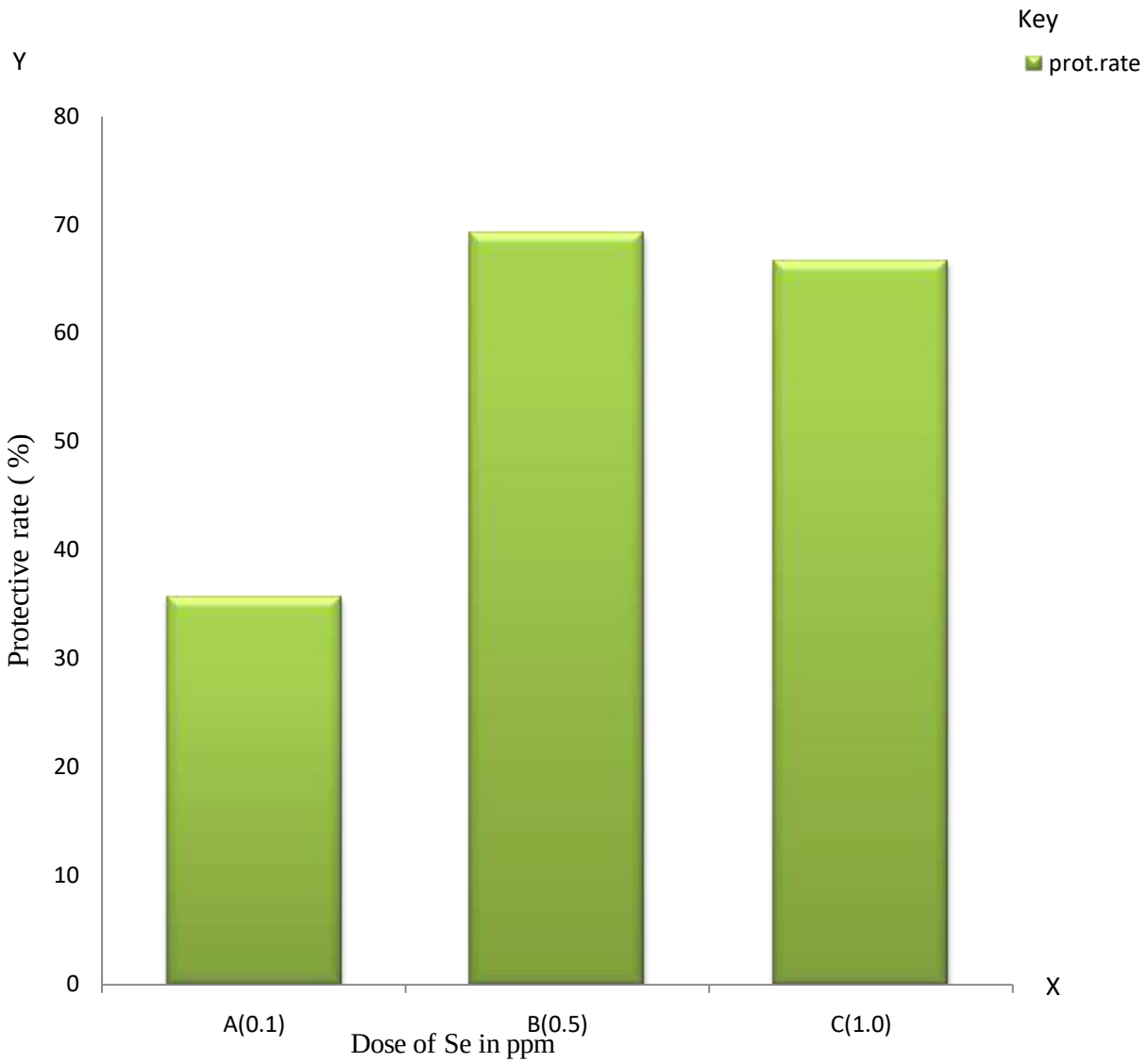


Figure 4.6: Graded levels of selenium supplementation and the protective rate of infectious bursal disease vaccine on Dominant black marshal cockerels infected with infectious bursal disease virus

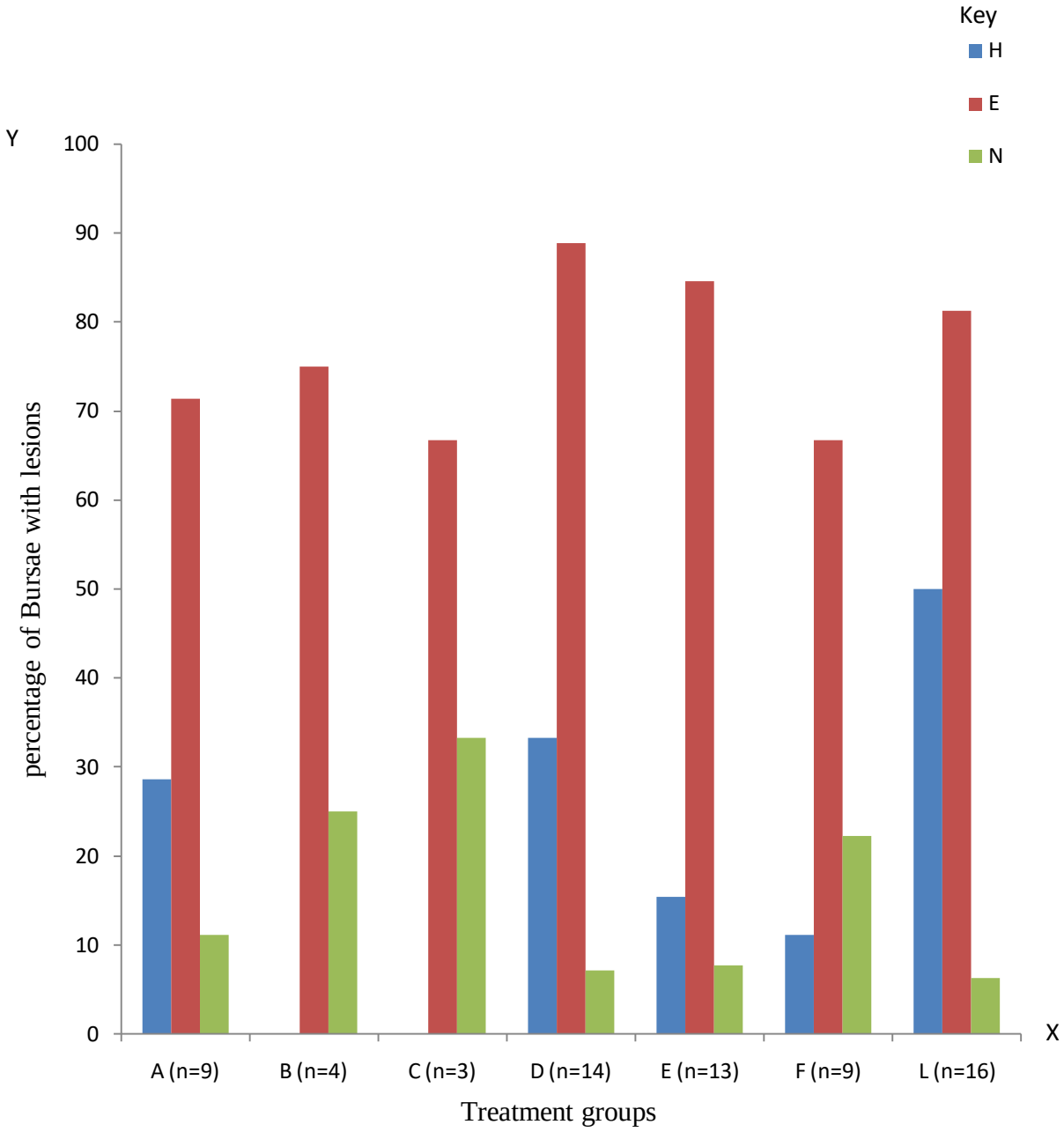


Figure 4.7: Selenium supplementation and the gross pathology of bursa of Fabricius of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and infected with infectious bursal disease virus.

Abbreviation: H= haemorrhagic, E= enlarged, N= normal

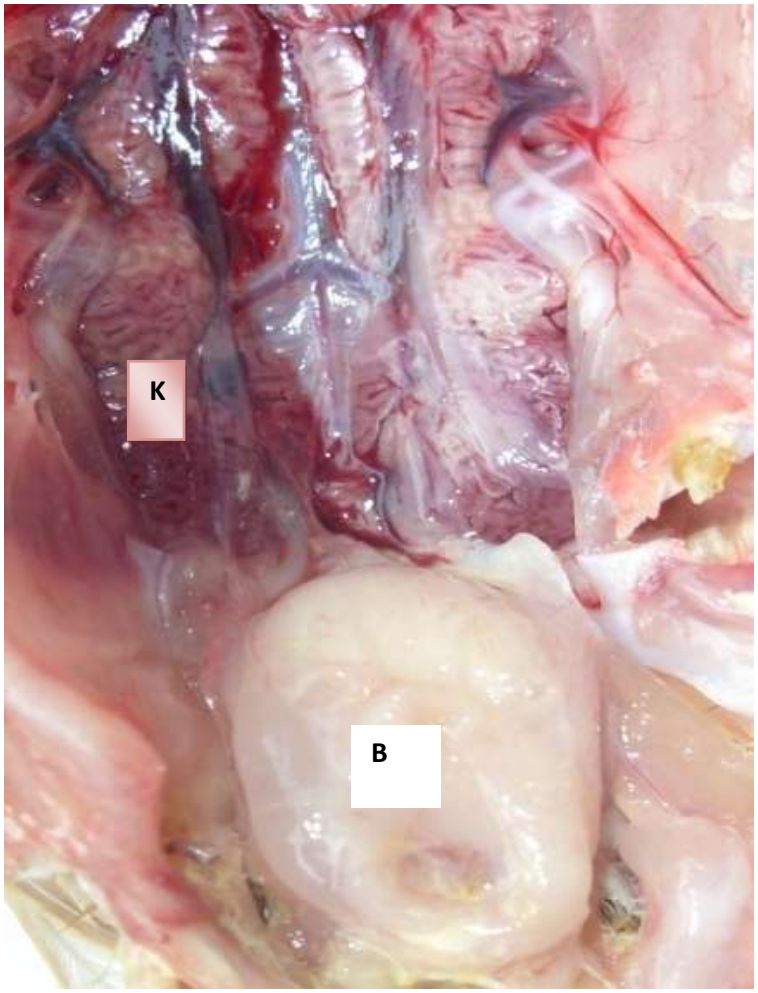


Plate I: Bursa of Fabricius and kidneys of a chick from group E (selenium supplemented at dose of 0.5 no vaccination, but challenged with infectious bursal disease virus at 6 weeks). Note the enlarged bursa (B) and kidneys (K)



Plate II:Incised bursa of Fabricius from a chicken from group L (no Se supplementation but challenged with infectious bursal disease virus at six week of age). Note the cherry red haemorrhages on entire mucosa.

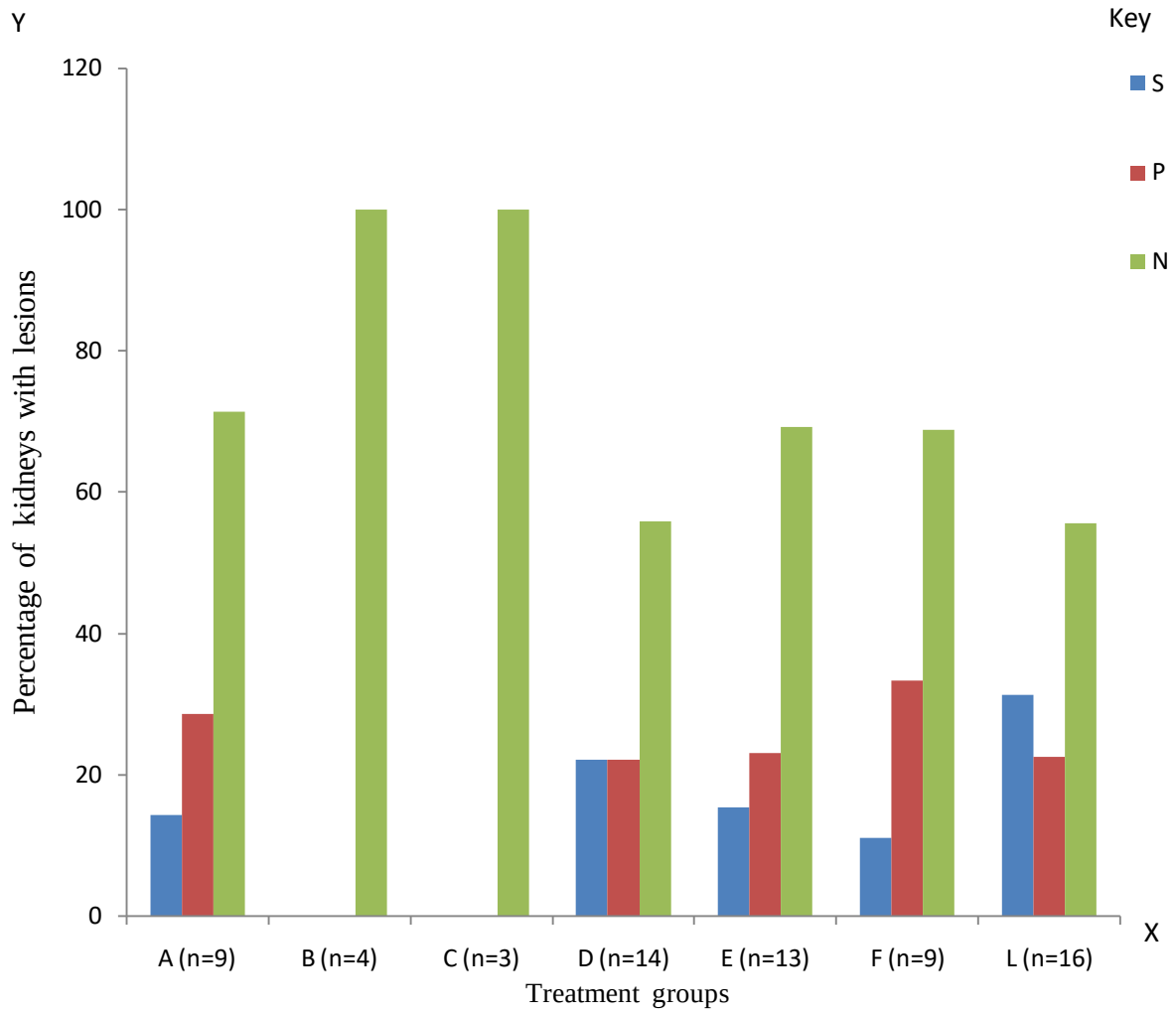


Figure 4.8: Selenium supplementation and gross pathology of kidneys of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and infected with infectious bursal disease virus.

Key: S = Swollen P = Pale, N = Normal

4.5.3 Liver: None of the livers in groups A, B, C, D, E and F showed any pathologic lesion, but 12.5% in group L were pale and 87.5% normal (Figure 4.9).

4.5.4 Leg muscles: In group A, 35% of leg muscles had petechial haemorrhages, 11.1% had ecchymotic haemorrhages and 53.9% normal. Leg muscles observed in group B had 26% petechiation and 74% of them were normal. In group C, 23.3% had petechial haemorrhages and the remaining 76.7% were normal. Group D, were observed to have 38.8% of their leg muscles with petechial haemorrhages, 25% with ecchymotic haemorrhages and 36.2% were normal. In group E, 25% had petechial haemorrhages, 21.4% had ecchymotic haemorrhages and 53.6% were normal. In group F, 35.3% had petechial haemorrhages and 15.4% had ecchymotic haemorrhages and 49.3 were normal. Group L, 44.4% of their leg muscles had petechial haemorrhages, 26.3% had ecchymotic haemorrhages while 29.3% were normal (Figure 4.10). Plate III shows petechial haemorrhages in the leg muscles.

4.5.5 Pectoral muscles: In group A, 7.1% were observed to have petechial haemorrhages, 11.1% with ecchymotic haemorrhages, 22.2% had congested pectoral muscles and 59.6% were normal. In group B, 25% had petechial haemorrhages and 75% were apparently normal. In group C, 33.3% had congestion in their pectoral muscles and 66.7% were normal. In group D, 22.2% had petechial haemorrhages, 21.4% had ecchymotic haemorrhages, 7.1% had congested pectoral muscles and 49.3% were normal. In groups E, 23.1% had petechial haemorrhages, 15.4% had ecchymotic haemorrhages, 7.7% had congested pectoral muscles and 53.8% were normal. In group F, 22.2% had petechial haemorrhages, 7.7% ecchymotic haemorrhages, 11.1% congestion and 59% were normal. In group L, 37.5% had petechial haemorrhages, 25% had ecchymotic haemorrhages, 12.5% congested while 38.5% were normal (Figure 4.11). Plate IV and V shows ecchymotic haemorrhages on the leg and pectoral muscles.

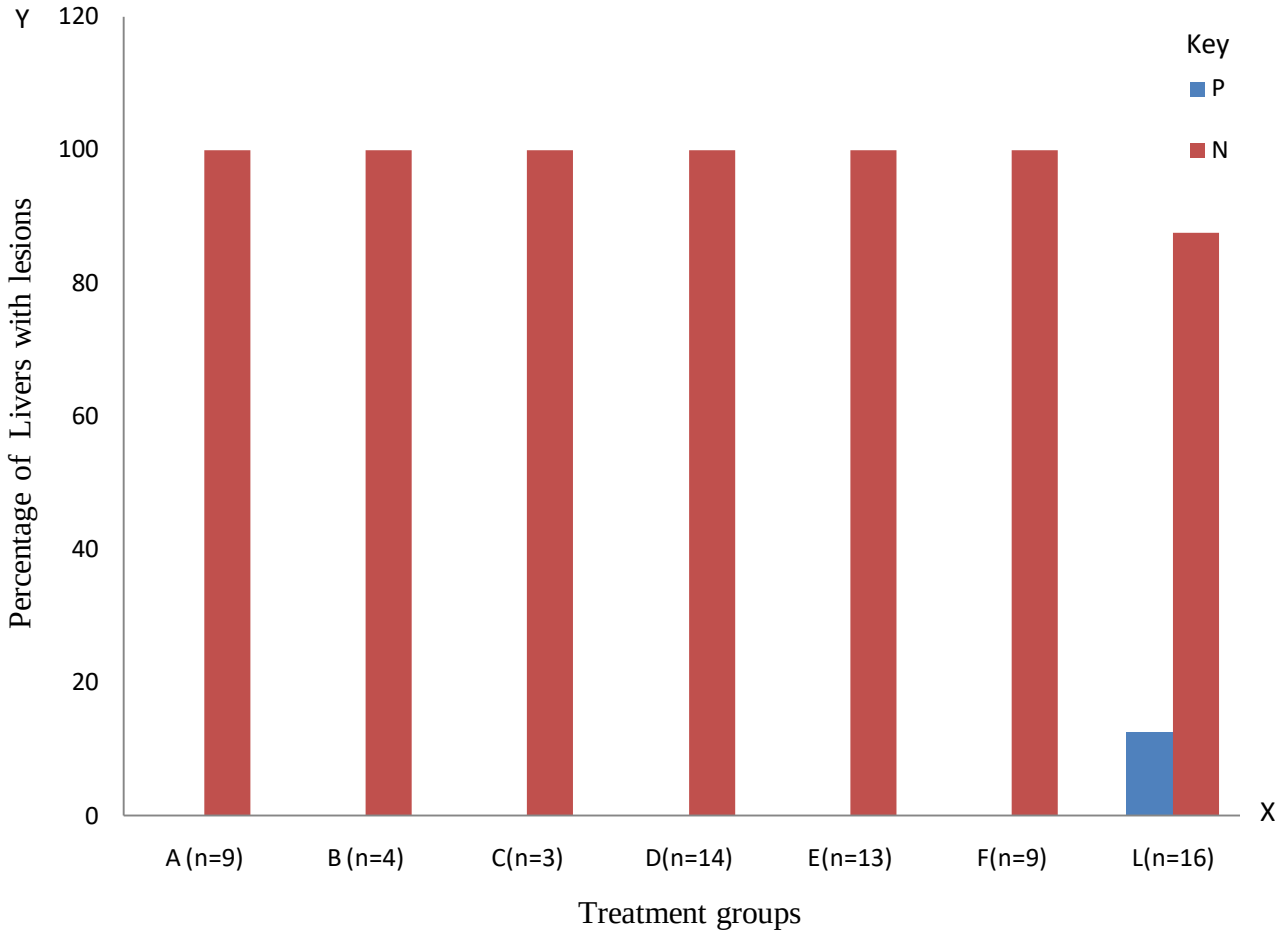


Figure 4.9: Selenium supplementation and gross pathology of liver of Dominant black Marshal cockerels vaccinated with infectious bursal disease vaccine and infected with infectious bursal disease virus.

Abbreviation: P = Pale, N = Normal

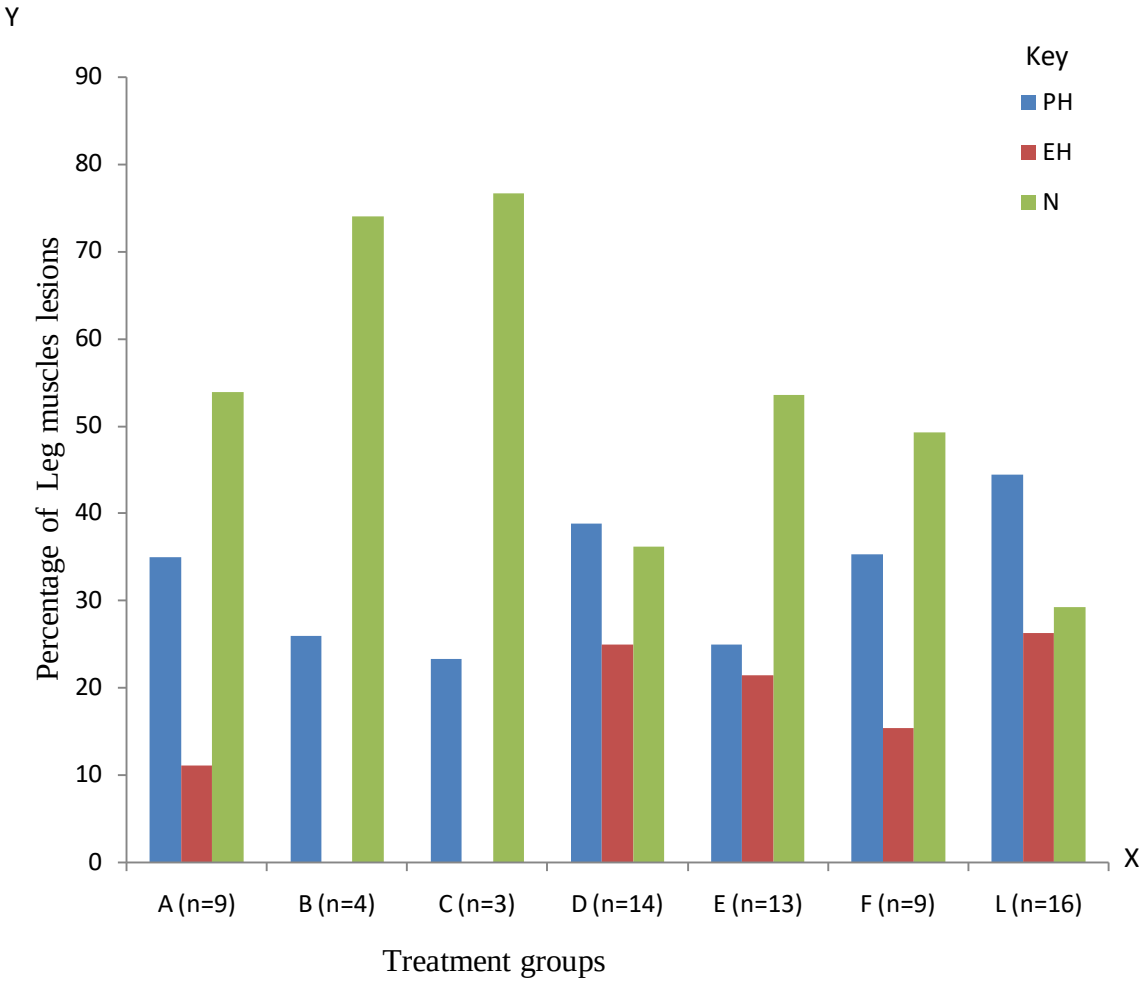


Figure 4.10: Selenium supplementation and gross pathology of leg muscles of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and infected with infectious bursal disease virus.

. Abbrevition: P H = Petechial haemorrhagic, EH = Ecchymotic haemorrhage, N = Normal

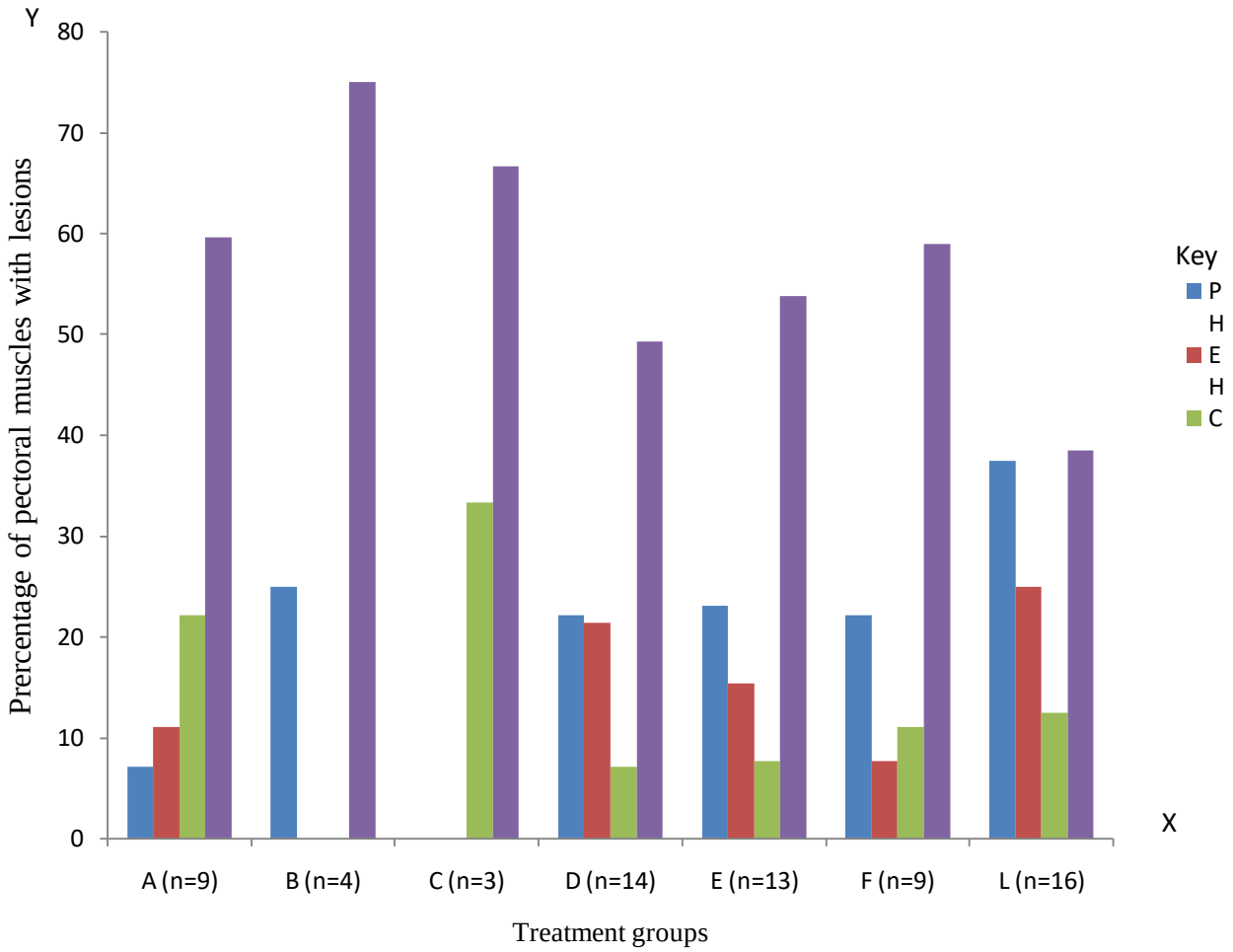


Figure 4.11: Selenium supplementation and gross pathology of the pectoral muscle of Dominant black marshal cockerels vaccinated with infectious bursal diseases vaccine and infected with infectious bursal disease virus.

Key: P H = Petechial haemorrhagic, EH = Ecchymotic haemorrhage, C = Congestion, N = Normal

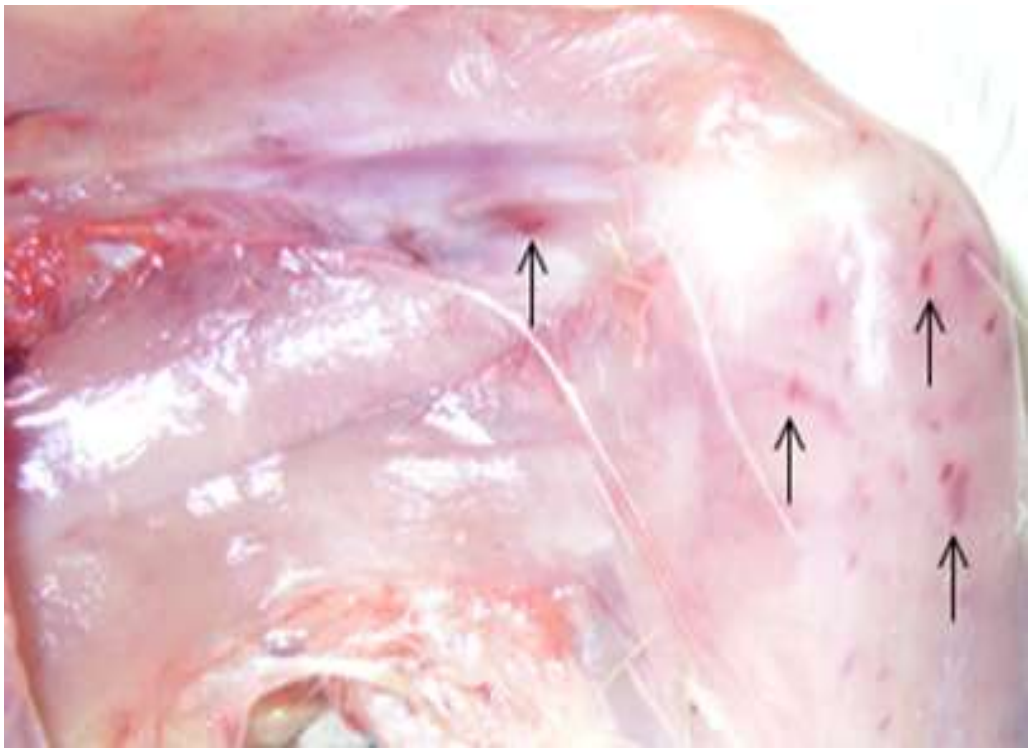


Plate III: Thigh and leg muscle of chick from group B (Birds received 0.5 ppm of Se, they were vaccinated and later challenged with infectious bursal disease virus at 6 weeks). Note petechial haemorrhages (arrows) on the leg muscles.

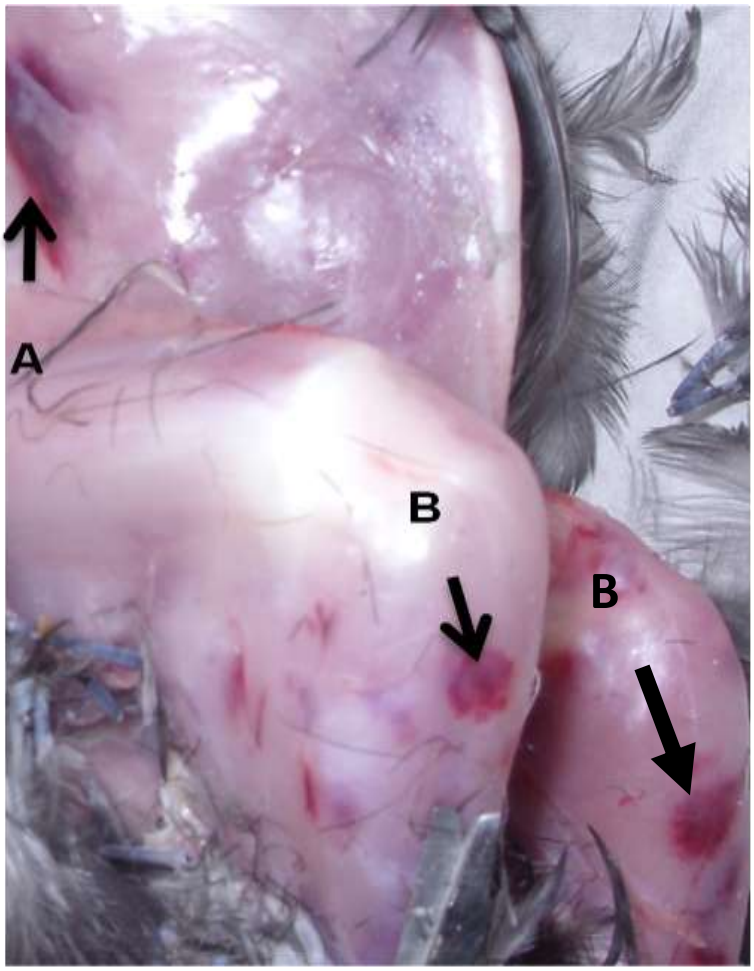


Plate IV: Leg and pectoral muscles of a chicken from group E (received Se Supplementation at a dose of 0.5 ppm, not vaccinated with infectious bursal disease vaccine, but challenged with infectious bursal disease virus at 6 weeks). Note echymotic haemorrhages on the pectoral muscles (arrow A) and leg muscles (arrow B).



Plate V: Leg and pectoral muscle of chick from group L (No Se supplementation, no vaccination but challenged with infectious bursal disease virus at 6 weeks). Note Echymotic haemorrhages on the Leg (A) and pectoral muscles (B)

4.5.6 Proventriculus: In group A, 21.4% had haemorrhages in their proventriculus-ventriculus junction, 21.4% had haemorrhages at the entire proventriculus mucosa and 57% were normal. In group B, 25% had haemorrhages at the proventriculus-ventriculus junction and 75% had normal proventriculus. In group C,, 33.3% had haemorrhages at proventriculus-ventriculus junction and 66.7% were normal. In group D, 35.7% had haemorrhages at their proventriculus-ventriculus junction and 23.1% in the proventricular mucosa, 41.2% of the proventriculi were normal. In group E, 38.5% had haemorrhages at the junction of proventriculus and ventriculus and 23.1% had haemorrhages at the entire proventriculus and 41.2% of the proventriculi were normal. Group F, had 33.3% of their proventriculus-ventriculus junction haemorrhagic, 22.2% at the entire proventriculus and 44.5% were normal. In group L, 50% had haemorrhages at the junction of proventriculus and ventriculus, 25% had haemorrhages at the mucosa of the proventriculus while 25% were normal (Figure 4.12). Plate VI shows petechial haemorrhages on the mucosa of proventriculus, while Plate VII shows haemorrhages at the proventriculus-ventriculus junction.

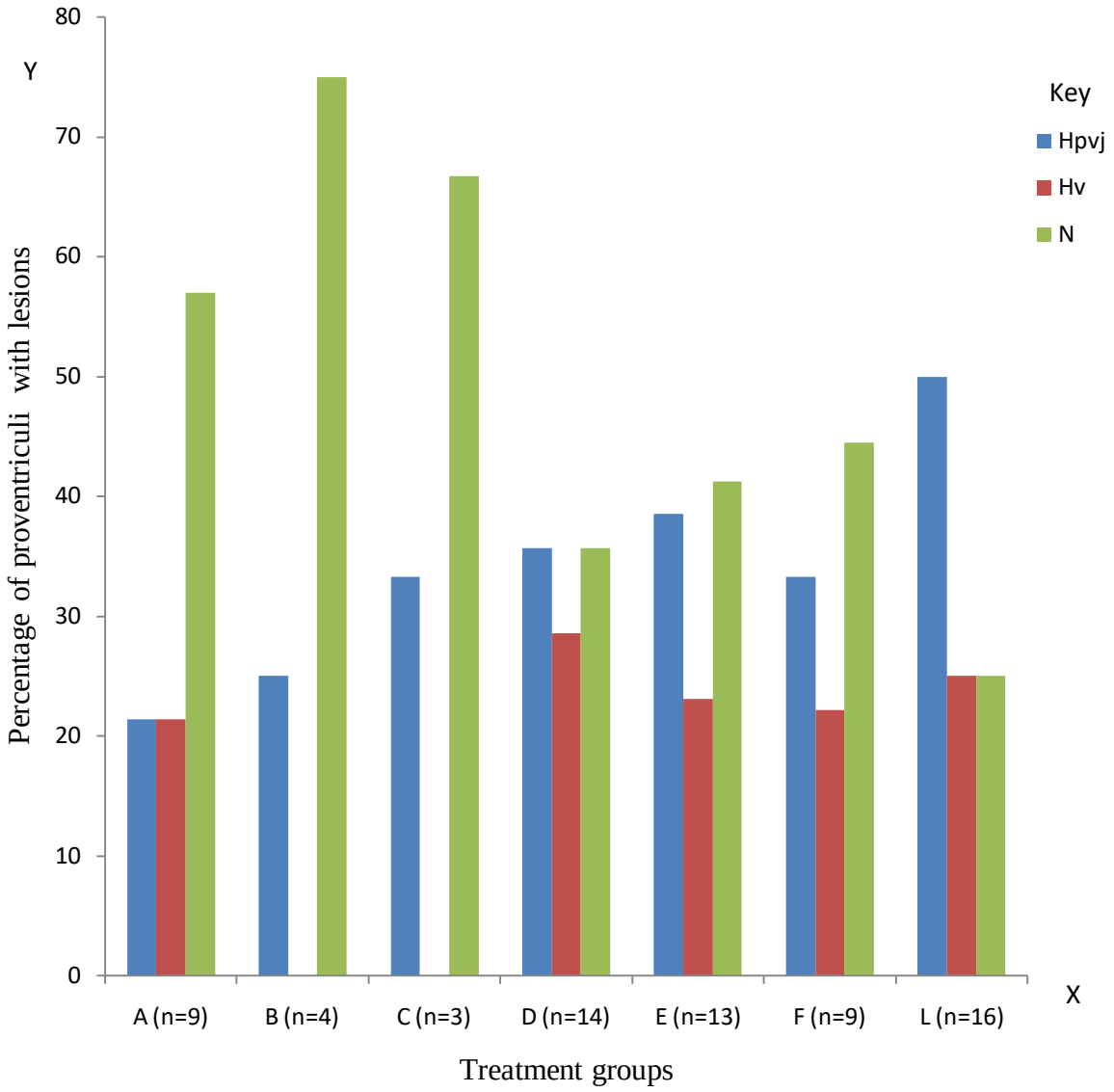


Figure 4.12: Selenium supplementation and gross pathology of proventriculus of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus.

Key: Hpvj = Haemorrhages at proventriculus–ventriculus junction,
Hv = Haemorrhages at ventriculus, N= Normal

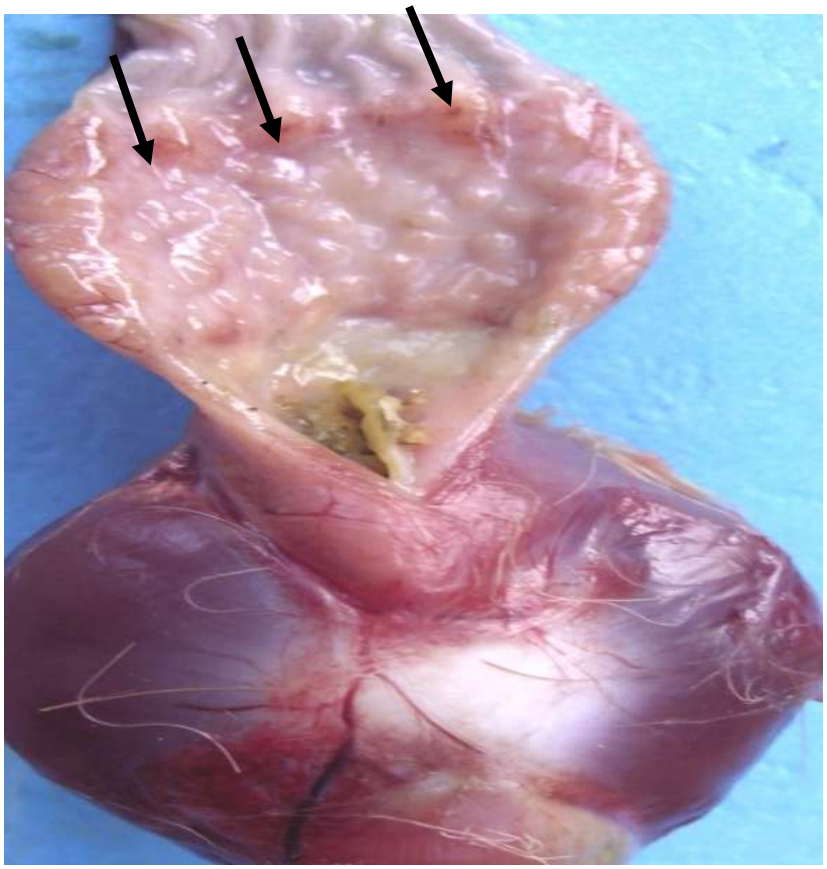


Plate VI: Proventriculus of a chick from group C (birds received 1 ppm of Se, they were vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus at 6 weeks of age). Note the petechial haemorrhages on the the proventriculus-ventricul junction (arrow head).

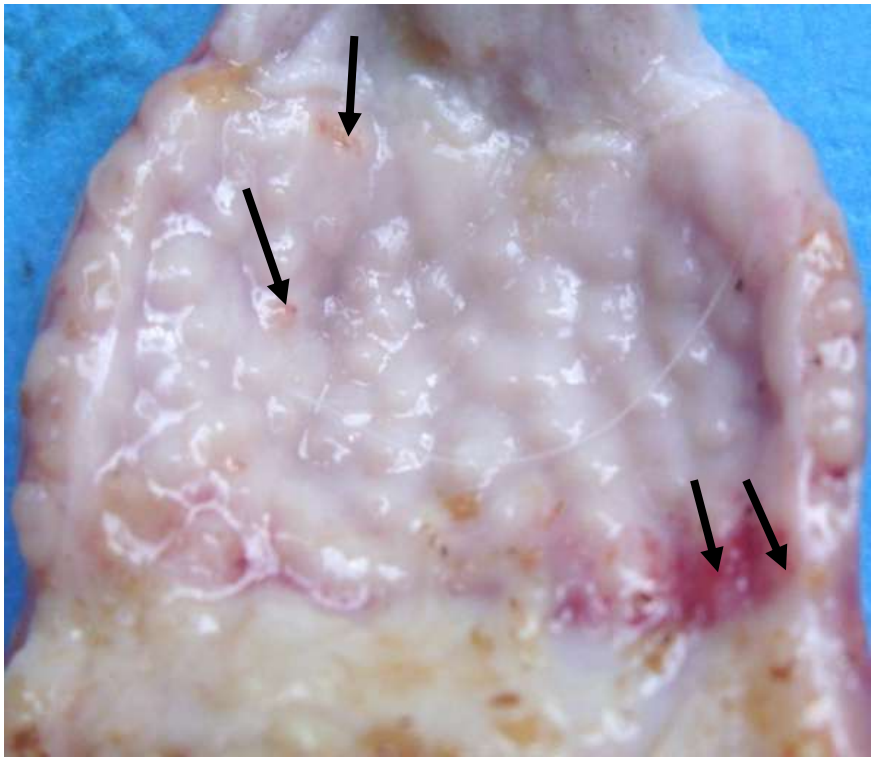


Plate VII: Proventriculus of a chick from group F (birds recieved 1 ppm of Se, they were not vaccinated with infectious bursal disease vaccine, but challenged with infectious bursal disease virus at 6 weeks). Note haemorrhages at the proventriculus mucosa (arrow heads)

4.6 GROSS LESION SCORE

The effect of Se supplementation on the gross lesions is as presented in Figure 4.13. The mean bursal lesion score in groups A, B, C, D, E, F and L was 3.1 ± 0.12, 3.0 ± 0, 3.0 ± 0, 3.5 ± 0.31, 3.4 ± 0.11, 3.3 ± 0.16 and 4.5 ± 0.46, respectively.

The mean lesions score in the kidneys in groups A, D, E, F and L was 2. ± 0, 3.5 ± 0.87, 3.0 ± 0.71, 3.0 ± 0.99 and 3.1 ± 0.51 respectively, there were no lesions in groups B and C. The liver had no lesions in groups A, B, C, D, E and F but in group L, the mean lesion score was 2 ± 0. In the leg muscles in groups A, D and L the mean lesion scores were 2.5 ± 0.23, 2.5 ± 0.22 and 2.5 ± 0.17, respectively. For groups B, C and E, the mean lesions score were 2 ±0, 2 ± 0 and 2.3 ± 0.35, respectively.

The mean lesions scores in the pectoral muscles in groups D and L, were 2.5 ± 0.40 and 2.7 ± 0.18 respectively, while in groups A, B, E, and F the mean lesions score were 1.5 ± 0.38, 2.0 ± 1.41, 2.0 ± 0.58 and 1.7 ± 0.33, respectively, in group C however, the mean lesions score was 1 ± 0.71. The mean lesions scores in the proventriculus in groups D and L were 2.5 ± 0.22 and 2.4 ± 0.18 respectively, but in groups A, B, C, E, and F the mean lesion scores were 2.3 ± 0.25, 2 ± 1.40, 2 ± 0, 2.0 ± 0 and 2.0 ± 0, respectively.

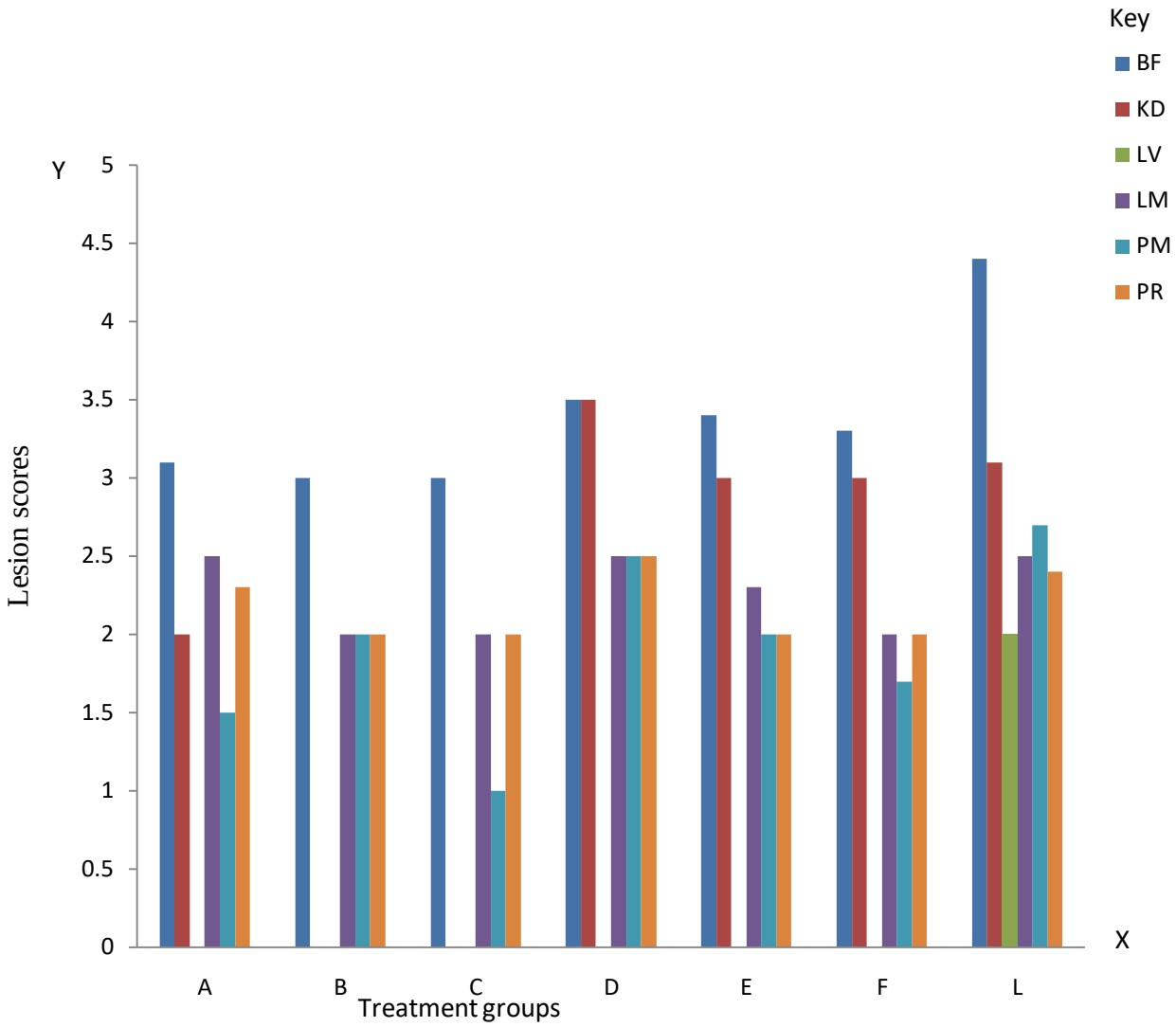


Figure 4.13: Gross pathological lesions scores in vaccinated and unvaccinated Dominant black marshal cockerels with and without selenium supplementation, infected with infectious bursal disease virus.

Key :BF- bursa of Fabricius, KD - kidneys, LV- liver, LM - leg muscles, PM – pectoral muscles, PR - proventriculus

4. 7 HISTOPATHOLOGY

The control group (unvaccinated, unsupplemented and uninfected), show normal lymphoid follicles in the bursa of Fabricius (Plate VIII). Group B (vaccinated, supplemented with 0.5 ppm Se and infected) showed lymphoid depletion and necrosis in less than 50% of cells, there was mild interfollicular fibrosis (Plate IX), lesions score 2. Group A (vaccinated, supplemented with 0.1 ppm Se and infected), showed marked lymphoid depletion in more than 50% of cells. It also showed areas of edema but with moderate interfollicular fibrosis (Plate X), lesion score 3. Group E (unvaccinated, supplemented and infected), showed massive depletion of lymphoid follicles in more than 50% of cells which was replace by edema (plate XI), lesion score 3.The infected unvaccinated and unsupplemented group (L), showed severe lymphoid depletion with glandular transformation of epithelium and marked interfollicular fibrosis (Plate XII), lesion score 4.

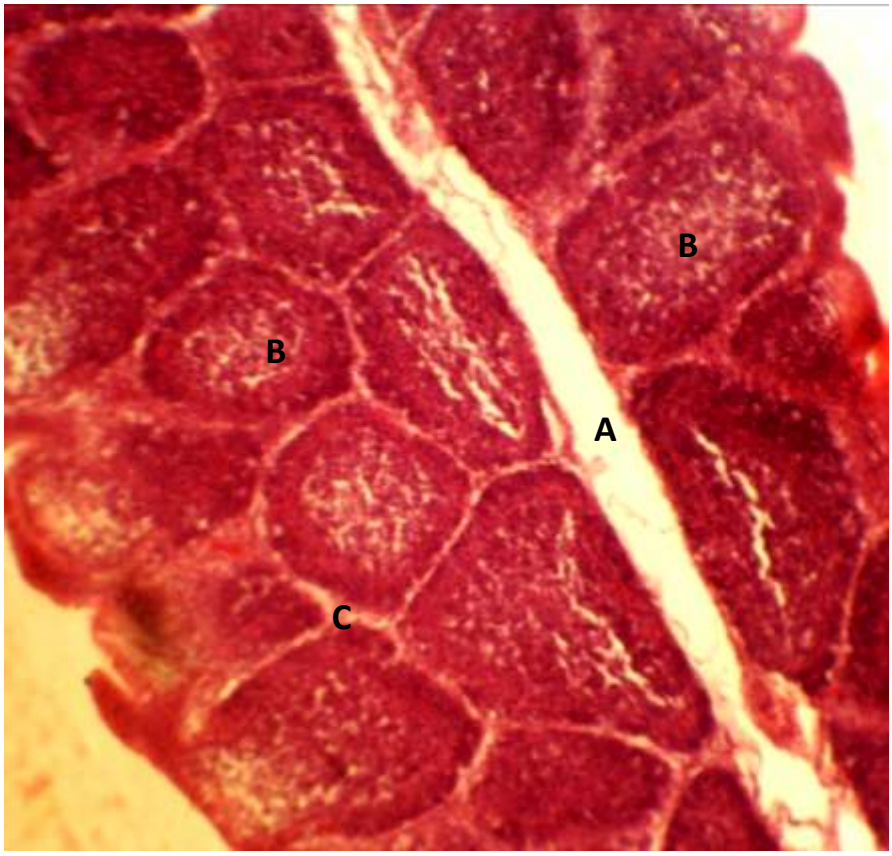


Plate VIII: Photomicrograph of the normal bursa of Fabricius from noninfected group showing the interfolicular space (A), lobule (B), and interlobular space (C). H & E X100

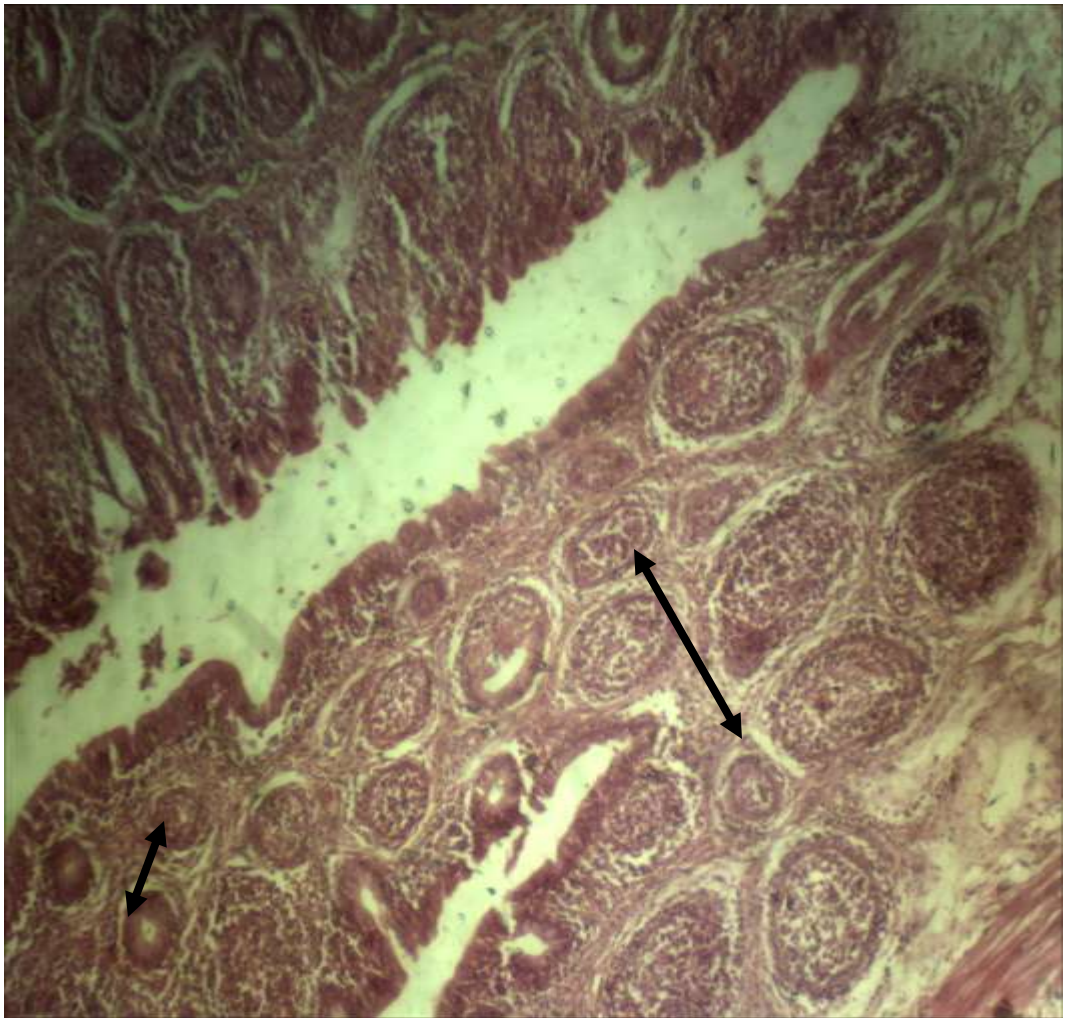


Plate IX: Photomicrograph of bursa of Fabricius from group B, vaccinated and supplemented with 0.5 ppm of Se and challenged. Note the degeneration of the follicular lobules (arrows). H & E X100. (Lesion score 2)

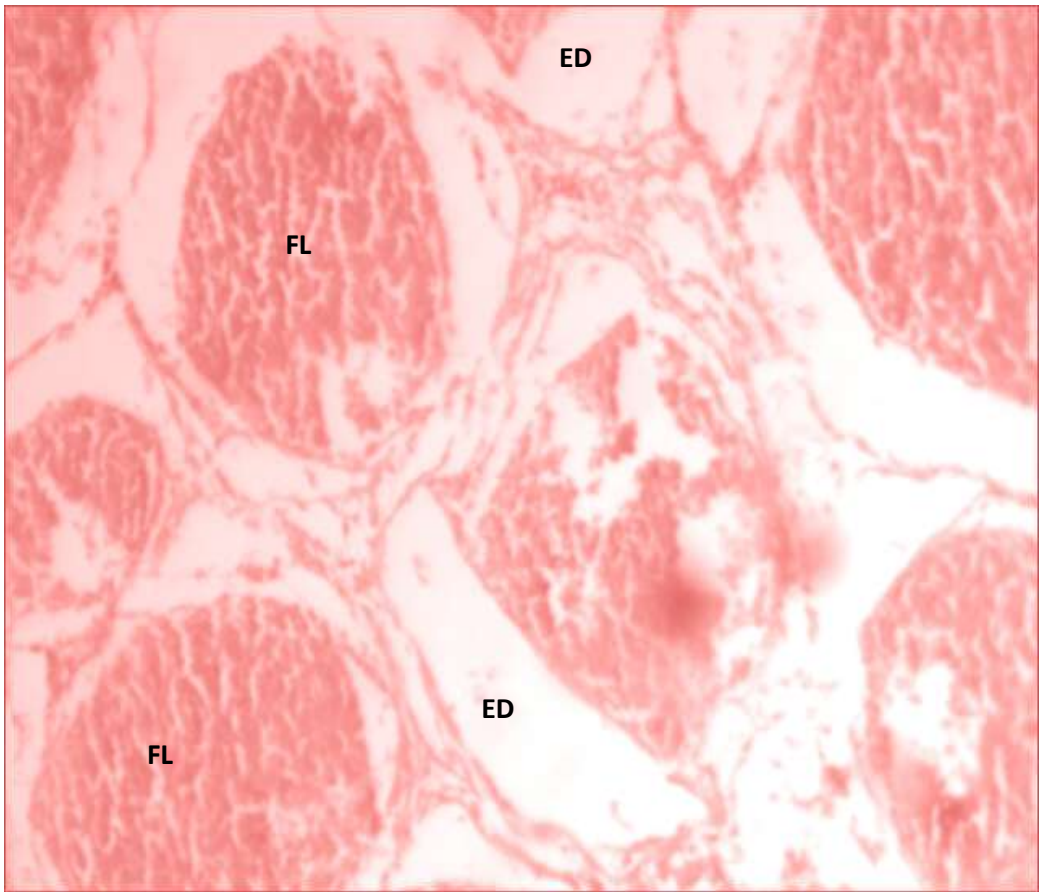


Plate X: Photomicrograph of bursa of Fabricius from group A (vaccinated and supplemented with 0.1 ppm 0f Se) sho wing area of edema (ED) and degeneration of follicular lobules (FL) H & E X100 (lesion score 3).

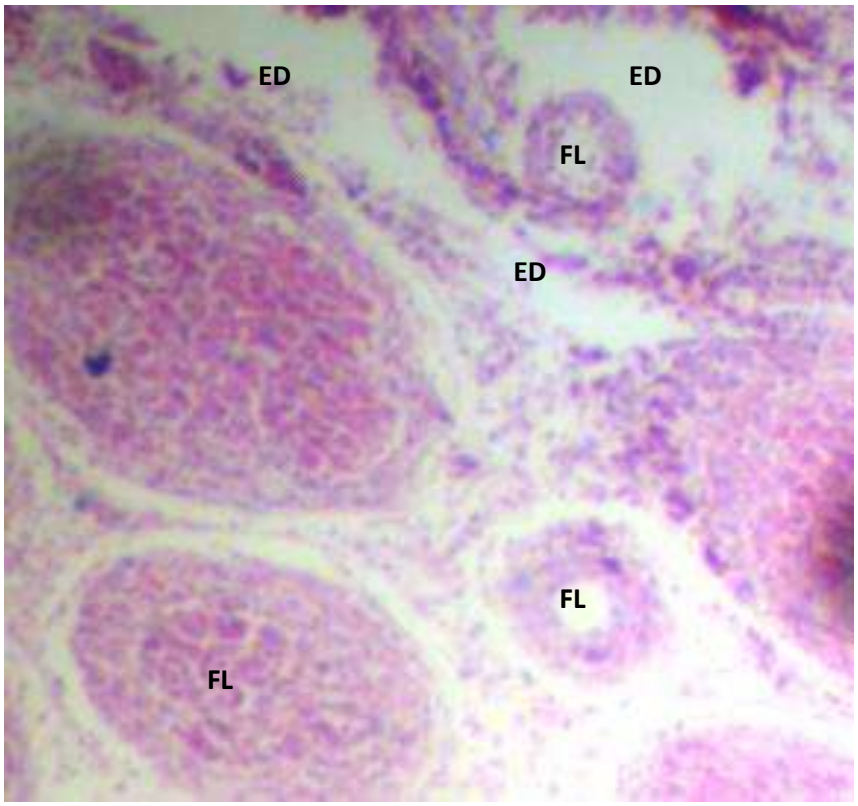


Plate XI: Photomicrograph of bursa of Fabricius from group E (not vaccinated but supplemented with 0.5 ppm 0f Se) showing area of edema (ED) and degeneration of follicular lobules (FL) H & E X100 (lesion score 3)

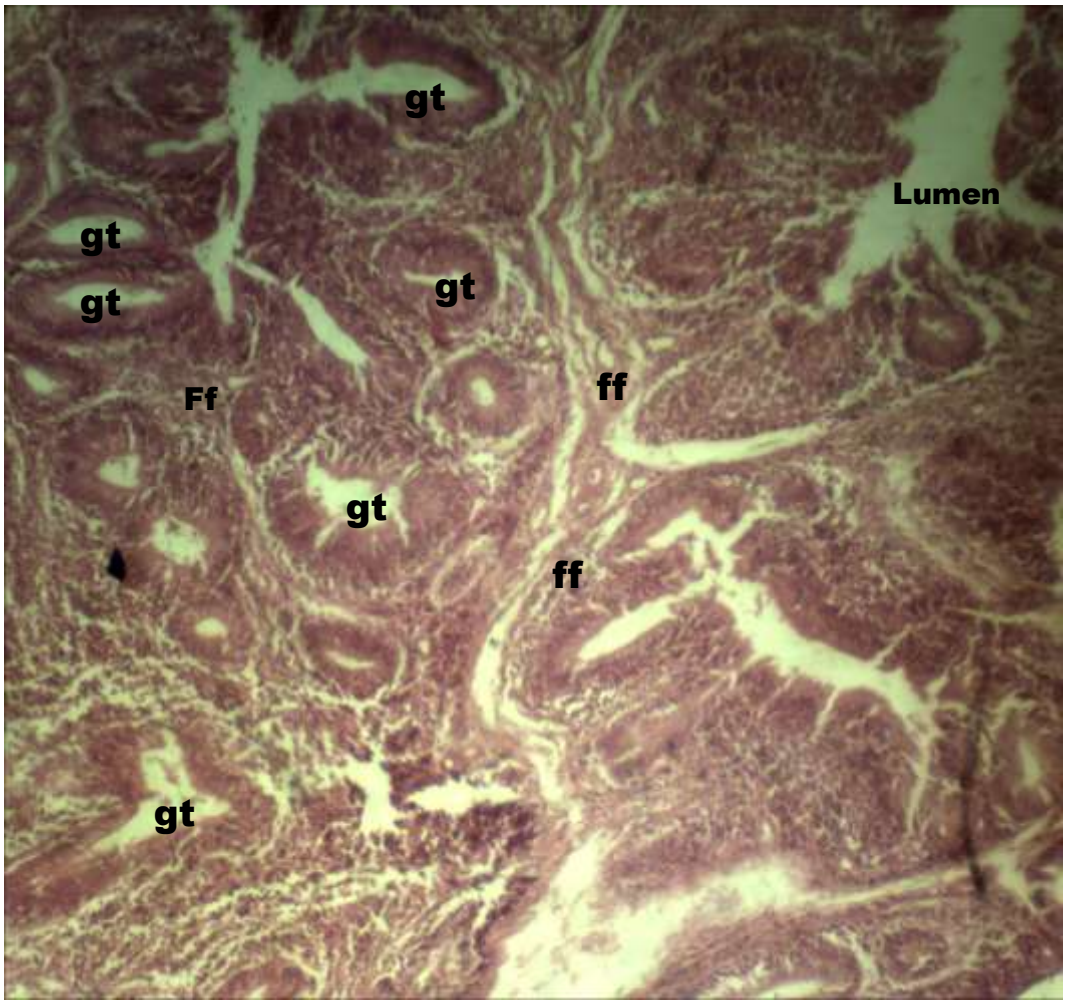


Plate XII: Photomicrograph of the bursa of Fabricius from group L, (not vaccinated, not supplemented but challenged) showing marked interfolicular fibrosis (ff) and glandular transformation of epithelium (gt). H & E X100 (lesion score 4)

4.8 HAEMATOLOGY

Table 4.3 shows the mean values of haematological parameters of day old DBMC cockerels. The mean values of RBC, HGB, PCV, LYM, HET and TP were 2.1 ± 0.71 x 10^{12}/l, 11.9 ± 0.86 g/dl, 32.5 ± 2.74%, 49.2 ± 3.77%, 50.4 ± 3.76% and 2.7 ± 0.43 g/dl, respectively.

Figures 4.14 - 4.19 shows the effect of selenium supplementation on the haematological values of birds challenged with IBDV. There was a positive correlation between the concentration of Se in feed and RBC count at weeks 2, 3, 4, 5, 6 and 7 with r = + 0.95, r = + 0.98, r = + 0.99, r = + 0.99, r = + 0.98 and r = + 0.97 respectively, (Figure 4.14). A positive correlation was also observed between the concentration of Se in feed and HGB concentration at weeks 1 and 2 with r = + 0.99 and r = + 0.95 respectively, (Figure 4.15). For PCV a positive correlation was observed between the concentration of Se in feed and PCV at weeks 1, 2 and 6 with r = + 0.95, r = + 0.90, r = + 0.97 respectively, (Figure 4.16). There was also a positive correlation between the concentration of Se in feed and percentage of LYM at weeks 4, 5, 6 and 7 with r = + 0.81, r = + 0.94, r = 0.93 and r = + 0.93 respectively, there was lymphopenia at week seven (Figure 4.17). There was a positive correlation between the concentration of Se in feed and HET count at weeks 3 and 4 with r = + 0.93, r = + 0.95 and r = - 0.99, respectively, there was heterophilia at week seven (Figure 4.18). There was no correlation between the concentration of Se in feed and TP (Figure 4.19).

Table: 4.3: Haematological parameters and total blood protein of day old Dominant black marshal cockerels.

n(5)	RBC ($\times 10^{12}$/l)	HGB (g/dl)	PCV (%)	LYM (%)	HET (%)	TP (g/dl)
	1.80	10.5	28.0	62	38	4.1
	1.87	10.2	28.1	52	48	1.8
	2.45	13.8	38.3	44	56	2.9
	1.96	10.8	27.9	40	60	2.7
	2.58	14.2	40.0	48	50	1.8
Mean	2.1	11.9	32.5	49.2	50.4	2.7
SEM	±0.71	±0.86	±2.74	±3.77	±3.76	±0.43

Abbreviation: RBC-Red blood cells, HGB-Hemoglobin, PCV-Packed cell volume, LYM-Lymphocyte, HET-Heterophil, TP-Total protein

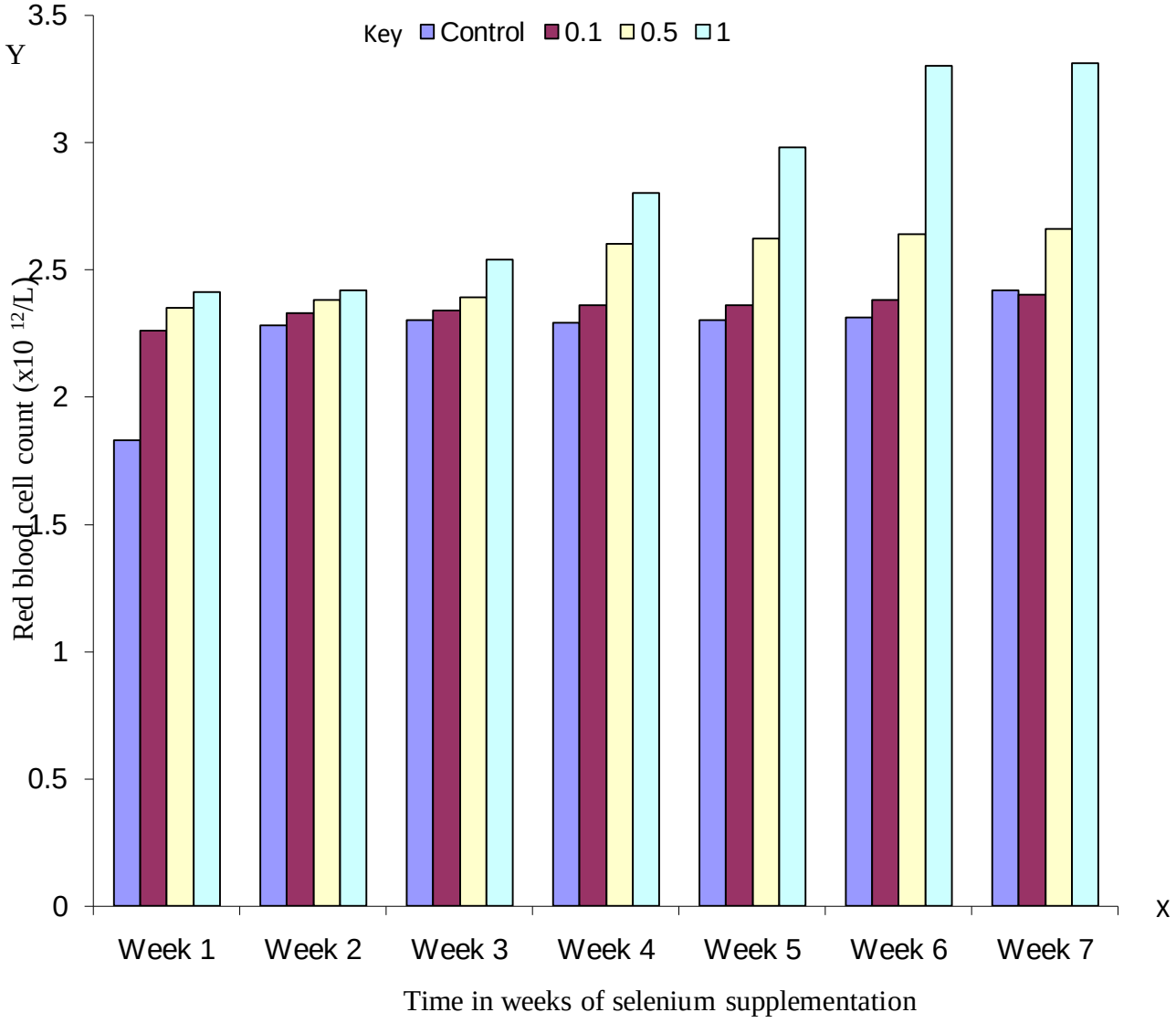


Figure 4.14: Graded levels of selenium supplementation and red blood cell count of Dominant black marshal cockerels

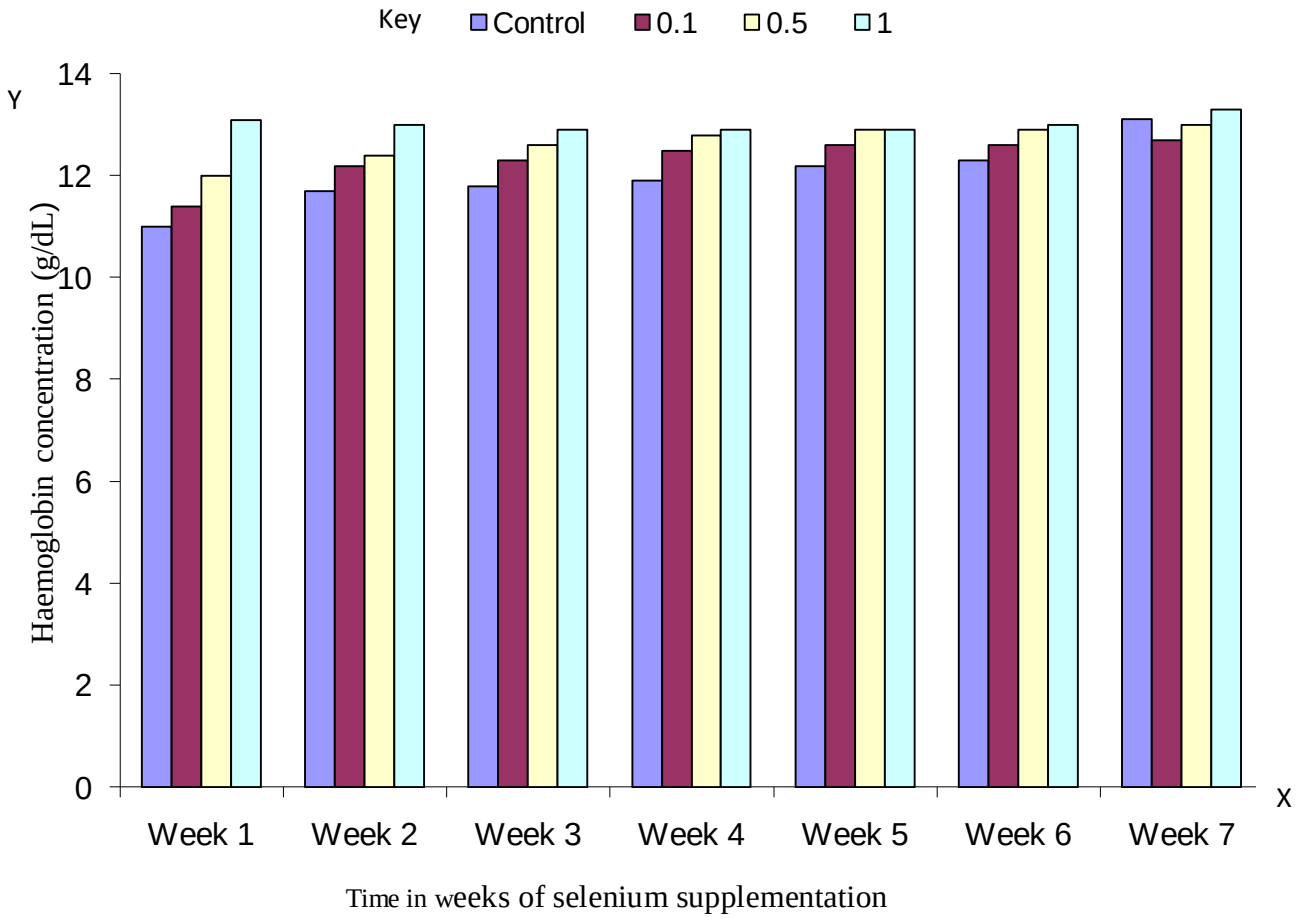


Figure 4.15: Graded levels of selenium supplementation and haemoglobin concentration concentration of Dominant black marshal cockerels

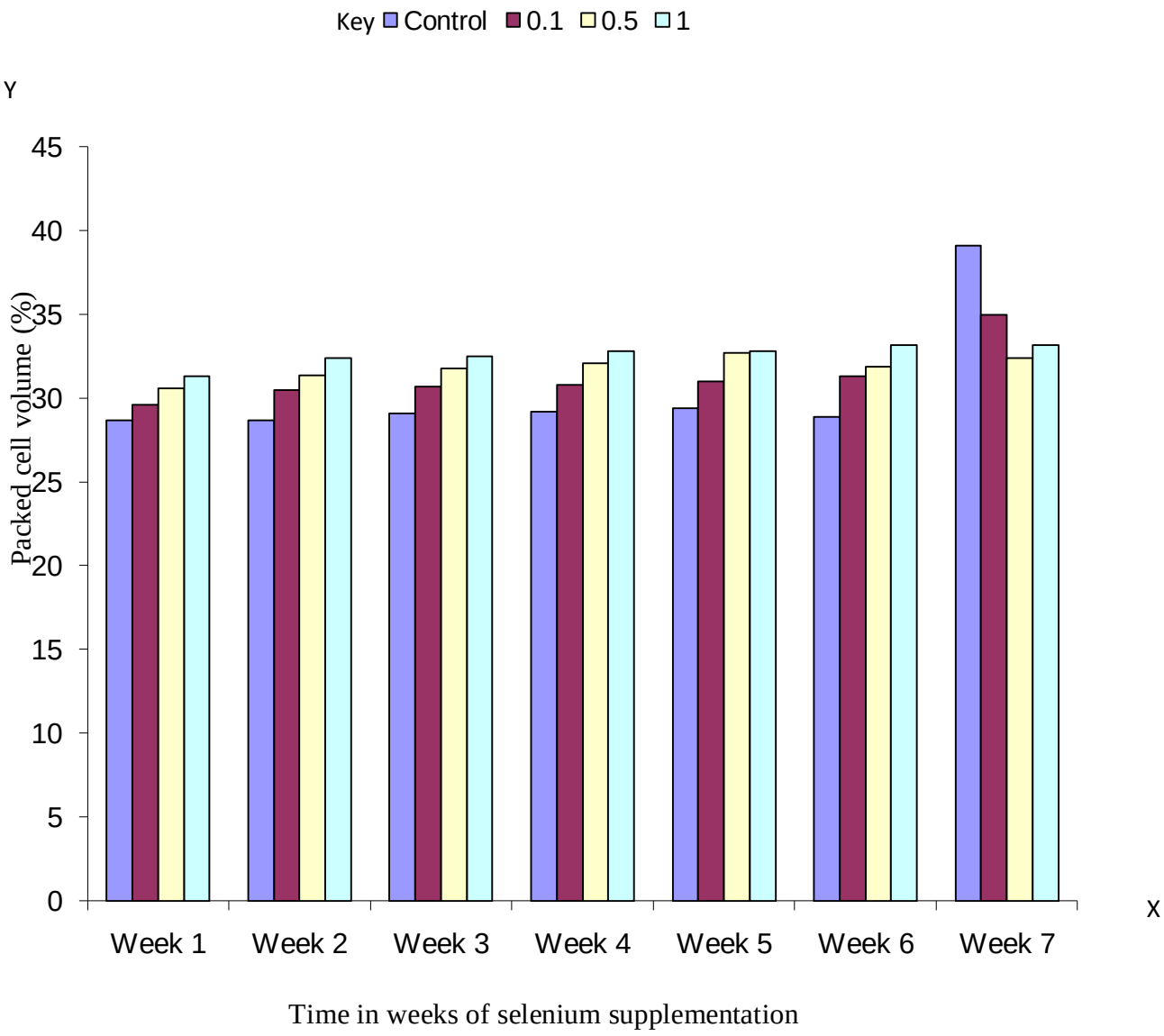


Figure 4.16: Graded levels of selenium supplementation and packed cell volume of Dominant black marshal cockerels

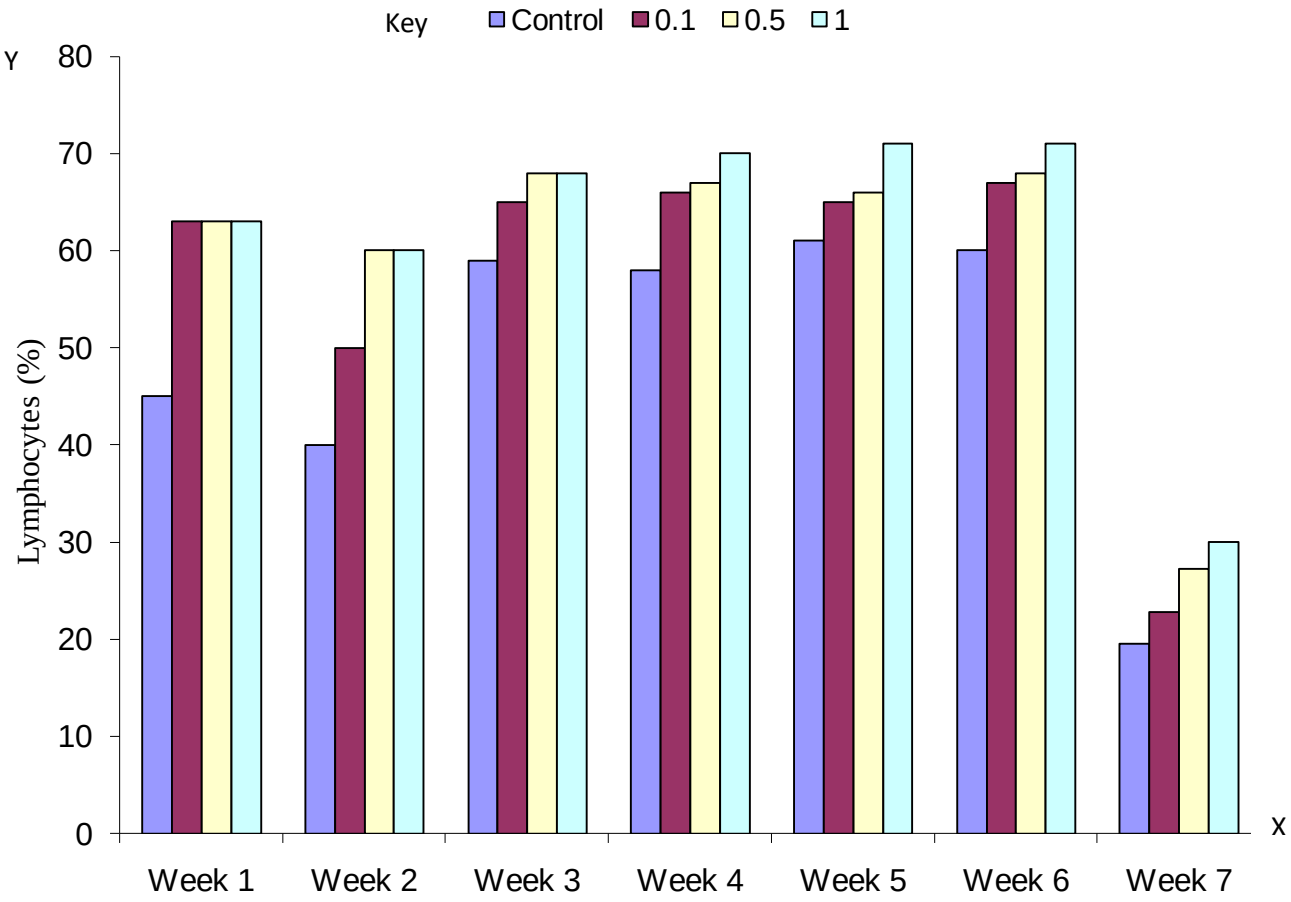


Figure 4.17: Graded levels of selenium supplementation and percentage lymphocyte of Dominant black marshal cockerels

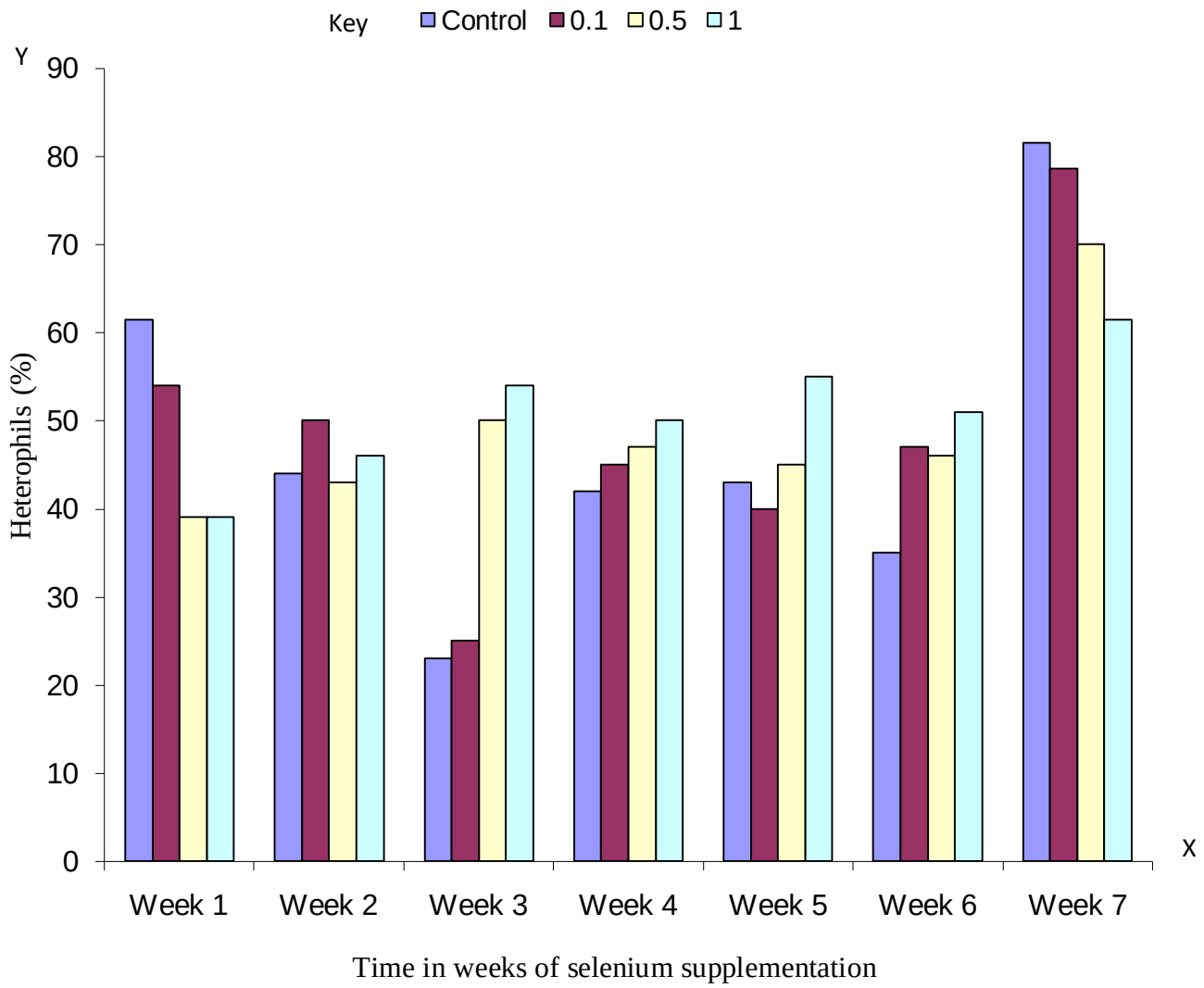


Figure 4.18: Graded levels of selenium supplementation and percentage heterophil of Dominant black marshal cockerels

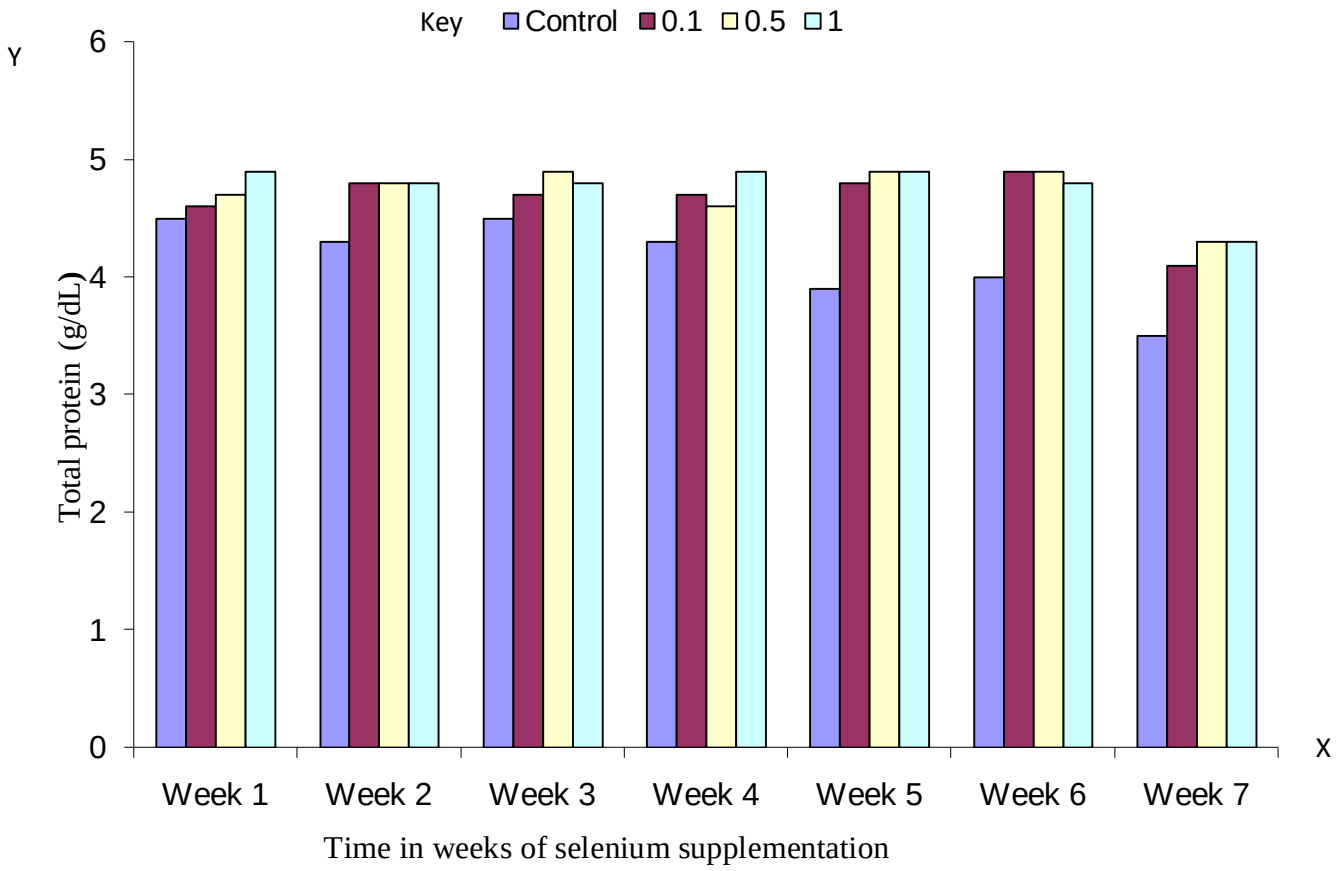


Figure 4.19: Graded levels of selenium supplementation and total blood protein of Dominant black marshal cockerels

CHAPTER 5

5.0 DISCUSSION

The results showed that only one of the four commercial feeds in Jos tested contained Se. The absence of Se in the three commercial feeds observed in this work may suggest that, the grains used in producing the feeds might have been grown in Se deficient soils. It has been documented that plants are the major sources of Se and the amount of Se present in the plant materials depends on the concentration of Se in the soil where the plant was grown (Longnecker *et al*, 1991). In previous report in Finland, it was observed that the concentration of Se in the soil is very heterogeneous and ranges from almost zero to 1 g/Kg (Bennet, 1983; Hartikainem, 2005).

Personal communication with the feed millers whose feeds tested negative for Se revealed that they sourced all raw materials locally in Nigeria. Also, discussion with the miller that produced the feeds which contained Se revealed that it used wheat which was imported from United States of America as the major source of energy in the feed. This imported wheat might have been grown in Se rich soil, hence its presence in the feed. Lenz and Lens (2008) described soil rich in Se to include those with trace concentrations, such as natural sulphides; like pyrites, chalcopyrite and sphalerite, and those with high concentration of sulphur coals, black shale and volcanic tuff. The same authors documented that utilization of rock phosphate fertilizer and the application of sewage sludge to agricultural land can be a source of selenium to the soil.

In this study, Se was found in the serum, blood, liver, muscles and kidneys from day old Dominant black marshal cockerels before they ingested the first feed. The presence of Se in day old chicks in this study, suggests that the Se was derived maternally. This is in agreement with the result of Surai (2002), who reported the presence of antioxidants in newly hatch chicks, such as fat soluble antioxidants, vitamin E and carotenoids, ascorbic acid as well as antioxidant

enzymes, GSH-px, catalase and Se. Surai (2006) further reported that Se content of egg depends on its concentration in the hen's diet, this Se in the diet when ingested by the hen is deposited in the egg yolk and during embryonic development the Se is transferred to the embryonic tissues.

In this study, varying concentrations of Se was observed in serum, blood, liver, muscles and kidneys before Se supplementation with the highest concentration in the muscles, followed by the kidneys, liver, blood and serum in descending order. This finding differ from that of Arnold *et al*. (1973), who found the concentration of Se in the tissue of birds in the following decreasing order; liver, kidneys, muscles and plasma.

After 10 weeks of Se supplementation in the diet, the tissue concentration was highest in the liver followed by the kidneys, muscles, blood and serum. These results agree with the findings of Arnold *et al*. (1973) and Echevarria *et al*. (1988) who reported the same decreasing order of concentrations of Se in similar tissues in chickens.

There was a positive correlation between increasing the concentration of Se in the feed and concentration observed in the kidneys. The significant increase in Se in the kidneys as the dietary dose increases as revealed in this study could suggest that excess Se ingested in the feed is store more in the kidney tissue as compare to other organs such as liver, muscles, serum and blood.

The lack of correlation between the dose of Se in feed and its concentration in muscles, liver, blood and serum in this study might be due to the fact that most of the excess Se that enters the body is excreted within 24 hours (ATSDR, 2003).

In this study, chicks screened for antibodies to IBDV at day old, showed that they had very low maternally derived antibody titre (MDA) to IBDV that is below the protective ComScore level of

3.0 and above (Biogal, 2009). This finding differed from those of Abdu (1986) and Babiker and Tawfeeg (2008) who reported low antibody titre to IBDV only after 17 days while Zaheer *et al.* (2003) reported that MDA became low at one week of age, and not at day old as observed in this study. The low level of MDA in chicks in the present study could be that the chicks came from old parent stock, whereby most of the antibodies have been shaded over time or the parent stock might not have received adequate booster dose of IBD vaccine (which is recommended to be given at the age of 18-22 weeks) or were given a chick doses (dose recommended by manufacturers for vaccination of chicks at 4 and 12 days of age) (Chansiripornchai and Sasipreeyajan, 2009). Ide *et al.* (1978) proposed the choice of a triple fold of chick dose for older birds at 18-22 weeks.

The implication of low MDA observed in this study, is that the birds may not be protected against IBDV in the event of any field challenge at an early age and this could predispose the chicks to subclinical infection and immunosupression (Faragher *et al.* 1972). This could also make the birds to respond poorly to other vaccines and consequently, making them susceptible to other disease agents, such as Newcastle disease virus to which the MDA titre was also low (Faragher *et al*. 1972).

In this study, an intermediate vaccine was used due to the low MDA observed at day old when the chicks were screened. It has been reported that the efficacy of IBD vaccination program was related to the level of MDA in the chickens (Tsukamoto *et al*., 1995; Haddad *et al*., 1997; Chansiripornchai and Sasipreeyajan, 2009). The viruses in mild and intermediate IBD vaccine can be neutralized by MDA in chicks that have high MDA titre; hence, the mild and intermediate vaccines should be used in the chicks that have a low level of MDA, while the intermediate-plus or hot vaccines are suitable for the high MDA titre chickens, this is because the virus in the

vaccines can break through the MDA and stimulate the production of antibodies against the IBDV (Tsukamoto *et al*., 1995; Haddad *et al*., 1997; Chansiripornchai and Sasipreeyajan, 2009).

In this study, there was an increase in CombScore (antibody titre) to IBDV in Se treated groups (A, B, C, G, H, I) than untreated group (J) which were vaccinated but not supplemented with Se. These findings agree with those of Arshad *et al*. (2005) who reported that inorganic Se (sodium selenite) supplementation helps to increase post vaccination humoral immune response against IBDV in broiler chickens. In a similar work conducted by previous workers there was an increase in humoral antibody titre to salmonella, coccidia and IBDV when Se was used in feed (Maldron and Vrzgulova, 1998; Calnago *et al.* 1984; Blood., *et al.*, 1995; Hegazy and Adachi, 2000; Schrauzer, 2000; DengHua *et al.*, 2001).

An increase in antibody titre to IBD vaccine with increase level of Se supplementation was also observed in this study. This finding supports the findings of El-Sheikh *et al*. (2010) who observed an increase in antibody response to IBDV in Bandarah chicks which received graded levels (0, 0.1, 0.2 and 0.3 ppm) of organic selenium supplementation.

A decrease in MDA to IBDV with increase in age was observed in this study. A similar work carried out by Ide *et al.* (1978) showed that at 18 days of age serum from most chicks did not contain demonstratable IBDV precipitating antibody as a result of MDA complete decay. In this study, there was detectable antibodies in week two in groups E and F, which were not vaccinated but received 0.5 and 1 ppm of Se respectively, no detectable antibodies in groups D, K and L which were also not vaccinated, but group D received 0.1 ppm of Se and group K and L did not received Se in their feed. This observation suggests that Se supplementation could delay MDA decay; this could help in preventing subclinical IBD infection at an early age of the chicks.

At three weeks of age no detectable antibodies were recorded in all the groups that were not vaccinated irrespective of Se supplementation in feed, this suggest complete MDA decay at three weeks of age. At seven weeks of age after they have been inoculated with IBDV at six weeks of age, antibodies to IBDV was detected in all the groups challenged, the antibodies detected here is as a result of response to the challenged virus, this buttresses the result of van den Berg *et al.* (2000), who found high morbidity, with up to 100% serological conversion, after infection with IBDV.

It is known that the susceptibility of chickens to IBD infection largely depends on the immune status of the birds to the virus, and that low antibody levels can result in high morbidity and mortality in the face of any challenge (El-Sheikh *et al*., 2010). In the present study, a negative correlation was observed ($r = -0.93$ and $r = -0.95$, for vaccinated and non vaccinated chickens, respectively) between Se concentration in feeds and the number of birds that died after challenge with IBDV at 6 weeks of age.

The low mortality rates observed in groups A, B, C compared to groups D, E, F and L could be attributed to the positive effect of intermediate IBD vaccine and Se treatments in groups A, B and C as against groups D, E and F which received Se but were not vaccinated against IBDV and group L which was not vaccinated and was not supplemented with Se. These findings support the results of previous authors, that Se supplementation enhance the immune system and increase natural resistance of chickens to salmonella and coccidia (Canalgo *et al.* 1994; Hegazy and Adachi, 2000). Mahmoud and Edens (2003) and Srimongkol (2003) also reported that Se, supplemented broilers chicks had significantly higher blood GSH-Px (a Se dependent enzyme) concentration than those of the control. Gluthathione peroxidase removes free radical from the system and enhances immunity (Brigelius-Flohe, 1999). Therefore, it is believed that Se

supplementation in the treated groups in this study is responsible for enhanced immunity that resulted in reduced mortality after challenge with IBDV.

Vaccinating birds with intermediate strain of IBD vaccine with Se supplementation did not confer complete immunity to birds in this study. This finding is in agreement with the reports of Abdu (1986), Zaheer *et al.* (2003) and Babiker *et al*. (2008), who observed outbreaks of IBD in flocks vaccinated with a variety of vaccines, but differed from the findings of Hassan *et al*. (2004), who reported that White Leghorn birds vaccinated with a live intermediate vaccine gave complete protection to the birds when challenged ten days later with vvIBDV.

In this study pathologic lesions were observed in all organs studied in the chicks that died in all the groups that were challenged, except the liver where lesions were observed only in chicks that died in group L (which was not vaccinated with IBD vaccine and not supplemented with Se but challenged with IBDV at six weeks of age) and the kidneys, where lesions were absent in groups B and C. The bursa of Fabricius had the highest percentage of observable lesions than other organs in all the groups, this finding supports the report of Rosenberger and Cloud (1986) who reported that the bursa of Fabricius is the most severely affected organ in IBDV infection.

It was observed in this study that there was a decrease in the percentage of organs with gross pathologic lesions and decrease in the severity of lesions with increase in Se concentrations in chicken feed in both the vaccinated groups and unvaccinated groups. The percentage of organs from chicks that died from the groups that were not vaccinated with IBD vaccine and not supplemented with Se in their feed was observed to be higher and lesions more severe than in Se supplemented groups. Similarly microscopic lesion were more severe in unvaccinated, unsupplemented groups infected with IBDV than those vaccinated and supplemented with Se.

Infections with IBDV have been reported to stimulate macrophages which produce NO and other cytokines with anti proliferative activity (Sharma *et al*, 2000). Heterophilia in IBDV infection results in an activation of cellular oxidative burst (Oladele *et al*, 2005 and Lam, 1998). Oxidative stress is a reflection of excess intracellular concentrations of reactive oxygen species and one of the important indications of cellular damage. Selenium transforms free radicals into less reactive species, thereby limiting their toxic effect (Hema *et al*, 2007). It was concluded that Se supplementation in chicken feed could reduce the severity of tissue damage in IBDV infection.

The effect of Se supplementation on the haematological values in this study showed a positive correlation between the concentration of Se in feed and RBC count, HGB concentration, PCV, LYM and HET values. This observation is in agreement with the result of El-Sheikh *et al.* (2010), who reported increase in RBC, HGB and WBC in Se treated Bandarah chicks when compared to the controls. In a similar study, Latshaw (1991), El-Sebai (2000) and Abaza (2002), found Se singly or in combination with vitamin E supplementation to broiler chickens and Japanese quail's diets led to a significant rise in RBC and WBC counts. In another study, Huang *et al*. (1999) found that Se improves markedly T-lymphocyte and natural killer cell activity in chickens. This general increase in the haematological parameters after Se supplementation might suggest that Se play a role in haematopoeisis, by increasing the activity of bone marrow cells (Chen and Te-Heisen, 2000).

The marked increase in PCV, marked lymphopenia and heterophilia at the 7^{th} week of age in all the groups inoculated with IBDV observed in this study is consistent with the pathogenesis of IBD in chicks as observed by Oladele *et al.* (2005). Increase in PCV values, as observed in infected chicks in this study; have previously been observed by Chineme and Cho (1984) and

Oladele *et al.* (2005). It is believed that dehydration, which is one of the clinical and postmortem findings in IBD, is a major cause of the increase in the PCV values.

The bursa of Fabricius is an organ where maturation and differentiation of B-lymphocyte takes place before it migrates into the blood stream and it is known that the IBDV has affinity for bursa of Fabricius (Weis and Kaufer, 1994). The lymphopenia observed post inoculation with IBDV in this study might be the result of massive destruction of B-lymphocyte in the bursa of Fabricius by IBDV; this could lead to immunosuppression in the chicks (Muller *et al.*, 2003).

Marked heterophilia was observed in this study after inoculation with IBDV. This could suggest massive tissue damage leading to infiltration of heterophils to clear the tissue debris, this support a similar study by Oladele *et al.* (2005) who reported an increase in the number of heterophils in chicks infected with IBDV.

CHAPTER 6

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

In the present study Se was found in varying concentrations in all the tissues examined in day old DBMC, with higher concentration in the muscles, follow by the kidneys then the liver, blood and the serum. Selenium supplementation in feed was found to increase antibody titre in cockerels vaccinated with intermediate IBD vaccine, increased haematological parameters of the chicks, reduced mortality of chicks to IBD infection and reduced severity of tissue damage in IBDV infection.

6.2 CONCLUSIONS

From this work it was concluded that:

i). Selenium was present in the tissue of the DBMC at one day old.

ii). The DBMC had MDA but the level was not protective.

iii). Selenium supplementation enhances the immune response of DBMC to IBD and it also reduces the severity of the disease.

6.3 RECOMMENDATIONS

It was recommended that:

i). Farmers and feedmillers should supplement Se in the poultry feed.

ii). More studies on the field on the efficacy of Se on IBD be carried out.

REFERENCES

Abaza, M. (2002). Immune system and some physiological aspects in Japanese quail affected by antioxidants. *Egyptian Journal of Poultry Science,* 22: 259 – 276.

Abdel-Alim, G. A. and Saif, Y. M. (2001[a]). Pathogenicity of cell culture-derived and Bursa derived infectious bursal disease viruses in specific-pathogen-free chickens. *Avian Diseases,* 45:844-852.

Abdel-Alim, G. A. and Saif, Y. M. (2001[b]). Immunogenicity and antigenicity of very virulent strains of infectious bursal disease viruses. *Avian Diseases,* 45:92-101.

Abdu, P.A. (1997). Studies on the problems associated with vaccination against infectious bursa disease in Nigeria. *PhD Thesis, A.B.U Zaria 129 Pp*

Abdu, P.A (1988). Infectious bursa disease in a flock of broilers and local Nigerian chikens. *Bulletin of Animal Health and Production Africa* 36: 269-271.

Abdu, P. A (1987). Infectious bursal disease and Spirochetosis in pullets chicks. *Avian Disease*. 31 (1) 204-205

Abdu, P.A. (1986). Infectious bursa disease immunization failures in chicken in Nigeria. *Tropical Animal Health and Production,* 18:123-125.

Abrams, C. K. Siram SM, Galsim C, Johnson-Hamilton H, Munford FL.and Mezghebe H. (1992). Selenium deficiency in long-term total parenteral nutrition. *Journal Nutrition in Clinical Practice,* 7: 175-178.

Agency for Toxic Substances and Disease Registry (ATSDR). (2003). *Toxicological Profile for selenium*. Atlanta, GA. U.S. Department of Health and Human Services, Public Health Service. P.71-137.

Allan, W. H., Faragher, J. T. and Cullen, G. A. (1972). Immunosuppression by the infectious ursal agent in chickens immunised against Newcastle disease. *Veterinaary Record,* 90:511- 512.

Alexander, D. J. and Chettle, N.J. (1998). Heat inactivation of serotype 1 infectious bursal isease virus. *Avian Pathology* 27:97-99.

Al-Natotur, M. Q., Ward, L. A., Saif, Y. M., Stewart-Brown B. and Keck, L. D. (2004). Effect of diffferent levels of maternally derived antibodies on protection against infectious bursal disease virus. *Avian Diseases,* 48:177-182.

Anderson, W. I., Reid W. M., Lukert, P. D. and Fletcher, O. J. (1977). Influence of infectious bursal disease on the development of immunity to *Eimeria tenella*. *Avian Diseases* 21:637-41.

Apsite, M., Pitrans, B., and Atlavin, A., (1994). Absorption of 75Se-Selenate and 75Se Selenite in chicks. In: Mengen- und Spurenelemente, Edited by Anke, M. and Meissener, D., Arbeitstagung, Jena.European. Food Safety Authority P. 188.

Arnér, E., and Holmgren, A. (2000). Physiological functions of thioredoxin and thioredoxin reductase. *Journal of the Federation of American Societies for Experimental Biology*, 267:6102-6109.

Arnold, R. L., Olson, I. E. and Carlson, C. W. (1973). Dietary selenium and arsenic additions and their effects on tissue and egg selenium. *Poultry Science*, 52:847-854.

Aro, A., Kumpulainen, J., Alfthan, G., Voshchenko, A. V. and Ivanov, V. N. (1994). Factors affecting the selenium intake of people in transbaikalian Russia. *Biological Trace Element research Journal,* 40: 277-285.

Arshad, M., Siddique, M., Ashraf, M. and Khan, H.A. (2005). Effect of selenium supplementation on antibody titers against Infectious bursal disease vaccine in broiler chicks.*Pakistan Veterinary Journal,* 25 (4): 203-204

Arteel, G. E., Briviba, K. and Sies, H. (2000). Protection against peroxynitrite. *Federation of European Biochemical Societies Letters,* 445:226-230.

Ayo, J.O and Oladele, S.B. (1996). Natural antioxidants and their uses in prophylaxis and therapy of disease conditions. *West African Journal of Pharmacology and Drug Research,* 12: 69-76

Azad, A. A., Barrett, S. A. and Fahey K. J. (1985). The characterization and molecular clonin of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus.*Virology Journal,* 143:35-144.

Azad, A. A., Jagadish, M. N., Brown, M. A. and Hudson, P. J. (1987). Deletion mapping and expression in Escherichia *coli* of the large genomic segment of a birnavirus. *Virology*, 161:145-52.

Babiker, M.A.A., Yahia, I.E., Noura, K. And Manal, M.E. (2008). Evaluation of four commercial anti-infectious bursal disease vaccines under Sudan condition *International Journalof Poultry Science,* 7 (6) 570-573.

Babiker M.A.A. and Tawfeeg, E. (2008). Role of administration routes of anti – infectious bursal disease virus (Gumboro) vaccine on immunization of chicken. *International Journal of Poultry Science,* 7 (3): 279-282

Barceloux, Donald G. and Barceloux, Donald, (1999). Selenium. *Clinical Toxicology*, 347 (2): 145-172.

Bartholomew, A., Latshaw, D. and Swayne, D. E. (1998). Changes in blood chemistry, hematology, and histology caused by a selenium/vitamin e deficiency and recovery in chicks. *Journal Biology Trace Element Research,* 62: 7-16.

Baum, M. K., Miguez-Burbano, M. J., Campa, A., and Shor-Posner, G. 2000. Selenium and interleukins in persons infected with human immunodeficiency virus type 1. *Journal of Infectious Diseases*, 182:69-73.

Bayliss, C. D., Peters, R. W., Cook, J. K., Reece R. L., Howes, K., Binns, M. M. and Boursnell, M. E. (1991). A recombinant fowlpox virus that expresses the VP2 antigen of infectious bursal disease virus induces protection against mortality caused by the Virus. *Archives of Virology,* 120:193-205.

Bayliss, C. D., Spies, U., Shaw, K., Peters, R. W., Papageorgiou, A., Muller, H. and Boursnell, M. E. (1990). A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. *Journal of General Virology,* 71 (6):1303-1312.

Becht, H. (1980). Infectious bursal disease virus. *Current Topical Microbiology Immunology* 90:107- 121.

Becht, H., Muller, H. and Muller, H. K. (1988). Comparative studies on structural and antigenic properties of two serotypes of infectious bursal disease virus. *Journal of General Virology,* 69 (3):631-640.

Becht, H. and Muller, H. (1991). Infectious bursal disease--B cell dependent immunodeficiency syndrome in chickens. *Behring Inst Mitt*: 217-225.

Beck, M. A. (1994). Increased virulence of a human enterovirus (coxsackievirus b3) in selenium deficient mice. *Journal of Infectious Diseases*, 170: 351-357.

Beck, M. A., Shi, Q., Morris, V. C., and Levander, O. A. (1995). Rapid genomic evolution of a non-virulent coxsackievirus b3 in selenium-deficient mice results in selection of identical virulent isolates. *Nature Medicine,* 1: 433-436.

Beckett, G., Russell, A., Nicol, F., Sahu, P., Wolf, C., and Arthur, J. (1992). Effect of selenium deficiency on hepatic type I 5-iodothyronine deiodinase activity and hepatic thyroid hormone levels in the rat. *Biochemistry. Journal,* 282:483-486.

Behne, D. and Wolters W. (1983). Distribution of selenium and glutathione peroxidase in the rat. *Journal of Nutrition,* 113:456-462.

Behne, D. and Kyriakopoulos, A. (2001). Mammalian selenium-containing proteins. *Annual Review Nutrition,* 21: 453-473.

Benjamin, M.M. (1978). Outline of Veterinary Clinical Pathology. The Iowa State University Press Ames, U.S.A., 361 pp.

Bennet, B.G. (1983). Exposure of man to environmental selenium-An exposure commitment assessment. *Journal of the Science of the Total Environment*, 331:117-127

Benton, W. J., Cover, M.S. and Rosenberger, J.K. (1967). Studies on the transmission of the infectious bursal agent of (IBA) of chickens. *Avian Diseases,* 11:430-438.

Beug, H., Muller, H., Grieser, S., Doederlein, G. and Graf, T. (1981). Hematopoetic cells transformed in vitro by REVT avian reticuloendotheliosis virus express characteristics of very immature lymphiod cells. *Journal of Virology,* 11:295-309.

Biogal, (2009). ImmunoComb® Poultry IBD-ND-IB Antibody Test Kit. *Catalog No 50PTV203/50PTV230*.

Blood, D. C., Radostits, O. M. and Gay, C. C. (1995). *Veterinary Medicine, A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses.* 8 th Ed. ELBS., pp: 1412-1416.

Boot, H. J., Huurne, A. A., Hoekman, A. J., Peeters, B. P. and Gielkens, A. L. (2000*).* Rescue of very virulent and mosaic infectious bursal disease virus from cloned cDNA: VP2 is no the sole determinant of the very virulent phenotype. *Journal Virology,* 74:6701-6711

Bottcher, B., Kiselev, N. A., Stel'Mashchuk, V. Y., Perevozchikova, N. A., Borisov, A.V. and Crowther, R. A. (1997). Three-dimensional structure of infectious bursal disease virus determined by electron cryomicroscopy. *Journal of Virology,* 71:325-30.

Bou-resil, M.N., Mathew, T.C., Dashti, H.M. and Al-zaid, N.S. (2002). Brain selenium accumulation in rat pups of selenium supplemented mothers. *Anatomy, Histology Embryology*, 31: 228-231.

Brandt, M., Yao, K., Liu M., Heckert R. A. and Vakharia, V. N. (2001). Molecular determinants of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. *Journal of Virology,* 75:74-82.

Brigelius-Flohe, R. (1999). Tissue-specific functions of individual glutathione peroxidases. *Free Radical Biology and Medicine*, 27:951-965.

Broke, E.A. and Loerch, S.C. (1989). Effect of parenterally selenium and vitamin E on performance, health and humoral immune response of steers new to feedlot environment. *Journal of Animal Science,* 67(6): 1350-1359.

Broome, C. S., McArdle, F., Kyle, J. A. M., Andrews, F., Lowe, N. M.., Hart, C. A., Arthur, J.A. and Jackson, M. J. (2004). An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. *America journal of Clinical Nutrition*, 80:154-162.

Brown, M. D., Green, P. and Skinner, M. A. (1994). VP2 sequences of recent European very virulent' isolates of infectious bursal disease virus are closely related to each other but are distinct from those of 'classical' strains. *Journal of General Virology,* 75 (3):675-680.

chaBurkhardt, E. and Muller, H. (1987). Susceptibility of chicken blood lymphoblasts and monocytes to infectious bursal disease virus (IBDV). *Archives of Virology,* 94:297- 303.

Calnago, G.L., Jensen, L.S. and Long, P.L. (1984). Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens. *Poultry Science,* 63(6): 1136-1143.

Cao, Y. C., Yeung, W. S., Law, M., Bi Y. Z., Leung, F. C., and Lim, B. L. (1998). Molecular characterization of seven Chinese isolates of infectious bursal disease virus: classical, very virulent and variant strains. *Avian Diseases,* 42:340-351

Capello, Z.J., King, L.K., Marcum, S.T. and Stanly, E.A. (1990). Chemical Analysis of Selenium and Copper Levels in Virginia Cattle Herds. Governor's School for Agriculture, Virginia Tech, Blacksburg, VA 24061.

Century, B., and Hurwitt M.K. (1964). Effect of dietary selenium on incidence of nutritional encephalomalacia in chicks. *Proceedings of the Society for Experimental Biology and Medicine,* 117:320.

Chang, H. C., Lin, T. L. and Wu, C. C. (2001). DNA-mediated vaccination against infectious bursal disease in chickens. *Vaccine,* 20:328-335.

Chansiripornchai, N. and Sasipreeyajan, J. (2009). Comparison of the efficacy of the immune complex and conventionally live vaccine in broilers against infectious bursal disease infection. *Thai Journal of Veterinary Medicine,* 39(2): 115-120.

Chen, C. and Te-Hsien, L. (2000). Effects of selenium dioxide on blood and femoral bone marrow of rats. *Journal of Toxicology and Environmental Health* Vol 59 No 7, p 553-560,

Chen, H. Y., Zhou, Q., Zhang, M. F. and Giambrone, J. J. (1998). Sequence analysis of the VP2 hypervariable region of nine infectious bursal disease virus isolates from mainland China. *Avian Diseases,* 42:762-769.

Cheng, Y. Y., and Qian, P. C. (1990). The effect of selenium-fortified table salt in the prevention of keshan disease on a population of 1.05 million. *Biomedical Environmental Science Journal,* 3: 422-428.

Chettle, N., Stuart, J. C. and Wyeth. P. J. (1989). Outbreak of virulent infectious bursal disease in East Anglia. *Veterinary Record,* 125:271-272.

Chettle, N. J., Eddy R. K. and Wyeth, P. J. (1985). Comparison of virus neutralizing and precipitating antibodies to infectious bursal disease virus and their effect on susceptibility to challenge. *British Veterinary Journal,* 141:146-150.

Cheville, N. F. (1967). Studies on the pathogenesis of Gumboro disease in the bursa of Fabricious spleen and thymus of the chicken. *America Journal of Pathology,* 51:527-551.

Chineme, C.N. and Cho, Y. (1984). Clinicopathological and morphological changes in chickens experimentally infected with infectious bursal disease virus. *Tropical Veterinarian*, 2:218-224

Cho, B. R. (1970). Experimental dual infections of chickens with infectious bursal and Marek's disease agents. I. Preliminary observation on the effect of infectious bursal agent on Marek's disease. *Avian Diseases,* 14:665-675.

Cho, Y. and Edgar, S. A. (1969). Characterization of infectious bursa agent. *Poultry Science Journal,* 48:2102-2109.

Clark, L. C., Combs, G. F., Tumbull, B. W., Slate, E. and Alberts D. (1996). The nutritional prevention of cancer with selenium 1983-1993; a randomized clinical trial. *Journal of America. Medical Association,* 276: 1957-1963.

Coles, H.E. (1986). *Veterinary Cclinical Pathology* 4th edition. Philadelphia, USA WB Saunders Pp 17-23.

Combs, G.F. (1994). Clinical implications of selenium and vitamin E in poultry nutrition. *Veterinary Clinical Nutrition,* 1:133-140.

Combs, G.F. Jr. and Scott, M.L. (1974). Dietary requirements for vitamin E and selenium measured at the cellular level in the chick. *Journal of Nutrition,* 104: 1292- 1296.

Combs, G. F. and Combs, S.B., (1984). The nutritional biochemistry of selenium. *Review of Nutrition,* 4: 257-280.

Combs, G.F. and Hady, M.M. (1991). Selenium involved with vitamin E in preventing encephalomalcia in the chick. *Federation of American Societies for Experimental Biology Journal,* 5:A714

Combs, G.F. Jr. and Gray, W.P. (1998). Chemopreventive agents: selenium. *Journal Pharmacological Therapy*, 79: 179-192.

Confer, A. W. and MacWilliams, P. S. (1982). Correlation of hematological changes and serum and monocyte inhibition with the early suppression of phytohemagglutinin stimulation of lymphocytes in experimental infectious bursal disease. *Canadian Journal of Comparative Medicine,* 46:169-175.

Confer, A. W., Springer, W. T., Shane, S. M. and Donovan, J. F. (1981). Sequential Mitogen stimulation of peripheral blood lymphocytes from chickens inoculated with infectious bursal disease virus. *American Journal of Veterinary Research*, 42:2109-2113.

Contreras, M., Fernandez, R., Motiel, E., Rivallan G. and Eterradossi, N. (2000). Gumboro threat to Latin American poultry. *International Poultry Production*., vol. 8, pp 7-8.

Cosgrove, A. S. (1962). An apparently new disease of chickens-Avian Nephrosis. *Avian Diseases,* 6:385-389.

Craft, D. W., Brown, J. and Lukert, P. D. (1990). Effects of standard and variant strains of infectious bursal disease virus on infections of chickens. *American Journal of Veterinary Research,* 51:1192- 1197.

Cruz Coy, J. S., Giambrone, J. J. and Panangala, V. S. (1993). Production and characterization characterization of monoclonal antibodies against variant A infectious bursal disease virus. *Avian Diseases,* 37:406-411.

Cullen, G. A. and Wyeth, P. J. (1975). Quantitation of antibodies to infectious bursal disease. *Veterinary Record,* 97:315.

Darteil, R., Bublo, M., Laplace, E., Bouquet, J. F., Audonnet, J. C. and Riviere, M. (1995). Herpesvirus of turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. *Journal of Virology,* 211:481-490.

Darling, G., Mathias, P., O'Regan, M. and Naughten, E. (1992). Serum selenium levels in individuals on pku diets. *Journal of Inherit Metabolic Disease,* 15: 769-773.

DengHua, Y.H., Yuqing, L. and Yong M. (2001). Effects of immunoenhancement with selenium on immune function in chickens. *Chinese Journal of Veterinary Science*, 21: 96-98.

Dobos, P., Hill, B. J., Hallett, R., Kells, D. T., Becht, H. and Teninges, D. (1979). Biophysical and biochemical characterization of five animal viruses with bisegmented doublestranded RNA genomes. *Journal of Virology,* 32:593-605.

Dohms, J. E., Lee, K. P. and Rosenberger, J. K. (1981). Plasma cell changes in the gland of Harder following infectious bursal disease virus infection of the chicken. *Avian Diseases,* 25:683-695.

Dohms, J. E., Lee, K. P., Rosenberger, J. K. and Metz, A. L. (1988). Plasma cell quantitation in the gland of Harder during infectious bursal disease virus infection of 3-week old broiler chickens. *Avian Diseases, 32:624*-631.

Dormitorio, T. V., Giambrone, J. J. and Duck, L. W. (1997). Sequence comparisons of the variable VP2 region of eight infectious bursal disease virus isolates. *Avian Diseases,* 41:36-44.

Dybing, J. K. and Jackwood, D. J. (1998). Antigenic and immunogenic properties of baculovirus-expressed infectious bursal disease viral proteins. *Avian Diseases,* 42:80-91.

Echevarria, M. G., Henry, P. R., Ammerman, C. B., Rao P. V. and. Miles, D. J., (1988). Estimation of the relative bioavailability of inorganic selenium sources for poultry. Tissue uptake of selenium from high dietary selenium concentrations. *Poultry Science Journal,* 67:1585-1592.

Edens, F.W. (2002). Practical applications for selenomethionine: broiler breeder reproduction. In: Biotechnology in the Feed Industry. *Proceedings of the 18th Alltech's Annual Symposium,* Edited by Lyons, T.P. and Jacques, K.A., Nottingham University Press, Nottingham, UK, pp. 29-42.

Edens, F.W. (1996). Organic selenium: from feathers to muscle integrity to drip loss. Five years onward: no more selenite! In: Biotechnology in the Feed Industry. *Proceedings of the 17th Alltech's Annual Symposium,* Edited by Lyons, T.P. and Jacques, K.A., Nottingham University Press, Nottingham, UK. pp. 349-376.

Edens, F. W., Burgos, S., Read-Snyder, J., Cantor, A., and Pescatore, A. (2007). Sel-Plex maintains small intestine integrity in reovirus-infected broiler chickens: IN: Proceedings of the 8th Asian Pacific Poultry Conference, Bangkokl, Thialand, pp 222-230.

Edens, F. W. and Gowdy, K. M. (2005). Involvement of the thioredoxin reductas system in the maintenance of cellular redox status. In: *Nutritional Biotechnology in the Food and Feed Industry Proceedings*. Alltech's 20th Ann. Symposium. T. P. Lyons, T. P., and Jacques, K. A., eds. Nottingham University Press, Nottingham, UK. Pp. 369-382.

Edgar, S. A. and Cho, Y. (1976). The epizootiology of infectious bursal disease and prevention of it by immunization. *Developments in Biological Standardization,* 33:349-356.

Edgar, S. A. and Cho, Y. (1965). Avian nephrosis (Gumboro disease) and its control by immunization. *Poultry Science*, 44:1366.

Eidson, C. S., Gelb, J., Villegas, P., Page, R. K., Lukert, P. D. and Kleven, S. H. (1980). Comparison of inactivated and live infectious bursal disease virus vaccines in White Leghorn breeder flock. *Poultry Science,* 59:2708-2716.

Ekermans, L. G. and Schneider, J. V. (1982). Selenium in livestock production: A review *Journal of South Africa Veterinary Association,* 53: 223-228.

El-Sebai, A. (2000). Influence of selenium and vitamin E as antioxidants on immune system and some physiological aspects in broiler chickens. *Egyptian Journal of Poultry Science,* 20:1065-1082.

El-Sheikh, A.M.H., Abdalla, E.A. Maysa, M.H. (2010). The effect of organic selenium supplementation on productive and physiological performance in a local strain of chicken. *Egyptian Poultry Science Journal,* 30: 517-533.

El-Zein, A., Chahwan, S. and Haddad, F. (1974). Isolation and identification of infectious bursal disease virus in Lebanon. *Avian Diseases,* 18:343-345.

Eterradossi, N. (2001). Major advances in infectious bursal disease virus (IBDV) research since the first international IBDV/CIAV symposium (RauischolGermany,1994). II .*International Symposium on Infectious Bursal Disease and Chicken Infecti Anaemia. Rauischholzhausen, Germa*ny, pp. 6-23.

Eterradossi, N., Arnauld, C., Tekaia, F., Toquin, D., Le Coq H., Rivallan, G., Guittet, M., Domenech, J., van den Berg T. P. and Skinner, M. A. (2001). Antigenic and genetic relationships between European very virulent infectious bursal disease viruses and an early West African isolate. *Avian Pathology,* 28:36-46.

Eterradossi, N., Arnauld, C., Toquin, D. and Rivallan, G. (1998). Critical amino acid changes in VP2 variable domain are associated with typical and atypical antigenicity in very virulent infectious bursal disease viruses. *Archives of Virology,* 143:1627-1636.

Eterradossi, N., Rivallan, G., Toquin, D. and Guittet, M. (1997). Limited antigenic variation among recent infectious bursal disease virus isolates from France. *Archives of Virology,* 142:2079-2087.

Ezeokonkwo, R.C. (1997). Infectious bursa disease outbreak in a flock of 4-week old pullets despite repeat vaccination with IBD vaccine. *Nigeria Veterinary Journal,* 18:253-256.

Fadly, A. M., Winterfield, R. W. and Olander, H. J. (1976). Role of the bursa of Fabricius in the pathogenicity of inclusion body hepatitis and infectious bursal disease viruses. *Avian Diseases,* 20:467-477.

Fahey, K. J., Erny, K. and Crooks, J. (1989). A conformational immunogen on VP-2 of infectious bursal disease virus that induces virus-neutralizing antibodies that passively protect chickens. *Journal of General Virology,* 70 (6):1473-1481.

Fahey, K. J., McWaters, P., Brown, M. A., Erny, K., Murphy, V. J. and Hewish, D. R. (1991). Virus-neutralizing and passively protective monoclonal antibodies to infectious bursal disease virus of chickens. *Avian Diseases,* 35:365-373.

Fahey, K. J., O'Donnell I. J. and Bagust, T. J. (1985). Antibody to the 32K structural protein of infectious bursal disease virus neutralizes viral infectivity in vitro and confers protection on young chickens. *Journal of General Virology* 66 (Pt 12):2693-26702

Faragher, J. T., Allan, W. H. and Cullen, G. A. (1972). Immunosuppressive effect of the infectious bursal agent in the chicken. *Nature New Biology,* 237:118-119.

Faragher, J. T., Allan, W. H. and Wyeth, P. J. (1974). Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease. *Veterinary Record,* 95:385-388.

Faure, P., Ramon, O., Favier, A. and Halimi, S. (2004). Selenium supplementation decreases nuclear factor-kappa B activity in peripheral blood mononuclear cells from type 2 diabetic patients. *European Journal of Clinical Investigation*, 34: 475-482.

Fernandez-Arias, A., Martinez, S. and Rodriguez, J. F. (1997). The major antigenic protein of infectious bursal disease virus, VP2, is an apoptotic inducer. *Journal of Virology*, 71:8014-8018.

Fernandez-Arias, A., Risco C., Martinez, S., Albar, J. P. and Rodriguez, J. F. (1998). Expression of ORF A1 of infectious bursal disease virus results in the formation of virus-like particles. *Journal of General Virology,* 79 (5):1047-1054.

Flint, S. J., Enquist, L. W., Krug, R. W., Racaniello, V. R. and Skalka, A. M. (2000). Principles of Virology, Molecular Biology, pathogenesis and Control. *American Society for Microbiology*. 34: 475-482.

Franke, K. W. (1934). A new toxicant occurring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminary feeding trials. *Journal of Nutrition,* 8:597-601.

Folorunsho, O.R. and Onibi, G.E. (2005). Assessment of the nutritional quality of eviscerate waste from selected chicken types. In Onibi, G.E., Agele, S.O. and Adekunle, V.A.J. (eds). 2005. *Proceedings of the 1st Annual Conference on Developments in Agriculture and Biological Sciences*. 27th April, 2005. School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria, Pp. 300.

Food and Agriculture Organisation of the United Nations (FAO) (2005). Nigeria Livestock Brief. Livestock Information, Sector Analysis and Policy Branch, AGAL.

Gagic, M., St Hill, C. A. and Sharma, J. M. (1999). In ovo vaccination of specific pathogenfree chickens with vaccines containing multiple agents. *Avian Diseases,* 43:293- 301.

Gary, D.B. and Richard, D.M. (2003). Infectious bursal disease (Gumboro) in commercial Broilers. In: VM84 Veterinary Medicine-larege clinic sciences Department, Florida cooperative Extension Service, Institute of Food and Agricultural Science, University of Florida. *http://idis.ifas.ufl.edu.16/10/2008* 5:52.

Gardner, H., Kerry, K., Riddle, M., Brouwer, S. and Gleeson, L. (1997). Poultry virus infection in Antarctic penguins. *Nature Medicine,* 387:245.

Giambrone, J. J. and Clay, R. P. (1986). Evaluation of the immunogenicity, stability, pathogenicity, and immunodepressive potential of four commercial live infectious bursal disease vaccines. *Poultry Science,* 65:1287-90.

Giambrone, J. J., Ewert, D. L. and Eidson, C. S. (1977). Effect of infectious bursal disease virus on the immunological responsiveness of the chicken. *Poultry Science,* 56:1591-1594.

Gladyshev, V., Factor, V., Housseau, F. and Hatfield, D. (1998). Contrasting patterns of regulation of the antioxidant selenoproteins, thioredoxin reductase, and glutathione peroxidase in cancer cells. *Biochemical and Biophysical Research Communications*, 251:488-493.

Gladyshev, V. N., Stadtman, D. L., Hatfield, D. L., and Jeang, K. T. (1999). Levels of major selenoproteins in T cells decrease during HIV infection and low molecular mass selenium compounds increase. *Proceedings for National Academic of Science,* U S A 96: 835-839.

Goehring, T. B., Palmer, I. S., Olson, O. E., Libal, G. W. and Wahlstrom, R. C. (1984). Effects of seleniferous grains and inorganic selenium on tissue and blood composition and growth performance of rats and swine. *Journal of Animal Science,* 59: 725-732.

Gouch, R. E., Drury, S. E., Cox W. J., Johnson, C. T. and Courtenay, A. E (1998). Isolation and identification of Birnavirus from Ostriches (*Struthio camelus*). *Veterinary Record,* 142:115-116.

Greenfield, C. L., Dohms, J. E. and Dietert, R. R. (1986). Infectious bursal disease virus infection in the quail-chicken hybrid. *Avian Diseases,* 30:536-542.

Gromer S., Eubel, J.K. and Lee, B.L. (2005). Human selonoproteins at a glance. *Cell Molecular Life Science,* 62:2412-2437.

Gukelberger, D., Ehrsam, H., Peterhans, E. and Wyler, R. (1977). Infectious bursitis in Switzerland: Seroepizootologic studies using counterimmunoelectrophoresis. *Schweiz Arch Tierheilkd,* 119:461-468.

Haddad, E. E., Whitfill, C. E., Avakian, A. P., Ricks, C. A., Andrews, P. D., Thoma, J. A and Wakenell, P. S. (1997). Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. *Avian Diseases,* 41:882-889.

Hair-Bejo, M.., Salina, S., Hafiza, H. and Julaida, S. (2000). In ovo vaccination against infectious bursa disease in broiler chickens. *Veterinary Journal Malaysia,* 12:63-69.

Hartikainem, H. (2005). Biogeochemistry of selenium and its impact on food chain quality and human health. *Journal of Trace Elements in Medicine and Biology,* 18:309-318

Hassan, M. K., Saif, Y. M. and Shawky, S. (1996) Comparison between antigen-capture ELISA nd conventional methods used for titration of infectious bursal disease virus. *Journal of Avian Diseases,* 40:562-566.

Hassan, S., Hakkarainen, J., Jonsson, L. and Tyopponen, J. (1990). Histopathological and biochemical changes associated with selenium and vitamin E deficiency in chicks. *Zentralbl Veterinarmed,* 37: 708-720.

Hassan, M.K., Amin, M.M., Khan, M.S.R., Souker, Y.A. and Rashid, H. (2004). Local field isolates of infectious bursal disease virus can induce immune response in chickens than that of commercial available vaccines. *Vaccine Journal*, 23:4736-4745.

Hegazy, S. M. and Adachi, Y. (2000). Comparison of the effects of dietary selenium and zinc supplementation on growth and immune response between chick groups that were inoculated with Salmonella and Aflatoxin. *Poultry Science,* 78: 331-335.

Heine, H. G. and Boyle, D. B. (1993). Infectious bursal disease virus structural protein VP2 expressed by a fowlpox virus recombinant confers protection against disease in chickens. *Archives of Virology,* 131:277-292.

Heine, H. G., Haritou, M., Failla, P., Fahey, K. and Azad, A. (1991). Sequence analysis and expression of the host-protective immunogen VP2 of a variant strain of infectious bursal disease virus which can circumvent vaccination with standard type I strains. *Journal of General Virology,* 72 (8):1835-1843.

Helmboldt, C. F. and Garner, E. (1964). Experimentally induced Gumboro disease (IBD). *Avian Diseases,* 8:561-575.

Hema, V., Faith D., Umamaheswari D., Deepa, B., Ryan, J., Jerry, T., Devin, G., Channa, C. and Sandeep K.P (2007**).** The Anti-inflammatory effects of selenium are mediated through 15-Deoxy-_12, 14-prostaglandin J2 in macrophages. The journal of biological chemistry vol. 282, no. 25, pp. 17964–17973.

Higashihara, M., Saijo, K., Fujisaki, Y. and Matumoto, M. (1991). Immunosuppressive effect of infectious bursal disease virus strains of variable virulence for chickens. *Veterinary Microbiology,* 26:241-248.

Hirai, K., Funakoshi, T., Nakai, T. and Shimakura, S. (1981). Sequential changes in the number of surface immunoglobulin-bearing B lymphocytes in infectious bursal disease virus-infected chickens. *Avian Diseases,* 25:484-496.

Hirai, K., Kunihiro, K. and Shimakura, S. (1979). Characterization of immunosuppression in chickens by infectious bursal disease virus. *Avian Diseases,* 23:950-965.

Hirai, K., Shimakura, S., Kawamoto, E., Taguchi, F., Kim, S. T., Chang, C. N. and Iritani, Y. (1974). The immunodepressive effect of infectious bursal disease virus in chickens. *Avian Diseases,* 18:50-57.

Hitchner, S. B. (1970). Infectivity of infectious bursal disease virus for embryonating eggs. *Poultry Science,* 49:511-16.

Hoffman, D.J., Heinz, G.H., LeCaptian, L.J., Bunck, C.M. and Green, D.E. (1991). Subchronic hepatotoxicity of selenomethionine ingestion in mallard ducks. *Journal of Toxicology and Environmental Health,* 32: 449-464.

Hurwitz, B., Klaus, J., Llabre, M., Gonzalez, A., Lawrence, P., Maher, K., Greeson, J., Baum, M., Posner-Shor, G., Skyler, J. and Schneiderman, N. (2007). Suppression of human immunodeficiency virus type 1 viral load with selenium supplementation. *Archives of Internal Medicine*, 167: 148-154.

Howie, R.I. and Thorsen, J. (1981). Identification of a strain of infectious bursal disease virus isolated from mosquitoes. *Canadian Journal of Comparative Medicine,* 45: 315-320.

Huang, K. H.; Chen, W.F.; Huang, K.H. and Chen, W.F. (1999). Effect of selenium on T-lymphocyte transformation rate and natural killer cell activities in chickens. *Journal of Nanjing Agricultural University*, 22:76-79.

Hudson, L., Pattison, M. and Thantrey, N. (1975). Specific B lymphocyte suppression by infectious bursal agent (Gumboro disease virus) in chickens. *European Journal of Immunology,* 5:675-679.

Ide, P. R. and Stevenson, R. G. (1973). Infectious bursal disease in New Brunswick. *Canadian Journal of Comparative Medicine,* 37:347-355.

Ide, P.R., Schulte-Nordholt, J.A., Dewit, W.F. and Smith, J.D. (1978). Broiler breeder vaccination against infectious bursa disease and persistence of maternal antibody in progeny. *Canadian Veterinary Journal*, 19: 123-127.

Inoue, M., Fukuda, M. and Miyano, K. (1994). Thymic lesions in chicken infected with infectious bursal disease virus. *Avian Diseases,* 38:839-846.

Inoue, M., Yamamoto, H., Matuo K. and Hihara, H. (1992). Susceptibility of chicken monocytic cell lines to infectious bursal disease virus. *Journal of Veterinary Medical Science,* 54:575-577.

Ismail, N. M., Fadly, A. M. and Chang, T. S. (1987). Effect of bursal cell number on the pathogenesis of infectious bursal disease in chickens. *Avian Diseases*, 31:546-555.

Ismail, N. M. and Saif, Y. M. (1990). Differentiation between antibodies to serotypes 1 and 2 infectious bursal disease viruses in chicken sera. *Avian Diseases,* 34:1002-1004.

Ismail, N. M. and Saif, Y. M. (1991). Immunogenicity of infectious bursal disease viruses in chickens. *Avian Diseases,* 35:460-469.

Ivanyi, J. (1975). Immunodeficiency in the chicken. II. Production of monomeric IgM following testosterone treatment or infection with Gumboro disease. *Journal Immunology,* 28:1015-1021.

Ivanyi, J. and Morris, R. (1976). Immunodeficiency in the chicken. An immunological study of infectious bursal disease. *Journal of Clinical Experimental Immunology,* 23:154-165.

Jackwood, D. J., Byerley, E. H. and Sommer, S. E. (2001). Use of a genetic marker for wild type potentially pathogenic infectious bursal disease viruses. *Avian Diseases,* 45:701-705.

Jackwood, D. J., Henderson, K. S. and Jackwood, R. J. (1996). Enzyme-linke immunosorbent assay-based detection of antibodies to antigenic subtypes of infectious bursal disease viruses of chickens. *Clinical Diagnostic Laboratory Immunology,* 3:456-463.

Jackwood, D. J. and Saif, Y. M. (1983). Prevalence of antibodies to infectious bursal disease virus serotypes I and II in 75 Ohio chicken flocks. *Avian Diseases,* 27:850-854.

Jackwood, D. J., Saif, Y. M. and Hughes, J. H. (1984). Nucleic acid and structural proteins of infectious bursal disease virus isolates belonging to serotypes I and II. *Avian Diseases,* 28:990-1006.

Jackwood, D. J., Saif, Y. M. and Moorhead, P. D. (1985). Immunogenicity and antigenicity of infectious bursal disease virus serotypes I and II in chickens. *Avian Diseases,* 29:1184-1194.

Jagadish, M. N., Staton, V. J., Hudson, P. J. and Azad, A. A. (1988). Birnavirus precursor polyprotein is processed in *Escherichia coli* by its own virus-encoded polypeptide. *Journal of Virology,* 62:1084-1087.

Jianhua, H., Ohtsuka, A. and Hayashi, K. (2000). Selenium influences growth via thyroid hormones status in broiler chickens. *British Journal of Nutrition,* 84: 727-732.

Johnson, T., Herbel, M., Bullen, T. and Zawislanski, P. (1999). Selenium isotope ratios as indicators of selenium sources and oxyanion reduction. *Geochimica et Cosmochimica Acta,* 63:2775-2783.

Jungmann, A., Nieper H. and Muller, H. (2001). Apoptosis is induced by infectious bursal disease virus replication in productively infected cells as well as in antigen negative cells in their vicinity. *Journal General Virology,* 82:1107-1115.

Kaufer, I. and Weiss, E. (1980). Significance of bursa of Fabricius as target organ in infectious bursal disease of chickens. *Journal Infectious Immunogy,* 27:364-367.

Kayanoki, Y., Fujii, J., Islam, K.N., Suzuki, K., Kawata, S., Matsuzawa, Y. and Taniguchi, N. (1996). The protective role of glutathione peroxidase in apoptosis induced by reactive oxygen species. *Journal of Biochemistry* (Tokyo), 119: 817-822.

Kelly, M. P. and Power, R. F. (1995). Fractionation and identification of the major selenium compounds in selenized yeast. *Journal of Dairy Science*, 78: 237.

Kibenge, F. S., Dhillon, A. S. and Russel, l R. G. (1988). Biochemistry and immunology of infectious bursal disease virus. *Journal of General Virology,* 69 (8):1757-1775.

Kibenge, F. S. and Dhama, V. (1997). Evidence that virion-associated VP1 of avibirnaviruses contain viral RNA sequences. *Archive of Virology,* 142:1227-1236.

Kibenge, F. S., Qian, B., Nagy, E. and Wadowska, D. (1999). Formation of virus- like particles when the polyprotein gene (segment A) of infectious bursal disease virus is expressed in insect cells. *Canadian Journal of Veterinary Research,* 63:49-55.

Kim, I. J., You S. K., Kim, H., Yeh, H. Y. and Sharma, J. M. (2000). Characteristics of bursal lymphocytes induced by infectious bursal disease virus. *Jounal of Virology,* 74:8884-8892.

Kim, Y. Y. and Mahan, D. C. (2001). Comparative effects of high dietary levels of organic and inorganic selenium on selenium toxicity of growing-finishing pigs. *Journal of Animal Science,* 79: 942-948.

Klaassen, C. D. (1985). Role of sulfhydryls in the hepatotoxicity of organic and metallic compounds. *Fundamental Applied Toxicology*, 5: 806-815.

Knoblich, H. V., Sommer, S. E. and Jackwood, D. J. (2000). Antibody titers to infectious bursal disease virus in broiler chicks after vaccination at one day of age with infectious bursal disease virus and Marek's disease virus. *Avian Diseases,* 44:874- 884.

Krishnamoorthy, P., Vairamuthu, S., Balachandran, C. and Muralimanohar, B. (2007). Pathology of Lymphoid Organs in Chlorpyriphos and T-2 Toxin Fed Broiler Chicken. *International Journal of Poultry Science* 6 (1): 71-76.

Kryukov, G.V., Castellano, S. and Novoselov, S.V. (2003). Characterization of mammalian selonoproteomes. *Science,* 300: 1439-1443.

Lam, K.M. (1998). Alteration of chicken heterophil and macrophage functions by the infectious bursal disease virus. *Journal of Microbiology and Pathology*, 3: 147-155.

Lam, K. M. (1997). Morphological evidence of apoptosis in chickens infected with infectious bursal disease virus. *Journal of Comparative Pathology,* 116:367-377.

Lam, K. M. (1991) IBDV type 1 induced suppression of chicken lymphocyte response to mitogen. *Avian Pathology,* 20:205-212.

Lam, K. M. (1988). Lysis of chicken lymphocytes by infectious bursal disease virus. *Avian Diseases,* 32:818-821.

Landgraf, H., Vielitz, E. and Kirsch, R. (1972). Occurrence of an infectious disease affecting the bursa of Fabricius (Gumboro disease). *Dtsch Tierarztl Wochenschr,* 74:6-10.

Laseinde, E.A.O. (1994). Terminology in Poultry Production. Tropical Agricultural Production Series Technical Guide to Poultry Production. *Division of Agricultural Colleges Newsletter*. 1:4 14-18.

Lasher, H. and Shane, S. (1994). Infectious bursal disease virus. *World's Poultry Science Journal,* 50:133-166.

Lasher, H. N. and Davis, V. S. (1997). History of infectious bursal disease in the U.S.A.—the first two decades. *Avian Diseases,* 41:11-19.

Latshaw, J.D., Ort, J.F. and Diesem, C.D. (1977). The selenium requirements of the hen and effects of a deficiency. *Poultry Science,* 56: 1876-1881.

Leeson, S., and Summers, J.D. (1991). Commercial Poultry Nutrition. *University Books*, Guelph, Ontario, Canada.

Lejal, N., Da Costa, B., Huet, J. C. and Delmas, B. (2000). Role of Ser-652 and Lys-692 in the protease activity of infectious bursal disease virus VP4 and identification of its substrate cleavage sites. *Journal General Virology,* 81:983-992.

Leng, L., Bobcek, R., Kuricová, S., Boldizárová, K., Grešáková, L., Ševcíková, Z., Révajová, V., Levkutová, M., and Levkut, M. (2003). Comparative metabolic and immune responses of chickens fed diets containing inorganic selenium and Sel-PlexTM organic selenium. In *Biotechnology in the Feed Industry- Beyond the Tornado: The Calm after the Storm,* pp. 131-145.

Lenz, M. and Lens, P.N.L. (2008). The essential toxin: The changing perception of selenium in environmental sciences. *Elsevier*

Li, Y. (2000). High prevalence of enteroviral genomic sequences in myocardium from cases of endemic cardiomyopathy (Keshan disease) in China. *Heart,* 83: 696-701.

Lim, B. L., Cao, Y., Yu, T. and Mo, C. W. (1999). Adaptation of very virulent infectious bursal disease virus to chicken embryonic fibroblasts by site-directed mutagenesis of residues 279 and 284 of viral coat protein VP2. *Journal of Virology,* 73:2854-2862.

Lin, Z., Kato A., Otaki, Y., Nakamura, T., Sasmaz, E. and Ueda, S. (1993). Sequence comparison of a highly virulent infectious bursal disease virus prevalent in Japan. A*vian Diseases,* 37:315-323.

Liu, M. and Vakharia, V. N. (2004). VP1 protein of infectious bursal disease virus modulates the virulence in vivo. *Journal of Virology,* 330:62-73.

Lombardo, E., Maraver, A., Espinosa, I., Fernandez-Arias, A. and Rodriguez, J. F. (2000). VP5, the nonstructural polypeptide of infectious bursal disease virus, accumulates within the host plasma membrane and induces cell lysis. *Journal of Virology*, 277:345-357.

Longnecker, M.P., Tailor, P.R., Levander, O.A., Howe, M., Weillon, C.M., and Adam, P.A. (1991).Selenium in diet, blood and toe nails in relation to human health in a selenoferous area. *America Journal of Clinical Nutrition*, 53:1288-1294.

Lucio, B. and Hitchner, S.B. (1979). Infectious bursal disease emulsified vaccine: Effect upon neutralizing -antibody levels in the dam and subsequent protection of the progeny. *Avian Diseases,* 23:466-478.

Lucio, B., Antillon, A. and Fernandez, P. (1972). Identification of the infectious bursal disease virus in Mexico. *Avian Diseases,* 16:241-248.

Lukert, P. D. and Davis, R. B. (1974). Infectious bursal disease virus: growth and characterization in cell cultures. *Avian Diseases,* 18:243-250.

Lukert, P. D. and Mazariegos, L. A. (1985). Virulence and immunosuppressive potential of intermediate vaccine strains of infectious bursal disease virus [abst]. *Journal of American Veterinary Medical Association*, 187-306.

Lukert , P. D. and Rifuliadi, D. (1982). Replication of virulent and attenuated infectious bursal disease virus in maternally immune day-old chickens [abst]. *Journal of American Veterinary Medical Association*, 187-306.

Lukert, P. D. and Saif, Y. M. (2003). Infectious bursal disease. In: *Diseases of Poultry,* 11th ed. M. Saif, H. J. Barnes., A. M. Fadly., J. R. Glisson., L. R. McDougald., and D.E Swayne., eds. Iowa State Press, Ames, Iowa, pp. 161-179.

Mackenzie, M. and Spradbrow, P. B. (1981). Persistence of infectious bursal disease virus in experimentally infected chickens. *Australian Veterinary Journal,* 57:534-535.

Machlin, L. J., Gordon, R. S., Marr, C. W. and Pope, C. W. (1962). Effect of antioxidants and unsaturated fatty acids on reproduction in the hen. *Journal of Nutrition,* 76: 284-290.

Macreadie, I. G., Vaughan, P. R., Chapman, A. J., McKern, N. M., Jagadish, M. N., Heine, H. G., Ward, C. W., Fahey, K. J. and Azad, A. A. (1990). Passive protection against infectious bursal disease virus by viral VP2 expressed in yeast. *Vaccine,* 8:549- 552.

Maldron, P. and Vrzgulova, N. (1998). Vitamin E and selenium increase the natural resistance of farm animals. *Veterinarstvi,* 38: 369-371

Mahan, D.C. and Moxon, A.L.. (1984). Effect of inorganic selenium supplementation on selenosis in postweaning swine. *Journal of Animal Science*, 58: 1216-1221.

Mahmoud, K. Z. and Edens, F.W. (2003). Influence of selenium sources on age-related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (*Gallus domesticus*). *Journal of Comparative Biochememistry and Physiology,* 136: 921-934.

Maina, G. A. (2008). Structure of poultry industry in Nigeria. *Workshop Organized by Ilei-IFPR I on the 17th June 2008, ILRI Campus Ibadan, Nigeria*, 3.

March, I.A., Combs, G.H., Whitacre, M.E. and Dietert, R.R. (1986). Effect of selenium and vitamin E dietary deficiencies on chick lymphoid organ development. In: *Proceeding on Experimental Biology and Medicine,* 182: 425-436

Marcus, R. W. (1993). Myopathy and cardiomyopathy associated with selenium deficiency: Case report, literature review and hypothesis. *Medical Journal,* 42: 669-674.

Mark, L.F. (2003). Trace element and host defense-recent advances and continuing challenges. *Journal of Nutrition,* 133:203-282.

Marin-Guzman, J., Mahan, D. C., Chung, Y. K., Pate, J. L. and Pope, W. F. (1997). Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality, and subsequent fertilization rates in mature gilts. *Journal of Animal Science,* 75: 2994-3003.

Marquardt, W. W., Johnson, R. B., Odenwald, W. F. and Schlotthober, B. A. (1980). An indirect enzyme-linked immunosorbent assay (ELISA) for measuring antibodies in chickens infected with infectious bursal disease virus. *Avian Diseases,* 24:375- 385.

Mazariegos, L. A., Lukert, P. D. and Brown, J. (1990). Pathogenicity and immunosuppressive properties of infectious bursal disease "intermediate" strains. *Avian Diseases,* 34:203-208.

Mbuko, I.J. Musa, W.I., Ibrahim, S., Sa'idu, L., Abdu1, P.A., Oladele, S.B and. Kazeem, H.M **(**2010). A Retrospective Analysis of Infectious bursal disease diagnosed at poultry unit of Ahmadu Bello University, Nigeria**.** *International Journal of Poultry Science* 9 (8): 784-790.

McFerran, J. B., McNulty, M.S., Mckillop, E.R., Conner, T.J., McCracken, R.M., Collins, D.S and Allan G.M. (1980). Isolation and serologic studies with infectious bursal disease virus from fowl, turkey and duck: Demonstration of a second serotype. *Avian Pathology,* 9:395-404.

McNeilly, F., Walker, I., Allan, G. M. and Adair, B. (1999). Bursal lymphocyte proliferation in the presence of phorbol myristate acetate: effect of IBDV strains on the proliferation response. *Avian Pathol*ogy, 28:301-303.

Mohammad, A. F., Abdulrahim, A., "Mo'ez Al-Islam" E. F., Ref'at A. A., Mohammad K. and yasser A. (2012). Effect of intermittent fasting on lipid profile and hematological parameters in healthy volunteers in Jordan. *Universal Journal of Medicine and Dentistry* Vol. 1(1) pp. 1005-1009,

Mohantey, G. C., Pandey, B.S. and Rajya, B.S. (1971). Infectiuos bursal disease virus in chickens. *Current Science Journal,* 40:181-184.

Moody, A., Sellers, S. and Bumstead, N. (2000). Measuring infectious bursal disease virus RNA in blood by multiplex real-time quantitative RT-PCR. *Journal of Virological Methods,* 85:55-64.

Moreno-Reyes, R., Egrise, D., Neve, J., Pasteels, J. L. and Schoutens, A. (2001). Selenium deficiency-induced growth retardation is associated with an impaired bone metabolism and osteopenia. *Journal of Bone Miner Resource,* 16: 1556-1563.

Moustafa, M. E. (1998). Overproduction of selenocysteine trna in Chinese hamster ovary cells following transfection of the mouse trna[ser]sec gene. *Rna,* 4: 1436- 1443.

Moxon, Alvin and Rhian, Morris. (1943). Selenium Poisoning. *Physiological Reviews*, October vol 23

Muller, H. (1986). Replication of infectious bursal disease virus in lymphoid cells. *Archives of Virology,* 87:191-203.

Muller, H. and Becht, H., (1982). Biosynthesis of virus-specific proteins in cells infected with infectious bursal disease virus and their significance as structural elements for infectious virus and incomplete particles. *Journal of Virology,* 44:384-392.

Muller, H., Islam, M. R. and Raue, R. (2003). Research on infectious bursal disease—the past, the present and the future. *Journal of Veterinary Microbiology,* 97:153-165.

Muller, H., Lange, H. and Becht, H. (1986). Formation, characterization and interfering capacity of a small plaque mutant and of incomplete virus particles of infectious bursal disease virus. *Virus Research Journal,* 4:297-309.

Mundt, E., de Haas, N. and van Loon, A. A. (2003). Development of a vaccine for immunization against classical as well as variant strains of infectious bursal disease virus using reverse genetics. *Vaccine,* 21:4616-4624.

National Research Council (NRC), (1989). *Recommended Dietary Allowances.* National Academy Press 10th edition, Pp. 195-246.

Nawathe, D.R. and Lamorde, A.G. (1982). Gumboro disease: Problems of control in Nigeria. *Review Science Techniques International Epizootic,* 1: 1182-1168.

Negash, T., al-Garib, S. O. and Gruys, E. (2004). Comparison of in ovo and post-hatch vaccination with particular reference to infectious bursal disease. *A Review of Veterinary ,Quaterly,* 26:76-87.

Nick, H., Cursiefen, D. and Becht, H. (1976). Structural and growth characteristics of infectious bursal disease virus. *Journal of Virology,* 18:227-234.

Nieper, H., Teifke, J. P., Jungmann, A., Lohr, C.and Muller H. (1999). Infected and apoptotic cells in the IBDV-infected bursa of Fabricius, studied by double labelling techniques. *Avian Pathology,* 28:279-285.

Nieper, H. and Muller, H. (1996). Susceptibility of chicken lymphoid cells to infectious bursal disease virus does not correlate with the presence of specific binding sites. *Journal General Virology,* 77 (6):1229-1237.

Nomura, K., Imai, H., Koumura, T., Arai M. and Nakagawa, Y. (1999). Mitochondrial phospholipid hydroperoxide glutathione peroxidase suppresses apoptosis mediated by mitochondrial death pathway. *Journal of Biology and Chemistry,* 274: 29294-29302.

Nunoya, T., Otaki, Y., Tajima, M., Hiraga, M. and Saito, T. (1992). Occurrence of acute infectious bursal disease with high mortality in Japan and pathogenicity of field isolates in specific-pathogen-free chickens. *Avian Diseases,* 36:597-609.

Obi, T.U., Olubukola, A. and Maina, G.A. (2008). Pro-poor highly pathogenic avian reduction strategies in Nigeria. *Africa/ Indonesia Team Working Paper* 5 Pp. 71.

Oei, H., Maas, R., Venema, S., Kant, A. and Claassen, I. (2004). Quantification of infectious bursal disease viral proteins 2 and 3 in inactivated vaccines as an indicator of serological response and measure of potency. *Avian Pathology,* 33:126-132.

OIE, 2004. Manual of Diagnostic Tests and vaccines for terrestrial animals 5th Edn. Chapter 2-7-1. part 2.

Okwori, A.E.J., Echeonwu, G.O.N., Olabode, A.O., Junaid, S.A., Chukwuedo, A. and Aba-Adulugba, E.P. (2006). Serological survey of local poultry for IBD antibodies using agar gel precipitation test in Jos Plateau State. *Nigeria Journal of Microbiology,* 20:1208-1213.

Oladele, O.A., Adene, D.F., Obi, T.U., Nottidge, H.O. and Aiyedun, A.I. (2005). A sequential haematological study of experimental infectious bursal disease virus infection in chickens, turkeys and ducks. *Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux,* 58 (4): 211-215.

Ojeda, F., Skardova, I., Guarda, M. I., Ulloa, J. and Folch, H. (1997). Proliferation and apoptosis in infection with infectious bursal disease virus: a flow cytometric study. *Avian Diseases,* 41:312-316.

Ojo, M.O., Oduye, O.O.B., Noibi, L.M. and Idowu, L.A. (1973). Gumboro like disease in Nigeria.*Tropical Animal Health and Production*, 5:52-57.

Okoye, J.O.A. and Uzoukwu, M. (1981). An outbreak of infectious bursal disease in a flock of 16-20 weeks old. *Avian Diseases,* 25, 1034-1037.

Okoye, J.O.A., Aba-Adulugba, E.P., Ezeokonkwo, R.C., Udem, S.C. and Orajaka, L.J.E. (1999). Susceptibility of Local Nigerian and Exotic Chickens to Infectious Bursal Disease by Contact Exposure. *Tropical AnimalHealth and Production,* 31 (1999) 75- 81

Oluyemi, J.A. and Roberts, F.A. (1979). *Poultry Production in warm wet Climate*. Macmillan Pres Ltd. London, Pp: 197.

Onunkwo, O. (1975). An outbreak of infectious bursal disease (IBD) of chickens in Nigeria. *Veterinary Record,* 97:433.

Oyedotun, A.A. and Durojaiye, O.A. (1999). Newcastle disease, infectious bursa disease and EDS76 antibodies in indigenous Nigeria local chickens. *Tropical Veterinarian,* 17: 4- 52.

Oyekunle, M.A. and Adeniji, O.O. (2008). On farm investigation to determine appropriate age to to vaccinate chicks with the. Nigerian infectious bursal disease vaccine Fibrogumbova®). Retrieved from: www.ajol.info/view article. 21/05/09 11:30 a.m.

Ozel, M. and Gelderblom, H. (1985). Capsid symmetry of viruses of the proposed Birnavirus group. *Archives of Virology*, 84:149-161.

Parkhurst, R. T. (1964). Avian nephrosis (Gumboro disease) in USA broilers: treatment trials. *World's Poultry Science Journal,* 20:208-211.

Patterson, E.L., Milstrey, R. and Stokstad, E.L.R. (1957). Effects of selenium in preventing exudative diathesis in chicks. In: *Preceedings of the Society for Experimental Biology and Medicine*. 95: 617-620.

Petek, M., D'Aprile, P. N. and Cancelloti, F. (1973).Biological and physiochemical properties of infectious bursal disease virus. *Journal of Avian Pathology*, 2:135-152.

Pesti, G. M. and Combs, G. F. Jr. (1976). Studies on the enteric absorption of selenium in the chick using localized coccidial infections. *Poultry Science*, 55:2265-2274.

Peters, G. (1967). Histology of Gumboro disease. *Berl Munch Tierarztl Wochenschr,* 80:394-396.

Peter, M. A., Lin, T. L. and Wu, C. C. (2004). Infectious bursal disease virus polyprotein expression arrests growth and mitogenic stimulation of B lymphocytes. *Archives of Virology,* 149:2413-2426.

Poonia, B. and Charan, S. (2005). Early and transient induction of nitric oxide (NO) in infectious bursal disease virus infection is T-cell dependent: a study in cyclosporin-A treated chicken- model. *Indian Journal of Experimental Biology,* 43:192-196

Proffitt, J. M., Bastin, D. A. and Lehrbach, P. R. (1999). Sequence analysis of Australian infectious bursal disease viruses. *Australian Veterinary Journal,* 77:186-188.

Ramaekers, V. T., Calomme, M., vanden Berghe, D., and Makropoulos, W. (1994). Selenium deficiency triggering intractable seizures. *Journal of Neuropediatrics,* 25: 217-223.

Raue, R., Islam, M. R., Islam, M. N., Islam, K. M., Badhy, S. C., Das, P. M., Muller, H. (2004). Reversion of molecularly engineered, partially attenuated, very virulent infectious bursal disease virus during infection of commercial chickens. *Avian Pathology,* 33:181-189.

Rayman, M.P. and Clark, L.C., (2000). Selenium in cancer prevention. In: Roussel, A.M. Favier A., Anderson, R.A., eds. Trace elements in man and animals 10. *Proceedings of the Tenth International Symposium on Trace Elements in Man and Animals*. *New York: Plenum Press,* 575-580.

Rayman, M. P. (2000). The importance of selenium to human health. *Lancet Journal,* 356: 233-241.

Regenmortel, V. (2003). Virus taxonomy. 7th *report of the international committee on taxonomy of virus,* Academic press.

Richard, A.H. (1986). Poultry nutritional requirements In: *The Mercks Veterinary Manual 6th edition*. Merck and Co., Inc. Rahway, NJ USA, Pp. 1202- 1291.

Rodenberg, J., Sharma, J. M., Belzer, S. W., Nordgren, R. M. and Naqi, S. (1994). Flow cytometric analysis of B cell and T cell subpopulations in specific-pathogen-free chickens infected with infectious bursal disease virus. *Avian Diseases,* 38:16-21.

Roessler, D. E. and Rosenberger, J. K. (1989). In vivo characterization of avian reoviruses. III. Host factors affecting virulence and persistence. *Avian Diseasse,* 33:555-565.

Rogel, A., Benvenisti, L., Sela, I., Edelbaum, O., Tanne, E., Shachar, Y., Gontmakher, T., Khayat, E. and Stram, Y. (2003). Vaccination with *E. coli* recombinant empty viral particles of infectious bursal disease virus (IBDV) confer protection. *Virus Genes Journal,* 27:169-175.

Rosenberger, J. K. and Gelb, Jr. J. (1978). Response to several avian respiratory viruses as affected by infectious bursal disease virus. *Avian Diseases,* 22:95-105.

Rosenberger, J. K., Klopp, S., Eckroade, R. J. and Krauss, W. C. (1975). The roles of the infectious bursal agent and several avian adenoviruses in the hemorrhagic aplastic-anemia syndrome and gangrenous dermatitis. *Avian Diseases,* 19:717-729.

Rosenberger, J. K. and Cloud, S.S. (1986). Isolation and characterization of variant infectious bursal disease virus. *Journal of American Veterinary Medical Association,* 189:357.

Saedi, M. S. (1988). Effect of selenium status on levels for glutathione peroxidase in rat liver *Biochemistry Biophysics Research Communication,* 153: 855-861.

Sah, R. L., Kataria, J. M., Arya, S. C. and Verma, K. C. (1995). Outbreaks of acute infectious bursa disease virus causing high mortality in chickens. *Indian Journal of Comparative Microbiology and Immunology of Infectious Diseases,* 16, 7-13.

Saif, Y. M. (1991). Immunosuppression induced by infectious bursal disease virus. *Veterinary Immunology and Immunopathology*. 30:45-50.

Saif, Y. M. (1984). Infectious bursal disease virus types. *Proceeding 19th National Meeting Poulryt Health Condemn*. Ocean city, MD, Pp. 105-107.

Savic, V., Bidin, Z., Cajavec, S., Stancic, M., Gjurcevic, D. and Savic, G. (1997). Epidemic of infectious bursal disease in Croatia during the period 1995-1996: field and experimental observations. *Veterinarski Arhiv,* 67:243-251.

Sayato, Y., Nakamuro, K. and Hasegawa, T. (1997). Selenium methylation and toxicity mechanism of selenocystine. *Yakugaku Zasshi,* 117: 665-672.

Schnitzler, D., Bernstein, F., Muller, H. and Becht, H. (1993). The genetic basis for the antigenicity of the VP2 protein of the infectious bursal disease virus. *Journal of General Virology,* 74 (8):1563-1571.

Schroder, A., van Loon, A. A., Goovaerts, D., Teifke, J. P. and Mundt, E. (2001). VP5 and the N terminus of VP2 are not responsible for the different pathotype of serotype I and II infectious bursal disease virus. *Journal of General Virology,* 82:159-169.

Schrauzer, G. N. (2000). Nutritional selenium supplements: Product types, quality and safety. *Journal of the American College of Nutrition*, 20:1-4.

Schrauzer, G.N., White, D.A. and Schneider, C.J. (1977). Cancer mortality correlation studies III. Statistical association with dietary selenium intakes. *Bioinorganic Chemistry*, 7: 35-6.

Schwarz, K. and Foltz, C. M. (1957). Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Journal of the American Chemical Society*, 79:3292-3293.

Shamberger, R.J., Frost, D.V. (1969). Possible protective effects of selenium against human cancer. *Canadian Medical Association Journal,* 100: 682.

Shamaila, A. (2005). Studies on infectious bursal disease. *PhD Thesis,* Graduate school of the Ohio State University. 61-71 Pp.

Sharma, J. M., Dohms, J., Walser, M. and Snyder, D. B., (1993). Presence of lesions without virus replication in the thymus of chickens exposed to infectious bursal disease virus. *Avian Diseases,* 37:741-748.

Sharma, J. M., Dohms, J. E. and Metz, A. L. (1989). Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of infectious bursal disease virus and their effect on humoral and cellular immune competence of specific-pathogen-free chickens. *Avian Diseases,* 33:112-124.

Sharma, J. M., Kim, I. J., Rautenschlein, S. and Yeh, H. Y. (2000). Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Developmental Comparative Immunology,* 24:223-235.

Sharma, J. M. and Lee, L. F. (1983). Effect of infectious bursal disease on natural killer cell activity and mitogenic response of chicken lymphoid cells: role of adherent cells in cellular immune suppression. *Journal of Infectious Immunology,* 42:747-754.

Sharma, J. M., Rautenschlein, S. and Yeh, H. Y. (2001). The role of T cells in immuno pathogenesis of infectious bursal disease virus.Cost 839.*Rauischholzhausen, Germany*, pp 324-328.

Sheppard, M., Werner, W., Tsatas, E. McCoy, R., Prowse, S. and Johnson, M. (1998). Fowl adenovirus recombinant expressing VP2 of infectious bursal disease virus induces rotective immunity against bursal disease. *Archives of Virology,* 143:915-930.

Shwed, P. S., Dobos, P., Cameron, L. A., Vakharia, V. N. and Duncan, R. (2002). Birnavirus VP1 proteins form a distinct subgroup of RNA-dependent RNA polymerases lacking a GDD motif. *Journal of Virology,* 296:241-250.

Silim, A. and Venne, D. (1989). Comparison of egg-yolk and serum antibody titers to four avian viruses by enzyme-linked immunosorbent assay using paired field samples. *Avian Diseases,* 33:643-648.

Sivanandan, V. and Maheswaran, S. K. (1981). Immune profile of infectious bursal disease. III. Effect of infectious bursal disease virus on the lymphocyte responses to phytomitogens and on mixed lymphocyte reaction of chickens. *Avian Diseases,* 25:112-120.

Sivanandan, V. and Maheswaran, S. K. (1980). Immune profile of infectious bursal disease (IBD). II. Effect of IBD virus on pokeweed-mitogen-stimulated peripheral blood lymphocytes of chickens. *Avain Diseases,* 24:734-742.

Skeeles, J. and Lukert, P. D. (1979). Studies with cell culture adapted IBDV: replicative sites and persistence of virus in SPF chickens. *Avian Diseases,* 24:43-47.

Skeeles, J. K., Lukert, P. D., De Buysscher, E. V. Fletcher, O. J. and Brown, J. (1979). Infectious bursal disease viral infections. The relationship of age, complement levels, virus-neutralizing antibody, clotting, and lesions. *Avian Diseases*, 23:107-117.

Snedeker, C., Wills, F. K. and Moulthrop, I. M. (1967). Some studies on the infectious bursal disease agent. *Avian Diseases,* 11:519-528.

Snyder, D. B., Vakharia, V. N., Mengel-Whereat, S. A., Edwards, G. H., Savage, P. K., Lutticken, D. and Goodwin, M. A. (1994). Active cross-protection induced by a recombinant baculovirus expressing chimeric infectious bursal disease virus structural proteins. *Avian Diseases,* 38:701-707.

Snyder, J. A. (2009). Genomic analysis of the influence of selenium sources in broiler chickens challenge10d with an enteric avian reovirus. *MSc. Thesis.* North Carolina State University. 6 Pp.

Spallholz, J. E. (1997). Free radical generation by selenium compounds and their pro-oxidant toxicity. *Biomedical and Environmental Sciences*, 10:260-270.

Spallholz, J. E., (1994). On the nature of selenium toxicity and carcinostatic activity, *Free Radical Biology and Medicine,* 17: 45-64.

Spallholz, J. E. and Hoffman, D. J. (2002). Selenium toxicity: Cause and effects in aquatic birds. *Journal of Aquatic Toxicology,* 57: 27-37.

Spies, U. and Muller, H. (1990). Demonstration of enzyme activities required for cap structure formation in infectious bursal disease virus, a member of the birnavirus group. *Journal of General Virology*, 71 (4): 977-981.

Spies, U., Muller, H. and Becht, H. (1987). Properties of RNA polymerase activity associated with infectious bursal disease virus and characterization of its reaction products. *Journal of Viral Research,* 8:127-140.

Srimongkol, C. (2003). Effects of selenium supplementation on growth performance, thyroid hormone (T3) Levels, antioxidant enzyme and disaccharidase activities in broiler chicks. *Thesis M.Sc*. Chulalongkorn University.

Stohs, S. J. and Bagchi. D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Journal of Free Radical Biology and Medicine,* 18: 321-336.

Surai, P.F. (2002). Selenium in poultry nutrition. Antioxidant properties deficiency and toxicity. W*orld Poultry Science Journal,* 58:333-346

Surai, P.F. (2006). *Selenium in Nutrtion and health*. Nottingham University Press Nottingham, U.K.

Survashe, B. D., Aitken, I. D. and Powell, J.R. (1979). The response of the Harderian glands of the fowl to antigen given by the ocular route, histological changes. *Avian Pathology,* 8:77-93.

Tanimura, N. and Sharma, J. M. (1998) In-situ apoptosis in chickens infected with infectious bursal disease virus. *Journal of Comparative Pathology,* 118:15-27.

Tanimura, N. and Sharma, J. M. (1997). Appearance of T cells in the bursa of Fabricius and caecal tonsils during the acute phase of infectious bursal disease virus infection in chickens. *Avian Diseases,* 41:638-645.

Tanimura, N., Tsukamoto, K., Nakamura, K., Narita, M. and Maeda, M. (1995). Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunohistochemistry. *Avian Diseases,* 39:9-20.

Thompson, J. N., and Scott, M. L. (1969). Role of selenium in the nutrition of the chick. *Journal of Nutrition,* 97: 335-342.

Tham, K. M. and Moon, C. D. (1996). Apoptosis in cell cultures induced by infectious bursal disease virus following in vitro infection. *Avian Diseases,* 40:109-113.

Tian, S. M., Ruan, K. C., Qian, J. F., Shao, G. Q. and Balny, C. (2000). Effects of hydrostatic pressure on the structure and biological activity of infectious bursal disease virus. *European Journal of Biochemistry,* 267:4486-4494.

Torrents, D., Maldonado, J., Saubi, N. and Pages-Mant. E. A. (2004). Dogs as potential carriers of infectious bursal disease virus. *Avian Pathology*. 33:205-209.

Tsukamoto, K., Matsumura, T., Mase, M. and Imai, K. (1995). A highly sensitive, broad pectrum infectivity assay for infectious bursal disease virus. *Avian Diseases,* 39:575- 586.

Ture, O., Saif, Y.M. and Jackword, D.J. (1998). Restriction fragment length polymorphism Analysis of highly virulent strains of infectious bursal disease viruses from Holland, Turkey and Taiwan. *Avian Diseases,* 42: 470-479.

Ursini, F. (2000). The world of gluthathione peroxidases. *Journal of Trace Elements Medical Biology,* 14: 116.

Vakharia, V. N., He J., Ahamed, B. and Snyder, D. B. (1994). Molecular basis of antigenic variation in infectious bursal disease virus. *Journal of Viral Research,* 31:265-273.

Vakharia, V. N., Snyder, D. B., He, J., Edwards, G. H., Savage, P. K. and Mengel-Whereat, S. A. (1993). Infectious bursal disease virus structural proteins expressed in a baculovirus recombinant confer protection in chickens. *Journal of General Virology,* 74 (6):1201-1206.

Vakharia, V. N., Snyder, D. B., Lutticken, D., Mengel-Whereat, S. A., Savage P. K., Edward, G. H. and Goodwin, M. A. (1994). Active and passive protection against variant and classic infectious bursal disease virus strains induced by baculovirusexpressed structural proteins. *Vaccine Journal,* 12:452-456.

van den Berg, T. P. (2000). Acute infectious bursal disease in poultry:a review. *Avian Pathology,* 29:175-194.

van den Berg, T. P., Eterradossi, N., Toquin, D. and Meulemans G. (2000). Infectious bursal disease (Gumboro disease). *Review of Science Technique*, 19:509-543.

van den Berg, T. P., Gonze, M. and Mueulemans, G. (1991). Acute infectious bursal disease in poultry:isolation and characterization of a highly virulent strain. *Avian Pathology,* 20:133-143.

Vasconcelos, A. C. and Lam K. M. (1995). Apoptosis in chicken embryos induced by the infectious bursal disease virus. *Journal of Comparative Pathology,* 112:327-338. .

Vendeland, S. C., Deagen, J. T., and Whanger, P. D. (1992). Uptake of selenotrisulfides of glutathione and cysteine bv brush borded membranes from rat intestines. *Journal of Inorganic Biochemistry,* 47:131-140.

Von Einem, U. I., Gorbalenya, A. E., Schirrmeier, H., Behrens, S. E., Letzel T. and Mundt, E. (2004). VP1 of infectious bursal disease virus is an RNA-dependent RNA polymerase. *Journal of General Virology,* 85:2221-2229.

Watanabe, T. and Endo, A. (1991). Nutrient requirements of laboratory animals.*The National academies press 4: 80-102*

Weis, E and Kaufer, I. (1994). Pathology and pathogenesis of infectious bursal disease. In: *Proceeding International symposium on infectious bursal disease and chicken infectious anemia*, Rauischholzhausen, Germany, 21-24 June, 1994.

Whitehead, C.C., and Portsmouth J.I. (1989). Vitamin requirements and allowances for poultry. In*: Recent Adavances in Animal Nutrition*. Edited by Haresign, W. And Cole, D.J.A., Butterworths, London, Pp. 35-86.

Winterfield, R. W. (1969). Immunity responses to the infectious bursal disease virus. A*vian Diseases,* 13:548-557.

Winterfield, R. W., Dhillon, A. S. and Thacker, H. L. (1981). Characteristics of apparent derivatives of the 2512 strain of infectious bursal disease virus when used as vaccines. *Avian Diseases,* 25:900-910.

Winterfield, R. W., Fadly, A. M. and Bickford, A. (1972). Infectivity and distribution of infectious bursal disease virus in the chicken. Persistence of the virus and lesions. *Avian Diseases,* 16:622-632.

Winterfield, R. W., Hitchner, S. B., Appleton, G. S. and Cosgrove, A. S. (1962). Avian Nephrosis, nephritis and Gumboro disease: Secondary.Avain nepfrosis, nephritis and Gumboro disease, in: *L&M news Views*. 1962, 3: Pp. 130.

Wolffram, S. (1999). Absorption and metabolism of Selenium: difference between organic and inorganic sources. In: *Biotechnology in the Feed Industry*. Lyons, T.P. and K.A. Jacques, Nottingham University Press, Nottingham NG11 0AX, United Kingdom. *Proceedings 15th Annual Symposium*, 15:547-566.

Wyeth, P. J. (1975). Effect of infectious bursal disease on the response of chickens to *S. typhimurium* and *E. coli* infections. *Veterinary Record*, 96:238-243.

Wyeth, P. J. and Cullen, G. A. (1978). Transmission of immunity from inactivated infectious bursal disease oil-emulsion vaccinated parent chickens to their chicks. *Veterinary Record*, 102:362-363.

Wyeth, P. J. and Cullen, G. A. (1979). The use of inactivated infectious bursal disease oil emulsion vaccine in commercial broiler parent chickens. *Veterinary Record*, 104:188-193.

Yang, C. (1993). Fulvic acid supplementation and selenium deficiency disturb the structural integrity of mouse skeletal tissue. An animal model to study the molecular defects of kashin-beck disease. *Biochemistry Journal*, 289 (Pt 3): 829-835.

Yao, K., Goodwin, M. A. and Vakharia, V. N. (1998). Generation of a mutant infectious bursal disease virus that does not cause bursal lesions. *Journal of Virology*, 72:2647-2654.

Zaheer, A.S., Inayat, K.N. and Malk, S.A. (2003). Comparative immune response pattern of commercial Infectious Bursal Disease vaccines against field isolates. *Pakistan International Journal of Poultry Science*, 2: 449-453.

Zorman-Rojs, O., Barlic-Maganja, D., Mitevski, D., Lubke, W. and Mundt, E. (2003). Very virulent infectious bursal disease virus in southeastern Europe. *Avian Diseases*, 47:186-192.

APPENDIX

Appendix 1: Concentration of selenium (in ppm) in the tissues of day old Dominant black marshal cockerels before selenium supplementation.

SR/NO	Serum	Blood	Muscle	Liver	Kidney
1	0	1.229	55.75	40.21	22.28
2	0.082	2.853	61.1	65.65	33.62
3	0.102	3.654	52.53	36.49	56.83
4	0.075	3.627	59.97	47.45	72.69
5	0.059	2.481	57.34	50.13	78.19
Total	0.383	14.204	286.69	239.93	263.61
Mean	0.08	2.84	57.34	48	52.72

Appendix 2: Concentration of selenium in the tissues of Dominant black marshal cockerels after graded levels of supplementation with selenium for ten weeks.

	TISSUES	SER	BLD	MM	LV	KD
Grp	SR/NO	Selenium concentations(ppm)				
A (0.1)	1	4.7	9.54	65.48	92.79	84.91
	2	3.14	8.96	86.38	96.72	92.12
	3	3.92	8.37	75.64	99.61	57.09
	4	4.15	7.25	81.01	87.45	99.33
	5	4.46	8.41	75.93	93.53	78.21
	Total	**20.37**	**42.53**	**384.44**	**470.1**	**411.66**
	Mean	2.8	3.1	76.9	94.0	82.3
B (0.5)	1	5.49	10.34	102.8	103.8	104.2
	2	4.74	9.63	86.34	105.9	83.66
	3	3.98	8.92	97.12	91.39	63.11
	4	4.02	7.33	94.57	104.85	101.52
	5	4.76	8.83	99.96	76.88	102.86
	Total	**22.99**	**45.05**	**480.79**	**482.82**	**455.35**
	Mean	4.1	8.5	96.2	96.6	91.1
C (1.0)	1	6.09	16.2	82.71	112.6	161.2
	2	5.14	11.105	97.79	99.89	101.6
	3	4.21	12.83	104.53	73.44	77.88
	4	5.15	11.97	89.34	106.25	54.15
	5	5.62	13.68	106.21	93.02	89.68
	Total	**26.21**	**65.79**	**480.58**	**485.2**	**484.51**
	Mean	4.6	13.2	96.1	97.0	96.9

KEY: SER -serum; BLD - blood; MM - muscles; LV- liver; KD - kidneys

Appendix 3: Graded level of Selenium supplementation and mean antibody titre to Infectious bursa disease vaccine in Dominant black marshal cockerels vaccinated with

intermediate infectious bursal disease vaccine at 1st and 3rd week and challenged with IBDV at 6th weeek.

	Treatment groups											
Age in weeks	A (0.1)	B(0.5)	C(1.0)	D(0.1)	E(0.5)	F(1.0)	G(0.1)	H(0.5)	I(1.0)	J(0.0)	K(0.0)	L(0.0)
1	1	1	1.1	1	1	1	1.2	1.2	1.2	1.3	1	1.2
2	2.8	3.2	3.6	0	0.2	0.4	3	3.4	3.6	2.8	0	0
3	1.4	4.6	5.2	0 0	0 0	0 0	3.8	4.6	5	4	0 0	0 0
4	4.6	4.8	5.4	0 0	0 0	0 0	4.4	5	5.2	4	0	0
5	5	5.4	5.6	0 0	0 0	0 0	5	5.6	5.8	4.6	0 0	0 0
6	5	5.6	5.8	0 0	0 0	0 0	5.4	5.8	6.2	5.1	0 0	0 0
7	5.4	5.8	5.8	6	6	6	5.8	5.1	6	5.8	0	6
CMC	4	4.3	4.6	1	1	1.1	4.1	4.4	4.7	3.9	0.1	1

Values $P < 0.05$ are significant

Groups A, B, C= were vaccinated with IBD vaccine at week 1 and week 3 of age, they were challenged at 6 weeks of age with IBDV.

Groups D, E, F = were not vaccinated with IBD vaacine but challenged at 6 weeks of age with IBDV.

Group J = vaccinated with IBD vaccine at 1 and 3 weeks of age but not challenged with IBDV.

Group K = not vaccinated with IBD vaccine and not challenged with IBDV.

Group L = not vaccinated with IBD vaccine but challenged with IBDV at 6 weeks of age.

Group G, H, I = were vaccinated with IBD vaccine at 1 and 3 weeks of age but not challenged

Abreviation, CMC = Cumulative comscore

Appendix 4: Graded level of selenium supplementation and mortality rate of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and challenged

with infectious bursal disease virus.

Group	Level of selenium (ppm)	Number of birds	Number of birds dead	Mortality rate
A	(0.1) Vi	25	9	36
B	(0.5) Vi	25	4	16
C	(1.0) Vi	25	3	12
D	(0.1) NVi	25	14	56
E	(0.5) NVi	25	13	52
F	(1.0) NVi	25	9	36
G	(0.1) VNi	24	0	0
H	(0.5) VNi	25	0	0
I	(1.0) VNi	24	0	0
J	(0.0) VNi	25	0	0
K	(0.0) NVNi	25	0	0
L	(0.0) NVi	25	16	64

Vaccinated, r = - 0.93, unvaccinated r = - 0.95

Abbreviation: Vi, vaccinated and infected; NVi, not vaccinated but infected; VNi, vaccinated not infected; NVNi, not vaccinated not infected.

,.

Appendix 5: Selenium supplementation and protective rate of infectious bursa disease vaccine

Selenium concentration	Protective rate
0.1	35.7
0.5	69.2
1.0	66.7

Appendix 6: Selenium supplementation and gross pathology of the bursa of Fabricius (a), kidneys (b), liver (c), leg muscles (d), pectoral muscles (e) and proventriculus (f)

	A(n=9)	B (n=4)	C (n=3)	D (n-14)	E (n-13)	F (n=9)	L(n=16)

a

H	28.6	0	0	33.3	15.4	11.1	50
E	71.4	75	66.7	88.9	84.6	66.7	81.3
N	11.1	25	33.3	7.1	7.7	22.2	6.3

b

	A(n=9)	B (n=4)	C (n=3)	D (n-14)	E (n-13)	F (n=9)	L(n=16)
S	14.3	0	0	22.2	15.4	11.1	31.3
P	28.6	0	0	22.2	23.1	33.3	22.5
N	71.4	100	100	55.9	69.2	68.8	55.6

c

d

	A(n=9)	B (n=4)	C (n=3)	D (n-14)	E (n-13)	F (n=9)	L(n=16)
L	0	0	0	0	0	0	12.5
PH	100	100	100	100	100	100	87.5
N	35	26	23.3	38.8	25	35.3	44.4
EH	11.1	0	0	25	21.4	15.4	26.3
N	53.9	74	76.7	36.2	53.6	49.3	29.3

e

	A(n=9)	B (n=4)	C (n=3)	D (n-14)	E (n-13)	F (n=9)	L(n=16)
PH	7.1	25	0	22.2	23.1	22.2	37.5
EH	11.1	0	0	21.4	15.4	7.7	25
C	22.2	0	33.3	7.1	7.7	11.1	12.5
N	59.6	75	66.7	49.3	53.8	59	38.5

f

	A(n=9)	B (n=4)	C (n=3)	D (n-14)	E (n-13)	F (n=9)	L(n=16)
Hpvj	21.4	25	33.3	35.7	38.5	33.3	50
Hv	21.4	0	0	28.6	23.1	22.2	25
N	57	75	66.7	35.7	41.2	44.5	25

Appendix 7: Selenium supplementation and gross mean lesion score of Dominant black marshal cockerels vaccinated with infectious bursal disease vaccine and challenged with infectious bursal disease virus

	TISSUE					
Group	BF	KD	LV	TM	PM	PR
A	3.1	2	0	2.5	1.5	2.3
B	3	0	0	2	2	2
C	3	0	0	2	1	2
D	3.5	3.5	0	2.5	2.5	2.5
E	3.4	3	0	2.3	2	2
E	3.3	3	0	2	1.7	2
L	4.4	3.1	2	2.5	2.7	2.4

Key: BF= bursa of Fabricius, KD = kidneys, LV = liver, LM = leg muscles, PM = pectoral muscles, PR = proventriculus

Appendix 8: Hematological parameters of day old Dominant black marshal cockerels.

n(5)	RBC ($\times 10^{12}$/l)	HGB (g/dl)	PCV (%)	LYM (%)	HET (%)	TP (g/dl)

	1.80	10.5	28.0	62	38	4.1
	1.87	10.2	28.1	52	48	1.8
	2.45	13.8	38.3	44	56	2.9
	1.96	10.8	27.9	40	60	2.7
	2.58	14.2	40.0	48	50	1.8
Mean	2.1	11.9	32.5	49.2	50.4	2.7
SEM	±0.71	±0.86	±2.74	±3.77	±3.76	±0.43

Appendix 9: Graded levels of selenium supplementation and mean haematological values of Dominant black marshal cockerels challenged with infectious bursal disease virus.

Age (wk)	Parameter	Control(n=5)	0.1 (ppm) (n=5)	0.5 (ppm) (n=5)	1.0 (ppm) (n=5)	Pearson	P-value
1	RBC($X10^{12}$/l)	1.83±.0.16	2.26±0.23	2.35±0.19	2.4±0.22	0.7514	0.2426
	HGB (g/dl)	11.0±9.11	11.4±9.34	12.0±2.57	13.1±2.85	0.9945	0.0055*
	PCV (%)	28.7±2.75	29.6.±2.74	30.6±8.11	31.3±7.67	0.9530	0.0470*
	LYM (%)	45±.3.71	63±2.92	63±3.77	63±3.77	0.8866	0.4134
	HET (%)	61.4±3.77	54±3.39	39±3.77	39±3.75	0.8803	0.1197
	T.P (g/dl)	4.5±0.43	4.6±0.39	4.7±0.23	4.9±0.43	0.9875	0.0125*
2	RBC($X10^{12}$/l)	2.28±0.37	2.33±0.05	2.38±0.05	2.42±0.08	0.9534	0.0466*
	HGB (g/dl)	11.7±2.71	12.2±2.11	12.4±3.70	13±4.75	0.9545	0.0455*
	PCV (%)	28±0.78	30.5±0.39	31.4±0.96	32.4±0.64	0.9098	0.0502*
	LYM (%)	40±2.52	50±4.31	60±4.51	60±2.98	0.8424	0.1576
	HET (%)	44±2.65	50±4.39	43±4.54	46±2.79	0.1658	0.8342
	T.P (g/dl)	4.3±1.88	4.8±0.38	4.8±0.09	4.8±0.07	0.5866	0.4134
3	RBC($X10^{12}$/l)	2.3±0.07	2.3±0.06	2.39±0.16	2.54±0.11	0.9846	0.0154*
	HGB (g/dl)	11.8±3.22	12.3±3.52	12.6±5.48	12.9±3.03	0.9223	0.0777
	PCV (%)	29.1±0.65	30.7±0.31	31.8±0.68	32.5±0.63	0.9055	0.0954
	LYM (%)	59±1.72	65±1.47	68±1.29	68±1.50	0.7777	0.0523*
	HET (%)	23±1.95	25±2.22	50±2.69	54±3.19	0.9330	0.0487*
	T.P (g/dl)	4.5±3.16	4.7±3.17	4.9±0.26	4.8±0.18	0.6869	0.3131
4	RBC($X10^{12}$/l)	2.29±0.05	2.36±0.07	2.6±0.09	2.80±0.05	0.9938	0.0062*
	HGB (g/dl)	11.9±2.98	12.5±2.90	12.8±3.98	12.9±4.19	0.8310	0.1690
	PCV (%)	29.2±0.49	30.8±0.86	32.1±0.79	32.8±1.562	0.9119	0.0881
	LYM (%)	58±1.46	66±1.49	67±2.91	70±2.43	0.8157	0.0483*
	HET (%)	42±1.66	45±0.97	47±1.29	50±1.53	0.9583	0.0417*
	T.P (g/dl)	4.3±0.30	4.7±0.23	4.6±0.15	4.9±0.15	0.7919	0.2081
5	RBC($X10^{12}$/l)	2.30±0.07	2.36±0.07	2.62±0.09	2.9±0.07	0.9995	0.0005*
	HGB (g/dl)	12.2±3.75	12.6±3.12	12.9±3.19	12.9±3.48	0.8180	0.1820
	PCV (%)	29.4±0.66	31.0±1.05	32.7±1.24	32.8±0.96	0.8601	0.1399
	LYM (%)	61±1.66	65±1.33	66±1.88	71±1.38	0.9448	0.0493*
	HET (%)	43±1.66	40±1.61	45±2.16	55±1.50	0.9363	0.0637
	TP (g/dl)	3.9±0.28	4.8±0.23	4.9±0.15	4.9±0.15	0.6492	0.3508
6	RBC($X10^{12}$/l)	2.31±0.05	2.38±0.07	2.64±0.06	3.30±0.06	0.9847	0.0153*
	HGB (g/dl)	12.3±3.81	12.6±2.49	12.9±4.07	13.0±4.29	0.9043	0.0957
	PCV (%)	28.9±1.85	31.3±1.08	31.9±2.27	33.2±0.18	0.9795	0.0492*
	LYM (%)	60±1.29	67±0.55	68±0.97	71±1.40	0.9349	0.0451*
	HET (%)	35±4.02	47±5.33	46±1.99	51±1.28	0.7600	0.2400
	T.P (g/dl)	4.0±0.18	4.9±0.15	4.9±0.15	4.8±0.18	0.5046	0.4954
7	RBC($X10^{12}$/l)	2.42±0.06	2.40±0.09	2.66±0.07	2.31±0.07	0.9729	0.0271*
	HGB (g/dl)	13.1±2.16	12.7±1.08	13.0±2.16	13.3±1.36	0.6455	0.3545
	PCV (%)	39.1±1.09	35.0±0.09	32.4±3.27	33.2±0.67	0.7312	0.2688
	LYM (%)	19.6±3.8340	22.8±2.3337	27.3±2.1409	30.0±4.09	0.9584	0.0416*
	HET (%)	81.5±1.69	78.6±2.44	70.0±3.49	61.4±0.59	0.9970	0.0030*
	T.P (g/dl)	3.5±0.32	4.1±0.26	4.3±0.15	4.3±0.18	0.7360	0.2640