EFFECT OF *MORINGA OLEIFERA* LEAF SUPPLEMENTATION OF FEED ON PERFORMANCE, ANTIBODY RESPONSE, HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN BROILERS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS

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DECLARATION

I declare that the work in this Thesis entitled **"Effect of *Moringa oleifera* Leaf Supplementation of Feed on Performance, Antibody Response, Haematological and Biochemical Parameters in Broilers Challenged with Infectious Bursal Disease Virus"** has been carried out by me in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria under the supervision of Professor P.A. Abdu, Dr. A.M. Wakawa and Dr. T. Aluwong. The work of other investigators referred to in this study was duly acknowledged. No part of this Thesis has been previously submitted for another degree, diploma or certificate at this or any other institution.

Arhyel Gana BALAMI

…….. …… ……

Signature Date

CERTIFICATION

This Thesis entitled **"EFFECT OF *MORINGA OLEIFERA* LEAF SUPPLEMENTATION OF FEED ON PERFORMANCE, ANTIBODY RESPONSE, HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN BROILERS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS"** by Arhyel Gana BALAMI meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This Thesis is dedicated to God Almighty for HIS grace over my life, my mother, for always being there for me, my ever loving and caring wife, Maryamu, our blessed and most cherish children John-Joel and Joy-Jadidah Arhyel Balami for their love, patience and understanding throughout the years of my research.

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ABSTRACT

This study was conducted to determine the effect of dietary *Moringa oleifera* leaf (MOL) supplementation of feed on performance, antibody response, haematological and biochemical parameters of broilers challenged with a very virulent infectious bursal disease virus (vvIBDV). Fresh MOL was collected from Potiskum, Yobe State and air dried for five days, ground into powder using a milling machine and analyzed for nutrients and elements using standard method of the Association of Official Analytical Chemists. The phytochemical constituents analysis of MOL was done using the method described by Sofowora. Two hundred and forty day-old Ross 308 hybrid broiler chicks were randomly assigned into four groups (A, B, C and D) of 60 chicks each and raised in a deep litter house consisting of 4 separate compartments. All birds were fed with broiler starter (BS) from 0 to 28 days of age and broiler finisher (BF) from 29 to 49 days of age. Broiler starter (22% crude protein CP) and BF (20% CP) mash were formulated each with 5% MOL included as part of the feed ingredient for broilers in groups A and B while BS and BF for broilers in groups C and D were formulated without MOL. Only birds in groups A and C were vaccinated intramuscularly with 0.5 ml of an inactivated intermediate strain infectious bursal disease (IBD) vaccine at 14 and 21 days of age. Inactivated Newcastle disease virus (NDV) vaccine (Komorov strain) was also administered (0.5 ml) intramuscularly at 18 days of age to groups A and C. Birds in groups A, B and C were challenged intraocularly at 35 days of age with vvIBDV while those in group D served as control. Daily feed intake (DFI), average daily weight gain (ADWG), feed conversion ratio (FCR) and selling price (SP) per bird were determined for each group. Five birds were randomly selected and euthanized from each group on 35, 38 and 42 days of age and the bursa of Fabricius, thymus, spleen

and Harderian glands were removed for evaluation of organ body weight index. Blood was collected from ten broilers in each group via the wing vein on 14, 21, 35, 38 and 42 days of age to determine the IBD antibody titre level, haematological and biochemical parameters. At 49 days of age, five birds from each group were slaughtered and their carcasses weighed to assess the performance of birds fed with MOL supplemented diets. The results of the nutrients analysis showed that MOL contained carbohydrate (55.14%), CP (25.9%), crude fibre (13.91%), moisture (7.94%), fat (5.85%), ash (3.72%) and energy (2930.63 Kcal/Kg). The phytochemical analysis of MOL revealed phytates (2.57%), tannins (2.19%), saponins (1.06%), oxalates (0.45%) and cyanides (0.1%). The elemental analysis on MOL revealed Ca (2.26%), P (0.35%), Mg (0.45%), K (1.9%), Na (0.11%), Zn (34 ppm), Cu (7.5 ppm), Mn (40.5 ppm), Fe (116.5 ppm) and Se (0.85 ppm). Broilers in group D correlated more strongly (Pearson correlation = 0.921; P = 0.000) in their feed intake than those in groups B (Pearson correlation = 0.875; P = 0.000), C (Pearson correlation = 0.863; P = 0.000) and A (Pearson correlation = 0.862; P = 0.000). Broilers in groups A and B had weaker correlation (Pearson correlation = □ 0.379; P = 0.000) in feed conversion ratio than those in groups C (Pearson correlation = □ 0.454; P = 0.000) and D (Pearson correlation = □ 108; P = 0.273). Broilers from groups D, A and B had a higher selling price/bird (₦ 1,151 ± 82.82, ₦1,093 ± 54.11 and ₦935.9 ± 70.69, respectively) than those in group C (₦908.3 ± 63.97). There was an increase in the enzyme linked immunosorbent assay (ELISA) IBD antibody titre level of broilers between 21 and 35 days of age in groups A (1,379.89 ± 829.98 to 2,836.83 ± 463.58), C (1,576.94 ± 566.51 to 3,290.51 ± 848.87) and D (1,542.43 ± 106.80 to 2,953.49 ± 561.88), and up to 42 days of age in broilers of group B (1,205.94 ± 612.32 to 3,193.10 ± 478.52). *Moringa oleifera* leaf feed supplementation improved bursa (1.4, 1.4), spleen (1.3, 1.1) and Harderian gland (2.0, 2.0) organ to body weight index of broilers of

groups A and B. Feeding broilers with 5% MOL supplemented diet without vaccination (group B) did not prevent vvIBDV from causing a decrease in lymphocyte count from 3.87 ± 1.52 to 2.67 ± 1.26 on day 3 pi. Supplementation of broiler feed with 5% MOL and vaccination with inactivated IBD vaccine did not prevent a decrease in PCV (28.60 ± 1.77 % to 21.80 ± 3.46 % and 25.22 ± 2.28 % to 21.44 ± 1.33 %) and RBC (2.44 ± 0.44 x 10^{12}/L to 2.01 ± 0.42 x 10^{12}/L and 2.25 ± 0.26 x 10^{12}/L to 1.79 ± 0.52 x 10^{12}/L), but caused an increase in Hb (10.55 ± 2.21 g/dl to 10.78 ± 1.95 g/dl and 9.81 ± 0.97 g/dl to 11.39 ± 1.17 g/dl) concentration on day 3 pi with vvIBDV in groups A and C. There were respective increase in aspartate aminotransferase and alanine aminotransferase levels in groups A (39.90 ± 3.96 IU L^{-1} to 45.10 ± 5.70 IU $L^{-1}$and 42.90 ± 3.21 IU L^{-1} to 49.60 ± 3.56 IU L^{-1}), B (39.80 ± 3.68 IU L^{-1} to 48.50 ± 4.22 IU L^{-1} and 43.30 ± 3.83 IU L^{-1} to 54.20 ± 5.53 IU L^{-1}) and C (38.40 ± 3.20 IU L^{-1} to 42.80 ± 4.02 IU L^{-1} and 42.70 ± 4.80 IU L^{-1} to 48.60 ± 4.45 IU L^{-1}). However, similar increase were observed in group D following challenge with vvIBDV (41.80 ± 3.85 IU L^{-1} to 45.30 ± 5.64 IU L^{-1} and 44.20 ± 4.52 IU L^{-1} to 51.60 ± 3.69 IU L^{-1}). Supplementing broilers feed with MOL without vaccination against vvIBDV could not prevent lipid peroxidation (from 1.37 ± 0.23 IU^{-1} to 1.51 ± 0.30 IU^{-1}) in broilers of group B following inoculation with vvIBDV. Supplementing broilers feed with MOL maintained the level of Na^{+} concentration in broilers of group A (140.00 ± 2.79 mg/dl to 139.90 ± 2.69 mg/dl) and B (140.10 ± 2.51 mg/dl to 138.30 ± 2.50 mg/dl) following inoculation with vvIBDV. Feed millers are encouraged to create awareness among poultry farmers on the nutritional and health benefits of MOL inclusion in the diets of broilers. Broilers fed with MOL supplemented diets can be vaccinated by farmers since it has no adverse effect against immune response to IBD.

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LIST OF ABBREVIATIONS

%	Percentage
<	Less than
>	Greater than
AGID	Agar gel immunodiffussion
ALP	Alkaline phosphatase
ALT	Alanine aminotrasferase
AOAC	Association of official analytical chemist
AST	Aspartate aminotransferase
BBI	Bursa to body index
BF	Bursa of Fabricius
Ca	Calcium
CAT	Catalase
CAV	Chicken anaemia virus
CEF	Chicken embryo fibroblast
CF	Crude fibre
CK	Creatine kinase
CP	Crude protein
Cu	Copper
DC	Dressing carcass
DFI	Daily feed intake
dsRNA	Double stranded ribonucleic acid
DXV	Drosophila X virus
ELISA	Enzyme linked immunosorbent assay

FAO	Food and Agriculture Organisation
FC	Feed cost
FCR	Feed conversion ratio
Fe	Iron
GMF	Gross margin of feed
GPx	Glutathion peroxidase
H/L	Heterophil to lymphocyte
H_2O_2	Hydrogen peroxide
Hb	haemoglobin
HBI	Harderian to body index
HDL	High density lipoprotein
IBD	Infectious bursal disease
IBD Abs	Infectious bursal disease antibodies
IBDV	Infectious bursal disease virus
IDL	Intermediate density lipoprotein
IPNV	Infectious pancreatic necrosis virus
K	Potassium
LBW	Live body weight
LCAT	Lecithin cholesterol acyltransferase
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume

MDAs	Maternally derived antibodies
MDA	Malondialdehyde
MDV	Marek's disease virus
Mg	Magnesium
Mn	Manganese
MO	*Moringa oleifera*
MOL	*Moringa oleifera* leave
MR	Mortality ratio
Na	Sodium
ND	Newcastle disease
NDV	Newcastle disease virus
O_2	Oxygen molecule
OIE	Office International des Epizootics
PBS	Phosphate buffered saline
PCV	Packed cell volume
Pi	Post inoculation
PPE	Personal protective equipment
RBC	Red blood cell
RBCs	Red blood cells
REDOX	Oxidation and reduction
RNA	Ribonucleic acid
ROS	Reactive oxygen specie
RPM	Revolutions per minute
S/P	Sample to positive ratio

SBI	Spleen to body index
SN	Serum neutralization
SOD	Superoxide dismutase
SP	Selling price
SPF	Specific pathogen free
TBI	Thymus to body index
TC	Total cholesterol
TG	Triglycerides
TWBC	Total white blood cell
VLDL	Very low density lipoprotein
vvIBDV	Very virulent infectious bursal disease virus
Zn	Zinc
µl	Microlitres

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Moringa oleifera belongs to the single genus monogeneric family *Moringaceae* and is indigenous to Northwest India, but at present, it is widely distributed in the tropics throughout the Pacific region, Central America, the Caribbean, as well as West Africa (Ramachandran *et al.*, 1980; Freiberger *et al.*, 1998; Foidl *et al.*, 1999; Makkar and Becker, 1999; Lockett *et al.*, 2000; Aregheore, 2002). In Nigeria, this plant is commonly and widely cultivated in the Northern region of the country and is known by most ethnic groups in the country (Anjorin *et al.*, 2010). *Moringa oleifera* is also referred to as Horseradish tree, Radish tree, Drumstick tree, Mother's best friend (English); *Gawara, Konamarade, Rini maka* (Fulfulde); *Zogale, Bagaruwa maka* (Hausa); *Ikwe oyibo* (Igbo); *Ewe ile, Ewe Igbale,* and *Idagbo monoye* ("the tree which grows crazily") (Yoruba) (Lowell and Sreeja, 2001).

The demand for *Moringa oleifera* products is on the increase due to the consideration of the tree as one of the world most useful plants known for its nutritional, medicinal and significant economic importance. The leaves, fruits, flowers and immature pods of the tree are edible and they form part of traditional diets in many countries and posses several nutrients, including; calcium, magnesium, potassium, iron, vitamin A, vitamin C and a crude protein content that varies from 16 to 40% (Foidl *et al.*, 2001*;* Marcu and Pharm, 2005; Rweyemamu, 2006). The *Moringa oleifera* leaves (MOL) have been reported to be a valuable source of both macro- and micronutrients, rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus

enhance the shelf-life of fat-containing foods (Dillard and Bruce German, 2000; Siddhuraju and Becker, 2003).

Moringa oleifera leaf has been reported to contain a high pepsin and total soluble protein, which makes it more suitable for monogastric animals such as poultry (Kakengi *et al.*, 2007). The use of MOL as a supplement can improve voluntary intake, digestability and performance (Aregheore, 2001). *Moringa oleifera* leaves are known to be very poor in anti-nutritional factors and have been used in poultry feeding with various performance results depending on their nutritional value and inclusion level in the diet (Foidl *et al*., 2001; Sarwatt *et al*., 2002; Kaijage *et al*., 2003; Soliva *et al*., 2005; Kakengi *et al*., 2007; Nuhu, 2010; Olugbemi *et al*., 2010a; 2010b).

In Nigeria, the state of nutrition of the populace is predominantly marked by inadequate protein intake both in quantity and quality (Taiwo *et al.,* 2005). Food and agriculture organization (FAO, 1992) recommended 27 g as the animal protein daily requirement for human beings. However, the intake per average Nigerian is grossly inadequate (3.24 g animal protein/day) (ILCA, 1980; FAO, 1986; FAO, 1992). Broiler production has been suggested as a means of massively producing animal protein because of its short generation interval and high growth rate (Larry, 1993; Dipeolu *et al.*, 1996; Nworgu *et al.*, 2000; Essen *et al.*, 2005), since they are prolific, possess a high feed conversion ratio and are accepted by all, irrespective of religion. Broilers are chickens reared from chick to slaughter weight in eight weeks (Smith, 2001). Broilers have several desirable nutritional characteristics such as high protein, low lipid content and high polyunsaturated fatty acids; these make them preferable, health wise, when compared to red meat (Mothershaw *et al.*, 2009).

Infectious bursal disease (IBD) has been considered as one of the important naturally occurring viral diseases of commercial chicken threatening the poultry industry worldwide (Müller *et al.,* 2003). In Nigeria, IBD was reported by Ojo *et al*. (1973), Onunkwo (1975) and Okoye (1984). The dreaded nature of IBD has rendered investment in poultry to be fearful and unrealistic to both organizations and individuals (Okoye, 1983; Abdu, 1986; Lukert and Saif, 1997; Shane, 1997). Shane (1997) and Sainsbury (2000) reported the disease as a setback to productivity and profitability in the poultry industries of both developing and industrialized nations. Infectious bursal disease is caused by an infectious bursal disease virus (IBDV) (Dobos, 1979; Kibenge *et al.,* 1988; Lukert and Saif, 2003) that is non-enveloped and has a bisegmented double-stranded RNA (dsRNA) genome (Kibenge *et al.,* 1988; Da Costa *et al.,* 2003). Infectious bursal disease virus is a member of the *Birnaviridae* family (Dobos, 1979).

The diagnostic application of haematology and biochemistry in human and veterinary medicine is a well established procedure (Ross *et al.,* 1976). The present organisation within the poultry industry, where large numbers of individuals of low genetic variance are maintained in controlled environments, present an ideal situation for the use of clinical chemistry. The circumstances are unique in that clinical examination and clinical chemistry estimates can readily be combined with postmortem examination on individuals sacrificed to complement disease investigation (Ross *et al.,* 1976). The introduction of rapid micro methods and automated line analyses to haematology and biochemistry has helped in the development of clinical chemistry and "profile" studies in poultry (Ross *et al.,* 1976).

Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids via a free radical chain mechanism, forming fatty acyl hydroperoxides, generally called peroxides or primary products of oxidation (Rasooli, 2007). The primary auto-oxidation is followed by a number of secondary reactions which lead to degradation of lipids and the development of oxidative rancidity (Ladikos and Lougovois, 1990). Lipid peroxidation is one of the primary causes of quality deterioration in meat and meat products, as it largely contributes to colour and flavour deterioration, loss of nutritional value and safety and generates compounds that may be detrimental to consumers (Min *et al*., 2008). The degree of lipid peroxidation is often used as an indicator of reactive oxygen species (ROS) mediated damage (Kuun and Borchert, 2002) and the concentration of MDA in blood and tissues are generally used as biomarkers of lipid peroxidation (Sehirli *et al.,* 2008; Yousef *et al.,* 2009).

1.2 Statement of Research Problem

Synthetic growth promoters and supplements in poultry nutrition are expensive, usually unavailable and possess detrimental effects in birds as well as humans (Portugaliza and Fernandez, 2012). The administration of antibiotics as growth enhancers in poultry at sub-therapeutic levels may result in development of antibiotic-resistant bacteria, which are hazardous to poultry health (Portugaliza and Fernandez, 2012). The continuous exposure of poultry to antibiotics have been found to have led to serious health problems such as allergies, spreading of drug resistant microorganisms, carcinogenic effect and a potential harmful effect on human intestinal microflora (Ferrinie *et al.,* 2006; Jafari *et al.,* 2007; Nonga *et al.,* 2010).

Broiler meat is one of the principal sources to fill the genuine gaps of the animal protein and can play a leading role in providing balanced diet (Alam and Khan, 2003). However, diseases constitute one of the major factors that limit broiler meat production (Fasanya, 1984). Infectious bursal disease is an important cause of economic losses in the poultry industry (de-Wit, 1998; Musa *et al.,* 2012). Infectious bursal disease have continued to be a major disease problem of commercial or rural chickens and constitute a major threat to poultry production in Nigeria (Okoye and Uzoukwu, 2001; Musa *et al.,* 2010). In Nigeria, despite rigorous vaccinations, outbreaks of IBD in commercial poultry have been reported to account for high loss (Abdu, 1986; Awolaja and Adene, 1995; El-Yuguda and Baba, 2004; Dashe *et al.,* 2009; Musa *et al.*, 2010; Musa *et al.,* 2012). The productivity of broilers in the tropics has been limited by scarcity and consequent high prices of conventional energy and protein sources. Protein sources are especially limiting factors in poultry feed production in the tropics and this hinders poultry production (D'Mello *et al*., 1987; Atawodi *et al.,* 2008).

Infectious bursal disease virus has been reported to be one of the very important immunosuppressive agents in modern poultry production. Infection with IBDV may induce a temporary or permanent destruction of the bursa of Fabricius (BF) and other lymphoid tissues (Sharma *et al.,* 2000; Lukert and Saif, 2003; Khatri *et al.,* 2005). Therefore, the main targets of the IBD virus are the lymphoid organs and the immune cells (Faragher, 1972). The disease is characterized by immune-deficiency and high mortality in chicks that are between 3 and 6 weeks old. Studies have shown that, IBD has acquired an endemic status in poultry farms in Nigeria (Nawathe *et al*., 1978; Durojaiye *et al*., 1984; Abdu, 1988). The prevention of IBD in Nigeria is largely dependent on vaccination with single

prototype indigenous and different types of imported IBD vaccines (Okoye and Uzoukwu, 2001). Regrettably, severe outbreaks still occur with high mortality rates both in vaccinated and unvaccinated flocks (Okoye, 1983; Abdu, 1986; Sainbury, 2000; Musa *et al.*, 2010), and these makes the control of IBD to be virtually impossible in most of the farms (Musa, 2009; Musa *et al.*, 2010).

Haematological values of avian species are significantly influenced by poultry diseases, such as IBD (Panigraphy *et al.*, 1986; Juranova *et al.*, 2001). Zeryehun *et al.* (2012) reported that IBDV causes alterations in different haematological parameters of poultry. In birds, clinical signs of illness are frequently delicate; therefore, clinical chemistry is necessary to evaluate cellular changes (Ritchie *et al*., 1994). Besides the severe clinical signs and high mortality rate that results from vvIBDV infection in susceptible chickens, it also causes many pathological changes that form part of the pathogenesis of the disease which could basically be explained in terms of the biochemical changes that occur in relation to the pathological effect of the virus in several organs such as the liver and kidneys (Ley *et al.,* 1983; Nunoya *et al.,* 1992; Lukert and Saif, 1997). Oxidative stress is the major cause of reduction in growth in broilers and increases incidence of infectious and metabolic diseases in poultry (Sen, 1995). Oxidative stress especially in broilers can results in damage to biomolecules, cells and tissues which decrease immunity and antioxidant status of birds (Peter, 2002; Yun-Zhong *et al.,* 2002; Joachim *et al.,* 2010).

1.3 Justification of the Study

The use of organic supplements such as probiotics and leaves, are generally believed to be safer, healthier, and less hazardous when compared to other synthetic products (Onyimonyi and Onu, 2009). Thus, plants are incorporated in livestock feeds in the form of leaf meal instead of synthetic products in order to improve weight gain, higher production and better feed efficiency (Onyimonyi and Onu, 2009). Moreover, plants contain active substances that can improve digestion and metabolism (Ghazalah and Ali, 2008). The incorporation of protein from leaf sources in diets for broilers is becoming popular because of its availability, abundance and relatively reduced cost (Onyimonyi and Onu, 2009). Based on the reports of Opara (1996), leaves in the diet of animals do not only serve as protein sources but provide necessary vitamins, minerals and oxycarotenoids which cause yellow colour of broiler skin, shank and egg yolk. Nutritional variations have been reported in several studies of MOL that includes; the genetic background of the plant in terms of ecotype and cultivar, environmental factors that include the soil and climate (Sanchez-Machado *et al.,* 2009). There is information concerning the nutritional value of MOL in different places and regions of Nigeria (Ojiako, 2014; Aja *et al.,* 2013; Bamishaiye *et al.,* 2011; Ogbe and Affiku, 2011), but little is known about the nutritional profile of MOL from Potiskum, Yobe State, Nigeria.

Moringa oleifera is one of the plants that contain bioceutical agents that could substitute synthetic growth enhancers and supplements in broiler and other livestock production due to the presence of vitamins and antimicrobial properties it possess, hence MOL are among the leaf meals that could be used as feed alternatives in commercial livestock and poultry in the tropics (Makkar and Becker 1997; Agbede, 2003). *In-vitro* studies have shown that

Moringa oleifera has a natural anti-helmintic, antibiotic, detoxifing and immune enhancing properties (Ratshilivha *et al.,* 2014; Dahot, 1998). It possesses hypocholesterolemic, blood boosting and antimicrobial properties, natural digestive enzymes, which can be used in feeds (Fuglie, 1999; Fahey *et al*., 2001; Sarwart *et al*., 2002; Greg, 2008; Olugbemi *et al*., 2010a). Increasing supplementation of *Moringa oleifera* decreases the contents of uric acids, triglycerides and albumin/globulin ratio in the serum of broilers hence, significantly increases the immune response of broilers (Du *et al.,* 2007).

One of the measures of immunity that have been commonly used in assessing poultry health is the lymphoid organ weights (Pope, 1991). Lymphoid organ weights are easily measured and reflect the body's ability to provide lymphoid cells during an immune response (Heckert *et al.,* 2002). To gain immunity, the animal needs energy and proteins for the manufacture of antibodies and cells as well as minerals (zinc, copper, iron and selenium) and vitamins (A and E) for transmitting signals in parts of the animal's body in other to fight infections (Conroy, 2005). Interestingly, *Moringa oliefera* leaves (MOL) has been reported to possess carbohydrate, proteins, minerals, vitamins and amino acids (Makkar and Becker, 1999; Kakengi *et al*., 2003; Oduro *et al*., 2008). *Moringa oleifera* are in high demand for their medicinal values as they have been reported to have the potential of boosting the immune systems (Ramachandran *et al.,* 1980; Atawodi *et al*., 2008; Sreelatha and Padma, 2009). The absence of available literature on evaluating the humoral immune response of broilers fed with *Moringa oleifera* feed supplement and vaccinated with a killed IBD vaccine necessitate this studies.

There is little information on the effect of *Moringa oleifera* on haematological parameters of broilers (Ewuola *et al.*, 2013). Ahmad *et al.* (2014) reported the potential use of MOL as antiviral substance against IBD. The absence of haematological values of broilers fed MOL supplemented diet, and subsequently, challenged with vvIBDV also necessitate this study. The few studies that have attempted to determine biochemical changes associated with IBDV infection (Ley *et al.*, 1983; Panigraphy *et al.*, 1986; Afaleq, 1998), reported variable biochemical profiles. Changes in various antioxidant enzyme activities can be used to estimate the level of oxidative stress and total antioxidant status (Rayman, 2000). *Moringa oleifera* leaf has been reported to possess an antioxidant known to have suppressive effects on formation of ROS (Sofidiya *et al.*, 2006; Ogbunugafor *et al.*, 2011). To the best of our knowledge, feeding broilers with supplemented MOL in their feeds and challenging them with a vvIBDV in order to determine biochemical changes that are associated with the disease has not been reported.

1.4 Aim of the Study

The aim of the study was to determine the effect of supplementation of feeds with *Moringa oleifera* on performance, antibody response, haematological and biochemical parameters of broilers challenged with a vvIBDV.

1.5 Objectives of the Study

The objectives of the study were to;

1. evaluate the haematological parameters of broilers fed with MOL feed supplementation and challenged with a vvIBDV.

2. evaluate the effect of MOL feed supplementation on the serum biochemical profile of broilers challenged with vvIBDV.
3. assess the performance and return on investment of broilers fed with MOL feed supplementation and challenged with vvIBDV.
4. evaluate the humoral immune response of broilers fed with MOL feed supplementation and vaccinated with an inactivated IBD vaccine.

1.6 Research Questions

1. Does MOL feed supplement in the diet of broilers have any effect on haematological parameters following infection with vvIBDV?
2. Does MOL feed supplement in the diet of broilers have any effect on serum biochemical profile following challenge with vvIBDV?
3. Does MOL feed supplement in the diet of broilers have any effect on the performance and return on investment following infection with vvIBDV?
4. Does MOL feed supplement in the diet of broilers have any effect on humoral immune response to an inactivated IBD vaccine?

CHAPTER TWO

LITERATURE REVIEW

2.1 *Moringa oleifera*

2.1.1 Historical background of *Moringa oleifera*

Moringa oleifera is the most widely cultivated species of a monogeneric family, the *Moringaceae* that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Mughal *et al.*, 1999). It is a softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. It is widely cultivated and naturalized in tropical Africa, tropical America, Sri Lanka, India, Mexico, Malabar, Malaysia and the Philippine Islands (Morton, 1991).

The name "Shigon" for *Moringa oleifera* was first mentioned in the "Shushruta Sanhita" which was written in the beginning of the first century A.D. There is evidence that the cultivation of MO in India dates back many thousands of years (Fuglie, 2001). In Nigeria, this plant is common in the north east and north central regions of the country and is known by most ethnic groups in the country (Ogbe and Affiku, 2011).

2.1.2 Taxonomic classification of *Moringa oleifera*

Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivision: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Subclass: Dilleniidae, Order: Capparales, Family: Moringaceae, Genus: Moringa Adans, Species: *Moringa oleifera* (Natural Resources Conservation, 2008).

2.1.3 Description and morphology of *Moringa oleifera* tree

Moringa oleifera tree is a short, slender, deciduous, perennial tree, of about 10 metre tall. It has a slender and drooping branch. The branches and stems are brittle, with corky bark. Their leaves are feathery, pale green, compound, tripinnate, and has a length of about 30–60 cm long, with many small leaflets that are 1.3–2 cm long, 0.6–0.3 cm wide, while the lateral ones are somewhat elliptic, the terminal ones, obovate and slightly larger than the lateral ones. The flowers are fragrant, white or creamy white in colour, and they have a diameter of about 2.5 cm, with borne in sprays, with five at the top of the flower. The stamens are yellow; the pods are pendulous, brown, triangular, splitting lengthwise into three parts when dry with a length of about 30–120 cm long, 1.8 cm wide, containing about 20 seeds embedded in the pith. The pod tapers at both ends, with 9-ribbed. The seeds are dark brown, with three papery wings. The main root of MO is thick (Appendix IV) (Kirtikar and Basu, 1980; Chatterjee and Prakash, 1994).

2.1.4 Uses of *Moringa oleifera* tree

Fuglie (2001), states the many uses of *Moringa oleifera* to include; alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), and water purification (powdered seeds).

Numerous medicinal properties have been ascribed to various parts of this highly esteemed tree (Estrella *et al.,* 2000; Siddhuraju and Becker, 2003). Almost all the parts of this plant; root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia (Wealth of India, 1962; Singh and Kumar, 1999; Morimitsu *et al.,* 2000; Siddhuraju and Becker, 2003).

The root of *Moringa oleifera* tree is used as an antilithic, rubefacient, vesicant and a carminative. The root of *Moringa oleifera* tree can be used in the treatment of infertility and inflammation, rheumatism, articular pains, lower back or kidney pain and constipation. It can also act as a stimulant in paralytic afflictions (act as a cardiac/circulatory tonic), and can be used as a laxative (Wealth of India, 1962; Dahort, 1998; Ruckmani *et al.,* 1998).

The *Moringa oleifera* leaf (MOL) is used as a purgative; applied as poultice to sores; rubbed on the temples for headaches; used in treatment of piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh. The leaf juice is believed to control glucose levels, and is applied to reduce glandular swelling (Wealth of India, 1962; Dahort, 1998; Morton, 1991; Makonen *et al.,* 1997; Fuglie, 2001). *Moringa oleifera* leaves have been reported to be a rich source of C-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Dillard and German, 2000; Siddhuraju and Becker, 2003). The stem bark is used in the treatment of eye diseases, ear-aches and pain killer in tooth aches. It destroys tumours and heals ulcers (Siddhuraju and Becker, 2003).

The flower of MO has a high medicinal value as a stimulant, aphrodisiac, abortifacient and cholagogue. It is used to cure inflammations, muscle diseases, hysteria, tumours, and enlargement of the spleen. It lowers the serum cholesterol, phospholipid, triglyceride, very low density lipoprotein (VLDL), low density lipoprotein (LDL), cholesterol to phospholipid ratio and atherogenic index; decrease lipid profile of liver, heart and aorta in hypercholesterolaemic rabbits and increases the excretion of faecal cholesterol (Nair and Subramanian, 1962; Dahort, 1998; Siddhuraju and Becker, 2003; Mehta *et al.,* 2003).

The seeds of MO are considered to be antipyretic (Oliveira *et al.,* 1999) and reported to show antimicrobial activity (Wealth of India, 1962). The seed can be consumed fresh as peas; or pounded, powdered and steeped for tea or used in curries roasted, or pressed into sweet, non-desiccating oil, commercially known as "Ben oil" of high quality. The unique property is the ability of its dry, crushed seed and seed press cake, which contain polypeptides, to serve as natural coagulants for water treatment (Gassenschmidt *et al.,* 1995; Ndabigengesere and Narasiah, 1998). *Moringa oleifera* seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication, and in the manufacture of perfume and hair care products (Tsaknis *et al.,* 1999). In West Africa, one of the best known uses of MO is the use of powdered seeds to flocculate contaminants and purify drinking water (Berger *et al.,* 1984; Olsen, 1987), the seeds are as effective as aluminum sulphate (alum) in removing suspended solids from tubid water (Donovan, 2007).



Figure 2.1: *Moringa olerfera* Tree.

2.1.5 Proximate analysis of *Moringa oleifera* leaves

Moringa oleifera leaf has been reported to contained appreciable amounts of crude protein (17.01%), carbohydrate (63.11%), crude fibre (7.09%), ash (7.93%), crude fat (2.11%), fatty acid (1.69%) and energy (1440.11kcal/kg) (Ogbe and Affiku, 2011). However, Aye and Adegun (2013) noticed that on a dry matter basis, MOL contained crude protein (22.23%), ash (7.96%) and crude fibre (6.77%). Oduro *et al*. (2008) reported that MOL contained crude protein (27.51%), crude fibre (19.25%), crude fat (2.23%), ash (7.13%), moisture (76.53%), carbohydrate (43.88%), and caloric value of 1296.00 kJ/g (305.62 cal/g). Foidl and Paull (2008) reported that the protein content of MOL is high (20–35% on a dry weight basis) and the protein is of high quality having significant quantities of all the essential amino acids.

In another study, Yameogo *et al.* (2011) reported that, on dry matter basis, protein, moisture, fat and carbohydrate contents of MOL were found to be 27.2%, 5.9%, 17.1% and 38.6%, respectively. However, Gupta *et al*. (1989) also reported that MOL leaves contained crude protein, crude lipids and ash values of 26.4%, 6.5% and 12%, respectively. Verma *et al.* (1976) and Hartwell (1971) had earlier reported MOL to contain per 100 g; 7.5 g H_2O, 6.7 g protein, 1.7 g fat, 14.3 g total carbohydrate, 0.9 g fiber, and 2.3 g ash. Fuglie (1999) reported the methanolic leaf extract of MO to contain 43.5%, 1.4% and 10.0% of crude protein, crude fat (lipid) and ash, respectively.

2.1.6 Phytochemical analysis of *Moringa oleifera* leaves

Phytochemicals are the non-nutritive plant chemicals that have protective or disease preventive properties which are generally found in plants. Examination of the

phytochemicals of MOL gives an opportunity of examining a range of fairly unique compounds that the specie contains (Fahey *et al*., 2001). Ogbe and Affiku (2011) reported that MOL contained low quantities of tannins (21.19%), phytates (2.57%), trypsin inhibitors (3.0%), saponins (1.6%), oxalates (0.45%) and low levels of cyanide (0.1%). Ojo (2011) reported the presence of useful compounds such as tannins, flavonoids, alkaloids, carbohydrate and phlobatannin, saponins, and cardiac glycoside from the aqueous leaf extract of MOL. Also, in another study, Didacus *et al*. (2013) reported that the concentrations of saponin, glycoside, steroid and reducing sugars were higher in the methanolic extract of MOL when compared to other chemical agents.

2.1.7 Mineral analysis of *Moringa oleifera* leaves

The quantities of minerals contained in MOL are higher when compared with the leaves from other plants. Aye and Adegun (2013) showed that minerals such as Na, Ca, Mg, K, Zn, Fe, Mn, Cu are higher in MOL than *Leucaena* and *Gliricidia* leaves. Ogbe and Affiku (2011), reported MOL to contain appreciable amount of minerals such as Mn (81.65), Zn (60.06) and Cu (6.1), while Mutayoba *et al*. (2011) reported values of 57.34, 21.70 and 5.73 parts per million (ppm) for Mn, Zn and Cu, respectively and Fe (318.81), Ca (2.47%), K (1.63%) and Mg (1.03%). Bouatene *et al*. (2011) reported a value of 1.36%, 0.44%, 3.60% and 0.02% for Ca, P, K, and Na, respectively, while Kakenji *et al*. (2003) observed that MOL contain 27.9% calcium and 0.26% phosphorus. Oduro *et al*. (2008) reported that calcium and iron content of MOL in mg/100 g (DM) were 20.09 and 28.29, respectively. The result of the mineral analysis of MOL by Ojo (2011) showed that Ca (30.44 ppm) had

the highest concentration, followed by Mg (5.67 ppm), Fe (3.06 ppm) and Zn (2.46 ppm) while, copper (Cu) was detected in trace quantity (0.07 ppm).

2.1.8 Nutritional evaluation of *Moringa oleifera* leaves inclusion in feeds of animals

Nutrition plays a major role in animal's ability to overcome the detrimental effects of parasitism and other diseases (Anwar *et al.,* 2007). A well-nourished animal resists diseases even when exposed to infection. When an animal is exposed to pathogens, the animal's immune system mounts a response to fight the infection. This includes raising antibodies to fight the infection, as well as using white blood cells to attack pathogens (FAO, 2002). *Moringa oleifera* tree is a typical multipurpose tree of significant economic importance and this makes it to be one of the world's most useful trees, because almost every part of the MO tree can either be used as food, medication or for industrial purposes (Ramachandran *et al.,* 1980; Khalafalla *et al.,* 2010).

A high degree of renewed interest was placed on the nutritional properties of MO in most countries where it was not native (Reyes *et al.,* 2006; Oduro *et al.,* 2008). This could be due to the claims that it increases animal productivity as it has nutritional, therapeutic and prophylactic properties (Fahey, 2005). The use of MO as an animal feed is not very common but the results of its chemical composition beside the absence of harmful objects make it as a successful animal feed ingredient (Mabruk *et al.,* 2010).

Moringa oleifera leaves are the preferable part of the tree to be used in the animal diet as leaf meal. Several researches were conducted to study the effect of this leaf meal on the growth performance of layer chicks (Aberra, 2011), on the productive performance of

laying hens (Kakengi *et al.,* 2007; Olugbemi *et al.,* 2010a; Abou-Elezz *et al.,* 2011) on broilers performance (Juniar *et al.,* 2008; Olugbemi *et al.,* 2010b) and on the growth, carcass and blood indices of weaner rabbits (Nuhu, 2010). The effect of MO seeds on broilers performance was also examined by Nuhu (2010). Other researches also conducted were the protective efficacy of *Moringa oleifera* during aflatoxin exposure in broilers (Rajendran *et al.,* 2012). The effects of *Moringa oleifera* methanolic leaf extract on the morbidity and mortality of chickens experimentally infected with Newcastle disease virus (Kudu 113) strain (Didacus *et al.,* 2013) was also reported.

Moringa oleifera leaf has been shown to have influence on weight gain, feed intake, feed conversion ratio, nitrogen digestibility and the economics of rabbit production when groundnut cake was replaced with MOL (Adeniji and Lawal, 2012). In another study conducted by Bouatene *et al*. (2011) to determine the effect of MOL on growth performance and health status of post-weaning rabbits, showed that the supplementation of MOL at 3% in the feed, gave the best results in terms of gross weight, growth rate and survival of young rabbits. Dougnon *et al*. (2012) showed that pellets of MOL can be substituted at a level of 15% to commercial feed with a positive effect on weight gains and carcass characteristics of rabbits. The replacement of *Centrosema pubescens* leaves with MOL gave no adverse effects on the reproductive performance of 20-week old rabbits (Odeyinka *et al*., 2008). The substitution of sunflower seed meal (SSM) with MOL as protein source in the diet of egg strain commercial chickens showed a better efficiency at 10% inclusion level (Kakengi *et al.,* 2007).

The effect of MOL on the performance and blood chemistry of starter broilers showed that MOL can be included at 7.5% in the diet of broilers without any deleterious effect on performance and blood characteristics (Onu and Aniebo, 2011). Olugbemi *et al.* (2010a) concluded that broilers can be safely fed cassava based diets containing MOL at a maximum level of 5% without deleterious effects. Exploratory study to investigate the effects of supplementing soya beans with MOL, showed that inclusion of MOL supplement in broiler diets at 25% inclusion level produces broilers of similar weight and growth rate compared to those fed with conventional commercial feeds (Gadzirayi *et al.*, 2012) and MO aqueous leaf extract given via drinking water regardless of concentration level significantly improved growth performance of Cobb broilers based on feed consumption, live weight, feed conversion ratio and return of investment (Portugaliza and Fernandez, 2012).

2.2 Free Radicals, Reactive Oxygen Species, and Oxidative Stress

An atom consists of a central nucleus with pairs of electrons orbiting around it. However, some of these atoms and molecules have unpaired electrons which are called free radicals (Kohen and Gati, 2000). These free radicals are usually unstable and are highly reactive because the unpaired electrons tend to form pairs with other electrons (Yoshikawa and Naito, 2002). Free radicals are types of reactive oxygen species (ROS), which include all highly reactive oxygen-containing molecules such as the hydroxyl radical and hydrogen peroxide (Kohen and Gati, 2000). These molecules are continuously generated inside the animal body as a consequence of exposure to a plethora of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving oxidation and reduction (redox) enzymes and bio-energetic electron transfer (Sreelatha and Padma, 2009).

Similarly, an oxygen molecule (O_2) undergoes four-electron reduction when it is metabolized *in vivo*. During this process, reactive oxygen metabolites are generated by the excitation of electrons secondary to addition of energy or interaction with transition elements. The reactive oxygen metabolites thus produced are more highly reactive than the original oxygen molecule and are called Reactive Oxygen Species (ROS). Superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen are ROS in the narrow sense. However, if ROS or free radicals are generated excessively, the balance between formation and removal is lost, resulting in oxidative stress (Yoshikawa and Naito, 2002). Oxidative stress occurs when the balance of formation of oxidant exceeds the ability of antioxidant systems to remove ROS (Stadtman, 1990; Dean *et al.*, 1997; Stadtman and Berlett, 1998).

Therefore, oxidative stress is an abnormal phenomenon occurring inside the cells or tissues of animals when production of oxygen radicals exceeds their antioxidant capacity. Excess of free radicals, damage essential macromolecules of the cells, leading to abnormal gene expression, disturbance in receptor activity, proliferation or cell death, immunity perturbation, mutagenesis, protein or lipofuscin deposition (Favier, 2006). Oxidative stress is the major cause of reduction in growth rate in broilers and increase incidence of infection and metabolic disease in poultry (Sen, 1995).

The conversion of oxygen to water produces high ROS that are known to induce the peroxidation of poly-unsaturated lipids in cell membranes and to cause degradation of structural proteins, enzymes and ribonucleic acids (Fridovich, 1978; Fridovich and Freeman, 1986). Also, oxidants are ROS which have the ability, either directly or indirectly, to damage all biomolecule, including proteins, lipids, DNA and carbohydrates.

Reactive oxygen species such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) are generated under numerous conditions *in vivo*. Under normal circumstances, there is a well-managed balance between formation and neutralization of ROS so that there is minimal modification of biomolecules. Reactive oxygen species was ascertained to play multiple and important roles in tissue damage and loss of function in a number of tissues and organs (Zheng and Huang, 2001).

Antioxidants are compounds capable of either delaying or inhibiting the oxidation processes which occur under the influence of atmospheric oxygen or ROS. Antioxidants are involved in the defence mechanism of the organism against the pathologies associated to the attack of free radicals. Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or nonenzymatic compounds, such as uric acid, bilirubin, albumin and metallothioneins. When endogenous factors cannot ensure a rigurous control and a complete protection of the organism against the ROS, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain active principle as antioxidant compound.

Amongst the most important exogenous antioxidants, vitamin E, vitamin C, β-carotene, flavonoids, mineral selenium are well known, but also vitamin D and vitamin K_3. Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc (Litescu *et al.*, 2011). There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed

deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs (Molyneux, 2004).

Antioxidants have attracted considerable attention in relation to free radicals and oxidative stress, cancer prophylaxis and therapy, and longevity (Kalcher *et al.*, 2009). Phenols and polyphenols are the target analytes in many such cases; they may be detected by enzymes like tyrosinase or other phenol oxidases, or even by plant tissues containing these enzymes (Ly, 2008). The recommendations based on epidemiological studies are such, that fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract (Halvorsen *et al.*, 2002).

There are numerous antioxidants in dietary plants: carotenoids, phenolic compounds, benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans, and lignins (Lindsay and Astley, 2002). Of the 50 analysed food products with high antioxidant content (Halvorsen *et al.*, 2006), thirteen were spices, eight fruits and vegetables, five berries, five chocolate based, five breakfast cereals, and four nuts or seeds. Antioxidants are known to play a key role in the protective influence exerted by plant foods (Liyana-Pathirana *et al.*, 2006; Rodríguez-Bernaldo de Quirós and Costa, 2006).

Moringa oleifera has been reported to possess some antioxidant properties (Sreelatha and Padma, 2009; Atawodi *et al*., 2010). Although there are several enzyme systems within the body which scavenge free radicals, the natural (vitamin) antioxidants are vitamin E, bêta-carotene, and vitamin C (Nair *et al*., 2003). These micronutrient antioxidants may be used

as defence system to prevent free radicals from damaging the animal's body. This therefore provides protection to animals against infections and degenerative diseases (Sreelatha and Padma, 2009; Verma *et al.*, 2009). A survey conducted by Yang *et al.* (2006) and Jung *et al*. (2010), on 120 edible plant species showed that *Moringa oleifera* was among the most promising species based on their high antioxidant activity, high contents of micro-nutrients and phytochemicals, processing properties, ease of growing, and also on palatability, stability and shelf life of meat products.

2.3 Infectious Bursal Disease

2.3.1 History of infectious bursal disease in Nigeria

Infectious bursal disease (IBD) was first observed in 1969 in Western and Midwestern States of Nigeria, and later reported by Ojo in 1973 (Okoye and Uzoukwu, 2001), eleven years after its initial appearance in Gumboro, Delaware, USA (Cosgrove, 1962). The first diagnosed outbreak of IBD occurred in Zaria in October 1972 (Ojo *et al*., 1973; Okoye and Uzoukwu, 2001). In 1975, Onunkwo further confirmed the presence of IBD in Nigeria at Vom, Plateau State. Previously, a disease with similar clinical signs and postmortem lesions had occurred at the poultry farm of the School of Agriculture, Ahmadu Bello University (ABU), Zaria, Nigeria in 1970 (Agbede, 1975). In 1977, it was included in the list of diseases to be compulsorily reported annually in Nigeria (Onukwo and Momoh, 1981). In the south of Nigeria, there was a report of about 140 outbreaks of disease involving 105 million chickens annually (Nawathe and Lamorde, 1982).

2.3.2 Transmission of infectious bursal disease virus

Only horizontal transmission of IBDV has been described, with healthy chickens being infected by the oral or respiratory routes. Infected chickens excrete the virus in faeces as early as 48 h after infection, and may transmit the disease by contact over a sixteen-day period (Vindevogel *et al.,* 1976), though the possibility of persistent infection in recovered birds has not been researched. The disease is transmitted by direct contact with excreting chickens, or by indirect contact with any inanimate or animate (farm staff, animals) contaminated vectors. Some researchers have suggested that insects may also act as vectors (Howie and Thorsen, 1981). The extreme resistance of the IBDV to the outside environment enhances the potential for indirect transmission. The virus can survive for four months in contaminated beddings and premises (Benton *et al.,* 1967), and up to fifty-six days in lesser mealworms *(Alphitobius* spp.) taken from a contaminated building (McAllister *et al.,* 1995). In the absence of effective cleaning, disinfection and insect control, the resistance of the virus leads to perennial contamination of infected farm buildings (McAllister *et al.,* 1995).

2.3.3 Aetiology of infectious bursal disease

Infectious bursal disease is caused by an IBDV (Dobos, 1979; Kibenge *et al.,* 1988; Lukert and Saif, 2003) that is non-enveloped and has a bisegmented double-stranded RNA (dsRNA) genome (Kibenge *et al.,* 1988; Da Costa *et al.,* 2003). Infectious bursal disease virus is a member of the family *Birnaviridae* (Dobos, 1979). The family consist of three genera that includes; *Aquabirnavirus* whose species includes the infectious pancreatic necrosis virus (IPNV) and infects fish, molluscs and crustaceans; *Avibirnavirus* whose species includes IBDV and infects birds and *Entomobirnavirus* whose species includes

Drosophila X virus (DXV) and infects insects (Delmas *et al.,* 2004). The virus in these genera is packaged into single-shelled, non-enveloped virions (Müller *et al.,* 1979; MacDonald, 1980).

Infectious bursal disease virus exists in two antigenically distinct serotypes, I and II. Serotype I strain are pathogenic to chickens and usually vary in their virulence whereas serotype II strains are not pathogenic and usually do not cause mortality or bursal lesions in specific pathogenic strains and are isolated from chickens and turkeys (Jackwood and Saif, 1983; Lukert and Saif, 2003). Based on their pathogenicity, IBDV antigenic serotype I can further be classified into 4 different strains that include classical, variant, attenuated and very virulent strains (Snyder, 1990; Lim *et al.,* 1999).

2.3.3.1 Classical strain of infectious bursal disease virus

Classical strain of IBDV has been isolated worldwide from affected poultry since the first reported case of Gumboro disease. Birds infected with classical strain of IBDV usually suffer severe lymphoid necrosis and inflammation of the BF, thereby resulting in immunodeficiency and moderate mortality of between 20-30% in specific pathogen free (SPF) chickens (Lim *et al.,* 1999).

2.3.3.2 Variant strain of infectious bursal disease virus

Antigenically, variant strain of IBDV are known for their ability to escape cross neutralization by antiserum against the classical strain and also cause a rapid and a severe bursal atrophy with no clinical signs of illness in contrast to the classical strain of IBDV (Vakharia *et al.,* 1994; Lim *et al.,* 1999).

2.3.3.3 Attenuated strain of infectious bursal disease virus

Attenuated strains of IBDV are apathogenic and have been used as live vaccines. They are generated as a result of adapting the classical and the variant strains of IBDV to chicken embryo fibroblast (CEF) or other cell lines (Lim *et al.,* 1999).

2.3.3.4 Very virulent strain of infectious bursal disease virus

The primary feature of vvIBDV is the ability to induce high mortality. The clinical sign induced by infection with a vvIBDV is similar to that observed when the classical strain of IBDV is involved except that infection with a vvIBDV is more acute and the disease and lesions are more pronounced in individual bird and are generalized in flocks. Haemorrhages are pronounced in the BF and muscles with a rapid bursal and thymic regression. However, the microscopic lesions of vvIBDV are similar to those observed in the classical strain of IBDV (van der Berg, 1991). Very virulent infectious bursal disease virus has an incubation period of 4 days and are characterised by a high mortality range of 5-25% in broilers and 60-100% in layers (van der Berg, 2000).

2.3.4 Pathogenesis of infectious bursal disease

The selected host of the IBDV is young chickens where a clinical disease occurs, while in older birds the infection is essentially subclinical. Susceptibility of different breeds has been described with higher mortality rates in the lighter (layers) than in the heavier (broilers) breeds (Bumstead *et al.*, 1993; Nielsen *et al.*, 1998). Inoculation of IBDV in other avian species fails to induce disease (McFerran, 1993). The target organ of IBDV is the BF at its maximum development, which is a specific source of B lymphocytes in avian species. Bursectomy can prevent illness in chicks infected with virulent IBDV (Hiraga *et al.*, 1994).

The severity of IBDV is directly related to the number of susceptible cells present in the BF.

Therefore, the highest age of susceptibility is between 3 and 6 weeks, when the BF is at its maximum development. This age susceptibility is broader in the case of vvIBDV strains (van den Berg *et al.*, 1991; Nunoya *et al.*, 1992). After oral infection or inhalation, the virus replicates primarily in the lymphocytes and macrophages of the gut-associated tissues. Then virus travels to the bursa via the blood stream, where replication will occur. By 13 h post inoculation (pi), most follicles are positive for virus and by 16 h pi, a second and pronounced viraemia occurs with secondary replication in other organs leading to disease and death (Müller *et al.*, 1979).

Similar kinetics is observed for vvIBDVs but replication at each step is amplified. Actively dividing, surface immunoglobulin M-bearing B cells are lysed by the virus (Hirai and Calnek, 1979; Hirai *et al.*, 1981; Rodenberg *et al.*, 1994). But cells of the monocyte–macrophage lineage can be infected in a persistent and productive manner, and play a crucial role in dissemination of the virus (Burkhardt and Müller, 1987; Inoue *et al.*, 1992; van den Berg *et al.*, 1994), and in the onset of the disease (Sharma and Lee, 1983; Kim *et al.*, 1998; Lam, 1998). The exact cause of clinical disease and death is still unclear but does not seem to be related only to the severity of the lesions and the bursal damage. Indeed, after infection, some birds with few bursal lesions can be found dead, while others can survive despite extensive bursal damage (Kim *et al.*, 1998; Lam, 1998).

Moreover, mortality rates are often variable and the establishment of median lethal dose for standardization has always been hazardous. In addition, the narrow age range for

susceptibility to clinical disease has not yet been clearly explained. Depletion of lymphoid cells in the BF after IBDV infection is due to necrosis. Apoptosis, or programmed cell death, is a process where, in response to specific stimuli, cells die in a controlled, programmed manner. Many different cell species can undergo apoptosis but immature B and T cells are particularly susceptible to apoptotic cell death (Sharma and Lee, 1983; Kim *et al.*, 1998; Lam, 1998).

2.3.5 Clinical signs and forms of infectious bursal disease

Clinical signs of IBD in chicken are; sudden onset of sickness including trembling, weakness, depression, somnolence, ruffled feathers, drooped wings, anorexia, prostration, yellow or whitish diarrhoea (Bishu *et al*., 1977; Okoye and Uzoukwu, 2001) and reluctance to move (Onukwo, 1975; Okoye and Uzoukwu, 2001). The clinical signs of IBD vary considerably from one farm, region, country or even continent to another. Schematically, the global situation can be divided into three principal clinical forms, as follows:

2.3.5.1 The classical form of infectious bursal disease

As described since the early 1960s, IBD is caused by the classical virulent strains of IBDV. Specific mortality is relatively low (20%), and the disease is most often subclinical, occurring after a decline in the level of passive antibodies (Faragher, 1972).

2.3.5.2 The immunosuppressive form of infectious bursal disease

Principally described in the USA, this form of IBD is caused by low-pathogenic strains of IBDV, as well as by variant strains, such as the Delaware variant E which partially resist

neutralisation by antibodies against the so-called 'classical' viruses (Jackwood and Saif, 1987; Snyder, 1990).

2.3.5.3 The acute form of infectious bursal disease

This form of IBD, first described in Europe and then in Asia and is caused by 'hypervirulent' strains of IBDV, and is characterised by an acute progressive clinical disease, leading to high mortality rates (50-70%) in affected farms (Chettle *et al.*, 1989; Stuart, 1989; van den Berg *et al.*, 1991).

2.3.6 Infectious bursal disease antibodies

Infectious bursal disease antibodies (IBDAbs) in breeders and maternal derived antibodies (MDAs) in chicks are prevalent and their concentration depends on the source and age of birds and the type of vaccine (live or attenuated) used (Okoye and Uzuokwu, 2001). It has been reported that chicks that are 3 or 4 weeks old are invariably devoid of MDA (Okoye and Uzuokwu, 2001). The presence of active immunity prevents antibody production or delays its rate of production and consequently the level produced (Okoye and Uzuokwu, 2001). Chicks should be vaccinated before the age of 6 weeks, preferably 3 and 4 weeks of age (Abdu *et al*., 2001).

Maternally derived antibody does interfere with vaccination preventing active antibody production (Abdu *et al.*, 2001). All vaccinated birds usually sero-convert at one (Okeke *et al*., 1982) two (Abdu, 1988) or three (Aba-Adulugba *et al*., 1992) weeks after vaccination. The level of antibodies produced depend on type of IBD vaccine administered (Okeke *et al*., 1982; Aba- Adulugba *et al*., 1992; Okoye and Uzuokwu, 2001) and ranged between 5 and 6 $\log_2$ and the highest antibody level recorded for vaccinated birds was 10.0 $\log_2$ (Aba-

Adulugba *et al*., 1992; Abdu *et al.*, 2001). A survey of antibody in vaccinated birds showed that 75 to 100% of chicks that are one, two, and three or four weeks old remained sero-negative despite vaccination but by the third week of age or older 53 to 100% of vaccinated bird had sero converted (Abdu, 1990). Results so far suggested that the outcome of vaccination depend on the type of vaccine administered, how the vaccine was handled and administered and the presence or absence of MDAs at time of vaccination (Abdu, 1987). Antibodies following IBDV challenge were detected seven days post infection in 100% of susceptible chicks aged 22-29 days in Zaria and the level of antibodies was highest in older birds (Abdu, 1987).

2.3.7 Serological test for detection of infectious bursal disease antibodies

In areas contaminated by IBDV, most broiler flocks have anti-IBDV antibodies when leaving the farm. Current serological tests cannot distinguish between the antibodies induced by pathogenic IBDV and those induced by attenuated vaccine viruses, so serological diagnosis is of little interest in endemic zones. Nonetheless, the quantification of IBDV induced antibodies is important for the medical prophylaxis of the disease in young birds, in order to measure the titre of passive antibodies and determine the appropriate date for vaccination (Muskett, 1979) or in laying hens to verify success of vaccination (Lucio, 1987). Serology is likewise essential to confirm the disease-free status of specific pathogen free (SPF) flocks. Each serological analysis must include a sufficient number (at least twenty) of individual serum samples representative of the flock under study (Lucio, 1987).

A kinetic study requires at least two serological analyses separated by an interval of three weeks (paired sera). The most widely used quantitative tests are the detection of precipitating antibodies by agar gel immunodiffusion (AGID) (Hirai *et al.*, 1997), enzyme-linked immunosorbent assay (ELISA) (Marquardt *et al.*, 1980), and serum neutralization (SN) in cell culture (Weisman and Hitchner, 1978). Agar gel immunodiffusion is the simplest, but least sensitive technique. Results are obtained after an incubation period of 48 hours. Variability in results may be due to the investigator, as well as the nature of the viral strain used as an antigen (Becht *et al.*, 1988). Serum neutralization presents the disadvantages of using specialised equipment and five days incubation are required (Weisman and Hitchner, 1978).

Serum neutralization technique is much more sensitive than AGID and correlates better with the level of protection of the subjects tested (Rooney and Freund, 1988). The ELISA is the most rapid and sensitive method, and presents the fewest variations due to the viral strain used as an antigen (Rooney and Freund, 1988). Considerable inter and intra-laboratory variability can occur with certain commercial kits. Although the correlation between results obtained using SN and ELISA is high, ELISA remains less sensitive compared to SN, and does not detect low neutralising titres which are sufficient to block vaccine administration (residual maternal antibodies) (Rooney and Freund, 1988). Enzyme-linked immunosorbent assays which use a recombinant VP2 protein as the sole antigen may be better correlated with protection (van den Berg *et al.*, 1997).

2.3.8 Prevention and control of infectious bursal disease

The high stability of IBDV to different environmental conditions has complicated attempts to control infection by hygienic means, and made vaccination inevitable (Lukert and

Hitchner, 1984). The IBDV can persist in poultry houses for several weeks or months even after thorough cleaning and disinfection has been done (Lukert and Hitchner, 1984). Its resistance to inactivation accounts for its persistent survival on poultry farms, despite disinfection (Benton *et al.,* 1967; ven der Berg *et al.,* 2000; Eterradossi and Saif, 2008). Infectious bursal disease virus is more resistant to heat and ultraviolet light than reovirus; more resistant to ether and chloroform, though it is inactivated at pH 12.0, and it can however, remain infectious at pH 2.0 (Benton *et al.,* 1967; Petek *et al.,* 1973). The virus is generally environmentally stable and is resistant to many physical and chemical agents (Cosgrove, 1962; Lukert and Saif, 2003).

It is a well known fact that IBDV is highly contagious and therefore any contact between IBDV infected and uninfected birds or uninfected and contaminated formite could result in the spread of the infection. Therefore, the application of standard biosecurity measures has to be implemented in order to stop the spread of the virus from one flock to another. Although, the integrated nature of commercial poultry operation and the activities of vectors like the lesser meal worm, mosquitoes and rats pose extra problems for the control of this infection (ven der Berg *et al.,* 2000).

There is still no therapy that has an effect on the course of the viral infection (Cosgrove, 1962; Lukert and Saif, 2003) and there are no reports of the use of any antiviral compounds and interferon inducers in the treatment of IBD (Lukert and Saif, 2003). Therefore, even with strict biosecurity programmes such as “down time” between broods; all-in/all-out production; cleaning and disinfecting of premises and equipment, vaccination remains an important practice that will reduce the incidence and impact of IBD in the poultry industry (ven der Berg *et al.,* 2000; Eterradossi and Saif, 2008).

Given the contagious nature of IBD and the resistance of IBDV to various agents, it is important that certain steps be adhered to during cleaning and disinfecting of poultry houses. Prior to cleaning, all insects and pests (e.g rats and mice) must be eliminated as soon as the farm premises are empty. Old bedding and dung must be eliminated and composted. All farm equipment must be disassembled and stored in clean rooms located outside the farm buildings. The buildings, immediate surroundings and farm equipment must be dry-cleaned first, in order to eliminate all dust, and then washed using hot water (60°C) with detergent. A second disinfection of full premises must be performed before the introduction of the chicks. Feed silos must be emptied completely and cleaned inside and outside. Under no circumstance may feed remain from previous flocks be reused. Disinfection is to be undertaken only after all the buildings have been cleaned. Most disinfectants are more active at a temperature above 20°C; however, chlorinated and iodinated disinfectants cannot be heated above 43°C (Meroz and Samberg, 1995).

2.3.9 Vaccination

Attenuated live virus vaccines and inactivated virus vaccines are the two types of vaccines used against IBDV (Thiry *et al.,* 1994). Thornton in 1977 (Thornton and Pattison, 1975) laid down a general principle governing the choice and use of vaccines which remains valid till this day. An ideal vaccine must offer the correct balance between efficacy and innocuity (Gough *et al.,* 1998), the vaccine must not cause disease or bursal lesions, and must not be immunosuppressive or excreted, but must confer long-lasting immunity even in birds with a high level of maternal immunity (McFerran, 1993). The dose and strains of the vaccine and challenge viruses, the route of administration, the appropriate vaccination time, as well as,

the levels of MDAs are important factors that determine the efficacy of a vaccine (McFerran, 1993).

2.3.9.1 Live infectious bursal disease vaccines

Live IBD vaccines are made by the attenuation of the strains of the IBDV through serial passages in embryonated eggs. The IBD vaccines are then classified as either mild, mild intermediate, intermediate, intermediate plus or "hot," depending on the degree of virus attenuation, and this attenuated vaccine strains can cause histopathological lesions of varying severity to the BF of SPF chickens (OIE, 2000; Eterradossi and Saif, 2008).

Live attenuated mild strains of IBD vaccines that are passaged in eggs were first developed in 1968. Some of the early vaccines included the viruses that were isolated by Edgar, Mouthrop-Snedecker and Winterfield. While Edgar's isolate was a moderately pathogenic bursa-origin virus strain used as a live vaccine (Mazariegos *et al.,* 1990), the other two isolates were attenuated by serial passage in eggs and became some of the first commercially available vaccines, Bursa Vac® (Lasher and Davis, 1997) and IBD.Blen™ (Winterfield *et al.,* 1981) respectively. These vaccines reduced clinical signs but caused significant bursal pathology (Winterfield *et al.,* 1981).

Lukert attenuated a field isolate by passaging in different cell culture systems and this strain served as the seed virus for many vaccines developed during the 1980's (Lasher and Davis, 1997). The use of tissue culture derived vaccines have been found to be less pathogenic than the embryo derived vaccines and are also less effective in stimulating active immunity

in chickens having MDA (Winterfield and Thacker, 1978). It is now known that the pathogenicity of the live IBD vaccines is inversely proportional to their attenuation (Lasher and Shane, 1994).

The use of live and killed vaccines made from classic strain of IBDV offered limited protection against variant strains of IBDV's but in contrast; the use of IBDV vaccine made from the variant strain gives protection against classical strain as well as the homologous and heterologous variant IBDV strains (Rosenberger *et al.,* 1987a; Rosenberger *et al.,* 1987b; Ismail and Saif, 1991). Live and killed vaccines that included both the classical and variant strains of IBDV have since been reformulated in order to broaden the range of antigenic subtypes and elicit a heightened immune response (Ismail and Saif 1991; Jackwood *et al.,* 2001; Jackwood and Sommer-Wagner, 2005).

The use of the classical live attenuated vaccines may induce a broad, lifelong protection, but has a disadvantage of retaining its residual pathogenicity as well as the potential to revert to virulence (Lukert and Rifuliadi 1982; Guittet *et al.,* 1992; van der Berg *et al.,* 2000). Mild strains of IBDV vaccines are predominantly used for breeder vaccinations, but due to their extreme sensitivity in interfering with homologous maternal antibody, they are normally administered between four and eight weeks of age when MDAs have waned depending on whether or not the grandparent flocks have been vaccinated with an oil-emulsion inactivated vaccine before lay (van der Berg *et al.,* 2000).

The intermediate strains of IBD vaccines are frequently administered to broilers and pullets, and also to chicks in the breeder flocks which are at risk of challenge by highly

pathogenic strains at an early age (Mazariegos *et al.*, 1990), and may also be administered by nebulisation to day-old broiler chicks in order to protect those who possess low levels of maternal antibodies at hatch. Some reasons for such early vaccination are to bring about replication of the vaccine virus in the chicks, and the dissemination of the virus within the farm; this would, at least partially, provide indirect vaccination to the other chicks at a time when they become sensitive to the infection (van der Berg *et al.*, 2000). Less attenuated (hot) strains of IBDV vaccines are known to cause histological lesions in SPF chickens and may provoke immunosuppression (van der Berg *et al.*, 2000). Live-attenuated IBDV vaccines are administered via drinking water application or nebulisation between the ages of 7 days and 2 or 3 weeks of age (van der Berg *et al.*, 2000; Eterradossi and Saif, 2008).

Live IBD vaccines are compatible with other avian vaccines. However, the IBDV strains that cause serious lesions to the BF may also provoke immunosuppression and exacerbate the pathogenicity of other immunosuppressive viruses such as Marek's disease virus (MDV) and chicken anaemia virus (CAV) which will jeopardise the immunisation of poultry against other diseases. Therefore, registration procedures for these vaccines must include tests to verify the absence of interference with other vaccinations as well as the absence of reversion to virulence in the course of serial passages in three- to six-week-old SPF chickens.

Infectious bursal disease can also be controlled by *in ovo* vaccination of embryos. This procedure involves using a mixture of IBD virus and a specific IBD antibody in a vaccine to inoculate 18-days-old chicken embryo (Whitfill *et al.*, 1995; Haddad *et al.*, 1997; Gagic *et al.*, 1999). This method helps to avoid interference by MDAs, while effectively initiating

a primary antibody response, and broiler chicks hatched from these eggs are immunised against IBDV throughout the growing period (Haddad *et al.,* 1997; Gagic *et al.,* 1999; Giambrone *et al.,* 2001). Various vaccines using recombinant viruses expressing the VP2 protein of IBDV have been described, and have proven efficacy in laboratory tests, although, no commercial version of these vaccines is currently available. The advantages of these vaccines are the absence of residual pathogenicity, sensitivity to MDAs and risk of selection of mutants, as well as the possibility of use *in-ovo* and of differentiation between infected and vaccinated animals (Bayliss *et al.,* 1991; Heine and Boyle 1993; Thiry *et al.,* 1994; Darteil *et al.,* 1995; Tsukamoto *et al.,* 1999).

2.3.9.2 Inactivated infectious bursal disease vaccines

Inactivated IBD vaccines in an oil adjuvant have the capacity of producing high, uniform and persistent antibody titres in breeder hens between the age of sixteen to twenty weeks (point of lay), thereby boosting the levels of MDAs that results in a longer-lasting immunity (Cullen and Wyeth, 1976; Wyeth and Cullen, 1978; Wyeth and Chettle, 1990; Guittet *et al.,* 1992). However, the duration and uniformity of this immunity may be influenced by the concentration and antigenic specificity of the vaccine strain (van de Berg *et al.,* 2000). Many of these IBD oil-adjuvant vaccines contain both classic and variant IBDV strains (Eterradossi and Saif, 2008).

The inactivated IBDV vaccine in an oil adjuvant does not stimulate a primary antibody response in chicks, so therefore, they are most effective when used only in birds that must have been "primed" with a live IBD vaccine or have had an exposure naturally to a field IBD virus (Wyeth and Cullen, 1978; Mazariegos *et al.,* 1990; Wyeth and Chettle, 1990;

Guittet *et al.,* 1992; Wyeth *et al.,* 1992; Eterradossi and Saif, 2008). These vaccines do not protect against vvIBDV strains (Mazariegos *et al.,* 1990; Van den Berg and Meulemans, 1991), and are usually administered by subcutaneous or intramuscular injection at the age of sixteen to twenty weeks (van de Berg *et al.,* 2000).

Breeder flocks are usually hyperimmunized with live and killed vaccines in order to confer high titre levels of MDAs to their progeny (van der Berg *et al.,* 2000). This passive immunity protects chicks against early immunosuppressive infections for 1 to 3 weeks of age; however, protection may be extended to 4 or 5 weeks by boosting the immunity in breeders with oil-adjuvanted vaccines (Lucio and Hitchner, 1979; Baxendale and Lutticken, 1981; Box, 1989; Wyeth and Chettle, 1990; Van den Berg and Meulemans, 1991; Wyeth *et al.,* 1992; Eterradossi and Saif, 2008). Young broiler chicks are sometimes actively immunized before the complete waning of MDAs (van der Berg *et al.,* 2000).

Maternal antibody titre levels may vary considerably due to differences in breeder vaccination programs, the age of the breeder flocks supplying progeny, and normal variation between hen titres in the same flock. This makes the timing of broiler vaccination in relation to waning MDAs critical, to prevent persistent MDAs from potentially neutralizing the vaccine virus (Lucio and Hitchner, 1979; Naqi *et al.,* 1983; Tsukamoto *et al.,* 1995; Fussell, 1998). Maternal antibody titre levels must fall below 1:64 before broiler chicks can be effectively vaccinated with a live-attenuated IBDV strain (Skeeles *et al.,* 1979). Serological monitoring of antibody levels in a breeder flock or its progeny is usually necessary to determine the proper time to vaccinate (van den Berg and Meulemans, 1991).

The progeny of hens that have been vaccinated with the inactivated IBD vaccines have protective antibodies until the age of approximately thirty days during which the chicks are only protected from been susceptible to the IBDV strains that only provoke immunosuppression (Wyeth and Cullen, 1976; Box, 1989; Wyeth and Chettle, 1990; Van den Berg and Meulemans, 1991; Wyeth *et al.,* 1992). However, the progeny are not protected from other highly pathogenic strains that may inflict high mortality rates at later stages (van den Berg and Meulemans, 1991; Wyeth and Cullen, 1979). The decision to use an inactivated vaccine will thus depend on the epidemiology of IBDV in the environment which include; the presence or absence of highly pathogenic strains that requires vaccination of broilers with live vaccines; where there is no risk of infection with vvIBDV; when boosting of laying hens with an inactivated vaccine just before lay is fully justified.

However, the duration and uniformity of the immunity conferred upon chicks will, to a great extent, depend on the concentration and the antigenic specificity of the virus present in the IBD vaccine. These vaccines are obtained either from bursal homogenates of infected chicks, or from viral cultures on embryonated eggs or fibroblasts, which are then inactivated by formaldehyde and presented as oil emulsions. Sub-unit vaccines produced in yeast or insect cell cultures have also been described, but are not currently in use (Fahey *et al.,* 1989; Macreadie *et al.,* 1990, Vakharia *et al.,* 1993).

2.3.10 Potential causes of vaccination failure

There are numerous causes or reasons why vaccination failure to live-vaccines is been experienced, amongst the most trivial causes are non-observance of the expiry date, inappropriate storage, non-observance of recommended doses, and incorrect or lack of

good vaccination techniques (Vakharia *et al.,* 1993). Freeze-dried live vaccines must be rehydrated immediately before use in distilled water. When using the spray technique, distilled water must be used in diluting the vaccines. When the vaccine is administered in drinking water, it is particularly important to deprive the birds of water for two to three hours before distributing the vaccine solution. Only fresh water, with no organic matter, chlorine or heavy metals, should be used. Adding powdered milk at a concentration of 2 g per litre helps to stabilise the vaccinal virus (Vakharia *et al.,* 1993).

Interference from MDAs is one of the most frequent causes of vaccination failure. The date of vaccination of the offspring must therefore be determined on the basis of the immune status of the chicks, and those of the vaccination protocol used for the parents. Vaccination failure with inactivated vaccines is rare, but may occur, either due to the absence of previous contact of some of the birds with a live virus (a vaccine virus or otherwise), or to the existence of antigenic variants not present in the vaccine. All suspected cases of antigenic variation in the field should be tested in isolation units on SPF birds after vaccination with classical strains (Vakharia *et al.,* 1993).

2.4 Haematology of Birds

Haematological and serum biochemistry parameters have been widely used in assessing the health and physiological status of animals (Uchegbu *et al.*, 2010; Graczyk *et al*., 2003). Changes in the haematological parameters are often used to determine various status of the body and stresses due to environmental, nutritional and/or pathological factors. Owen *et al.* (2009) and Akpodiete and Ologhodo, (1998) noted that significant changes in the blood

parameters can be used to assess both the pathological and nutritional status of individual animals. In poultry, haematological values are influenced by age, sex, breed, climate, geographical location, season, day length, time of day and nutritional status (Islam *et al.*, 2004). Although, chickens have been used as research models for determining physiological parameters of different bird species, little is known about their hematological parameters in general (Wakenell, 2006). Most of the current information about hematological parameters in chickens is extrapolated from the values established for other species (Phillip *et al.*, 2009). The evaluation of blood profiles of broiler strains have been assessed by several studies (Hauptmanova *et al.*, 2002; Onifade and Odunsi, 1998; Uko and Ataja, 1996; Levi *et al.*, 1989). Talebi *et al.* (2005) demonstrated a significant increase in the values of red blood cell and leukocyte count of broiler chicks. Islam *et al.* (2004) illustrated that, the amount of red blood cell, haemoglobin and packed cell volume rises along with animal growth. Nirmalan and Robinson (1971) found the amount of lymphocytes, monocytes, heterophils, eosinophils and basophiles in poultry and quails at various ages, and demonstrated that, with increase in age, the number of all mentioned parameters except heterophils will be increased. Mitruka and Rawnsley (1997) , also reported that high packed cell volume (PCV) and high haemoglobin content (HB) are associated with high feed conversion efficiency, while high percentage of white blood cells especially lymphocytes are associated with the ability of the chicken to perform well under very stressful conditions.

2.5 *Moringa oleifera* in Ethnoveterinary Medicine

Ethnoveterinary medicine (EVM), which is also known as traditional animal health care practices, is defined as local or indigenous knowledge and methods for caring, healing, and managing livestock (Guéye, 1999). It is the study of indigenous knowledge system of animal health care. The EVM drugs are used in the management of bacterial, viral, protozoan, fungal, parasitic and non-infectious causes of diseases. Ethnoveterinary practices concern to animal health care comprises of beliefs, knowledge, practices and skills pertaining to health care and management of livestock. Worldwide the people have traditionally relied on a whole range of indigenous practices to keep their flock health and to treat them when they are sick (Anon, 1996). Medicinal plants have for several centuries been widely used as a primary source of prevention and control of livestock diseases (Hoareau and DaSilva, 1999). In Botswana, *Aloe vera* and *Nicotiana tabacum* are the most commonly utilized remedies used against diseases and parasites of poultry. Mwale *et al.* (2005) reported that *Aloe vera* and *Aloe spicata* were the predominantly used plant species for chicken health management in Zimbabwe. Tobacco (*Nicotiana tabacum*) leave has been used by poultry farmers for the treatment of chickens against Newcastle disease (Deeba, 2009; Ranwedzi, 2002). *Moringa oleifera* tree itself represents an important health care and economic resource of biodiversity. Almost all plant parts are used in traditional medicine and as vegetables for dietary purposes (Iqbal and Bhanger, 2006). Root-bark extracts are used as anthelmintic, analgesic, and astringent, and for ulcers, tumours, earache, tuberculous glands in the neck, etc. (Nikkon *et al.*, 2003). Due to the importance uses of *Moringa oleifera* in traditional medicine, many investigations have previously reported on pharmacological properties such as antifertility (Shukla *et al.*, 1988), anti-inflammatory, antispasmodic, and diuretic activities (Cáceres *et al.*, 1992). Ghasi *et al.* (1999) reported

that juice extracted from *Moringa oleifera* leaves was a potent hypocholesterolemic agent. In their research using Wister rat, they concluded that even when given at the relatively low dose of 1 mg/g, co-administered with a high fat diet daily over a period of 30 days, cholesterol was reduced in serum, liver and kidney. Moreeng (2008) reported that *Moringa oleifera* leaves in the feeds of chickens are effective against intestinal parasites. Yang *et al.* (2006) reported that *Moringa oleifera* leaves are potential plant material that enhances immune responses and improve intestinal health of broilers. The bursa of fabricius of birds infected with IBD is usually cut and dressed with salt or a mixture of salt and powdered tobacco (*Nicotiana tabacum*) in order to stop bleeding. The practice of trimming bursa of fabricius is said to be effective against IBD (Moreki *et al*., 2010; Simainga *et al.,* 2010).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Location

The study was conducted at the animal pen of the department of Veterinary Public health of the Faculty of Veterinary Medicine, Ahmadu Bello University Samaru, Zaria, located in Sabon Gari Local Government Area of Kaduna State. Samaru is located within the Northern Guinea Savannah zone of North western Nigeria. It lies between latitude 7° and 11° N, and longitude 7° 44 E and has an average rainfall of between 1,000 to 1,250 mm and an average temperature of between 17°C to 33°C and a vegetation cover of predominantly trees and grasses (KDSG, 2011).

3.2 Collection and Processing of *Moringa oleifera* Leaf

Moringa oleifera leaves (MOL) were harvested (between the months of August and September, 2013) from an orchard at an early flowering stage in Potiskum, Yobe State. The stem and branches were cut from the Moringa trees and spread out to dry under shade at room temperature for five days. The MOL were then removed manually by hand and grounded into powder using a locally manufactured milling machine.

3.3 Mineral Analyses of *Moringa oleifera* Leaf

To determine the presence of calcium, phosphorus, magnesium, iron, sodium, zinc, copper, selenium, potassium, and manganese components in the MOL;

I. Weigh 1 g of the powdered MOL into a 250 ml capacity micro Kjeldahl flask and add 20 ml digestion acid and about 3 glass beads. Fix the flask in a clamp to stay over night.

II. When the initial reaction subsided, increase the temperature of hot plate or micro digestion bench slowly to 180^{o} to 200^{o} C.

III. Continua the digestion at this temperature with occatiotional swirling until there are no visible particles and the digestion acid is quite clear. If the solution darkens when the volume is reduced, remove the Kjeldahl flask from the heating source, add 1 or 2 ml nitric acid and continue the digestion.

IV. Allow the temperature to rise of the heating source to 240^{o} C and evaporate the digestion acid until dense white fumes are formed within the digestion flask.

V. After completing the digestion, the flask is removed from the heating source. Filter the content of the flask through acid wash filter paper in a 100 ml capacity volumetric flask using deionised water.

VI. At the end, suitable aliquot of digested material may be tranfered into washed polyethylene bottles and keep it in a dust proof glass chamber.

To test for the presence of minerals, the supernatant was decanted and the liquid was analysed for the levels of calcium, copper, potasium, magnesium, manganese, sodium, iron, phosphorus, zinc and Se using standard procedure described by Harris (1970). Sodium and potassium were determined using the vanadate/molybdate yellow method by Allen and Deits (1953). Calcium, magnesium, copper, zinc and iron levels were analysed using atomic absorption spectrophotometer. In order to avoid various interference in the determinations, the mixtures were made to contain the same acid concentrations. To overcome potential interference when determing the presence of calcium and magnesium, a modifier 1% Lanthanium (w/v) was added to the final sample dilution and /all standards and blank which served as releasing agent. In the determination of sodium and potassium, ionization interference was dealt by adding caesium to both the sample and the standard.

The concentration or levels of each element in the sample solution was determined by reference to a calibration curve.

3.4 Phytochemical Analyses of *Moringa oleifera* Leaf

Preparation of the aqueous plant extract:

The preparation of the aqueous plant extract of MOL was carried out as described by Yakubu *et al.* (2008). Approximately 218 g of the powder was extracted with 500 ml distilled water using soxhlet apparatus and concentrated by rotator evaporator 50ºC. This was transferred into a suitable container and lyophilized (freeze dried). The yield of the crude aqueous plant extract was 8.75 g. The dried extract was stored in desiccators until required for use. The extract was dissolved in appropriate volume of distilled water to the desired concentration.

Preparation of the ethanolic plant extract:

The method used was as described by Oyagbemi and Odetola (2010). Air-dried powder (1 kg) of fresh matured MOL was extracted by percolation at room temperature with 70% ethanol. Leaf extract of MOL was concentrated under reduced pressure (bath temperature 50ºC) and finally defatted with n-hexane. The extract was evaporated to dryness. The dried mass yielded 69.9 g.

Phytochemical screening:

Phytochemical screening for major constituents was undertaken using standard qualitative procedures as described by Sofowora, (1993), Trease and Evans (1989) and Harborne, (1973).

Identification of sterols and triterpenes:

Three grams of the powdered *Moringa oleifera* leaf was placed in a test tube and 10 ml of 50% alcohol was added, the tube was then placed on a water bath and heated for 3 min. It was then allowed to cool to room temperature and filtered. The filtrate was then evaporated in an evaporating dish to dryness and 5 ml of petroleum ether was added to the dish and stirred for 5 min, the petroleum ether portion was then decanted and discarded. 10 ml of chloroform was then added and stirred for about 5 min, it was then transferred into test tube and 0.5 mg of anhydrous sodium sulphate was added and shaken gently and filtered, the filtrate was then divided into two test tubes and used for the following tests.

Lieberman-Burchard's reaction: To test tube I, equal volume of acetic anhydride was added and gently mixed. Then 1 ml of concentrated H_2S0_4 was added down the side of the tube. The appearance of a brownish-red ring at the contact zone of the two liquids and a greenish colour in the separation layer indicates the presence of sterols and triterpenes.

Salwoski's test: To test tube II, 2 to 3 drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown colour at the inter phase indicates the presence of steroidal ring.

Identification of alkaloids:

Two grams of the powdered *Moringa oleifera* leaf (2 g) was boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The

lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute sulphuric acid. The various extracts were then used for the following test:

Mayer's test: To the filtrate in test tube I, 1 ml of mayer‟ s reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids (Evans, 2002).

Dragendoff's test: To the filtrate in test tube II, 1 ml of dragendoff‟ s reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Wagner's test: To the filtrate in tube III, 1 ml of wagner‟ s reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

Identification of tannins:

Two grams of *Moringa oleifera* leaf was extracted with 10 ml of 50% alcohol, it was then filtered and the filtrate was divided into three portions for the following tests.

Ferric chloride test: Three drops of diluted solution of $FeCl_3$ was added to the test tube I, production of a blue or greenish-black colour that changes to olive green as more ferric chloride is added indicates the presence of tannins.

Bromine water test: Three drops of bromine water was added to the second portion of the filtrate. A buff coloured precipitate indicates condensed tannins while hydrolysable tannins gave none.

Lead sub-acetate test: Three drops of lead sub acetate solution was added to the third portion. Occurrence of a coloured precipitate indicates the presence of tannins.

Identification of saponins:

Frothing test: About 0.5 g of the powdered *Moringa oleifera* leaf was placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 30 min and observed. Formation of honey comb froth indicates the presence of saponins.

Identification of flavonoids:

Two gram of the powdered *Moringa oleifera* leaf sample was completely detanned with acetone. The residue was extracted with warm water after evaporating the acetone on a water bath. The mixture was then filtered while hot; the filtrate was allowed to cool and used for the following test:

Shinoda's test: Few magnesium chips were added to 3 ml of the aqueous solution and 2 drops of dilute hydrochloric acid was added and warmed. A pink or red colour indicates the presence of flavonoids.

Sodium hydroxide test: To test tube II, 2 mls of 10% NaOH solution was added, yellow solution indicates the presence of flavonoids which on adding dilute hydrochloric acid becomes colourless.

$FeCl_3$ test: To test tube III, 3 drops of FeCl3 solution was added, production of greenish-black colour indicates the presence of phenolic nucleus.

Identification of Carbohydrates:

Four grams (4g) of the powdered *Moringa oleifera* leaf was boiled in 50ml of distilled water on a hot plate for 3 minutes.

The mixture was filtered with Whatman filter paper No. 1. The hot filtrate was allowed to cool and used for the following tests:

General Test: Molisch's Test:

A few drops of Molisch's reagent was added to 2ml of aqueous solution of the extract, thereafter 1ml concentrated sulphuric acid was allowed to run down the side of the inclined test tube to form a lower layer without shaking. The interface was observed for colour change. Purple colour is indicative of the presence carbohydrate.

General Test for Monosaccharides: Barfoed's test. One ml aqueous solution of the extract and 1ml of Barfoed's reagent were added into a test tube, heated in a water bath for about 2 minutes. The test tube content was observed for red precipitate which is indicative of carbohydrate.

Identification of Phenols:

Zero point five grammes (0.5 g) of the dried *Moringa oleifera* leaf powdered sample was boiled in 20 ml of water in a test tube and then filtered. 1 ml of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

3.5 Proximate Analyses of *Moringa oleifera* Leaf

The estimation of the various parameters such as the moisture content, crude fat, crude fibre, crude protein total carbohydrate on dry matter basis were carried out according to the standard procedure described by Harris (1970).

Determination of Crude protein:

The Nitrogen in the samples was determined by the routine semi-micro Kjeldahl procedure/technique (Peason, 1976). This consists of three stages of analysis namely; Digestion, Distillation and Titration.

Digestion:

Measure 0.5 g of each finely ground dried sample (for samples with high concentration of protein) or 1.0 g of the samples and other raw materials weighed carefully into the Kjeldahl digestion tubes to ensure that all sample materials get to the bottom of the tubes. 1 Kjeldahl catalyst tablet is added to each of the tubes followed by addition of 12 to 15ml of conc. H_2SO_4. These are set in the appropriate hole of the digestion block heaters in a fume cupboard. The digestion is left on for 1 hour 15minutes after which a clear colourless solution remains in the tube. The digest is left in the fume cupboard for cooling and is diluted with some quantities of distilled water to lower the high concentration of the acid.

Distillation:

Measure 25 mls of boric acid into a clean and dry 250 mls conical flask and the digestion tube with its content are placed in the distillation machine (pre-warmed). 50 mls of NaOH (10N) solution is added to the digest in the tube through the alkali container. The

distillation is allowed till the content of the conical flask is up to 150 mls. The red colouration of boric acid will change to green in the course of distillation.

Titration:

The green colour solution obtained is then titrated against 0.1N H_2S0_4 contained in a 50 ml burette. At the end point or equivalent point, the green colouration of the distillate turns to wine which indicates that all the Nitrogen trapped as Ammonium Borate [$(NH_4)_2BO_3$] have been removed as Ammonium sulphate ($(NH_4)_2SO_4$).

$$\% \text{ Nitrogen} = \frac{(\text{Vol. of acid - Blank}) \times 14.01 \times 100 \times \text{Normality of acid}}{1000 \times \text{weight of sample}}$$

$$\% \text{ Protein} = \text{Value of Nitrogen} \times 6.25 (\text{conversion factor})$$

Moisture content, crude fat/ether extract, crude fibre and total ash content of the MOL were determined by the method described by A.O.A.C (1990).

Determination of fat / Ether extract:

Weigh 1g of the sample and place it into the thimble (a white paper cup for fat extraction). Cover it with defatted cotton wool and label it.

Place it into the oven for about 1 hr to further dry it. Place the empty and clean fat cups into the ovens. Remove and cool in the desiccators. Take the weight and record.

Add 50 mls of petroleum ether or ethanol into the cup. Fix the thimbles and cups into the fat extraction machine and extract for $1^1/_2$ hours. Remove the cups and further dry in the oven for 1 hr. Place it in the desiccator and take the weight while still warm.

$$\% \text{ Fat} = \frac{\text{Final weight of cup- Initial weight of cup}}{\text{Weight of sample}} \times 100$$

Determination of fibre:

Weigh the fibre cap and record. Take 1 g of the sample, pour it into the fibre cap and dip into a solution of dilute H_2SO_4 (1.25 %). Place on the hot plate and digest for 35mins. Remove and wash with hot distill/deionized water. Repeat the procedure placing the fibre cap into 1.25 % KOH for another 35 mins.

Remove and rinse with hot water. Place it into the oven for 3 to 4 hrs to dry. Weigh an empty crucible and record it. Also take the weight of the fibre cap + dry sample. Put the dry cap into the crucible and put into the heated furnace for about 1 hr.

Remove after the expiration of 1 hr and put in the desiccator for cooling. Weigh and record.

The fibre level is determined by the formula:

$$\% \text{ Fibre} = \frac{\text{W3 - (W5 – W4) – (0.9987xWt of empty crucible) – 0.002}}{\text{Weight of the sample}} \times 100$$

Determination of ash:

Weigh the clean, empty and dry crucible on analytical balance. Tare (Zero) it and add 2 g of the sample into the crucible. Keep in the heated furnace for 7 hrs. Remove the crucible into the desiccator for cooling. Weigh while warm.

Note: Muffle furnace operates at a temperature of about 600° C, but there is thermostatic control for temperature regulation.

$$\% \text{ Ash} = \frac{\text{Final weight of crucible} - \text{Initial weight of crucible} \times 100}{\text{Weight of sample}}$$

Determination of moisture:

Place the clean, empty and dry crucible on the analytical balance, zero it and add 5 g of the sample. Remove the crucible, zero it and take the weight (i.e. crucible + sample). Record the weight and keep in the oven for 4 hrs at 104° C. Remove the crucible after the expiration of 4 hrs and keep in the desiccator to cool. Weigh while still warm and record the reading.

$$\% \text{ Moisture} = \frac{(\text{Final weight of crucible + sample}) - (\text{Initial weight of crucible + sample}) \times 100\%}{\text{Weight of sample}}$$

To determine the total carbohydrate in the MOL, the method described by James (1995) was employed using the equation;

Total carbohydrate = 100 □ (% crude protein + % crude fat + % crude fibre + % total ash)

To determine the energy or calorific value;

The total energy value in the MOL in Kcal/100 g was estimated using the method by FAO (2003) as shown below;

Metabolizable Energy = (% crude protein × 4.0) + (% crude fat × 9.0) + (carbohydrate × 4.0)

3.6 Feed Formulation and Analyses

The dried MOL was milled with a hammer mill and sieved with 3 mm mesh siever to obtain *Moringa oleifera* leaf meal. Broiler starter (22% crude protein) and broiler finisher (20% crude protein) were formulated with 5% MOL inclusion as described by the methods of Olugbemi *et al.,* 2010a using pearson square. The feed was subjected to proximate and mineral analysis based on the method described by the AOAC (1990) in the Feed Analysis Laboratory of the Department of Animal Science, Ahmadu Bello University Zaria, to determine the level of metabolizable energy, crude protein, crude fibre, moisture, ash content, and dry matter (Table 3.1).

3.7 Experimental Chicks and Housing

A total of 240 day old Ross 308 hybrid broiler chicks were obtained from a commercial hatchery located in Yola, Nigeria. The chicks were brooded in a deep litter house which was properly cleaned and disinfected before the arrival of the chicks with wood shavings as litter material and feeders and drinkers were provided. The chicks were individually weighed and assigned in a complete randomised design into four different groups A, B, C and D of 60 chicks each. A 100-watt bulb was provided in each of the compartment to supply light and heat during brooding.

3.8 Feeds and Feeding

All the broilers were fed with broiler starter for 28 days (from 0 to 4 weeks of age) and broiler finisher for 21 days (from 5 weeks to 7 weeks). Feed and water were provided *ad libitum* (using plastic drinkers and galvanised feeders).

3.9 Experimental Design

Groups A and B were fed with broiler starter and finisher diets each containing 5% MOL, while groups C and D were fed with broiler starter and finisher feed without MOL. Groups A, B and C were challenged at 35 days of age with a vvIBDV. All the groups were fed for 49 days (7 weeks) (Table 3.2).

Table 3.1: Composition of Experimental Diets of broilers starter and finisher diets per 100 kg feed.

	Broiler starter (A and B) (%)	Broiler finisher (A and B) (%)	Broiler starter (C and D) (%)	Broiler finisher (C and D) (%)
Maize	50.14	52	50.14	52
Maize offal	9.2	10	9.2	10
Soyabean cake	11.695	8.4875	14.1925	10.18
Ground nut cake	11.69	13.9875	14.1925	17.295
***Moringa oleifera* leaf meal**	5	5	0	0
Fish meal	5	5	5	5
Salt	0.3	0.3	0.3	0.3
Lime stone	1.5	0.5	1.5	0.5
Bone meal	3.5	3.5	3.5	3.5
Lysine	0.85	0.5	0.85	0.5
Methionine	0.85	0.375	0.85	0.375
Broiler starter/finisher premix	0.25	0.25	0.25	0.25
Enzyme	0.025	0.1	0.025	0.1
Total:	100	100	100	100
Proximate analysis				
ME Kcal/Kg DM	2798.45	2752.55	2687.88	2664.83
Crude protein	22.50	20.69	22.31	20.63
Crude fiber	5.53	5.15	5.06	5.24
Ether extract	16.45	16.69	16.01	15.93

Key:

Premix used contained: vitamin A – 15,000.00 iu Vitamin D3 - 3, 000,000 iu, Vitamin E-30,000 iu Vitamin K- 3,000 mg Vitamin B1 3000 mg, Vitamin B2 6000 mg, Vitamin B6 5,000 mg, Vitamin B 40 mg, Biotin 200 mg, Niacah-40,000 mg, Pantothenic 15,000 mg, Folic acid 2,000 mg, choline 300,000 mg, Iron 60,000 mg, manganese 80,000 mg, copper 25,000 mg, Zinc 80,000 mg, cobalt 150 mg, iodine 500 mg, selenium 310 mg, Antioxidant 20,000 mg.

Table 3.2: Experimental design for assessing the effect of *Moringa oleifera* leaf supplementation of feed on performance, antibody response and health of broilers.

Group (%MOLM)	Age in days						
	14	18	21	35	38	42	49
	Activity						
A (5%)	IBD killed vaccine, Serology	NDV killed vaccine	IBD killed vaccine, Serology	Serology, Haematology, Serum biochemistry, Challenge with IBD virus	Serology, Haematology Serum biochemistry	Serology, Haematology, Serum biochemistry	Performance indices
B (5%)	Serology	No vaccination	Serology	Serology, Haematology, Serum biochemistry, Challenge with IBD virus	Serology, Haematology Serum biochemistry	Serology, Haematology, Serum biochemistry	Performance indices
C (0%)	IBD killed vaccine, Serology	NDV killed vaccine	IBD killed vaccine, -Serology	Serology, Haematology, Serum biochemistry, Challenge with IBD virus	Serology, Haematology Serum biochemistry	Serology, Haematology Serum biochemistry	Performance indices
D (0%)	Serology	No vaccination	Serology	Serology, Haematology Serum biochemistry	Serology, Haematology Serum biochemistry	Serology, Haematology Serum biochemistry	Performance indices

3.10 Vaccines and Vaccination

Inactivated killed vaccine against IBD (inactivated intermediate strain, Virsin 122, oil emulsion, Biovac Limited, Isreal, Batch 1- 382222) and inactivated killed vaccine against Newcastle disease (ND) (oil emulsion Komorov strain, Biovac Limited, Isreal, Batch 1-422222) were obtained from a Veterinary Pharmaceutical store in Jos, Nigeria. Broilers in groups A and C were vaccinated through the thigh muscles intramuscularly with 0.5 ml of the killed IBD vaccine at 14 and 21 days of age, while vaccination against ND was done with the killed ND vaccine (0.5 ml) through the thigh muscles intramuscularly at 18 days of age (Table 3.2).

3.11 Challenge with Infectious Bursal Disease Virus

At 35 days of age, all the broilers in groups A, B and C were challenged intraocularly with 0.05 ml of a live vvIBD virus. The IBD virus used for the challenge was a field strain (uncharacterized) of vvIBDV isolated from chickens in Zaria. (It was obtained from Prof. Paul Abdu of Department of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria).

3.12 Observation of Challenged Birds

Following inoculation of broilers with vvIBDV, the onset of clinical signs, morbidity, mortality, case fatality rates and gross lesions at postmortem were observed daily and recorded. The morbidity, mortality and case fatality rates were calculated as follows;

$$\text{Morbidity rate} = \frac{\text{Number of sick birds}}{\text{Total number of birds}} \times 100$$

Mortality rate = $\frac{\text{Number of dead birds}}{\text{Total number of birds}} \times 100$

Case fatality rate = $\frac{\text{Number of dead birds}}{\text{Total number of sick birds}} \times 100$

3.13 Assessment of Performance Parameters

At 49th day of age, all the birds from each group were slaughtered and carcasses weighed to assess the performance of the birds fed with or without MOL supplemented diets. Before weighing the carcasses, the slaughtered birds per group were dipped in hot water, defeathered and eviscerated. The liver, heart, lungs, drumsticks, thighs, and gall bladder were weighed and recorded for each bird. The colouration of the skin of the legs and beaks of each of the carcasses for each group were measured using a scoring technique applied by Kaijage *et al.* (2003) and Onibi *et al.* (2008) in which the score varied from 1 to 4 according to the intensity of the yellowing. Thus 1) no yellow colour, 2) light to moderate yellow, 3) enough to well yellow and 4) intense to dark yellow colour observed.

3.13.1 Return on investment

Feed consumption was assessed based on daily feed intake (DFI), average daily weight gain (ADWG), feed conversion ratio (FCR), dressing carcass (DC), mortality rate (MR), feed cost (FC), and selling price (SP) generated per bird and per kilogram carcass and recorded for each group according to the formular by Ayssiwede *et al.* (2011). Where;

DFI (g/bird/day) = {(Quantity of feed offered – Quantity of feed left)/day ÷ Number of birds}

ADWG (g/day) = Weight Gain of the period (g) ÷ Length of the period (days)

FCR = Feed intake during a period (g) ÷ Weight gain of the period (g)

DC (%) = (Carcass weight of the bird ÷ Live body weight of the bird) × 100

MR (%) = {(Initial number of birds – Final number of birds) ÷ Initial number of birds} × 100

Feed Cost/bird (#) = FCR × Feed price/ Kg diet × Live body weight of bird (Kg)

Feed Cost/Kg carcass (#) = {(Feed Cost/bird) ÷ Carcass weight of bird (Kg)}

Selling price/bird carcass (#) = Carcass weight of bird (Kg) × Selling price/Kg carcass

Gross Margins of Feed (GMF)/bird carcass = Selling, price/bird carcass - Feed cost/bird

Gross Margins of Feed (GMF)/Kg carcass = Selling price/Kg carcass - Feed cost/Kg carcass

3.14 Collection of Immune Organs

Five birds were randomly selected from each group at 35, 38 and 42 days of age. The selected birds were euthenised and the bursa of Fabricius, thymus, spleen and Harderian gland removed for evaluation of organ body weight index (Lucio and Hitchner, 1979)

The organ body index was obtained by employing the formula:

$$\text{Organ: body index} = \frac{\text{organ: body weight ratio of groups}}{\text{mean organ: body weight ratio of control}}$$

$$\text{where, organ:body weight ratio} = \frac{\text{organ weight in grams}}{\text{body weight in grams}} \times 100$$

3.15 Collection and Processing of Blood

3.15.1 Collection of blood for haematology and serology

Blood samples of the broilers were collected on days 14, 21, 35, 38 and 42 of age from all the groups for haematological and serological assay. On each blood collection day, 10 broilers from each group that were previously randomly selected and marked were bled via the brachial vein using a 25 gauge sterile needle on a plastic disposable 5 ml syringe. Two millilitres of blood were collected after the birds were properly restrained by an assistant. The blood sample collected on days 35, 38 and 42 of age were divided into two parts. One part was emptied into a commercially available sample bottle containing ethylene diamine tetra acetic acid (EDTA) for haematology and the other into plain test tubes (without anticoagulant) and allowed to coagulate to produce sera according to the methods described by Okeudo *et al.* (2003). Serum was separated by centrifugation at 447.2 g for 10 min and stored at -20°C until analysed for antibody. Each of the sample bottles was labelled using a permanent marker.Prior to this; the area around the brachial vein was swabbed with 70% methanol to allow for easy access to the vein and for collection of blood. Direct contact with blood was avoided by the use of hand gloves and laboratory coat.

3.15.2 Determination of packed cell volume

The packed cell volume (PCV) was determined using standard technique as described by Rehman *et al*. (2003). Non-heparinized capillary tubes were filled up to about ¾ of its length from one end and the second end was heat-sealed using Bunsen burner. The blood in the sealed capillary tubes was then centrifuged for 5 minutes at 4,383 x *g* using the Saitexiangyi TG12MX® Microhaematocrit centrifuge machine. Then the proportion of cells

in the total volume of blood was measured and recorded as a percentage using the Hawksley® Micro-haematocrit Reader.

3.15.3 Estimation of haemoglobin concentration

Haemoglobin concentration was assayed colorimetrically as cyanomethhaemoglobin (Drabkin, 1945). Five millilitres of HICN (Drabkin) solution were measured using a 5 ml syringe into plastic test tubes. Twenty microlitres of blood were measured using a micropipette and added to the Drabkin solution in the test tube and properly mixed by gently shaking the test tube. It was centrifuged at 1,509 x*g* for 15 minutes to separate the empty RBC from interfering with the reading. The supernatant was separated into a sample bottle. The supernatant was absorbed into the haemoglobin meter (XF–C, China). After the wiggling pump stops working, the value displayed on the screen was recorded in g/dl as the haemoglobin concentration.

3.15.4 Determination of red blood cell and total white blood cell count

Red blood cell (RBC) and total WBC (or TWBC) counts were determined with the Natt-Herrick solution (1:200 dilution) and the improved Neubauer haemocytometer (Campbell and Ellis, 2007) as both counts can be prepared directly from the same sample placed in the haemocytometer. The heparinised blood samples were slightly agitated and the RBC diluting pipette was used to pipette the blood to the 0.5 marking. The tip of the pipette was cleaned properly using a tissue paper without touching the distal opening of the pipette tip with tissue, as this will cause capillary shift of blood into the tissue. The diluting solution (Natt-Herrick) was also pipette to the 101 marking (1:200) without entirely immersing the pipette tip into the diluting fluid. The mixture was well shaken for 1 minute to obtain equal

distribution then emptied into a clean sample bottle. The Neubauer haemocytometer and cover slip were cleaned using a dry, lint free cloth. The cover slip was properly placed on the haemocytometer.

The mixture of Natt-Herrick solution and the blood sample was then agitated a little and a capillary tube was used to withdraw a small aliquot. Both sides of the haemocytometer were filled up (charged) by gently touching the intersection between the cover slip and haemocytometer with the loaded capillary tube avoiding air bubbles and under-filling or over-filling, and then left for 5 minutes for cells to settle down. The light microscope (Olympus-XSZ-107BN), at low power magnification (X40) was used to view the cells and counting was done using the tally counter.

For TWBC count, the WBC in the four outer large squares of the haemocytometer were counted and calculated using the formula:

$$N/20 = WBC \times 10^9 /L$$

Where N = Number of WBC counted in the four outer large squares (or in 64 small squares)

For RBC count, the cells contained in the four corner and central squares in the mid section of the haemocytometer were counted. Following the "**L**" rule: cells that touch the centre triple lines of the ruling on the left and the bottom sides were counted but cells that touch the centre triple lines of the ruling on the right and the top sides were not counted. The RBC count was calculated using the formula:

$$N/100 = RBC \times 10^{12} /L$$

Where N = Number of RBC counted in the five squares in the mid section of the haemocytometer (or in 160 squares).

Note that both charged sides of the haemocytometer were counted for both the RBC and TWBC and the average calculated.

3.15.5 Preparation of smears for differential leucocyte count and thrombocytes estimation

From the blood sample collected in all the birds, a pair of smears for each blood sample was made. A small drop of blood was immediately used for the preparation of blood smears each using the standard slide-to-slide technique. The air-dried smears were properly labelled using a pencil on the frosted end of the slide and then fixed in a fixing jar containing 70% methanol for 3 minutes and air-dried.

Staining was done by flooding the smears with Wright-Giemsa stain for 3 minutes. An equal amount of Sørensen's buffer (pH 6.8) was added then mixed gently by blowing using a pipette until green metallic sheen forms on the surface. The smear was allowed to stand for further 6 minutes. The smears were rinsed with the Sørensen's buffer and allowed to stand for a minute for differentiation. The stained slides were then washed copiously with the Sørensen's buffer and the back of the smears were wiped with tissue paper to remove the excess stain and allowed to air dry. The slides were then neatly packed into a slide box until viewed.

Examination of the stained blood smears for differential leucocytes count was done using a light microscope (Olympus-XSZ-107BN) under high-power magnification with oil immersion (X1, 000). One hundred WBC were counted and classified based on their morphologic features (Campbell, 1998; Hawkey and Dennet, 1989; Campbell and Ellis,

2007). The counting was done using the Marble® Blood Cell Calculator. The differential WBC count was then expressed as a percentage of the individual cell group. The percentage of each cell was then converted into absolute numbers by reference to the total WBC using the formula:

$$\frac{\text{Percentage of WBC counted}}{100} \times \text{TWBC} = \text{Absolute Number} \times 10^9/\text{l}$$

An estimated thrombocyte count was obtained from the stained blood film using the same formula for the indirect estimation of total WBC (Campbell and Ellis, 2007). Valid and reliable results were not obtained where there was evidence of thrombocytes clumping. The absolute number of thrombocytes was estimated by using the formula:

$$\frac{\text{Number of thrombocytes counted}}{100} \times \text{TWBC} = \text{Absolute Thrombocytes} \times 10^9/\text{l}$$

3.15.6 Enzyme-linked immunosorbent assay

The enzyme linked immunosorbent assay (ELISA) was carried out according to the methods described by IDEXX laboratories, USA. Briefly the antigen coated plates and the ELISA kit reagents were adjusted at room temperature prior to the test. The test sample was diluted to five hundred folds (1:500) with sample diluents prior to the assay. A 100 µl of diluted sample was then added into each well of the plate. This was followed by 100 µl of undiluted negative control into the well A1 and A2, 100 µl of undiluted positive control was also dropped into well A3 and A4. The plate was then incubated for 30 minutes at room temperature. Each well was then washed with approximately 350 µl of distilled water 3 times. Goat anti-chicken conjugate (100 µl) was dispensed into each well. The plate was again incubated for another 30 minutes, followed by washing each well with 350 µl of distilled water 3 times. Tetramethylbenzidine (TBM) solution (100 µl) was dispensed into

each well. The plate was then incubated at room temperature for 15 minutes. Finally, 100 µl of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650 nm using ELISA reader. Infectious bursal disease antibody titre was calculated automatically, using software by Blankford and Silk (Blankford and Silk, 1989).

3.15.7 Biochemical analyses

Once the serum thawed, blood urea nitrogen, creatinine kinase, total protein, albumin, chloride, hydrogen carbonate, potassium, magnesium and sodium were assayed using an Audiocomb Serum Auto-analyser (Bayer Express Plus, Bayer Germany, Serial Number 15950) in the Chemical Pathology Laboratory, Ahmadu Bello University Teaching Hospital (ABUTH) Shika. The globulin fraction was calculated by subtracting the albumin fraction from the total protein.

The levels of antioxidant enzymes (SOD, CAT and GPx) in the serum, were evaluated using commercial test kits for SOD, CAT and GPx obtained from North West Life Science specialties LLC, Vancouver, WA98662, Canada, while the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine kinase (CK) was determined using an auto-analyzer (Audiocomb Serum Auto-analyser, Bayer Express Plus, Bayer Germany, Serial Number 15950).

The level of thiobarbituric acid reactive substance (TBA), melondialdehyde (MDA), as an index of lipid peroxidation was evaluated in the serum using the double heating method of Draper and Hadley (1990) as modified by Yavuz *et al.* (2004). The concentration of MDA

in the sera were calculated by the absorbance coefficient of MDA-TBA complex 1.56 x 105/cm/M and expressed as nmol/mg of protein.

3.15.8 Serum cholesterol and triglyceride assay

Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-cholesterol) and triglyceride (TG) was determined in the sera by colorimetric methods of Allain *et al.* (1974), Burstein *et al.* (1970) and Trinder (1969), respectively, using enzymatic diagnostic kits (AGAPPE Diagnostic Switzerland Gmbh). Low-density lipoprotein cholesterol (LDL-cholesterol) was calculated according to the formula by Friedewald *et al.* (1972) where;

$$\text{(LDL-chol)} = \frac{\text{(Total chol)} - \text{(HDL-chol)} - \text{Triglyceride}}{5}$$

3.16 Data Analyses

The results of the phytochemical, mineral and proximate analysis were analysed by descriptive statistics. Data obtained from the performance parameter were analysed using pearson correlation. Data obtained from returns on investment, haematological and ELISA mean optical density values and biochemical analyses were expressed as means (± standard deviation). They were further subjected to repeated measure one way analysis of variance (ANOVA) followed by tukeys post-hoc test for multiple comparism. T test was used to compare the mean between the initial and final body weight, initial and final feed consumption. The values obtained from the organ to body weight index were compared with that of the control. Morbidity, mortality and case fatality rates were expressed as percentages. Values of $p < 0.05$ were considered significant using Statistical Package for Social Science (SPSS) version 20 for windows.

CHAPTER FOUR

RESULTS

4.1 Nutrients, Elemental and Phytochemical Constituents of *Moringa oleifera* Leaf

The results of the MOL analysis revealed that it contained appreciable quantity of crude protein (25.9%) and carbohydrate (55.14%), fats (5.85%) and fibre (13.91%) (Table 4.1). The moisture content of MOL was 7.94%, while the ash content was 3.72%. The qualitative phytochemical analyses of the MOL using different solvents confirmed the presence of alkaloids, carbohydrates, flavonoids, saponins, steroids, tannins, terpenoids, phenol and phylobatanin. Aqueous extract of MOL did not contain steroids, terpenoids, and phylobatannin. Tannins and terpenoids were absent from ethanolic extract of MOL. Methanolic extract did not contain steroids, tannins, terpenoids and phylobotannins (Table 4.2). The quantitative phytochemical analysis of MOL detected cyanide (0.1%), oxalate (0.45%), saponins (1.06%), phytates (2.57%) and tannins (2.19%) (Table 4.3). Mineral analysis showed that MOL contained minerals such as Ca (2.26%), P (0.35%), Mg (0.45%), Fe (116.5 ppm), Na (0.11%), Zn (34 ppm), Cu (7.5 ppm) and Se (0.85 ppm) (Table 4.4).

Table 4.1: Proximate composition of *Moringa oleifera* leaves harvested from Potiskum, Yobe State, Nigeria.

Metabolite	Composition
Carbohydrate	55.14 (%)
Crude protein	25.9 (%)
Crude fibre	13.91 (%)
Moisture	7.94 (%)
Fat	5.85 (%)
Ash	3.72 (%)
Energy	2930.63 (KCal/Kg)

Table 4.2: Qualitative phytochemical composition of *Moringa oleifera* leaves harvested from Potiskum, Yobe State, Nigeria.

	Extract		
Metabolite	**Aqueous**	**Methanolic**	**Ethanolic**
Alkaloids	+	+	+
Carbohydrate	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	-	-	+
Tannins	+	-	-
Terpenoids	-	-	-
Phenol	+	+	+
Phylobatanin	-	+	+

Key: + = present
□ = Absent

Table 4.3: Quantitative phytochemical composition of *Moringa oleifera* leaves harvested from Potiskum, Yobe State, Nigeria.

Phytochemical	Concentration (%)
Phytates	2.57
Tannins	2.19
Saponins	1.06
Oxalates	0.45
Cyanides	0.1

Table 4.4: Mineral composition of *Moringa oleifera* leaves harvested from Potiskum, Yobe State, Nigeria.

Element	Concentration
Ca	2.26 %
P	0.35 %
Mg	0.45 %
K	1.9 %
Na	0.11 %
Zn	34 ppm
Cu	7.5 ppm
Mn	40.5 ppm
Fe	116.5 ppm
Se	0.85 ppm

ppm = parts per million (1 mg/kg = 1 ppm)

4.2 Assessing the Performance of Broilers Fed with *Moringa oleifera* Feed Supplementation and Challenged with Very Viruluent Infectious Bursal Disease Virus.

4.3

Broilers in all the groups showed a positive correlation in their daily intake of feed with respect to age. However, broilers in group D strongly correlates (Pearson correlation = 0.921; P = 0.000) than those in groups B (Pearson correlation = 0.875; P = 0.000), C (Pearson correlation = 0.863; P = 0.000) and A (Pearson correlation = 0.862; P = 0.000) (Figure 4.1).

Broilers in all the groups had a positive correlation in their average daily weight gain with respect to age. However, broilers in group D shows strong correlation (Pearson correlation = 0.971; P = 0.000) when compared to broilers in groups A (Pearson correlation = 0.961; P = 0.000), B (Pearson correlation = 0.954; P = 0.000) and C (Pearson correlation = 0.908; P = 0.000) (Figure 4.2).

While broilers in all the groups showed a negative correlation in feed conversion ratio with respect to age throughout the study period, broilers in groups A and B correlates strongly (Pearson correlation = 0.379; P = 0.000) than those in groups C (Pearson correlation = 0.454; P = 0.000) and D (Pearson correlation = 108; P = 0.273) (Figure 4.3).

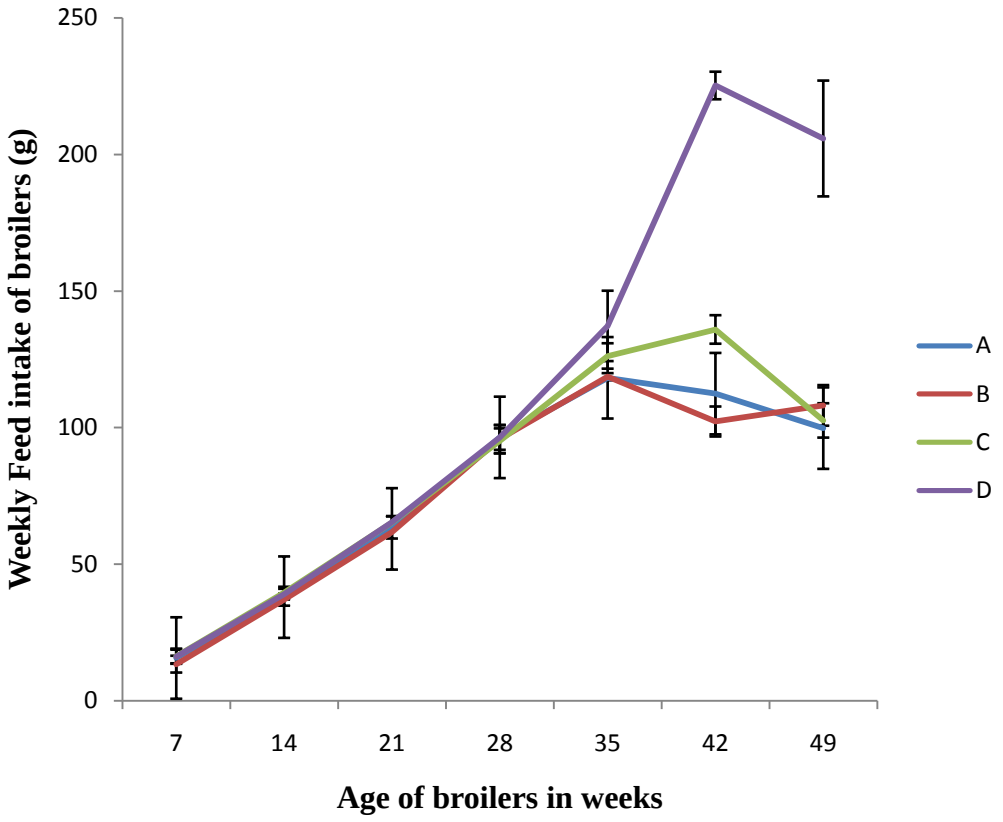


Figure 4.1: Feed intake of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

Key: A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.

C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

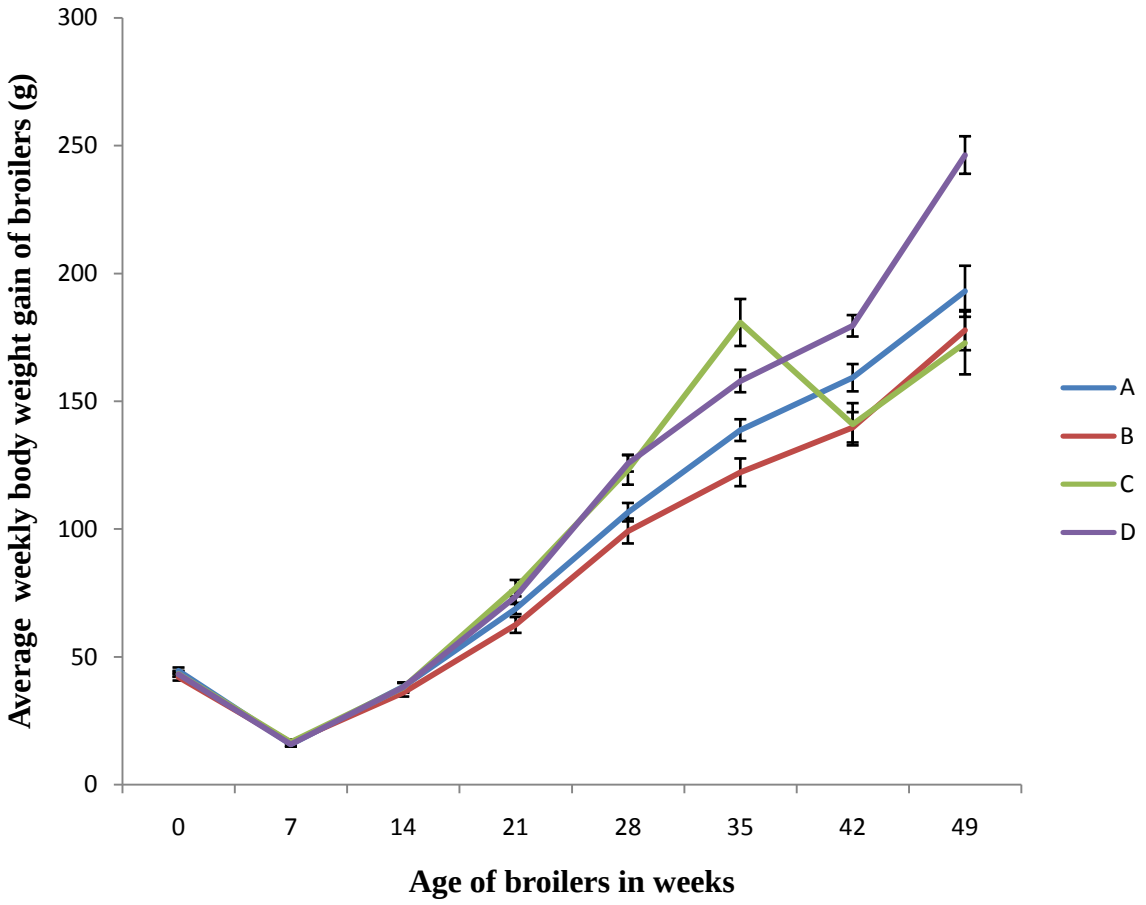


Figure 4.2: Average daily body weight gain of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

Key: A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.

C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

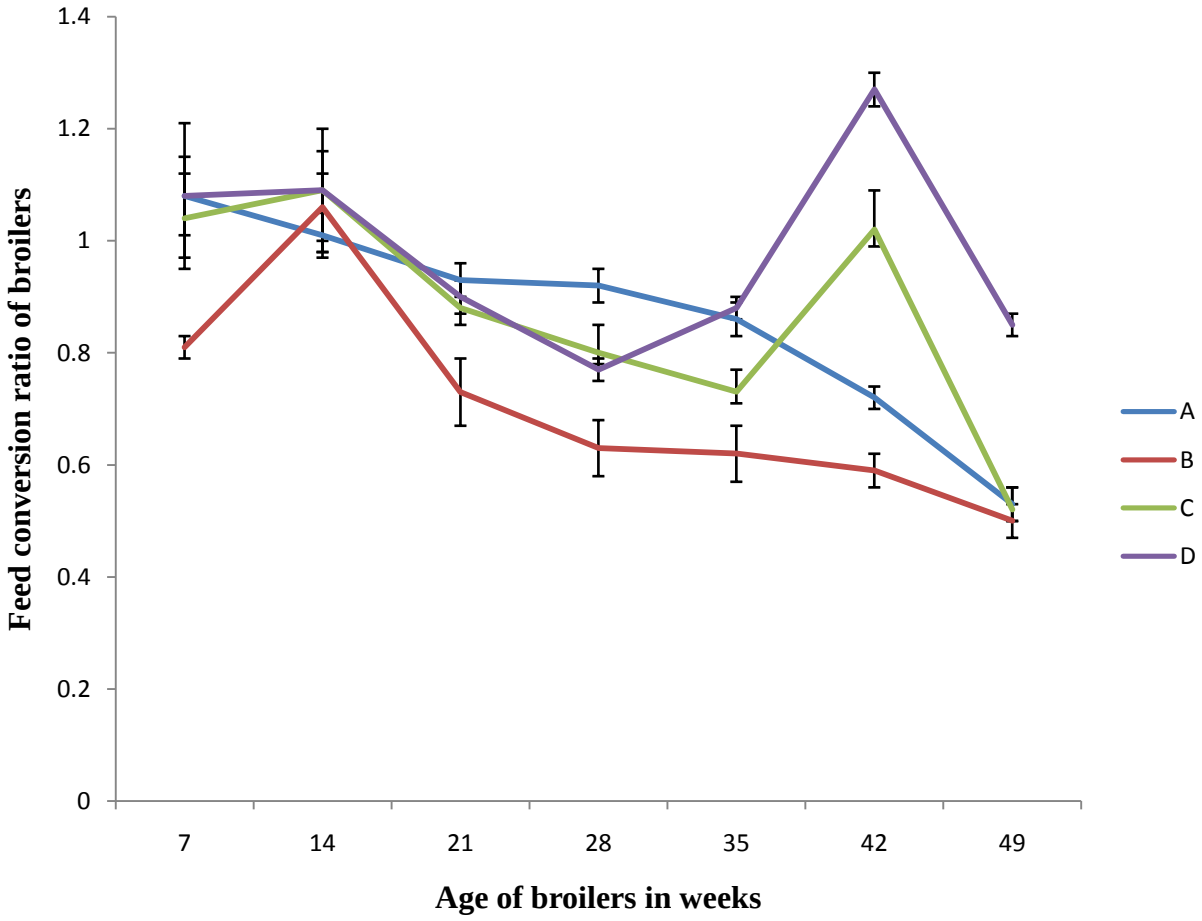


Figure 4.3: Feed conversion ratio of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

Key: A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.

C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

At 49 days of age, the live and carcass weight of birds in group D were much heavier followed by that of groups A, B and C, though not statistically significant (Table 4.5). There was no significant difference ($p = 0.06$) in the weight of the crop and gizzard between groups. The proventiculus, abdominal fat and liver were significantly ($p = 0.001$) heavier in group A when compared with those of group C. The weight of the gall bladder, heart, lungs and drumstick did not show any significant difference ($p = 0.06$) between groups. The weight of the thigh was significantly heavier ($p = 0.01$) in groups D than in C.

The MOL in the diet of broilers in groups A and B produced a significant ($p = 0.000$) yellow colouration of the skin of legs and beak (3.00 ± 0.0 and 3.40 ± 0.24, respectively) when compared to those of groups C and D. The feed cost per Kg of carcass did not show significant difference ($p = 0.92$) between groups. However, birds in groups C and D had a higher feed cost (₦195 and ₦195.3 per Kg carcass, respectively) than those of groups A and B (₦182 and ₦183 per Kg carcass, respectively). For a selling price of ₦650/Kg of carcass, the gross margins of feed (GMF) per Kg carcass generated per group were significantly different ($p = 0.001$) between group A (₦467.4 ± 0.63) with groups C and D, and between group B ($p = 0.0001$) (₦466.9 ± 0.53) with groups C and D (Table 4.5).

Table 4.5: Performance parameters, carcass and organ weights of broilers (at 49 days of age) fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
Organs weight (g)	A (n = 5)	B (n = 5)	C (n = 5)	D (n = 5)
Live weight	1739.0 ± 198.95	1493.2 ± 250.3	1453.0 ± 231.1	1844.0 ± 291.9
Carcass weight	1681.0 ± 186.1	1439.8 ± 243.0	1397.4 ± 220.1	1771.4 ± 284.9
Dress carcass (%)	96.72 ± 0.74	96.42 ± 0.61	96.22 ± 0.73	96.04 ± 0.53
Crop	22.00 ± 4.39	28.60 ± 6.38	12.80 ± 2.13	27.80 ± 8.754
Proventiculus	10.40 ± 0.55^{a}	9.80 ± 1.30^{a}	7.00 ± 2.00^{cd}	8.20 ± 1.92^{cd}
Gizzard	48.80 ± 2.54	49.00 ± 3.05	46.40 ± 5.34	50.60 ± 4.67
Abdominal fat	26.00 ± 9.85^{a}	17.40 ± 5.37^{bd}	8.40 ± 5.68^{c}	15.60 ± 6.94^{bd}
Liver	45.20 ± 8.20^{a}	34.20 ± 8.07	29.20 ± 5.26^{c}	41.00 ± 7.87
Gall bladder	2.00 ± 0.0	1.40 ± 0.24	2.20 ± 0.20	1.70 ± 0.44
Heart	9.00 ± 0.89	7.20 ± 1.32	6.80 ± 0.80	8.00 ± 0.55
Lung	9.60 ± 0.68	8.00 ± 0.89	7.20 ± 0.58	9.20 ± 0.80
Thigh	181.20 ± 37.08	166.00 ± 35.93^{b}	137.60 ± 25.03^{c}	204.00 ± 28.12^{d}
Drum stick	159.8 ± 8.63	132.0 ± 13.22	135.2 ± 11.68	171.0 ± 11.61
Yellow legs	3.00 ± 0.00	3.40 ± 0.55^{b}	1.00 ± 0.00^{cd}	1.00 ± 0.00^{cd}
Yellow skin	2.60 ± 0.55^{a}	1.80 ± 0.45^{b}	1.00 ± 0.00^{c}	1.00 ± 0.00^{d}
Feed Cost/bird (₦)	307.0±15.71	263.6±19.76	272.5±19.39	345.9±24.49
Feed Cost/Kg (₦)	182.6 ± 0.63	183.1 ± 0.53	195.0 ± 0.66	195.3 ± 0.48
Selling price/Kg (₦)	650	650	650	650
Selling price/bird (₦)	1093 ± 54.11	935.9 ± 70.69	908.3 ± 63.97	1151 ± 82.82
GMF/bird (₦)	785.6 ± 38.44	672.2 ± 50.89	635.8 ± 44.61	805.5 ± 58.35
GMF/Kg (₦)	467.4 ± 0.63^{a}	466.9 ± 0.53^{a}	455.0 ± 0.66^{cd}	454.7 ± 0.48^{cd}

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabets on the same row differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.

C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

4.3 Evaluating the Humoral Immune Response of Broilers Fed with *Moringa oleifera* feed Supplementation and Vaccinated with an Inactivated Infectious Bursal Disease vaccine.

There was a highly significant decrease ($p = 0.001$) in the mean IBD antibody titre of birds in group A at 21 days of age and significant increase at 35 days of age. Birds in group B showed a highly significant decrease ($p = 0.000$) in ELISA antibody mean titre at 21 days of age and a significant ($P = 0.002$) increase at 35, 38 and 42 days of age. A very highly significant decrease ($p = 0.000$) was observed in the ELISA mean antibody titre of broilers in group C at 21 days of age and a significant increase ($p = 0.022$) was observed at 35 days of age. The ELISA antibody mean titre of broilers in group D showed a significant decrease ($p = 0.000$) at 21 days of age, and a significant ($p = 0.000$) increase at 35 and 42 days of age (Table 4.6).

The bursa to body index (BBI) of broilers in group A was 1.4, 1.57 and 0.71 at 35, 38 and 42 days of age, respectively. Group B had a BBI of 1.4, 0.71 and 0.86 at 35, 38 and 42 days of age, respectively. Birds in group C had a BBI of 0.86, 1 and 0.71 at 35, 38 and 42 days of age, respectively (Table 4.7).

The spleen to body index (SBI) of birds in group A was 1.3, 0.9 and 0.8 at 35, 38 and 42 days of age, respectively. Birds in group B had a SBI of 1.1, 0.9 and 1.1 at 35, 38 and 42 days of age, respectively, while those in group C had a SBI of 0.77, 1, and 0.8 at 35, 38 and 42 days of age, respectively (Table 4.7).

Harderian gland to body weight index (HBI) in birds of group A was 2, 2.5 and 0.3 at 35, 38 and 42 days of age, respectively. Birds in group B had a HBI of 2, 2 and 0.66 at 35, 38

and 42 days of age, respectively, while those in group C had a HBI of 1.25, 1.5 and 0.66 at 35, 38 and 42 days of age, respectively (Table 4.7). The thymus to body weight index (TBI) of birds in group A was 1.09, 1.05 and 1.03 at 35, 38 and 42 days of age, respectively, while those in group B had a TBI of 0.84, 1.02 and 0.89 at 35, 38 and 42 days of age, respectively. The TBI of birds in group C were 1.04, 1.22 and 1.29 at 35, 38 and 42 days of age, respectively (Table 4.7).

Signs of somnolence, ruffled feathers, depression, anorexia and whitish-yellowish diarrhoea were observed from the different groups. Signs of sickness (somnolence, anorexia) were observed at day 1 post inoculation (pi) in group B (1 bird), day 2 pi in group A (1 bird), B (3 birds) and C (1 bird). At day 3 pi, signs of sickness were observed in 11 of the birds with 1 dead in group B, while signs of sickness were observed only in group A (7 birds) and group C (8 birds). By day 4 pi, 4 and 5 birds were observed to be sick in group A and C, respectively, while 7 birds were sick in group B. By day 5 pi, only 1, 4 and 3 birds from group A, B and C, respectively, were sick, while 2, 3 and 2 birds showed sign of sickness on day 6 pi in group A, B and C, respectively. By day 7 pi, 3 and 1 bird were sick in group B and C, respectively while only 1 bird in group B showed sign of sickness on day 8 pi (Table 4.8).

Postmortem examination of the dead bird from group B showed pathological lesions suggestive of IBD and these include; haemorrhages on the thigh, breast muscles and mucosa of the proventiculus, an empty crop, oedematous bursa of Fabricious, congested liver and spleen.

Table 4.6: Changes in enzyme linked immunosorbent assay infectious bursal disease antibody titre level of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) IBD antibody titre (%)			
14	3285.71 ± 920.30[a]	3363.95 ± 660.15[a]	3434.89 ± 476.89[a]	2701.24 ± 829.95[a]
21	1379.89 ± 829.98[b]	1205.94 ± 612.32[b]	1576.94 ± 566.51[b]	1542.43 ± 106.80[b]
35	2836.83 ± 463.58[c]	2224.54 ± 636.35[c]	2853.42 ± 544.64[c]	2953.49 ± 561.88[c]
38	2545.13 ± 1102.17[d]	3061.87 ± 617.75[d]	2722.18 ± 570.80[d]	688.16 ± 821.56[d]
42	2226.01 ± 1074.36[e]	3193.10± 478.52[e]	3290.51 ± 848.87[e]	3152.77 ± 1221.87[e]
F statistic	702.947	542.503	751.57	209.391
P value	0.006	0.000	0.000	0.000

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

Group A: Fed 5% *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group B: Fed 5% *Moringa oleifera* leaf supplemented feed, non vaccinated and challenged at 35 days old with very virulent infectious bursal disease virus.
Group C: Fed feed with out *Moringa oleifera* leaf, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group D: Fed feed with out *Moringa oleifera* leaf inclusion, non vaccinated and non challenged with very virulent infectious bursal disease virus.

Table 4.7: Immune organs to body weight index of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 5	B n = 5	C n = 5	D n = 5
Age in days	Bursa to body weight index			
35	1.4	1.4	0.86	1
38	1.57	0.71	1	1
42	0.71	0.86	0.71	1
	Spleen to body weight index			
35	1.3	1.1	0.77	1
38	0.9	0.9	1.0	1
42	0.8	1.1	0.8	1
	Harderian gland to body weight index			
35	2.0	2.0	1.25	1
38	2.5	2.0	1.5	1
42	0.33	0.66	0.66	1
	Thymus to body weight index			
35	1.09	0.84	1.04	1
38	1.05	1.02	1.22	1
42	1.03	0.89	1.29	1

Key: n = total number of birds sampled

Group A: Fed 5% *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

Group B: Fed 5% *Moringa oleifera* leaf supplemented feed, non vaccinated and challenged at 35 days old with very virulent infectious bursal disease virus.

Group C: Fed feed with out *Moringa oleifera* leaf, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

Group D: Fed feed with out *Moringa oleifera* leaf inclusion, non vaccinated and non challenged with very virulent infectious bursal disease virus.

Table 4.8: Morbidity and mortality rates of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

Group	Number challenged	Morbidity rate (%)	Mortality rate (%)	Case Fatality rate (%)
A	45	15 (33.3)	0.0	0.0
B	45	33 (73.3)	2.2	3.0
C	45	20 (44.4)	0.0	0.0
D	0	0 (0.0)	0.0	0.0

	Days post inoculate with very virulent infectious bursal disease virus							
	1	2	3	4	5	6	7	8
	Number (%) of birds sick							
A	0	1 (2.2)	7 (15.6)	4 (8.9)	1 (2.2)	2 (4.4)	0	0
B	1 (2.2)	3 (6.7)	11(24.4)	7 (15.9)	4 (9.1)	3 (6.8)	3 (6.8)	1 (2.3)
C	0	1 (2.2)	8 (17.8)	5 (11.1)	3 (6.7)	2 (4.4)	1 (2.2)	0
D	0	0	0	0	0	0	0	0

Group A: Fed 5% *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

Group B: Fed 5% *Moringa oleifera* leaf supplemented feed, non vaccinated and challenged at 35 days old with very virulent infectious bursal disease virus.

Group C: Fed feed with out *Moringa oleifera* leaf, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

Group D: Fed feed with out *Moringa oleifera* leaf inclusion, non vaccinated and non challenged with very virulent infectious bursal disease virus.

4.4 Evaluating the Haematological Parameters of Broilers Fed with *Moringa oleifera* Feed Supplementation and Challenged with a Very Viruluent Infectious Bursal Disease Virus.

Haematology result showed a highly significant decrease in the values of PCV in group A at 38 ($p = 0.001$) and 42 ($p = 0.000$) days of age. The values of PCV were also significantly decreased in group B ($p = 0.0002$), C ($p = 0.0004$) and D ($p = 0.0180$) at 38 days of age, but significantly increased ($p = 0.019$) by 42 days of age in group B (Table 4.9). Haemoglobin concentration significantly increased in group B ($p = 0.02$) and group C ($p = 0.002$) at 38 days of age and decreases significantly ($p = 0.015$) at 38 days of age in group D (Table 4.10). The value of RBC significantly ($p = 0.01$) decreased at 38 days of age and increased significantly ($p = 0.039$) at 42 days of age in group B (Table 4.11), while TWBC count was observed to significantly increase ($p = 0.034$) between groups A and C, and B and C at 42 days of age (Table 4.12).

The result of this study also showed a significant increase ($p = 0.025$) in the values of eosinophils count among broilers in group B at 42 days of age, but no significant increase ($p = 0.7719$) was however observed among broilers in groups A, C and D at 35, 38 and 42 days of age (Table 4.13). A significant decrease ($p = 0.002$) was observed in lymphocyte count at 38 days of age in group B (Table 4.14). The values of heterophil/lymphocyte ratio significantly ($p = 0.005$) decrease at 38 days of age and a significant ($p = 0.011$) increase was observed between 35 and 42 days of age in group D (Table 4.15).

4.5 Evaluating the Effect of *Moringa oleifera* Feed Supplementation on the Serum Biochemical Profile of Broilers Challenged with Very Viruluent Infectious Bursal Disease Virus.

The level of MDA was observed to significantly decrease between 35 and 38 days of age in group A ($p = 0.028$), and between 35 and 42 days of age in group C ($p = 0.008$). There was a significant increase in the level of MDA among broilers in group B ($p = 0.044$) and D ($p = 0.000$) at 38 days of age and a subsequent significant decrease in the same groups (B and D) when the broilers were 42 days of age (Table 4.16).

Table 4.9: Packed cell volume of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) packed cell volume (%)			
35	28.60 ± 1.77^{a}	26.30 ± 1.94^{a}	25.22 ± 2.28^{a}	25.50 ± 3.54^{a}
38	21.80 ± 3.46^{b}	21.10 ± 1.91^{b}	21.44 ± 1.33^{b}	23.30 ± 3.06^{a}
42	19.90 ± 2.73^{b}	23.80 ± 3.01^{c}	22.67 ± 1.87^{b}	26.80 ± 2.04^{c}
F statistics	25.528	12.585	8.952	3.028
P value	0.0000	0.001	0.003	0.079

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabet on the same column differ significantly $p<0.05$

Group A: Fed 5% *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group B: Fed 5% *Moringa oleifera* leaf supplemented feed, non vaccinated and challenged at 35 days old with very virulent infectious bursal disease virus.
Group C: Fed feed with out *Moringa oleifera* leaf, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group D: Fed feed with out *Moringa oleifera* leaf inclusion, non vaccinated and non challenged with very virulent infectious bursal disease virus.

Table 4.10: Haemoglobin concentration of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) haemoglobin concentration (g/dl)			
35	10.55 ± 2.21	10.01 ± 1.77^{ac}	9.81 ± 0.97^{a}	13.38 ± 1.47^{a}
38	10.78 ± 1.95	10.32 ± 0.93^{b}	11.39 ± 1.17^{b}	11.14 ± 1.47^{a}
42	10.77 ± 1.50	12.06 ± 2.04^{ac}	11.77 ± 2.00^{b}	13.38 ± 1.47^{c}
F statistics	0.053	5.499	4.280	8.924
P value	0.940	0.015	0.040	0.015

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

Group A: Fed 5% *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group B: Fed 5% *Moringa oleifera* leaf supplemented feed, non vaccinated and challenged at 35 days old with very virulent infectious bursal disease virus.
Group C: Fed feed with out *Moringa oleifera* leaf, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group D: Fed feed with out *Moringa oleifera* leaf inclusion, non vaccinated and non challenged with very virulent infectious bursal disease virus.

Table 4.11: Red blood cell count of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) red blood cell count (x 10^{12}/L)			
35	2.44 ± 0.44	2.49 ± 0.34^{ac}	2.25 ± 0.26	2.44 ± 0.63
38	2.01 ± 0.42	1.78 ± 0.35^{b}	1.79 ± 0.52	2.19 ± 0.82
42	2.04 ± 0.49	2.19 ± 0.51^{ac}	2.09 ± 0.29	2.10 ± 0.51
F statistics	2.210	8.011	3.399	0.542
P value	0.140	0.006	0.077	0.572

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

Group A: Fed 5% *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group B: Fed 5% *Moringa oleifera* leaf supplemented feed, non vaccinated and challenged at 35 days old with very virulent infectious bursal disease virus.
Group C: Fed feed with out *Moringa oleifera* leaf, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group D: Fed feed with out *Moringa oleifera* leaf inclusion, non vaccinated and non challenged with very virulent infectious bursal disease virus.

Table 4.12: White blood cell count of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) white blood cell count (x 10^{12}/L)			
35	3.86 ± 0.92	4.70 ± 1.97	4.70 ± 1.45	4.06 ± 2.59
38	4.12 ± 1.22	3.49 ± 1.35	4.98 ± 1.89	5.09 ± 2.05
42	4.22 ± 2.00[a]	4.76 ± 1.37[a]	6.94 ± 2.58[c]	4.05 ± 2.05[a]
F statistics	3.205			
P value	0.034			

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabets on the same row differ significantly $p<0.05$

Group A: Fed 5% *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group B: Fed 5% *Moringa oleifera* leaf supplemented feed, non vaccinated and challenged at 35 days old with very virulent infectious bursal disease virus.
Group C: Fed feed with out *Moringa oleifera* leaf, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
Group D: Fed feed with out *Moringa oleifera* leaf inclusion, non vaccinated and non challenged with very virulent infectious bursal disease virus.

Table 4.13: Eosinophil count of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) eosinophil count x 10^9 /L			
35	0.05 ± 0.06	0.01 ± 0.02[a]	0.15 ± 0.17	0.16 ± 0.21
38	0.10 ± 0.12	0.03 ± 0.03[a]	0.19 ± 0.24	0.12 ± 0.12
42	0.12 ± 0.12	0.05 ± 0.05[b]	0.13 ± 0.11	0.09 ± 0.12
F statistic	4.205	7.25	0.389	0.570
P value	6.07	0.025	0.618	0.556

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 4.14: Lymphocyte count of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) lymphocyte count (x 10^9 /L)			
35	3.2 ± 0.26	3.87 ± 1.52[a]	3.5 ± 0.34	2.2 ± 0.44
38	3.1 ± 0.32	2.67 ± 1.26[b]	4.1 ± 0.40	4.0 ± 0.60
42	3.6 ± 0.62	3.77 ± 0.82[a]	4.7 ± 0.70	3.3 ± 0.51
F statistic	0.335	1.338	1.165	2.160
P value	0.653	0.023	0.328	0.153

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 4.15: Heterophil/lymphocyte ratio of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) heterophil/lymphocyte ratio			
35	0.15 ± 0.05	0.13 ± 0.06	0.19 ± 0.07	0.68 ± 0.42[a]
38	0.22 ± 0.20	0.18 ± 0.10	0.14 ± 0.06	0.17 ± 0.07[b]
42	0.22 ± 0.10	0.19 ± 0.09	0.20 ± 0.12	0.22 ± 0.11[b]
F statistic	0.877	1.511	2.442	11.568
P value	0.387	0.251	0.118	0.007

Key: n = total number of birds sampled, Mean (± SD) = standard deviation of the mean
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 4.16: Malondialdehyde concentration of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) malondialdehyde concentration (IU^{-1})			
35	1.61 ± 0.21^{a}	1.37 ± 0.23	1.76 ± 0.36^{a}	1.48 ± 0.28^{ab}
38	1.27 ± 0.27^{b}	1.51 ± 0.30^{b}	1.45 ± 0.28^{b}	1.65 ± 0.18^{ab}
42	1.34 ± 0.38	1.13 ± 0.35^{c}	1.23 ± 0.37^{c}	0.80 ± 0.18^{c}
F statistics	4.854	3.847	8.033	7.132
P value	0.028	0.044	0.008	0.000

Key: n = total number of birds sampled, Mean (± SD) = standard deviation
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

A significant decrease and a subsequent significant decrease was observed in the level of urea in group D at 38 ($p = 0.008$) and 42 ($p = 0.031$) days of age, respectively (Table 4.17). A significant decrease ($p = 0.028$) was also observed in the values of sodium at 42 days of age in group C (Table 4.18). Glucose was observed to significantly increase ($p = 0.004$) at 42 days of age in group B (Figure 4.4). A significant decrease was observed in the values of globulin in group B ($p = 0.016$) and C ($p = 0.024$) at 42 days of age (Figure 4.5).

A significant increase in the level of creatinine kinase was observed between 35 and 42 days of age in group B ($p = 0.018$) (Table 4.19). There was a significant decrease and increase in the level of AST between 38 and 42 days of age in group A ($p = 0.028$), B ($p = 0.001$), C ($p = 0.005$) and D ($p = 0.029$) (Table 4.20). The values of ALT significantly decreased and increased between 38 and 42 days of age in group A ($p = 0.000$), B ($p = 0.000$), C ($p = 0.000$) and D ($p = 0.000$) (Table 4.21). The level of ALP significantly increased and decreased between 38 and 42 days of age in group A ($p = 0.000$) and B ($p = 0.002$) (Table 4.22).

High density lipoprotein cholesterol was observed to significantly decreased ($p = 0.033$) at 38 days of age and increased ($p = 0.011$) at 42 days of age in group D (Figure 4.6). Triglycerides were however observed to significantly decreased ($p = 0.002$) at 38 days of age and increased ($p = 0.043$) at 42 days of age in group C (Figure 4.7)

Table 4.17: Urea level of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Groups			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) UREA level (IU^{-1})			
35	2.94 ± 0.59	3.03 ± 0.45	2.94 ± 0.51	3.96 ± 0.23^{a}
38	3.16 ± 0.65	3.29 ± 0.88	3.21 ± 0.72	3.26 ± 0.61^{bc}
42	3. 28 ± 0.49	3.58 ± 0.84	3.03 ± 0.49	3.32 ± 0.74^{bc}
F statistics	0.874	0.987	0.419	5.634
P value	0.433	0.361	0.636	0.014

Key: n = total number of birds sampled, Mean (± SD) = standard deviation
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 4.18: Sodium concentration in broilers fed 5% *Moringa oleifera* leaf supplemented feed.

Age in days	Group A n = 10	B n = 10	C n = 10	D n = 10
	Mean (± SD) sodium concentration (mg/dl)			
35	140.60 ± 2.76	139.70 ± 2.67	141.60 ± 2.46[a]	139.89 ± 2.93
38	140.00 ± 2.79	140.10 ± 2.51	140.10 ± 3.07	139.33 ± 2.55
42	139.90 ± 2.69	138.30 ± 2.50	137.60 ± 3.41[c]	139.56 ± 3.57
F statistic	0.183	1.147	4.031	0.076
P value	0.833	0.337	0.044	0.863

Key: n = total number of birds sampled, Mean (± SD) = standard deviation
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

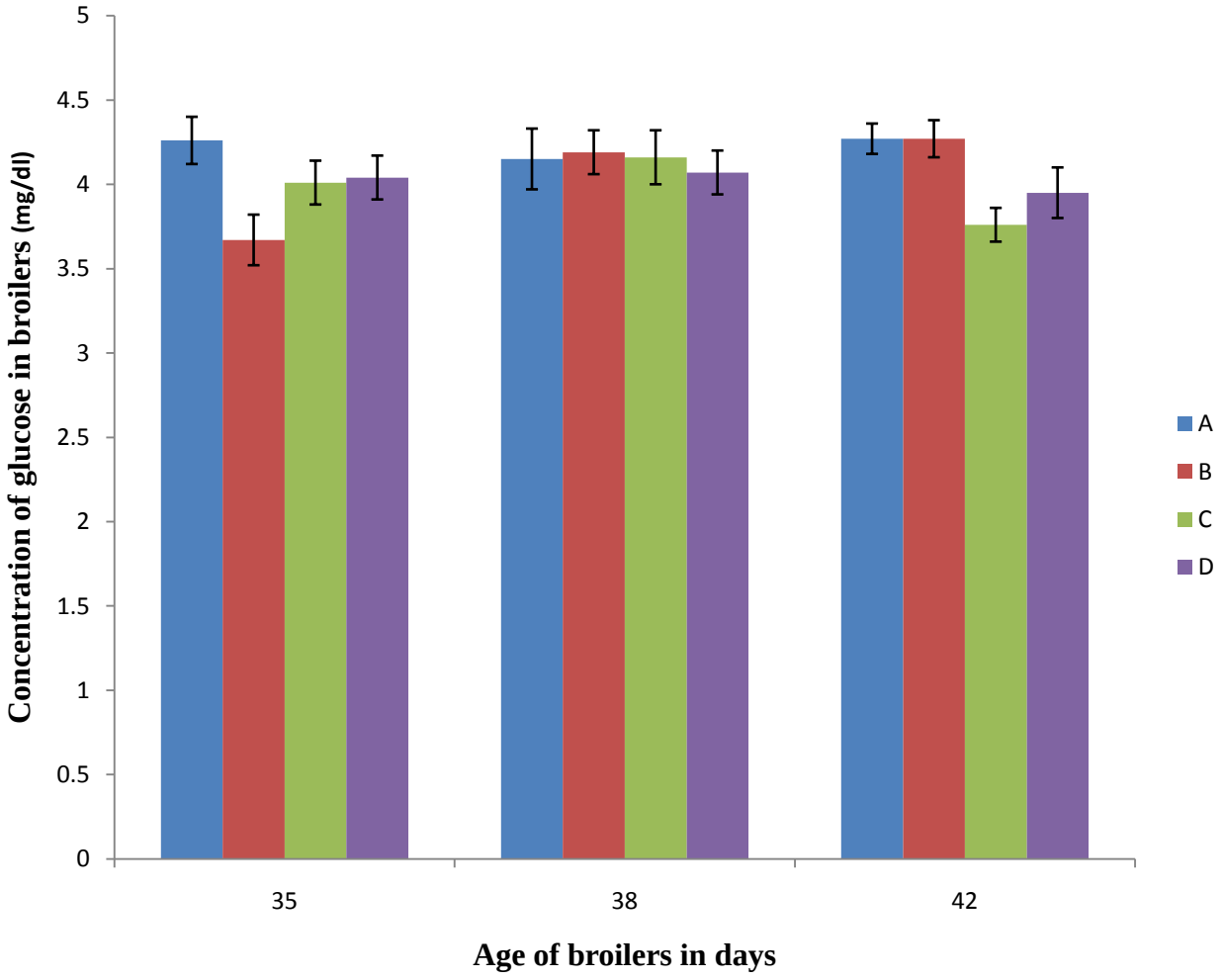


Figure 4.4: The glucose level of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.

C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

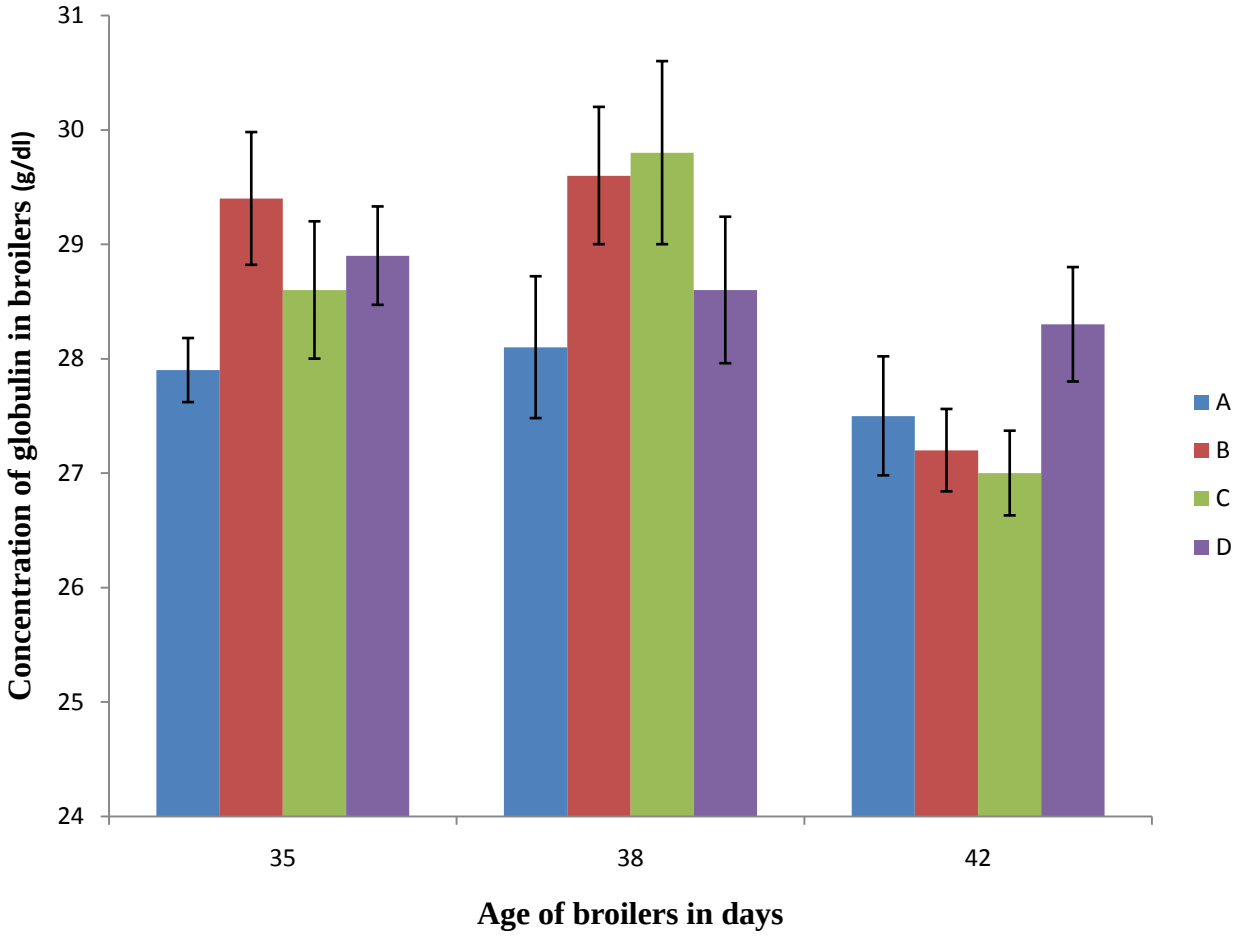


Figure 4.5: The globulin level of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.

C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 4.19: Creatinine kinase enzyme activity of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

Age in days	Group A n = 10	B n = 10	C n = 10	D n = 10
	Mean (± SD) creatinine kinase (IU L^{-1})			
35	150.80 ± 10.32	139.80 ± 2.39[a]	142.90 ± 7.48	143.63 ± 6.72
38	142.40 ± 6.99	146.70 ± 4.74[bc]	139.50 ± 6.67	151.38 ± 12.19
42	151.20 ± 10.57	150.70 ± 1.17[bc]	144.40 ± 8.97	144.88 ± 8.11
F statistics	2.910	7.035	1.083	1.200
P value	0.095	0.018	0.351	0.329

Key: n = total number of birds sampled, Mean (± SD) = standard deviation

Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 4.20: Aspertate aminotransferase enzyme activity of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SD) aspertate aminotransferase (IU L^{-1})			
35	39.90 ± 3.96^{ab}	39.80 ± 3.68^{ab}	38.40 ± 3.20^{a}	41.80 ± 3.85
38	37.50 ± 4.83^{ab}	36.80 ± 6.11^{ab}	35.50 ± 3.27^{b}	37.40 ± 4.43^{b}
42	45.10 ± 5.70^{c}	48.50 ± 4.22^{c}	42.80 ± 4.02^{c}	45.30 ± 5.64^{c}
F statistics	5.762	14.161	9.265	5.460
P value	0.028	0.001	0.005	0.029

Key: n = total number of birds sampled, Mean (± SD) = standard deviation
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 4.21: Alanine aminotransferase enzyme activity of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (±SE) alanine aminotransferase (IU L^{-1})			
35	42.90 ± 3.21^{ab}	43.30 ± 3.83^{a}	42.70 ± 4.80^{ab}	44.20 ± 4.52^{ab}
38	41.00 ± 3.20^{ab}	40.10 ± 3.03^{b}	40.30 ± 2.53^{ab}	40.40 ± 1.54^{ab}
42	49.60 ± 3.56^{c}	54.20 ± 5.53^{c}	48.60 ± 4.45^{c}	51.60 ± 3.69^{c}
F statistics	23.380	23.924	14.126	12.101
P value	0.000	0.000	0.000	0.003

Key: n = total number of birds sampled, Mean (± SD) = standard deviation
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

Table 4.22: Alkaline phosphatase enzyme activity of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
Age in days	Mean (± SE) alkaline phosphatase (IU L^{-1})			
35	82.60 ± 6.31^{ab}	84.40 ± 4.62^{ac}	83.80 ± 7.18	81.50 ± 5.40
38	79.20 ± 6.61^{ab}	77.70 ± 5.96^{b}	82.90 ± 7.29	81.10 ± 8.42
42	96.70 ± 6.04^{c}	88.20 ± 6.70^{ac}	84.90 ± 6.67	78.70 ± 5.14
F statistics	23.994	10.311	0.217	0.443
P value	0.000	0.002	0.803	0.636

Key: n = total number of birds sampled, Mean (± SE) = standard error of the mean
Means having different superscripts alphabets on the same column differ significantly $p<0.05$

A= Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
B= Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.
C= Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.
D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

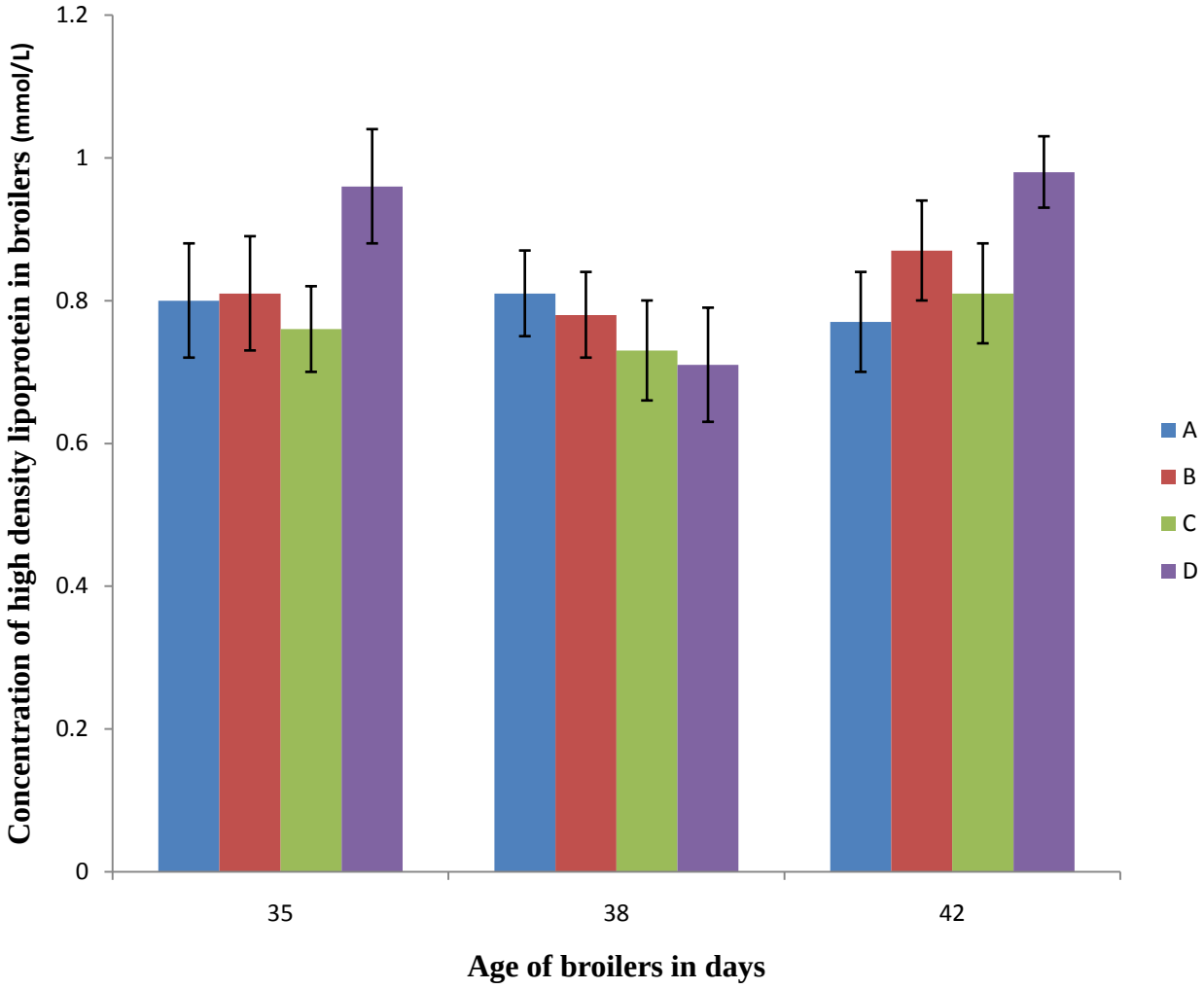


Figure 4.6: High density lipoprotein level of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

Key:A = Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

B = Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.

C = Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

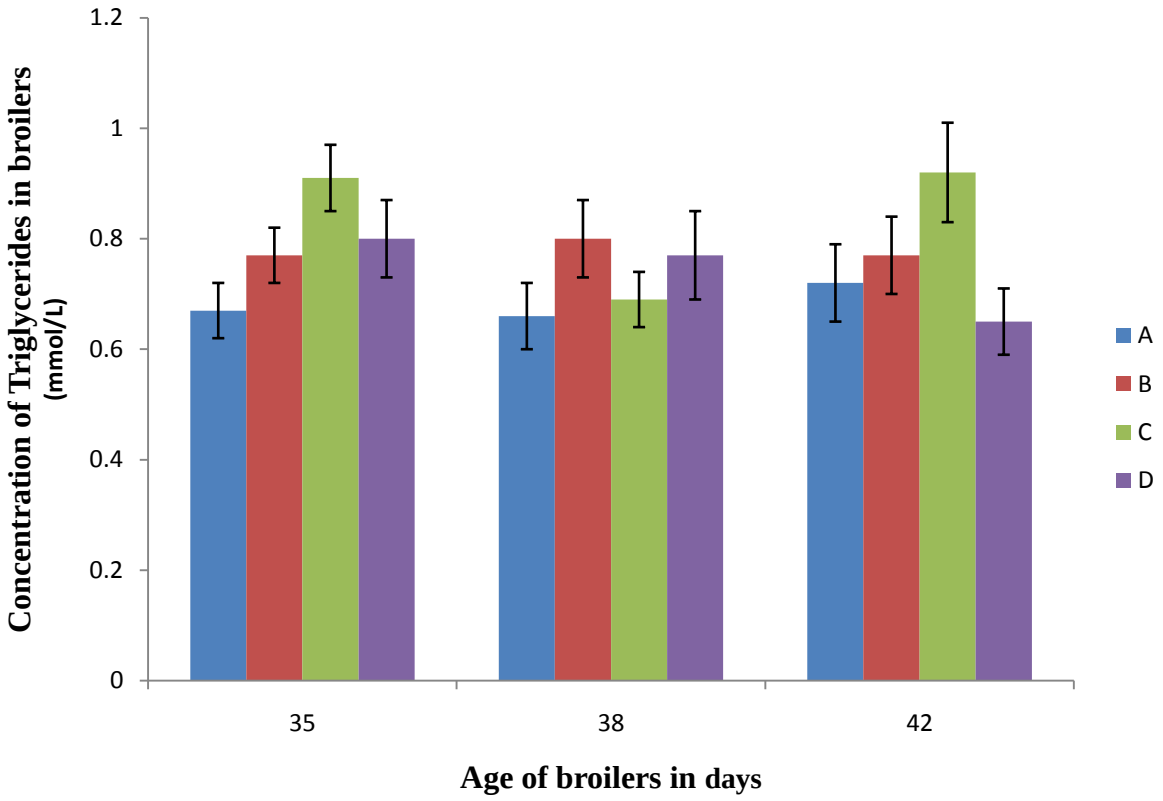


Figure 4.7: Triglycerides' level of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

Key: A = Broilers fed *Moringa oleifera* leaf supplemented feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

B = Broilers fed *Moringa oleifera* leaf supplemented feed, not vaccinated but challenged at 35 days old with very virulent infectious bursal disease virus.

C = Broilers fed control feed, vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old and challenged at 35 days old with very virulent infectious bursal disease virus.

D = Broilers fed control feed, not vaccinated and not challenged at 35 days old with very virulent infectious bursal disease virus.

CHAPTER FIVE

DISCUSSION

The 25.9 % crude protein (CP) obtained in this study showed MOL to be a potential source of supplementary protein in chicken diet as proteins serves as essential building blocks of body tissue. This agrees with the findings of Makkar and Becker (1997) and Sarwatt *et al.* (2002), that MOL are a rich source of protein. The level of crude protein (25.9 %) is of particular nutritional significance as it is capable of meeting the chicken's protein requirements and boosting the immune system against diseases (Makker and Becker, 1997; Kyriazakis and Houdijk, 2006; Brisibe *et al.,* 2009).

The quantity of CP in MOL used in this study means that it could be used as feed supplement to prevent protein deficiency in the diet of chickens as their amino acid requirement is proportional to the CP content of their diet (NRC, 1994). Amino acids are needed in direct proportion to the dietary protein level. The level of feed consumption in chickens depends on the level of the protein in diet (Morris *et al.,* 1987; Mendonca and Jensen, 1989). The 25.9% CP of MOL recorded in this study is higher than 17.01% reported by Ogbe and Affiku (2011), 22.23%, by Aye and Adegun (2013), 1.40%, by Aja *et al.* (2013) and 18.92% by Nkechinyere and Nwafor (2014). However, the findings of this study is lower than 28.0% and 27.61% (for late and early maturation, respectively) reported by Bamishaiye *et al.* (2011), 39.13%, by Sodamade *et al.* (2013), (27.44%), Olugbemi *et al.* (2010a), (30.27%) Moyo *et al.* (2011), (27.2%), Yameogo *et al.* (2011) and Mutoyoba *et al.* (2011) (30.65%).

The variations in the values of the CP may be as a result of different climatic conditions of the various locations, different soil types where the leaves were collected and the stage of maturity, in addition to edaphic factors as reported by Bamishaiye *et al.* (2011).

The high carbohydrate content of MOL in this study (55.14%) agrees with the report of Bamishaiye *et al.* (2011). Though the value for carbohydrate in this study is higher than those of Oluduro (2012) who reported 45.43%, Sodamade *et al.* (2013) (38.21%) and Yameogo *et al.* (2011) (38.6%), it is however lower than the 63.11% reported by Ogbe and Affiku (2011) and 57.01% reported by Nkechinyere and Nwafor (2014). The high content of carbohydrate indicates that MOL can provide energy when incorporated in feeds. The nutritional variations observed in the several studies of MOL could be attributed to the genetic background of the plant, in terms of ecotype and cultivar, environmental factors that include the soil and climate (Sanchez-Machado *et al.,* 2009). In addition, the cultivation method used encompasses the frequency of harvesting and age of the plant or leaves, and the method of conservation between collection and analysis (drying, refrigeration, freezing) might influence MOL nutritional composition (Barminas *et al.*, 1998; Broin, 2006).

The result of the qualitative phytochemical analysis of MOL agrees with the work of several authors on the presence of most of the metabolites using different solvents (Bamishaiye *et al.*, 2011; Oluduro, 2012; Ojiako, 2014), but differ from the work of Ojiako (2014) and Oluduro (2012) who reported the absent of phlobotannins and steroid, respectively using ethanolic extract of MOL. The findings in this study agree with earlier

studies by Majorie (1999), Tijjani *et al*. (2009) and Ayinde *et al*. (2007) who reported that extracting phytochemicals from plant parts depends on the extraction solvent used.

The quantitative analysis showed an appreciable quantity of tannins, saponins, phytates, oxalate and cyanide using aqueous extract of MOL. Aqueous extract has been shown to contain more phytochemicals than ethanolic extract of MOL (Oluduro, 2012; Nkechinyere and Nwafor, 2014). The values of the concentration of oxalate obtained in this study agrees with the work of Ogbe and Affiku (2011), but differ from the work of Ojiako (2014) who reported 8.22% for tannins and 1.75% for saponins. The low quantity of anti-nutrients detected in this study means that, the MOL used for this study can improve growth performance and health status of poultry. The presence of flavonoids observed in the MOL indicates the antifungal activity and antioxidant capability of the MOL. Flavonoids have been shown to possess an *in vitro* antifungal activity (Galeotti *et al.*, 2008), and Alan and Miller (1996) revealed that, scavenging hydroxyl radicals, superoxide anion and lipid peroxy radicals are the most important function of flavonoids.

The level of saponin in MOL found in this study is of significant health benefit to poultry. Saponins have been shown to possess beneficial properties (such as lowering of cholesterol level, bone health and stimulation of immune system) when consumed in low concentration (Price *et al.,* 1987; Oakenful and Sidhu, 1989) and a deleterious effect (cytotoxic, permeabilization of the intestine) when present in high concentration although acute poisoning is relatively rare in both animal and man (Osagie, 1988). High concentration of saponin have been shown to reduce feed intake and growth rate in poultry (Dei *et al.*, 2007)

and the reduction in feed intake has been ascribed to the bitter taste of saponins (Cheeke, 1971).

The composition of minerals found in MOL plays a significant role in nutritional, medicinal and therapeutic values (Al-kharusi *et al.,* 2009). The presence of iron in MOL will assist in the production of haemoglobin and myoglobin which is important in transporting oxygen and further cellular processes of growth and division (Kozat, 2007). Umar *et al.* (2007) found that iron is an essential trace element for the normal functioning of the central nervous system and in the oxidation of carbohydrate, proteins and fats. The result of the current study showed that MOL contains appreciable quantity of zinc.

The presence of high amount of zinc in the MOL found in this study is of particular interest because of the importance of its inclusion in the diet of chickens. Zinc is essential for the synthesis of DNA, RNA, insulin and function in the structure of several enzymes, cell reproduction and growth, especially sperm cells in addition to its antiviral, antibacterial, antifungal and anticancer properties (Brisibe *et al.,* 2009). The values obtained in this study for zinc were higher than that reported by Moyo *et al.* (2011), but was lower than the report of Ogbe and Affiku (2011).

The level of manganese (40.5 ppm) found in the MOL for this study will assist in building the immune system, regulation of blood sugar level and production of energy (Suttle, 2010). Manganese help in preventing bone abnormalities known as perosis, which is why the requirement for manganese is much higher in broilers and turkeys than any other animal (Suttle, 2010). Reduced manganese supplementation in the diet of broilers reduces the

percentage of abdominal fats (Lu *et al.,* 2007). The quantity of manganese found in this study was lower than that reported by Moyo *et al.* (2011) and Ogbe and Affiku (2011).

The significant positive correlation observed in the daily feed intake (DFI) of broilers in all the groups on day 42 (7 days post infection) might have been due to the challenge with the vvIBDV. Reduced feed intake observed in groups A and B could either be as a result of the change of feed from broiler starter to broiler finisher at 28 days of age or it could be associated with the presence of tannins found in the MOL which has been reported to reduced palatability of the MOL in feeds (Kakengi *et al.,* 2003). This was further aggravated in this present study by challenge with vvIBDV, as IBD is known to be associated with anorexia (Tsukamoto *et al.,* 1995; Islam *et al.,* 2001).

The result of the DFI appears not to have greatly affected the live body weight (LBW) of birds in groups A and B, though; LBW of birds is dependent on feed intake. This is in agreement with the findings of Portugaliza and Fernandes (2012) who reported a similar trend. The result of this study also showed that, even after challenge with vvIBDV, there was considerable increase in the LBW of birds in groups A and B, while those in group C experienced a significant decrease in their LBW. The increase in the LBW of birds in groups A and B despite challenge with vvIBDV could be attributed to the presence of amino acids, vitamins, minerals, antioxidants, immunostimulants and antibacterials found in the MOL which could have aided the increase in the LBW (Makkar and Becker, 1997; Fahey, 2005; Anwar *et al.,* 2007).

It was also observed that the FCR of broilers in groups A and B were lower than those in groups C and D. It therefore implies that birds fed MOL supplemented diets will adequately

utilize the nutrients in the feeds they consume. This could probably be the reason for the increase in LBW of birds in groups A and B when compared with those in group C. Ebenebi *et al.* (2012) and Safa and El Tazi (2012) also recorded lower FCR in broilers fed MOL supplemented diet when compared with the controls.

The study showed a steady and an uninterrupted increase in the LBW of broilers in groups A and B even after challenge with vvIBDV. This was in contrast to the birds in group C that experienced a decrease in LBW after challenge with vvIBDV which consequently negatively affected their final LBW. The lower FCR and increase in LBW of birds observed in groups A and B may be attributed to the rich nutrient contents (Kakengi *et al.,* 2003; Sarwatt *et al.,* 2004) and antimicrobial properties of MOL (Fahey *et al.,* 2001; Ratshilivha *et al.,* 2014).

The final live weight, carcass weight and percentage dressed carcass weight were found to be higher in the control (group D) followed by groups A, B and C. This difference observed in the weight of the birds in groups A, B and C as compared to D was due to the challenge of the birds in groups A, B and C with vvIBDV. Although, to the best of our knowledge, no literature had reported final live and carcass weight of broilers fed with MOL and challenged with vvIBDV, Maroufyan *et al.* (2010) had earlier recorded a decrease in the live and carcass weight of broilers after challenge with vvIBDV.

The increase observed in the live and carcass weight of broilers in groups A and B when compared with those from group C could be that, birds from groups A and B could have recovered earlier from the IBD than those from group C due to the immune

boosting/antiviral properties of MOL (Ratshilivha *et al.*, 2014; Fuglie, 1999) and they resumed eating faster as MOL have been reported to aid digestability, absorption and performance (Aregheore, 2001). The improved weight gain of birds fed MOL supplemented diets could be attributed to the higher protein content of the feeds which were efficiently metabolized for growth.

The result of inclusion of MOL for broilers in groups A and B when compared to those without MOL inclusion (group C) is in conformity with the works of Kakengi *et al.* (2003); Olugbemi *et al.* (2010a) and Banjo (2012) who in their separate studies reported that inclusion of MOL in the diet of broilers enhances their weight gain when compared to those without MOL in their diet. Ologhobo *et al.* (2014) also reported that higher mean values of slaughter weight were obtained for birds fed diet containing 5% MOL as compared to those fed with control diet. The result of this study is also in agreement with the work of Onu and Aneibo (2011) who reported that broilers fed with 5% MOL in feed recorded a significantly higher body weight which could be attributed to the higher protein content of the diet. The none challenge of broilers in group D with vvIBDV could possibly be the reason for higher weight observed when compared with those in groups A, B and C.

Also, inclusion of the MOL in the diet of birds in groups A and B increased the weight of organs in them when compared to the birds in group C. This is evidenced in the weight of the proventiculus, gizzard, liver, heart and lungs. The increase in the weight of these organs is proportional to the LBW in the various groups. This result is in agreement with that of Safa and El Tazi (2014) who reported that birds fed MOL in their diet had a higher organ (proventiculus, gizzard, liver, spleen, heart, lungs) weight when compared with controls.

Similar result have also been reported by Preston and William (1973) who indicated that heavier birds at slaughter would have higher dressing percentage as well as organ weight.

An intense yellowish colouration of the legs, skin and beak of broilers in group A and B were observed. This may be due to the high content of beta-carotene and xanthophylls in the MOL which the birds efficiently absorbed and utilized. Diets rich in xanthophylls pigmentation influence the yellowing of skin, abdominal fats and egg yolk (Talpin *et al.*, 1981; D'mello *et al.*, 1987; Surai *et al.*, 2001; Agbede and Aletor, 2003). The yellow colouration observed in this study is in agreement with the report of Olugbemi *et al*. (2010 a, b) and Etalem *et al.* (2013), who also observed that, intense yellow colouration of the beak, legs, abdominal fat, skin and egg yolk of broilers increased when MOL was included in their diets.

The high cost of purchasing and processing of the harvested MOL greatly increased the price of feed/kg diet of the birds in groups A and B which ultimately increased cost of feed per bird. This agrees with the work of Ayssiwede *et al.* (2011) who reported an increase in the cost of feed per bird as a result of cost of MOL in the diet of chickens. The increase in the cost of feed/kg carcass in groups C and D is also in conformity with the report of Ayssiwede *et al.* (2011). This increase in the cost of feed/kg carcass ultimately increases the gross margin of feed (GMF)/kg carcass in groups A and B when compared with those in groups C and D. This report is in line with the findings of Onibi *et al.* (2008) in Nigeria and that of Tendonkeng *et al.* (2008) in Cameroon who in their separate studies indicated that feed cost/kg of LBW of broiler finishers were increased with the inclusion of leuceana leaf or MOL, respectively in their diets.

At the selling price/bird carcass of ₦650, the result of this study showed that birds from control group D had a higher return on investment, followed by the birds from groups A, B and C. Although, there is no available literature that reported selling price/bird carcass of broilers challenged with vvIBDV, the result of this work shows that, despite challenge with vvIBDV, birds that were fed with MOL included in their diet (groups A and B) had a significantly higher return on investment than those from group C which had no MOL in their diet.

The significant decrease observed in the ELISA antibody titre in birds of groups A, B, C and D at 21 days of age indicates a decline in the maternally derive antibody (MDA) level in the birds. Hair-Bejo *et al.* (2004) and Babiker *et al.* (2008) reported a decline in the MDA level of broilers at 14 and 17 days. Another reason for the decline observed could be that the inactivated IBD vaccine used in vaccinating the birds of groups A and C may have delayed in inducing an immune response.

The result of this study agrees with the experimental findings of Faragher (1972) and Phatek (2000), who in their separate studies reported that inactivated vaccine induces an antibody response more slowly than a live vaccine. However, the significant increase observed in the ELISA antibody titre level of birds in groups A and C at 35 days of age, showed a better seroconversion and immune response to the second dose of the inactivated IBD vaccine administered at 21 days of age. This finding is in agreement with the report of Ahmed and Akhter (2003) who reported that higher antibody titre level against IBD was achieved by using two doses of inactivated IBD vaccine at 10 and 21 days of age.

The increase in the ELISA antibody titre level in the birds of group B at 35 days of age could suggest that, the MOL inclusion in the diet of broilers in that group may have been responsible for the increase. *Moringa oleifera* leaves have been reported to be an immune modulator in poultry (Jayavardhanan *et al.,* 1994; Olugbemi *et al.,* 2010a). Although, there has not been any report on the humoral immune response against IBDV in broilers fed MOL, Didacus *et al.* (2013) reported an increase in the haemagglutination inhibition (HI) titre against Newcastle disease in unvaccinated broilers using methanolic extract of MOL. The significant increase observed in the ELISA antibody titre level in birds of group B at 38 (3 dpi) days of age, could indicate a stimulation of an active immunity (Haddad *et al.*, 1997). Since the birds in group B were not vaccinated against IBD with any of the inactivated IBD vaccines, it can be suggested that, the MOL included in their diet may have aided in stimulating active immunity, since it possess immune modulatory properties (Jayavardhanan *et al.*, 1994; Olugbemi *et al.,* 2010a). The significant decrease observed in the ELISA antibody titre level of birds in group D at 21 and 35 days of age, could be as a result of weaning of the antibody titre against IBD, as the birds in this group were not vaccinated against IBD.

The higher bursal, spleen, Harderian and thymus to body weight index observed in the birds of groups A and B before the challenge with vvIBDV (35 days of age) could be an affirmation to the immune properties of MOL that has been reported (Jayavardhanan *et al.*, 1994; Olugbemi *et al.,* 2010a), and could also be that MOL included in the diet of birds in groups A and B must have stimulated the infiltration of more lymphoid cells into the various organs. Increase in the bursal and Harderian to body weight index observed in group A at 38 days of age indicates the production of more B cells by these organs and also

signify the importance of MOL with respect to immune stimulation. The decrease in the bursal and Harderian to body weight index observed at 42 days of age indicates that both the MOL in the diet of birds in groups A and B and the inactivated IBD vaccine given to birds in groups A and C could not prevent the atrophy caused by vvIBDV of these organs.

An observed increase in the TBI of birds in group A may suggest that both the inactivated IBD vaccine and MOL in the diet of the birds may have been responsible for the increase. This is because of the immune modulatory properties of the MOL (Olugbemi *et al.*, 2010a) and the immune response due to the vaccination with inactivated IBD vaccine.

The lower TBI of birds in group B could either be due to the non vaccination of the birds with inactivated IBD vaccine or that the immune modulatory properties of MOL alone (without vaccination with IBD vaccine) could not cause an increase in the TBI. Very virulent infectious bursal disease virus is known to cause the destruction of the B lymphocytes and has little or no effect on the T lymphocytes and the thymus is responsible for the production of T cells (Cooper *et al.,* 1966; Boehm and Bleul, 2007). This was observed from the findings of this study where the vvIBDV was shown not to cause atrophy of the thymus in birds of groups A and C.

The clinical signs and gross lesions that were observed in this study were as earlier reported in several IBD outbreaks (Abdu, 1997; Lukert and Saif, 1997; Cereno, 2008; de Wit and Baxendale, 2013). The morbidity rate of IBD for the birds in group B agrees with the work of Jindal *et al.* (2004) who reported a lower IBD morbidity rate (7%) in unvaccinated broilers of ages between 31 to 40 days of age. Still, the findings of this work showed that

the mortality rate for birds in group B is far lower than the 25-30% reported for broilers by Nunoya *et al.* (1992) and Van den Berg *et al.* (1991). The absence of mortality observed in birds from groups A and C could be as a result of vaccination with inactivated IBD vaccine at 14 and 21 days of age. Inactivated IBD vaccine has been reported to be efficacious against IBD (Angani *et al.*, 2014). The result of the findings also revealed that more birds in group B showed clinical signs of IBD than those in groups A and C. This may not be far from the fact that they were the first group to show the clinical signs of sickness and that consistently, the daily morbidity rate was higher in the birds of group B starting from 35 days of age to 43 days of age. The low mortality rate observed in birds from group B could be that the immune-modulatory properties of MOL (Olugbemi *et al.*, 2010a) included in their diet must have helped in reducing the high mortality associated with vvIBDV.

The significant decrease in PCV and RBC observed in groups A, B and C at 3 dpi could be due to anaemia resulting from haemorrhage which is usually associated with IBD infection (Moss, 1999; Skeeles *et al.*, 1980). This finding agrees with the works of Panigraphy *et al.* (1986) and Kassim, (2014) who in their separate studies reported a significant reduction in the values of PCV and RBC at 5 dpi with vvIBDV, when 4 weeks old broilers and cockerels were challenged, respectively. This result is however, contrary to the findings of Oladele *et al.* (2005) who reported an increase in PCV of broilers challenged with vvIBDV at 32 days of age. The significant increase in PCV at 42 days of age observed in group B could suggest that minerals such as iron that were found in high quantity in the MOL used for supplementing the feed fed to broilers in group B may have possibly helped in increasing their PCV.

The increase in the concentration of haemoglobin observed in groups B and C could indicate polycythaemia that could be due to dehydration, which is a characteristic finding in IBD (Panigraphy *et al.,* 1986). It could also imply that the 5% MOL supplemented diet and the feeds without MOL were rich in iron which is responsible for the synthesis of haemoglobin.

The increase in TWBC count observed in group C could be a response to subclinical bacterial infections such as *E coli*. Infectious bursal diasease have been reported to increase the susceptibility to various bacterial infections (Niki, 1996; Shane, 1997). The significant increase observed in the values of eosinophils in group B was probably as a result of an increase from an initial absence of eosinophils at 35 days of age (pre infection with vvIBDV).

The significant decrease observed in the values of lymphocyte in group B, showed a marked lymphopenia, at 3 dpi with vvIBDV. It is well known that viral infections in birds are associated with lymphopenia (Jain, 1986). This is because IBDV causes the destruction of B-lymphocytes within the bursa of Fabricius before their migration into the blood stream, thus causing the reduction in the number of lymphocytes in the blood (Weiss and Kaufer-weiss, 1994). After infection with IBDV, an increase in the percentage of lymphocyte without lysosomes and a decrease in lymphocytes with large single and large multiple lysosomes have been reported at 3 dpi (Klucinski *et al.,* 1984). The finding of this work agrees with that of Oladele *et al*. (2005) and Kassim (2014) who separately reported lymphopenia at 6, 12, and 48 hours, and a subsequent, increase in lymphocyte counts between 120 and 144 hours post infection with vvIBDV in 4 weeks old broilers and

cockerels, respectively. The result of this study implies that despite feeding broilers with 5% MOL supplemented feed; the vvIBDV was able to cause destruction of B-lymphocytes.

Increase H/L ratio has been used as an important indicator of stress in birds (Gross and Siegel, 1983). Stress in birds which may vary from food or water deprivation, temperature extremes, constant light or diseases usually elevates the number of heterophils and depresses the number of lymphocytes (Gross, 1989; McFarlanje and Cutis, 1989). The highly significant increase in H/L ratio observed in broilers of group D may not necessarily be due to the above mentioned stressors, because broilers used for this study were not deliberately disturbed in anyway. However, it was observed that broilers in group D had more males than females, though not deliberately apportioned. This high number of males could probably be the reason for the higher H/L ratio observed. This finding is in agreement with the findings of Al-Murrani *et al.* (1997) where he reported that male broilers had a higher H/L ratio when compared to female broilers and suggested that, the additional stress of higher body weight in males must have attributed to the increase in the H/L ratio.

Malondialdehyde (MDA) is an indicator of lipid peroxidation which usually occurs in birds as a result of high oxidative stress. The significant MDA decrease among the broilers of groups A and C at 38 days of age indicates that the IBDV vaccine administered to them was able to prevent lipid peroxidation due to oxidative stress that is associated with vvIBDV. The significant increase in the level of MDA in group B at 38 days of age showed that the MOL could not prevent lipid peroxidation that might have taken place as a result of oxidative stress following challenge with vvIBDV. Comparison between groups showed

that, broilers in group A significantly had lower levels of MDA at 38 days of age than those in groups B, C and D.

The finding (above) implies that supplementing broilers feed with MOL without vaccination against IBDV may not prevent lipid peroxidation in broilers following infection with vvIBDV. The decrease in MDA level observed in group A when compared with those in groups B, C and D could be associated with the amount of Zn (34 ppm) contained in the MOL that was used in supplementing the diet fed to broilers of groups A and B. This is because Zn has been reported to induce the production of metallothionein, which is said to be effective in scavenging hydroxyl radical (Sahin *et al.*, 2009). In other studies, inclusion of Zn in the diet of broilers has been shown to result in decrease in the level of MDA (Tawfeek *et al.*, 2014).

The significant decrease in Na^+ concentration (hyponatermia) observed in group C could be as a result of the dehydration, anorexia, diarrhoea, reduced water intake that is associated with IBD infection. These signs were observed in the present study commencing at 2 dpi in groups A, B and C. The reduction in the concentration of Na^+ observed in this study agrees with the findings of Tesfaheywet *et al.* (2012), who reported a reduction in the concentration of Na^+ in the serum of broilers at 5 and 7 dpi with vvIBDV. The findings of this study could also suggest that the amount of Na^+ (0.11%) contained in the MOL used for this study may have been responsible for the maintainance of Na^+ concentration of broilers in groups A and B following challenge with vvIBDV.

The increase in the uric acid concentration observed in group D could be as a result of an impaired kidney function which may be due to an immune-mediated glomerulonephritis compatible with immune-complexemia (Ley *et al.,* 1983). Renal function in chickens is indicated by serum uric acid concentration. This is because the uric acid is the major nitrogenous end product of chickens excreted in to the urine through the renal tubules (Sturkie, 1986).

The significant increase observed in the values of globulin in groups B and C at 3 dpi shows that vvIBDV has not affected the concentrations of globulin. This finding agrees with that of Afaleq (1998), and Panigraphy *et al.* (1986) following infection with vvIBDV. Significant difference was not observed in the values of total proteins and albumin within the group between 38 and 42 days of age. However, total protein and albumin significantly increased in group C when compared to groups A, B and D at 35 days of age. This finding contradicts that of Afaleq (1998) and Panigraphy *et al.* (1986) who reported a decrease in total proteins and albumin, respectively in the serum of birds following challenge with vvIBDV. The finding of this study also imply that MOL supplementation in the diet of groups A and B did not significantly increased their serum total proteins, which is contrary to the findings of Onu and Aniebo (2011), who reported a significant increase in total proteins when broilers were fed with MOL at 5% inclusion rate.

The significant increase in glucose level observed in group B following challenge with vvIBDV may be associated with the high available energy contained in the MOL that was used in the supplementation of the diet used for this study. In broilers, CK is believed to be

released into circulation following changes in the permeability of the sarcolemma (muscle membrane) in response to various pathologies or physiological changes in the body (Mitchell *et al.,* 1992; Mitchell and Sandercock, 1995). The significant increase in CK observed in groups B and D could not be as a result of challenge with vvIBDV, because IBD infection is not associated with muscle damage (Holland *et al.,* 1980), but could either be due to increase in liver metabolism or due to the significant muscle development that usually occur at this age. This is evident in the significant weight gain earlier observed in all the groups in the course of this study. This finding agrees with an observation made by Szabo *et al.* (2005) in turkeys of commercial strain.

The significant increase in serum concentrations of AST and ALT at 42 days of age in groups A, B and C is suggestive of pathology involving the liver and kidneys, respectively which is common sequelae in IBDV infection, especially following secondary viraemia (Hair-Bejo *et al.,* 2004; Roosevien, 2006). Liver and kidney injuries are postulated to result from hypoxic state caused by aplastic bone marrow following IBDV infection (Nunoya *et al.,* 1992). The finding of this study is in agreement with that of Tesfaheywet *et al.* (2012) who reported an increase in AST, ALT and ALP at 3, 5 and 7 dpi with vvIBDV in 32 day old broilers. The finding of this study therefore implied that MOL did not protect the liver and kidneys from the pathological damage caused by vvIBDV.

The significant decrease observed in the values of TG in group C at 38 days of age could be associated with the anorexia and diarrhoea that usually accompanied IBDV infection. This condition will cause a reduced availability and absorption of fatty acid (Dhawale, 2007). Similar finding was reported by Tesfaheywet *et al.* (2012) when 32 day old broilers were challenged with a vvIBDV. The significant decrease and increase observed in HDL-

cholesterol in group D could be due to the high energy demand at this stage of their growth due to high body development (Almeida *et al.,* 2006).

High density lipoproteins are sets of lipoproteins that vary in sizes from 8 to 11 nm in diameter. Lipoproteins aids in the transportation of fatty acids and cholesterol from the body's tissue to the liver. They help in preventing the accumulation of cholesterol by taking excess cholesterol away and are therefore known as the 'good' cholesterol (Blake *et al.,* 2002). The findings of this study therefore showed that, MOL supplementation in the diet of broilers had no influence on the lipid profile of broiler chickens. This agrees with the findings of Zanu *et al.* (2012) and Gakuya *et al.* (2014) who in their separate studies reported that MOL had no significant influence on the lipid profile of broiler chickens. However, the findings of this study is contrary to the reports of Olugbemi *et al.* (2010a) who noted that MOL possesses hypocholesterolemic properties in broilers, and that of Ashong and Brown (2011) who reported MOL to have significantly decreased the levels of cholesterol and triglycerides in White Leghorn.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Based on the findings of the study, it can be concluded that:

1. *Moringa oleifera* leaves harvested from Potiskum Yobe State, contained; carbohydrate (55.14%), CP (25.9%), crude fibre (13.91%), moisture (7.94%), fat (5.85%), ash (3.72%), energy (2930.63 Kcal/Kg), phytates (2.57%), tannins (2.19%), saponins (1.06%), oxalates (0.45%), cyanides (0.1%) and Ca (2.26%), P (0.35%), Mg (0.45%), K (1.9%), Na (0.11%), Zn 34 ppm, Cu (7.5 ppm), Mn (40.5 ppm), Fe (116.5 ppm), Se (0.85 ppm),
2. Final mean live body and carcass weight were higher in broiler fed with MOL and challenged with vvIBDV (A and B) (1,739 g ± 88.97 and 1,493 g ± 111.9, respectively) than those not fed with MOL but were challenged (group C) (1,453 g ± 103.4),
3. *Moringa oleifera* leaf inclusion in the diet of broilers remarkably increased the yellowish colouration of the skin of the legs and beak of broilers in groups A (3.00 ± 0.0) and B (3.40 ± 0.24) out of a maximum scale of 4.
4. Feed conversion ratio was lower in group B (0.59 ± 0.03) followed by birds in group A (0.72 ± 0.02), C (1.02 ± 0.07) and D (1.27 ± 0.03).
5. *Moringa oleifera* leaf inclusion in the diet of broilers resulted in reduced feed intake, (99.77 g ± 6.43 and 108.1 g ± 7.43) and increased average daily weight gain (1,739 g ± 88.97 and 1,493 g ± 111.9) of broilers in groups A and B, respectively,
6. Broilers from groups A and B had a higher return on investment (₦1, 093 ± 54.11 and ₦935.9 ± 70.69, respectively) than those in groups C (₦908.3 ± 63.97).

7. *Moringa oleifera* leaf feed supplementation improved the bursa (1.4, 1.4) spleen (1.3, 1.1) and Harderian gland (2, 2) to body weight index of broilers of group A and B, respectively.
8. The MOL feed supplementation and inactivated vaccine did not prevent the atrophy of bursa (0.71, 0.86) and harderian gland (0.33, 0.66) against the negative effect of vvIBDV at 7 dpi in groups A, B and C.
9. Challenging broilers with vvIBDV did not cause a reduction in the TBI of birds in group A (1.05), B (1.02) and C (1.22) 3 dpi.
10. Supplementing broiler feed with MOL and vaccinating them against IBD decreased the TBI of birds in group A (1.05, 1.03 and 1.09) at 35, 38 and 42 days of age, respectively.
11. Morbidity rate was consistently higher in group B (2.2%, 6.7%, 24.4%, 15.9%, 9.1%, 6.8% and 2.3%) than in group A (2.2%, 15.6%, 8.9%, 1.1%, 4.4%) and C (2.2%, 17.8%, 11.1%, 6.7%, 4.4%, 2.2%) from 2 to 8 dpi.
12. Feeding broilers with 5% MOL supplemented diet (group B) without vaccination did not prevent vvIBDV from causing a significant decrease in B-lymphocyte count from 3.9 ± 0.48 to 3.1 ± 0.40 3 dpi.
13. Supplementation of broiler feed with 5% MOL and vaccination with inactivated IBD vaccine did not prevent a decrease in PCV (21.80 ± 1.0 and 21.10 ± 0.60) and RBC (2.01 ± 0.13 and 1.78 ± 0.11), but caused a decrease in Hb (10.78 ± 0.2 and 10.32 ± 0.29) concentration 3 dpi with vvIBDV in groups A and B.
14. Feeding broilers with 5% MOL supplemented diet without vaccination caused a significant decrease in lymphocyte count (from 3.9 ± 0.48 to 3.1 ± 0.40) in group B at 3 dpi with vvIBDV than those in groups A and C.

15. Supplementing broilers feed with or without MOL could not protect the liver from pathological damage as evident by increase in the AST and ALT in group A (39.9 ± 1.25 to 45.1 ± 1.80 and 42.9 ± 1.02 to 49.6 ± 1.13), B (39.8 ± 1.16 to 48.5 ± 1.34 and 43.3 ± 1.21 to 54.2 ± 1.75) and C (38.4 ± 1.01 to 42.8 ± 1.27 and 42.7 ± 1.29 to 48.6 ± 1.41) following challenge with vvIBDV.
16. Supplementing broilers feed with MOL without vaccination against vvIBDV could not prevent lipid peroxidation in broilers of group B (from 1.37 ± 0.07 to 1.51 ± 0.09) following inoculation with vvIBDV.
17. Supplementing broilers feed with MOL maintained the level of Na^+ concentration in broilers of group A (141 ± 0.87 to 140 ± 0.88) and B (140 ± 0.84 to 140 ± 0.79) following inoculation with vvIBDV.

6.2 Recommendations

Based on the findings of the study, it is recommended that:

1. Feed millers be encouraged to create awareness among poultry farmers on the nutritional and health benefits of MOL inclusion in poultry feeds.
2. Crop farmers should be encouraged to cultivate more *Moringa oleifera* plants in order to reduce the cost of inclusion of MOL in broiler feed.
3. The inclusion of *Moringa oleifera* leaf in feeds should be recommended to farmers to reduce the feed conversion ratio of chickens.
4. Histopathological studies should be conducted on the immune organs of broilers fed MOL to determine the type and extent of tissue and cellular changes.

5. *Moringa oleifera* leaf in the diet of broilers should not be used as vaccination against vvIBDV.
6. Feed millers are encouraged to incorporate 5% MOL in the diets of broilers in order to guide against electrolyte imbalance.
7. Further studies should be conducted to determine the effect of MOL supplemented broiler diet on serum amino acids, vitamins and other minerals (Se, Zn, Fe, Cl, P, S, Cu, Cb, Mn, and I) not analysed in the present study following challenge with vvIBDV.

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