EVALUATING THE EFFECT OF *MOMORDICA BALSAMINA LINN*, SELENIUM AND LAMIVUDINE FOR THE TREATMENT OF NEWCASTLE DISEASE IN PULLETS

BY

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DECLARATION

I declare that the work in this thesis titled **"Evaluating the Effect** of ***Momordica Balsamina Linn*, Selenium and Lamivudine for the Treatment of Newcastle Disease in Pullets"** has been performed by me in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree, diploma or certificate at any other University.

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CERTIFICATION

This thesis titled **"EVALUATING THE EFFECT** OF ***MOMORDICA BALSAMINA LINN*****, SELENIUM AND LAMIVUDINE FOR THE TREATMENT OF NEWCASTLE DISEASE IN PULLETS''** Ishaya AGANG, meets the regulation governing the award of the degree of Master of Science 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 the ALMIGHTY GOD for seeing me throughout my stay and study in the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria . Dedicated to my wife and children.

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May the Almighty God help us, AMEN.

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ABSTRACT

Newcastle disease virus is an avian paramyxovirus that causes significant economic losses to the poultry industry worldwide. Aim of the study was to evaluate the effect of *M. balsamina*, selenium,lamivudine and nutritional substances while the objectives of the study were to : Determine the phytochemical of *M. balsamina*, determine the nutritional substances of *M. balsamina*, determine the median lethal dose of *M. balsamina* in pullets and evaluate the effect of *M. balsamina*, selenium and lamivudine weather they can be used in the treatment of Newcastle disease. A total of three hundred day-old dominant black pullets obtained from a commercial hatchery in Ibadan Nigeria were randomly assigned into six groups. Group 1 was pretreated orally with 300 mg of aqueous extract of *Momordica balsamina* per bird for 1 week before challenge with Newcastle disease virus (NDV) Kudu 113. Birds in group II were inoculated with NDV Kudu 113 and then treated immediately with 300 mg of extract of *M. balsamina* for 2 weeks. Birds in group III were inoculated with NDV Kudu 113 and immediately treated with lamivudine (300 mg /litre) of water per 40 birds for 2 weeks/40 birds. Group IV was inoculated with NDV Kudu 113 and treated immediately with selenium 1 ml/litre of water and group V and VI were negative and positive controls respectively. All negative and positive control groups were treated with drinking water for 2 weeks orally. Birds in week V were given 25 ml/ bird for 2 weeks. Birds were bled on weekly basis on day- old, 4th week, 5th week, 6th week and 7th week. Serum was obtained by allowing the blood collected from each bird to stand for 3 hours at room temperature. Proximate composition of *M. balsamina* was performed at a temperature of 500-600 Ċ. The Phytochemical composition of *M. balsamina* fruit showed that it contains tannins (0.0008 %), saponins (23.99 %) and alkaloids (6.94 %). Proximate composition of *M. balsa mina* indicated high amount of carbohydrate (27.07 %) compare to low crude protein, crude fibre (15.80 %) and ash (11.10 %). Clinical signs of ND recorded in groups due to Newcastle disease in pullets were : Group I inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, salivation, mortality 5,9,8,1,7. Group II : Inactive, emaciation, dollness, ruffle

feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, rales , mortality 12, 7, 5, 1. Group III : inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, salivation, mortality 12, 13, 3. Group IV inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, mortality 4, 3, 2, 2. Group VI : inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, mortality 13, 9, 9, 7. The morbidity rate recorded after challenge for groups I, II, III, IV and VI were 25 %, 27.5 %, 47.5 %, 20 %, 32.5 % while the mortality recorded in group I, II and IV were 88.10 %, 68.10 % and 37.10 % respectively. Postmortem lesions observed in groups I, II, III, IV, and VI were: Generalized haemorrhages in the proventriculus, intestine, trachea and caecal tonsils. There was congestion of the visceral organs (Liver, lungs intestine) while the air sacs were cloudy. At day-old, there was no statistical significant difference ($P > 0.05$) in the level of antibody titre in all the birds. Similarly, at 4 weeks of age post challenge, there was no significant difference ($p = 0.2626$) in the level of ND antibody titre in challenged birds. Therefore, it may conclude that Selenium can be used to control ND in pullets. The study recommend further research on the leaves, stems, flowers, whole plant and roots to determine the effect or benefit of *M. balsamina* in the control of ND. Further studies on the potential benefits of *M. balsamina*, selenium and lamivudine in the control of Newcastle disease in pullets and their mechanism or mode of action recommended.

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LIST OF ABBREVIATIONS AND SYMBOLS

Ab	Antibody
APMV I-9	Avian Paramyxovirus type 1-9
Arg	Arginine
CHO	Carbohydrate
Ċ	Celsius
CM	Centimetre
CBN	Central Bank of Nigeria
CRD	Chronic respiratory disease
CF	Crude fibre
CL	Crude Lipid
Dept	Department
Dev	Development *M. balsamina*
DC	Disease of Erysihecichoracearum affecting powdering mildew
Econs	Economics
Edn	Edition
EID_{70}	Embryo infective dose/ $_{50}$
=	Equal to
Feb	February
FDLPCS	Federal Department of Livestock Pest and Control Services

FO	Precursor Protein
F	Fusin
F1 & F2	Fusin fragments
F-Protein	Fusion- protein
>	Greater than
GP	Group
GDP	Gross Domestic Product
HA	Haemagglutination
HI	Haemagglutination Inhibition
HAU	Haemagglutination unit
HN	Haemagglutinin-Neuraminidase
Ha	Hecta
Hrs	Hours
Kg	Kilogram
Kdv	Kudu virus
Lam.	Lamivudine
<	Less than
LD50	Lethal Median Dose $_{50}$

MDAs	Maternal derived antibody
Mg	Milligram
Ml	Millimeter
NAS	National Academy of Science
NVRI	National Veterinary Research Institute
ND	Newcastle disease
NDV	Newcastle disease virus
Nig	Nigeria
NPK	Nitrogen, phosphorus & potassium fertilizer
NP	Nucleoprotein
Obs	Observation
OIE	Office International des Epizootics
PP	Pages
%	Percentage
P.OS	Per os
Prod	Production
P.i.	Post infection
PM	Post mortem

PBS	Phosphate Buffered Saline
RBC	Red blood cells
RNA	Ribonucleic acid
S.E.M.	Sample Error of Mean
Se	Selenium
Solv.	Solven
T	Tone of *M. balsamina*
UK	United Kingdom
USA	United State of America
Vet	Veterinary
Who	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Poultry production is the fastest growing segments of the agricultural subsector in Nigeria. This is due to increased demand of animal protein in Nigeria as the population is growing (Salami *et al*., 1989). However, one of the constraints to the development of the poultry industry is outbreak of diseases (Salami *et al.*, 1989).

Newcastle disease (ND) is one of the most economically important diseases of poultry and other birds (Okeke and Lamorde, 1988). The disease is distributed worldwide (Alders and Spradbrow, 2001). The causative agent is avian paramyxovirus type 1 (APMV-1). Newcastle disease virus (NDV) infection in birds have devastating consequences with flock mortality reaching up to 100 % as well as the economic impact of trade restrictions and embargo placed on areas and countries where outbreaks have occurred (Alders and Spradbrow, 2001). Newcastle disease is a highly contagious disease primarily of chickens and turkeys. Other domestic poultry, various species of wild birds and humans are susceptible to it, but in man it is usually mild and is characterized by inflammation of one eye, seldom both (Aldous and Alexander, 2001; Knipe *et al.*, 2007). Many species of domestic, semi domestic and wild birds have been found to be susceptible to ND (Arshad *et al.*, 1988; Kaleta and Baldouf, 1988; Alexander *et al.*, 1997; Aldous and Alexander, 2001). Newcastle disease virus contains a non-segmented single stranded RNA genome of negative polarity and replicates entirely in the cytoplasm (Emmerson, 1999; Knipe *et al*., 2007). The envelope contains lipid and surface glycoproteins, which surround the virion, Haemagglutinin-

Neuraminidase (HN) and fusin (F) (Alexander, 1988).The penetration of the host cell by NDV occurs by fusion and is mediated by a protein of the external viral envelope, called fusion protein (F = protein) (Rott and Klenk, 1988). It is synthesized as a precursor (FO) and needs to be cleaved into two smaller fragments in order to be active (F1 and F2) (Rott and Klenk, 1988).

Studies have shown that the pathogenicity of the NDV strains is determined by the amino acid sequence at the site of F-protein cleavage (Rott and Klenk, 1988; Lee *et al.*, 2004). The virus that causes ND is present in actively infected individuals and in apparent healthy carriers (Lee *et al*., 2004). About 250 thousand of NDV measure an inch (Lee *et al*., 2004). Nasal secretions, saliva and droppings of infected birds contain the virus 1 or 2 days after they are exposed to the infection (Lee *et al.*, 2004). Birds that survived attacks of ND seldom shede the virus for more than 3 or 4 weeks, but the virus has been recovered from the lungs of a few chickens 2 or 3 months after recovery (Lee *et al.*, 2004).

There are four forms of the disease caused by different strains of the virus. The Doyle's form (Viscerotropic Velogenic), Beach's form (Viscerotropic Neurogenic), Beaudettes form (Mesogenic) and Hitchner's form (Lentogenic) (Dobson, 1939). In the Doyle's form all ages are susceptible. Morbidity may reach up to 100 % and mortality is very high usually 90 % (Dobson, 1939). In the Beach's form (Viscerotropic Neurogenic), morbidity is also high and mortality variable. About 10 % mortality in adult is very common though may be higher (Elam, 1993). Among immature chickens, mortality is as high as 90 %. Mortality in the adult in the case of the Beaudettes form (mesogenic) is

rare and morbidity is variable (Elam, 1993). The Hitchner's form (lentogenic) of the disease has negligible death (Elam, 1993). The mortality rate in young birds especially when complicated with other infectious diseases can reach 30 % (Elam, 1993).

The simplest and most logical control measure against ND and other infections is to prevent contact of the virus with susceptible birds and to vaccinate (Brandly, 1952). Newcastle disease is also reported to be more common during the harmattan (November to March) (seasonality) (Abdu *et al.,* 1992; Sa'idu *et al.,* 1994; Abdu *et al.,* 2005). The first documented outbreak of ND in Nigeria occurred between December, 1952 and February, 1953 in and around Ibadan (Hill *et al*., 1953). Since then, the disease became the most important viral disease of chicken and is widely spread throughout the country with annual epidemics being recorded in highly susceptible poultry flocks (Adu *et al*., 1986; Sa'idu *et al*., 1994; Halle *et al*., 1999; Orajaka *et al*., 1999). Subsequently, ND appears to be wide spread in local and exotic chickens (Fatumbi and Adene, 1979; Gomwalk *et al*., 1985; Nwosu and Okeke, 1989; Sa'idu *et al*., 1994; Baba *et al*., 1995). Newcastle disease virus was isolated from apparently healthy ducks in and around Jos but there is no report of clinical ND in the ducks (Majiyagbe and Nawathe, 1981; Echeonwu *et al.,* 1993).

Due to the annual nature of the ND outbreak in local chickens, farmers in many parts of Nigeria used several plants and plant products to treat ND. Local names for the plant are Garafuni (Hausa), Ejirin (Yoruba), and Akban ndene (Igbo). Saponins obtained from *M. balsamina* were found to have effect on fertilization, egg production, hatchability and growth of newly hatched *Biompharia Alexandria* snails (Faddah, 1994). *M. balsamina* contains a bitter principle called momordocin (Faddah, 1994).

The young leaf contains 3.6 µg/100 g of Vitamin C and yields two resin acids and momordocin (Faddah, 1994). The plant contains a highly aromatic volatile oil, fixed oil, carotene, a resin, two alkaloids one of which is momordocin and saponins. Momordocin is an amaroid and contains 0.038 % of alkaloid (Faddah, 1994). African traditional herbalists claimed that *M. balsamina* increased the strength of nails and hair in people taking the plant and bone structure is strong and healthy (WHO, 1992). The plant has high concentration of calcium, phosphorus and vitamins which help to increase bone density (Attisso, 1978). Also, women are especially at risk of contacting osteoporosis, in particular after menopause, when estrogen levels dropped and are therefore advice to take *M. balsamina* regularly (Attisso, 1978; WHO, 1992). In nutritionally deprived areas and in winter, postnatal mothers eat the leaves to stimulate milk production (Attisso, 1978). In southern parts of Mozambique the leaves are taken as an anti-inflammatory remedy and particularly indicated for urinary track inflammations (Grover and Gupta, 1990). The Portuguese used the leaves in tea form as remedy for diabetes, digestive disorders, fever, ulcers and a mild form of malaria "Paludismo" and sometimes referred to as "yellow fever, tree sickness" (WHO, 1992). In West Africa the plant is used as a medicine for both humans and animals, particularly as a bitter stomachic, for fever and yaws, and as a purgative (Khanna *et al.,* 1981; Yeung *et al.*, 1986). The root of *M. balsamina* is sometimes used as an ingredient in an aphrodisiac preparation, in the treatment of urethral discharges, jaundice and diseases of the liver (Tiangda *et al.,* 1987).

Due to climatic changes in different parts of the world, the medicinal properties of the plant changes according to geographical location of the plant (WHO, 1992). Climate

and soil play an important role in the concentration of the ingredients used for medicinal properties (WHO, 1992).

1.2 Statement of the Research Problem

Nigeria has the largest poultry population in Africa (Nawathe and Abegunde, 1980). The poultry sub-sector contributes 9-10 % to the Nigerian Agricultural Gross Domestic Product (GDP) with net worth of $250 million dollars (FDLPCS, 2007).The economic losses due to very virulent Newcastle disease virus (vNDV) pose a threat to the fast growing poultry industry worldwide (Max Burgh *et al*., 1978). The disease which affects poultry and other birds is an economically important disease because of its high morbidity, mortality and drop in egg production (Alexander, 1990). The disease continues to be a serious economic threat to the poultry industry resulting in high morbidity and mortality rates and reduce egg production for both breeding and human consumption (Jungher and Markham, 1962; Phillips, 1973; Abdu *et al*., 1992). Newcastle disease poses a serious threat as it has economic, sociological and ecological impact on pets, free-living, as well as domestic birds (Okeke and Lamorde, 1988). An average of 200-250 outbreaks of the disease is reported in Nigeria annually (Okeke and Lamorde, 1988). There is very little or no veterinary intervention against ND particulally for the local poultry farmers. Therefore, farmers are forced to use other alternative ways to control ND.

1.3 Justification of the Study

Newcastle disease remains a constant threat to poultry production and has a devastating effect on the production of commercial and rural poultry industry (Shamaki *et al.*, 1989). Improvement in the poultry industry should incorporate emphasis on the prevention and control of diseases that cause economic losses (Okwor *et al.,* 2009). Newcastle disease has caused great losses in Nigerian poultry industry within the last several years and still poses a threat to the poultry farmers. Vaccination to prevent or reduce losses due to ND is common (Beard *et al*., 1975). Where vaccines are not available the farmers developed traditional remedy such as the use of *M. balsamina* in treating sick birds.

It was based on the knowledge of the farmers on the beneficial effect of *M. balsamina* in chicken infected with Newcastle disease and previous work done by scientists in that area that this study was designed to compare the potentials of *M. balsamina,* selenium and lamivudine in the treatment of Newcastle disease (ND) and the best means of delivery to chickens.

1.4 Aim of the Study

The aim of the study was to evaluate the effect of *M. balsamina,* selenium and lamivudine and nutritional substances in the treatment of Newcastle disease in pullets.

1.5 Objectives of the Study

The objectives of the study are to:

i. Determine the phytochemical of *M. balsamina.*

ii. Determine the nutritional substances of *M. balsamina.*

iii. Determine the median lethal dose of M. *balsamina* in pullets.

iv. Evaluate the effect of *M. balsamina,* selenium and lamivudine whether they can be used in the treatment of Newcastle disease.

1.6 Research Questions

i Does *M. balsamina* fruit ethanol extract cause acute toxicity effect in pullets.

ii Does *M. balsamina* effective in the treatment of Newcastle disease in pullets.

iii Does *M. balsamina,* Selenium and lamivudine effective in the control of Newcastle disease in pullets.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction Newcastle Disease

Newcastle disease (ND) is a highly contagious viral disease affecting wild and domestic avian species cause by a paramyxovirus which is characterized by : ruffle feathers, anorexia, lost weight, coughing, sneezing, rales, greenish watering diarrhea, swollen of the face and neck (Alexander, 2003). The impact of ND is most notable in domestic poultry due to the high susceptibility of poultry and the severe consequences of virulent strain on the poultry industries. In fact, it has been argued that ND may represent a bigger drain on the world economy than any other animal viral disease (Alexander, 2003) although the epizootics of H5N1 avian influenza in Southeast Asia are challenging (if not surpassing) this status (Alexander, 2003). Newcastle disease is a negative single-stranded RNA virus with a 15kb genome that contains six genes: nucleoprotein (Neucloprotein), Phosphoprotein (P) (RNA editing of the p gene can also result in expression of V and W), matrix (M), fusion (F), haemagglutinin-neuraminidase (HN) and polymerase (L) (Alexander and Senne, 2008). Over 200 avian species are naturally or experimentally susceptible to NDV (Alexander and Senne, 2008). Isolates are of a single serotype, but have a wide range of naturally occurring pathogenicities from a less mildy virulent (lentogenic), to mildy virulent (mesogenic) and highly virulent (velogenic) (Alexander and Senne, 2008).

2.2 Aetiology and Classification of Newcastle Disease Virus

Newcastle disease virus belongs to the order *mononegavirales*, family *paramyxoviridae*, subfamily *Paramyxovirinae* and genus *Avulavirus*. The *avulavirus*

includes 9 serologically distinct avian *paramyxoviruses* APMV-1-APMV-9 (Alexander *et al.*,1997; Beard and Hanson, 1997; Knipe *et al*., 2007; Alexander and Jones, 2008). This virus contians non- segmented single –stranded RNA genomes of negative polarity (Emmerson, 1999) and replicates entirely in the cytoplasm (Emmerson, 1999; Knipe *et al*., 2007). A lipid envelope contains two surface glycoproteins, which surround the virion, haemagglutinin-neuraminidase (HN) and fusion (F). The natural hosts of APMV-1 are all domestic and many wild avian species (Alexander, 1988; Kaleta and Baldouf, 1988; Alexander *et al.*, 1997; Aldous and Alexander, 2001; Maw *et al.*, 2003).

2.2.1 Pathogenesis of newcastle disease virus

The penetration of the ND virus in the host cell occurs by fusion and is mediated by a protein of the external viral envelope, called fusion–protein (F-protein). It is synthesized as a precursor (FO) and need to be cleaved into two smaller fragments in order to be active (F1 and F2): Studies showed that the pathogenicity of the ND strains is determined by the amino acid sequence at the site of F- protein cleavage (Rott and Klenk, 1988; Lee *et al*., 2004). The proteins with single basic amino acid sequences, at the cleavage site could be hydrolyzed only by trypsin and similar enzymes, present in a restricted number of tissues and organs in susceptible animals (Maw *et al.*, 2003; Lee *et al*., 2004). Thus, viruses with such F-Protein possess a limited tropism and cause subclinical or inapparent infections. Unlike the proteins with several basic amino acid (Arginine ``R'' and Lysine's-``K'') at the site of F-protein cleavage as well as phenylalanine at position 117 (Kohama *et al.*, 1981) could be cleaved by proteases, present in cells every where in the microorganism (Kohama *et al*., 1981).

2.3 Incubation Period of Newcastle Disease

The incubation period of ND varies with strain of virus, route and dose of infection; typically for VND it is 4-5 days, but it may be from 2-15 days (Lancaster, 1966; Beard and Hanson, 1984).

2.4 Clinical Findings of Newcastle Disease

The disease is mostly characterized by moderate to high mortality, somnolence, weakness, severe depression, anorexia, yellowish-green diarrhea, dehydration, abnormal respiration, tracheal rales, sneezing, coughing, gasping, swollen eye lids, severe haemorrhage and cyanosis of the legs and shanks, trembling, paralysis of wing and legs, torticollis, star gazing and drastic decline in egg production (Echeonwu *et al*.,1993; Haruna *et al.,* 1993).

2.5 Gross Lesions of Newcastle Disease

The pathology usually observed during postmortem examination in ND outbreaks were: congestion of skeletal muscles, haemorrhages in the proventricular mucosa, intestines, caecal tonsils and caecum. Also pneumonic trachea, froth discharges in the bronchi,congested trachea, liver, spleen, pancreas, thymus and bursa of Fabricius (Haruna *et al*.,1993; Sa'idu *et al.*,2006).

1.6 Diagnosis

2.6.1 Diagnosis of newcastle disease

A tentative diagnosis of ND can be made based on clinical signs and gross lesions (Yusoff and Tan, 2001). In the field, ND can also be suspected when nervous signs are manifested like torticollis, star gazing, incoordination, paralysis of the wings and/or legs (McFerran and McCracken, 1988; Alexander, 1995; Yusoff and Tan, 2001).

2.6.2 Differential diagnosis of newcastle disease

Disease of birds affecting respiratory and gastrointestinal systems may be confused with the less severe form of ND (Alexander *et al.,* 1997).

2.6.2.1 Avian influenza

Avian influenza initially was recognized as a highly lethal systemic disease (Swayne and Halvorson, 2008). Infection of domestic poultry by AI viruses typically produces syndromes ranging from asymptomatic infection to respiratory disease and drops in egg production to severe systematic disease with near 100 % mortality (Easterday *et al*., 1997).

2.6.2.2 Infectious laryngotracheitis

This disease is manifested by acute respiratory infection of poultry and characterized by nasal discharges and moist rales followed by coughing, gasping, marked dyspnea and blood stained mucus expectorants (Guy and Garcia, 2008).

2.6.2.3 Infectious bronchitis

The characteristic respiratory signs of infectious bronchitis in chicks are gasping, coughing, sneezing, tracheal rales and nasal discharge (David and Jack, 2008). The course of the disease varies with the severity of the lesions. However, most chickens have been reported to recover in 10-14 days (Guy and Garcia, 2008).

2.6.2.4 Fowl cholera

It is a disease that is of acute nature and rapid onset with high mortality in the affected poultry indicating cyanosis and oedema of the combs and wattles. Haemorrhages in the proventriculus and abdominal fat may be seen in fowl cholera as may be seen in ND and AI (Stubbs, 1965).

2.6.3 Molecular techniques in diagnosis of Newcastle disease

In addition to the use of revers transcriptase polymerase chain reaction (RT-PCR) and other similar techniques for the determination of the virulence of ND viruses or for phylogenetic studies, there has been increasing use of such molecular techniques to detect NDV in clinical specimens, the advantage being the extremely rapid demonstration of the presence of ND virus. Care should be taken in the selection of clinical samples as some studies have demonstrated lack of sensitivity in detecting virus in some organs and particularly in faeces (Gohm *et al.*, 2000; Creelan *et al.*, 2002; Koch, 2003). One further important problem of ND virus is that while the vast majority of NDV isolates are genetically quite close, some have been shown to be genetically

distinct. For example, one group of viruses, which were placed in genogroup 6 by Aldous *et al*. (2003) and subsequently class 1 by Czegledi *et al*. (2006).

2.6.4 Pathogenicity index

The extreme variation in virulence of different NDV isolates and the widespread use of live vaccines means that the identification of an isolate as NDV from birds showing clinical signs does not confirm a diagnosis of ND, so that an assessment of the virulence of the isolate is also required. In the past tests such as the mean death time in eggs, the intravenous pathogenicity test and variations of these tests have been used for ND (Hanson, 1980).

2.7 Monoclonal Antibodies of Newcastle Disease

Mouse monoclonal antibodies (Mabs) directed against strains of NDV have been used in haemagglutination inhibition test (HIT) to allow rapid identification of NDV without the possible cross-reactions with other APMV serotypes that may occur with polyclonal sera. Mabs have been produced that give reaction in HI test that are specific for particular strains or variant NDV isolates (Alexander *et al.*, 1997; Alexander, 2003).

2.8 Phylogenetic Studies of Newcastle Disease

Development of improved techniques for nucleotide sequencing, the availability of sequence data of more ND viruses in computer database and the demonstration that even relatively short sequence lengths could give meaningful results in phylogenetic

analyses have led to a considerable increase in such studies in recent years (Aldous *et al.,* 2003). Considerable genetic diversity has been detected, but viruses sharing temporal, geographical, antigenic or epidemiological parameters tend to fall into specific lineages or cades and this has proven valuable in accessing both the global epidemiology and local spread of ND (Aldous *et al.,* 2003). Although in the past phylogenetic studies have been impracticable as a routine tool, the greater availability and increased speed of production of results obtained using sophisticated, commercial available kits for revers transcriptase polymerase chain reaction (RT-PCR) and automatic sequencers now means such studies are within the capabilities of many more diagnostic laboratories and can give meaningful results that are contemporaneous rather than retrospective (Aldous and Alexander, 2001).

2.9 Vaccines Available for Newcastle Disease and Vaccination

The duration of immunity depends on the vaccination programme chosen. One of the most important considerations affecting vaccination programmes is the level of maternal immunity in young chickens, which may vary considerably from farm to farm, batc to batch, and among individual chickens. For this reason, one of several strategies is employed either the birds are not vaccinated until 2-4 weeks of age when most of them will be susceptible or 1-day-old birds are vaccinated by conjunctival installation or by the application of a course spray is then carried out 3-4 weeks later. It has been demonstrated that inactivated vaccines may also be usefully employed to vaccinate 1-day-old chicks that have a degree of maternal immunity (Box *et al.,* 1976). Hitchner-B_1 by conjunctival or spray administration at 1 day of age, live Hitchner-B1 or la sota at 18-21 days of age in the drinking water; live la sota in the drinking water at 10 weeks of

age, and an inactivated of emulsion vaccine at point of lay. For the second example, when the disease is severe and more widespread, the same protocol as above is adopted up to 21 days fo age and this is followed by revaccination at 35-42 days of age with live la sota in the drinking water or as anaerosol, this revaccination is repeated at 10 weeks of age with an inactivated vaccine (or a mesogenic live vaccine) and again repeated at point of lay (Allan *et al.*,1978).

2.10 Duration of Immunity to Newcastle Disease

The level of immunity reached with any single dose or regimen of ND vaccination will vary enormously with both vaccine and host species. The level of immunity required in a given host (i.e. to protect from death, disease or egg production losses) is extremely complex and difficult to evaluate. Generally some assessment of the longevity of serum antibodies should be made and vaccine regimens adopted to maintain these above an acceptable level (Allan *et al.*, 1978).

2.11 Prevention and Treatment of Newcastle Disease

In response to the threat presented by ND, several countries have put in place vaccination campaigns to prevent epizootics of the disease (Burridge *et al*., 1975; Alexander, 2003). However, outbreaks have been reported in vaccinated populations despite the fact that vaccination is widely applied as for example in the Netherlands in 1992-1993, the UK in 1997 and USA in 2002 (Burridge *et al*., 1975; Alexander, 2003). It is known that vaccination of poultry provides an excellent means to lessen clinical signs of infection caused by virulent NDV (Alexander, 2003; Senne *et al*., 2004). It has also been known for a long time that vaccination itself (with live vaccines based on

non-virulent virus strains) may cause disease and reduced growth in vaccinated birds (Alexander, 2003). Consequently, there has been a trend to use less virulent strains as the seed viruses for vaccine production. Although this strategy has reduced the disease rates after vaccination, it also might have contributed to the fact that current vaccines and vaccination campaigns are not maximally effective in preventing infection and transmission (Burridge *et al*., 1975; Voeten *et a*l., 1987; Alexander, 2003; Senne *et al*., 2004). Hence, it is not clear whether the ultimate goal of prevention of major outbreaks of Newcastle disease after primary virus introductions can be achieved with current vaccines and vaccination programme

2.13 Chickens

2.13.1 Pullets

The rearing of pullets was the most favoured form of poultry production by majority (70%) of the farmers particularly women (Amballi *et al*., 2005; Ajala *et al*., 2007). The rearing of pullets by majority of women could be attributed to the desire to raise money to supplement the family income and to a lesser extent, to improve family nutrition (Amballi *et al*., 2005; Ajala *et al*., 2007). In Asia, similar results have been reported among women poultry farmers in rural areas of Indian, Nepal, Pakistan and Sri Lanka (Herath, 2008).

2.13.2 Village chickens

Village chickens are free-ranging poultry, mostly unimproved indigenous breeds (Martin, 1991). Local chicken's production, which is important in low income families in Nigeria, is a cheap source of protein for the fast growing human population in the

country (Obi, 2007). According to Mariner (1999), livestock owners are no longer seen as an inert substrate upon which development is to be practiced; they are active participants who can and must bring important intellectual contribution to development. Rural poultry makes up a major part of the total poultry production in fewer developing countries as about 80 % of the rural populace in Africa keep family poultry (Sonaiya, 2007). Nigeria has the largest poultry population in Africa estimated at about 130-150 million chickens with over 80 % being village chickens (Nawathe and Abegunde, 1980; Abdu *et al*., 1985; Ibrahim and Abdu, 1992; Lamorde, 1996; Sa'idu *et al.*, 1994). In spite of the importance of rural poultry, mortality due to ND is one of the most important factors militating against its development in Nigeria (Nwanta, 2003).

A serological study carried out in rural chickens by Ezeokoli *et al.* (1984) showed 73 % prevalence of antibodies against NDV in traditionally managed backyard flocks in Zaria while 63 % seroprevalence was reported by Orajaka *et al*. (1999) in southern Nigeria. In southwestern Nigeria around Ibadan, 38 % seroprevalence was reported by Oyewole *et al*. (1996). In North central Nigeria (Plateau state) (Musa *et al.,* 2009) reported a prevalence of 51.9 %. Because of the importance attached to ND by rural farmers, various local names have been given to the disease by the people in many countries (Alders and Spradbrow, 2001). In northern Nigeria, ND is known in Hausa language as 'Haukan kaji' (Ibrahim and Abdu, 1992). The Yorubas in western Nigeria called it 'Koli' (Dipeolu *et al*., 1998) while it is called 'Ogbunakpo' in Igbo, in Estern Nigeria (Nwanta *et al*., 2006). Although, Newcastle disease have been recognized as the most important disease of village chickens in Zaria, Nigeria (Abdu *et al*., 1992) and as the principle factor limiting village chicken production in Africa (Spradbrow, 1988). The mortality of these chickens affects the economy of the poor, especially, women who

largely own these birds (Gu'eye, 2000). Due to the annual epidemic of the disease and the associated economic lost due to the disease there are several traditional remedies for the disease in almost every community.

2.15 Ethnoveterinary Medicine

Ethnoveterinary medicine covers people's knowledge, skills, methods, practices and beliefs about the care of their animals (McCorkle, 1986). It also involves more than just using medical herbs. It also involves information; stock raisers commonly know when their animals are sick. They can describe the disease signs, which season the disease commonly strikes, and what types of animals are affected. Plants and their products have been used for treating Newcastle disease, worms and bacterial infections. Examples of other plants used for ND treatment are: (i) Red pepper (*Capsicum frutenscens*) and (ii) Gauta kaji (*Solanum nodiflorum*). In Yoruba, ND is known as '*Lukuluku'* or '*Yirunyirun*' and in Hausa 'Haukan kaji' (Ibrahim and Abdu, 1992). Yoruba language went further to call infertility as '*Soso*' in local chicken production.

2.16 Selenium

During the last decade, it has been demonstrated in several species that selenium influence immune responses (Nockels, 1986). Early research on the effects of nutrients on immunity involves measuring antibody production (Marsh *et al*., 1981). More contemporary immune response measures involve quantifying the ability of lymphocytes to proliferate when stimulated by mitogens (Bendich *et al.*,1983, 1986)

and the abilities of polymorphonuclear cells (PMN) to phagocytize (engulf) and or kill invading organisms Bendick *et al.,* 1983, 1986; Boyne and Arthur, 1986).

2.17 Lamivudine

Lamivudine is often used as part of a regimen that decreases mother- to child transmission of HIV and is generally well tolerated by the breastfed infant (WHO, 2007; Dao *et al.,* 2006). Breastfed infants whose mothers receive highly active antiretroviral therapy (HAART) have higher rates of neutropenia during the first month and severe anemia during the first 6 months of life. Lamivudine has not been studied in HIV-negative nursing mothers being treated for hepatitis B infection, but the low doses used would not be expected to cause any serious adverse effects in breastfed infant. Some sources recommending during lamivudine therapy for hepatitis B (Bzowej, 2012). In a survey, 226 physicians with a practice interest in liver disease in the United States responded. Of these, 31 % stated that they recommend breastfeeding for their patients with hepatitis B who are taking antiviral therapy, 44 % stated that they do not recommend breastfeeding during antiviral therapy and 25 % stated that they were unsure (Ahn *et al.,* 2010).

2.18 *Momordica balsamina* Plant

The general botanical characteristics of the cultivated members of this family (*Cucurbitaceae)* have been outlined by Purseglove. (1968) as follows: tendril-climbing or postrate annuals, occasionally rapidly growing perennials, annual herb, climbing, vines growing to 3-4m in length.

2.18.1 Physiology of the family (*Cucurbitaceae*)

Cobley (1977) grouped the family *Cucurbitaceae* as a *dicotyledonous* crop, and it has been cultivated more than 4,000 years in the dried part of the world and the crop is made of stems, leaves, flowers, fruits and seeds.

2.18.2 The stems of the Cucurbitaceous plant

The stems may reach great length, either trailing along the ground or climbing any supporting materials with their tendrils a modified floral brack (Steel, 1977). The stems are about 2.3 cm thick for a matured plant and about 3-4 m in length. There is also a node from which the leaf exit, the flower bud, a vegetative bud and a tendril developed (Steel, 1977).

2.18.3 The leaves of the Cucurbitaceous plant

Generally, the family has large leaves which are dark green and kidney shaped with large nodes and entire margins (Cobley and Steel, 1977). Its tendrils are fairly stout and are divided about half way along their length into many branches. The leaves are also very hairy on the under surface and are simple but often deeply lobed alternate or spirally arranged (Cobley, 1977).

2.18.4 The flowers of the Cucurbitaceous plant

The plants are monoecious or dioecious with unisexual flowers occurring singly in the leave axil, the leaf is divided at the top into five lobes with corolla being five in number

that are commonly fused in the male flower, the androecium is variable to be massive with three clited carpels, carpels with numerous ovules carried on three thick fleshy apparently parentally pariental placenta which later grows out to the wall of the ovary during the development and carrying the ovules with them (Steel,1977).

2.18.5 The fruits of the Cucurbitaceous plant

The fruits of the family or plant are pendulous green, becoming orange or yellow when mature in size and shape variable, often pear-shaped or oblong tapering, 10-25 cm in length, 5-8 cm in diameter, ribbed with numerous protuberances (Steel, 1977).

2.18.6 The seeds of the Cucurbitaceous plant

The seed of the cucurbitaceous plant or matured plant is brown in colour, with scarlet aril, and is flattened, oval 1-5 cm in length containing 32 % oil content in a seed, and the weight of 1,000 seeds is 60 g (Steel, 1977).

2.18.7 Environmental response of the Cucurbitaceous plant

The cucurbitaceous plant normally adapted to a wide variation of rainfall, and the plant normally grows in the hot, humid areas at elevations up to 5000 m and the plant requires a soil with a high organic content and water retaining capacity are required for optimum yield of the plants (Martin, 1979).

2.18.8 Cultural requirement of the Cucurbitaceous plant

In the cultural requirement, this is propagation and planting of the seeds. Seeds are sown directly on beds or ridges 60-75 cm apart from each other and 30-38 cm between plants or 50 cm times 50 cm each way. The plant requires support by poles or trellis. The seeds require 4-5.5 kg/ ha for a density of 40,000 plants/ ha (Knott and Deanon, 1967).

2.18.9 The nutrient requirement of the Cucurbitaceous plant

For a cucurbitaceous plant to grow and produce many fruits there must be sufficient nutrient supply. Nitrogen, phosphorus and potassium (N. P. K.) is required before sowing, and after sowing, followed by application of nitrogenous fertilizer at intervals during the growing period in other to enhance growth and multiplication of the plant (Knott and Deanon, 1967).

2.18.10 Growth and harvesting of the Cucurbitaceous plant

The immature fruits may be harvested at 50-70 days from sowing. Up to 15 tones/ ha can be obtained but an average yield would be approximately 8-10 tones/ ha (National Academic of Science, 1975).

2.18.11 Storage of the Cucurbitaceous plant

Although rarely stored for any appreciable period, fruits of *M. balsamina* may be maintained at a temperature of 1-2℃ for up to 20-30 days (Grubben, 1977).

2.18.12 Pest and diseases of the Cucurbitaceous plant

There are some specific diseases that affect the cucurbitaceous plant for example, disease like *Erysihe cichoracearum DC* normally affects the Powdering mildew and Dacus *Cucurbit COQ* normally affects the fruit, and is called Melon fruit fly. This *M. balsamina* plant has been used by local poultry farmers to treat ND.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Source of Newcastle disease virus

Newcastle disease virus Kudu 113 strain was sourced from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The virus was transported to Zaria using a flask containing ice blocks. The titre of the NDV Kudu 113 was$10^{8.5}$ ELD_{50}, dilution of NDV Kudu virus 1:100 ml of phosphate buffered solution and 0.1 ml administered per bird intranasally and the number of viruses was 31,622,776.6 million.

3.1.2 Pullets

Day old pullets (Dominant black) were purchased from a reputable commercial hatchery in Ibadan Nigeria. Commercial chick mash from vital feed (Grand cereal composed of (300-400 kg) maize, (calcium = 1.0 %) bone meal, (23 %) crude protein, (10 %) fat, (10 %) crude fibre, (0.46 %) available phosphorus, (3 000 kcal/kg metabolisable energy). Vitalyte® (Anupco, England) a preparation containing vitamins and minerals was given at a dose rate of 5 g/4 litre of water. Neomycin sulphate (Neotreat$^{(R)}$) at a dose rate of 60 mg/200 litres of water was administered to the birds during the brooding period. Birds were allowed to stay only on clean drinking water for one week after the last drug and vitamin administration for the birds to stabilize.

3.1.3 Source of vaccines

The birds were vaccinated against infectious bursal disease (Gumboro) at the second week of age using the Georgia strain (Intermediate strain) manufactured by (Indovax - India). Two vials of the vaccines (200 doses/ vial) each were reconstituted in 4 litres of diluents (saline) for administration through drinking water (orally). Vaccination was carried out using milk which traped debrise and was administered in the morning.

3.1.4 Heat

The chickens were brooded together or separately in small groups for 4 weeks to enable them obtain source of heat. Heat was provided using six (6) 200 watt electric bulbs with a range of temperature range of 36-37 ℃ was applied (Demerchi *et al.,* 2008).

3.1.5 Chemicals and reagents

3.1.6 Source of selenium

Selenium a product of Biovac Company in Israel was purchased from Rabiah Company Nigeria Ltd in Jos, Plateau State, Nigeria. Ingredients were; Selenium. It was given at a dose rate of 1 ml/1litre of water.

3.1.7 Source of Tween 80 (diluant)

Tween 80, a product of William and Sons Company England was obtained from a reputable chemical store in Zaria, Nigeria. It was used at 2-3 drops to dissolve the *M. balsamina* fruit ethanol extract.

3.1.8 Source of lamivudine

Lamivudine, a product of Dana dam's Pharmaceutical Company, Accra, Ghana was also obtained from a reputable Pharmaceutical store in Zaria and given at a dose rate of 150 mg/ kg (2 tablets /40 chickens) in drinking water (7.5 mg/chicken).

3.1.9 Housing

The chicks were housed in the experimental Animal House of the departments of Public Health and Preventive Medicine and Veterinary Anatomy in the faculty of veterinary medicine ABU Zaria.. The chicks on arrival were randomly divided into six groups (Groups 1-6) of 51 chicks each for brooding. At the end of the brooding period, 40 pullets were assigned to every group in the same house but separate under deep litter system with a floor space of 0.14 sqm^2/ bird.

3.2 Methods

3.2.1 Collection of plant

Fresh fruits of *M. balsamina* were collected between the month of February to April, 2013 (seasonal) at the National Veterinary Research Institute (NVRI), Vom in Jos South Local Government Area of Plateau State, Nigeria (Plate I and II). The collected fruit was transported to Zaria, Kaduna State, Nigeria. The identity of the plant was confirmed by Mallam Mohammed Musa (Taxonomist) and a Voucher specimen Number. 1139 was deposited at the Department of Biological Sciences, Ahmadu Bello

University Zaria. The fruit was air dried in the laboratory at room temperature, pounded using mortar and pestle and stored in plastic containers until required.

3.2.2 Collection of blood sample

In day-old chicks about 2 ml of blood was collected from the heart of each day-old chick using insulin syrings. Cocks were also sampled for HI. The blood samples were transferred into 5 ml plastic tubes and left overnight in a refrigerator at a temperature of 4℃. Serum samples were extracted using a plastic micropipette and transferred into sample bottles and were frozen until tested. The sera were used for haemagglutination inhibition test (HI) as described by OIE (2009).

3.2.3 Extraction and phytochemical composition of *M. balsamina*

Two thousand eight hundred and fifty (2,850 g) grams of air dried powdered sample was sequentially extracted in a soxhlet extractor with 75 % ethanol. The crude ethanol extract was concentrated in a vacuum using rotary evaporator, labeled and stored in the refrigerator at 4^0C until used. The extraction process was done at the Department of Pharmaceutical and Medicinal chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. The extract was subjected to phytochemical analysis to detect and quantify secondary metabolites in the extract according to the method of Trease and Evans (1983). Phytochemical screening was done at the National Research Institute for Chemical Technology (NARICT), Basawa Zaria, Nigeria.

3.2.4 Proximate composition of *M. balsamina*

3.2.4.1 Procedure for proximate composition

M. balsamina fruits were cut into small pieces and air dried at room temperature of 36℃ for 2 weeks until a constant dry weight was obtained. The plant sample was ashed at 500-600^{o} C for 4 h where organic compounds are removed. The weight loss was calculated from the dry matter to obtain the weight of the crude ash as established by Kjeldhl, (1883).

3.2.5 Determination of the median lethal dose

A total of 12 pullets of 4 weeks old were used for the experiment. The birds were divided into two groups (A&B). Group A was further divided into 3 subgroups of 3 pullets each. Subgroups A1, A 2 and A3 were orally treated with 10, 100, and 1000 mg/kg body weight respectively. The birds were observed for 2 days for signs of toxicity and mortality. The birds in group B were further subdivided into 3 groups (B1, B2 and B3) of 1 bird each. Based on the initial results, further doses of 1600, 2900 and 5000 mg/kg body weight were administered to calculate an LD_{50} determine that as described by (Lorke's method, 1983).

Table 3.1: Experimental design of groups and ages (in weeks) of pullets inoculated and treatment.

Group	(Weeks) of pullets or inoculation & Treatment		
	4	5	6
TI	*M.b*	Kdv	Solv
T2	Kdv+*M. b*	*M.b*	Solv
T3	Kdv+Lam	Lam	Solv
T4	Kdv+Se	Se	Solv
T5	Solv	Solv	Solv
T6	Kdv	Solv	Solv

Key: Lam = lamivudine (antiretroviral drug), *M. balsamina*, Kdv = Newcastle disease virus (kudu 113), Solv = solvent, Se = selenium.

3.3 Clinical signs

Clinical signs were monitored and recorded. Group I : Inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, rales mortality 5, 9, 8, 1, 7. Group II : Inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, rales mortality 12, 7, 5,1. Group III : Inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, rales mortality 12, 13, 3. Group IV : Inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, rales mortality 4, 4, 2, 2. Goup V : Inactive, emaciation, dollness, ruffle feather, anorexia, weakness, coughing, sneezing, greenish white diarrhea, rales mortality 13, 9, 9, 7.

3.4 Haemagglutintion Test and Haemagglutination Inhibition Test

Haemmagglutination inhibition test was used to determine antibody titres to Newcastle Disease using methods described by (OIE, 2009). This was done for all the groups.

3.4.1 Preparation of 1% chicken red blood cell

An equal volume 2 ml of alsever's solution was added to 2 ml of blood that was collected from mature cocks. The red blood cell was washed 3 times with phosphate buffered saline (PBS). Thereafter, 1 ml of the washed RBC was added to 99 ml of PBS to obtain 1 % RBC solution.

3.4.2 Antigen

Newcastle disease virus la-sota obtained from National Veterinary Research Institute (NVRI) Vom was used as the antigen for the HI test.

3.4.3 Haemagglutination inhibition test

Sera collected from pullets were tested for NDV specific antibody by the Hae magglutination Inhibition test (HI) as described by Allan and Gough (1974). The HI test was performed using beta technique (constant virus and varing serum) against 4 HA units of the virus computed from the HI titration. Two fold serial dilution of 25μl serum was made with phosphate buffer saline (PBS) in V-bottomed microtitre plates up to 10th well. Twenty five microlitres of 4 haemagglutinating (HA) units of NDV virus or antigen (La sota) was added up to 11th well. The plates were kept at room temperature for more than 30 minutes to facilitate antigen-antibody reaction. Then 50μl of 1 % (v/v) chicken RBC suspension was added to each well. The 11th well contains antigen and RBCs as the positive control and the 12th well contains only RBCs as the negative control. After gentle mixing, the RBCs were allowed to settle at room temperature for 40 minutes and agglutination was assessed by tilting the plates. The samples showing peculiar central button shaped settling of RBCs were recorded as positive and maximum dilution of each sample causing haemagglutination inhibition was considered as the end point, which was used to estimate the HI titre. The HI titre of each serum sample was expressed as reciprocal of the serum dilution and most conveniently expressed as the logarithm to the $base_2$

The HI test is based on the principle that the haemagglutinin on the viral envelope can bring about the agglutination of chicken red blood cells abd that this can be inhibited by specific antibodies. In the absence of any antibody against the virus, haemagglutination occurs, appearing as a diffuse red colour at the bottom of the well. In the wells where the antibody against the virus is of a sufficient level, haemagglutination is inhibited and the red blood cells sediment and appear as a small pellet at the bottom of the well. The presence or absence of agglutination is accurately assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing RBCs and PBS only) should be considered to show inhibition.

3.5 Data Analyses

Mean antibody titre values were expressed as mean ± S.E.M, and were subjected to two way analysis of variance (ANOVA) followed by Tukey's post-hoc test using GraphPad prism version 4.0. For windows from graphpad software, San Diego, California, USA. Value of $P \leq 0.05$ was considered significant. Mortality rate was calculated (number of dead÷ total number (N) × 100.

CHAPTER FOUR

RESULTS

4.1 Proximate Composition

The result of proximate composition showed that ethanol extract of fruit of *M. balsamina* contained : carbohydrate (27.07 %), moisture (26.23 %), crude fibre (15.80 %), crude lipid (12.80 %), ash (11.10 %) and crude protein (7.00 %) (Table 4.2).

4.2 Phytochemical Composition

Phytochemical constituents of the ethanol extract include tannins (0.0008 %), alkaloids (64.90 %), flavonoids (16.31 %) and saponins (23.99 %) (Table 4.3).

4.3 Clinical Signs

Common clinical signs were: ruffled feathers, seclusion, dullness, inactive, anorexia, weakness, coughing, sneezing, greenish watering diarrhea, torticolis, salivation, leg paralysis, emaciation, and rales (Appendices VI-X).

4.4 Mortality Rate

Generally mortality observed in T1 (88.10 %), T3 (68.60 %), T3 (73.70 %), T4 (37.10 %) and T6 (78.90 %) (Table 4.4).

4.5 Gross Lesions

Gross lesions were: T1: Congestion of the lungs haemorrhages of the intestinal lining, liver, thymus and spleen. Haemorrhagic bands in the proventriculus, necrosis of the intestinal lining and the trachea, mucoid in the trachea, severely haemorrhagic

proventriculus, cloudy air sacs, emaciation of the muscles, blood clot in the thorac and heart, pus in the proventriculus, distended gall blader, and pale kidneys. T2: Congestion of the lungs haemorrhages of the intestinal lining, liver, thymus and spleen. Necrosis of the intestinal lining and the trachea, mucoid in the trachea, cloudy air sacs, emaciation of the muscles, pus in the proventriculus, distended gall blader, and pale kidneys. T3: Congestion of the lungs, haemorrhages of the intestinal lining, liver, thymus and spleen. Haemorrhagic bands in the proventriculus, mucoid in the trachea, severely haemorrhagic proventriculus, cloudy air sacs, emaciation of the muscles, distended gall blader, and pale kidneys. T4: Congestion of the lungs, haemorrhages of the intestinal lining, liver, thymus and spleen. Necrosis of the intestinal lining and the trachea, mucoid in the trachea, severely haemorrhagic proventriculus, cloudy air sacs, emaciation of the muscles, blood clot in the thorac and heart, pus in the proventriculus, and pale kidneys. T6: Congestion of the lungs, haemorrhages of the intestinal lining, liver, thymus and spleen. Haemorrhagic bands in the proventriculus, necrosis of the intestinal lining and the trachea, mucoid in the trachea, severely haemorrhagic proventriculus, cloudy air sacs, emaciation of the muscles, blood clot in the thorac and heart, pus in the proventriculus, distended gall blader (Appendices XI-XV).

4.6 Haemagglutination Inhibition titre

Generally, the HI titre was significantly higher in group 1 compared to the other groups (Table 4.1). Figure 4.1 showed the level of HI titre, which appeared higher in group 1.

Table 4.1: Antibody titre

Group	**Age in weeks**				
	Day-old	4 weeks	5 weeks	6 weeks	7 weeks
	P= 0.2626	P= 0.01506	P< 0.01	P< 0.01	P = 0.2387
T1	2.20± 0.49	0.00± 0.00	10.00 ± 0.00	5.38 ± 1.39	7.56 ± 1.25
T2	3.14± 0.34	3.00 ± 0.58	4.00 ± 1.16	9.5± 0.24	9.50 ± 0.34
T3	3.17 ± 0.54	3.50 ± 0.50	4.60 ± 0.68	9.50 ± 0.31	9.00 ± 0.21
T4	2.00 ± 0.45	2.75 ± 1.18	8.29 ± 0.18	6.22 ± 0.78	10.00 ± 0.00
T5	2.38 ± 0.33	2.00 ± 0.00	1.00 ± 0.00	8.13 ± 0.81	9.25 ± 0.75
T6	2.43 ± 0.37	3.00 ± 1.00	5.17 ± 0.91	6.00 ± 0.0	8.50 ± 1.50

Table 4.2: Proximate composition of *M. balsamina* fruit ethanolic extract

Nutritional substances	**Values**
Carbohydrate	27.07 %
Moisture	26.23 %
Crude fibre	15.80 %
Crude lipid	12.80 %
Ash	11.10 %
Crude protein	7.00 %

Table 4.3: Quatitative Phytochemical constituents of *M. balsamina* fruit ethanolic extract and their concentrations

Phytochemical Constituents	**Concentration mean values**
Tannins	0.0008 %
Saponins	23.99 %
Alkaloids	64.90 %
Flavonoids	16.31 %

Key:
+ = Present
- = Absent

Table 4.4: Mortality rate in four week old pullets following administration with *M. balsamina* and challenge with virulent NDV Kudu 113 of group I-VI

Group	Number infected	Number dead	Mortality rate %
T1	10	37	88.1
T2	14	27	68.6
T3	23	29	73.7
T4	11	15	37.1
T5	0	0	0.0
T 6	4	31	78.9

Mortality rate % = n/N ×100/1

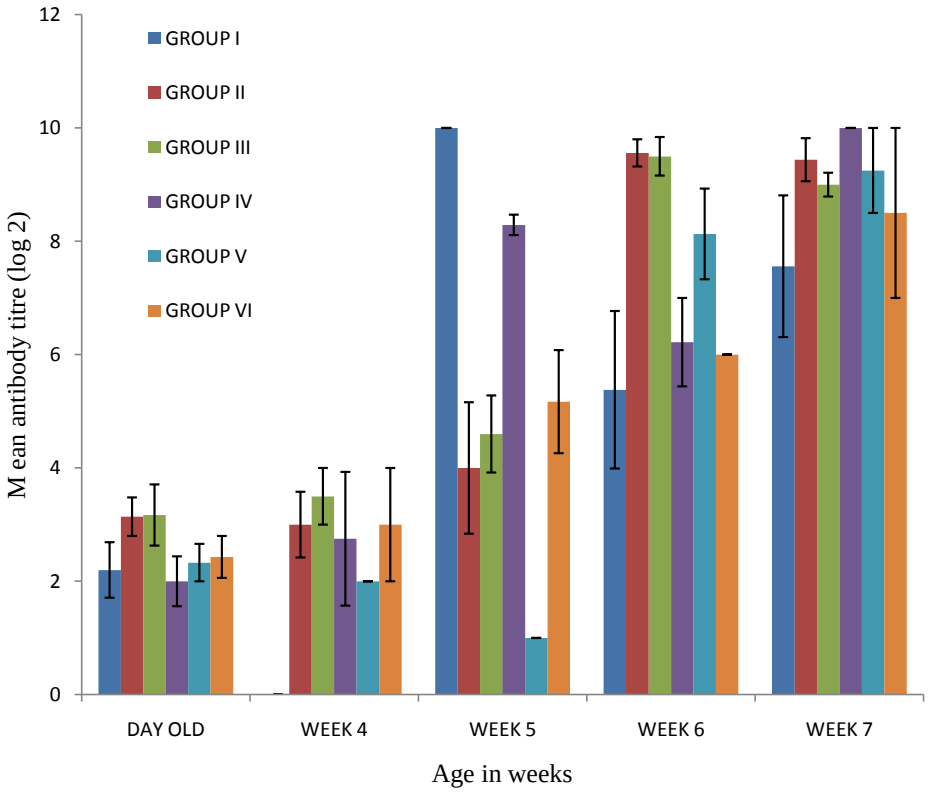

Figure 4.1: Newcastle disease antibody titre and ages of pullets administered *M. balsamina* and challenge with Newcastle disease virus 113 (group's I-VI)

CHAPTER FIVE

DISCUSSION

Rearing of pullets could be attributed to the desire to raise money to supplement the family nutrition. This agrees with the report of Amballi., *et al* (2005) and Ajala., *et al* (2007) where it was indicated that most poultry farmers do so to raise income. In Asia, similar results have been reported among poultry farmers in rural areas of India, Nepal, Pakistan, and Sri lanka.

(Herath, 2008) Analyses results showed that (NDV kudu 113 strain used) to be the major poultry diseases affecting pullets. Management and high cost of orthodux drugs lower pullet's production too. These findings are in agreement with results of similar studies in other parts of the country (Nigeria) (Ibrahim and Tanya, 2001; Bukar-Kolo *et al*., 2006).

Median lethal does$_{50}$ (LD_{50}) was determined in this study to be LD_{50} > 5 000 mg/kg with a dose of 300 mg/kg body weight. This disagreed with the work of Mgbojkwe *et al*. (2002) who adopted 100 mg/ml as dose for his experiment at National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria.

Phytochemical composition revealed the presence of alkaloids in high concentration (64.90 %) followed by saponins (23.99 %), tannins (0.0008 %) respectively. This agreed with Zwag and Sherma (1972) that a substance can be directly identified from its

Rf value. These classes of chemical compounds and elements have been kwon to exert pharmacological and antagonistic effects in the herb from decomposing either chemically or physically. Saponins (23.99 %) which were found to be also high. This agreed with the work done by Faddah (1994) who worked on *cucurbitaceous* plants that contained high amount of saponins that had effect on egg production, egg fertilization, hatchability and growth of newly hatched Biopharia alexandria snails. Also the work of Hassan and Umar (2006) disagreed with the work that *M. balsamina* had high saponins that had anti-inflammatory, antiviral, antibacterial and antioxidant activities.

Proximate composition of *M. balsamina 's* Carbohydrate (27.07 %) was found to be very high when pretreated for one week in group I and post treated for two weeks in group II (231 g) while other groups (Groups III, IV, V and Group VI was (173.7 g). There was gained in Weights. This indicated tremendous increased in weights of groups I and group II probably due to the effect of feed plus *M. balsamina*. This agreed with the work of Weende (1860) whose sample was processed to carbohydrate, crude fibre, crude protein, ash, crude lipid, and moisture. Therefore, carbohydrate can be used in feed formulation and this reserved of maize and guinea corn could be used by human consumption.

Group I before treated with *M. balsamina* for 1 week had increased in feed consumption and the pullets were active probably due to high amount of calcium, phosphorus and vitamins in ash (11.10 %) of *M. balsamina.* These minerals and vitamins were required for increased bone density and muscles. This agreed with the

work of Hassan and Umar (2006) that *M. balsamina* has high calcium, phosphorus and vitamins.

Mortality rate observed first: Group I on the 5th day (30), group II on the 6th day (25), group III on the 6th day (28) group IV on the 6th day (11) and group VI on the 5th day (38). This agreed with the work of Wakamatsu (2006a) who worked with an isolate of high virulence NDV virus that caused 100 % mortality post infection. There was significant difference between group I before treatment and group II after treated (Group I before treatment using *M. balsamina* and group II after treatment with *M. balsamina* for 2 weeks respectively. These Group I mortality was 88.10 % and group II 68.60 % probably due to a mild effect of Infectious bursal disease used at the second week of vaccination While group IV had the least mortality rate of 37.10 % probably due to the antiviral activities of Selenium used for treatment in group IV which had clinical signs of Torticolis and toe paralysis and were signs of survival but remained deform due to nervous involment, while other groups did not indicated such signs.

In this study, mean antibody titre increased from day-old up to the 7th week. At day old mean ND antibody titre range from 2.00 ± 0.45 to 3.17 ± 0.54 $\log_2$ while after challenge mean antibody titre range increase to 10.00 ± 0.00 $\log_2$ for group 1. The mean HI antibody titres measured for all the groups were above the cutoff point of

4$\log_2$ and above, indicating protection of the chickens against Newcastle disease. This is similar to what was reported previously by others (Allan *et al*., 1978; Verma *et al*., 1985) that HI antibody titre of 4$\log_2$ or higher is considered as protective. This titre obtained at day-old could be ascribd as maternal derived antibodies (MDAs). The

significantly increased mean HI titre obtained at first and second weeks for all the experimental groups could be attributed to the first active dose of IBD vaccine administered. This is because specific immunity against ND develops within a week of age or older (Brandly, 1952). Moreover, the mean Ab titre for group I was significantly higher than that of group II, 10.00 $\pm$ 0.00 and 4.00 $\pm$ 1.16 respectively. This is in agreement with the finding of Kouwenhoven, (1993) and Alexander, (1995) which stated that eye drop application is the best for uniformly high degree of protection. The ND virus usually replicates in the Harderian glands resulting in the production of lachrymal IgG, IgA and IgM (Russell, 1993). Group I that had highest mean antibody titres agreed with Saeed *et* al., (1988) who also mentioned that immune response was absent at high titre of MDAs. There was a significant difference between group I, II and other groups. No significant difference ($P<0.01$) between group I and group IV in terms of antibody titre probably due to chicks producing more antibody when before treatment of group I and group IV due to treatment after with selenium. In this case, *M. balsamina* was considered more prophylactic than chemotherapeutic comparing group I and group II (group I before treatment and group II after treatment). In addition to *M. balsamina*, despite the fact that group I had the highest mean antibody titre at the 5^{th} week (10.00$\pm$ 0.00) than groups II, III, IV, V and VI but had the highest mortality rate of 88.10 % probably due to the cutoff point of $4\log_2$ was low. This agreed with the work of Baba *et al*., (1998) used or got or had a cutoff point of $3\log_2$ in his work. The general increased in mean antibody titre from day old to the 7th week, agreed with the work of Wakamatsu (2006b) that a subset of genes of Newcastle disease virus isolates had a concomitant increased in mean antibody titre post infection.

Clinical signs and lesions observed showed low level of protection as indicated in all the groups. This might be attributed to the induced immune response by clone-30 at the

mucosal surface. Another reason could be the route of challenge may enhance the clinical signs and gross lesions observed in most cases: muscle congestion and neurological signs. This is in line with the findings of Beard and Hanson, (1984) who found that intramuscular or intravenous routes of NDV infection enhance neurological signs. Whereas natural routes of infection (nasal, oral and ocular) appear to emphasize the respiratory nature of the disease (Beard and Easterday, 1967; Alexander, 2000). Although the role of cell-mediated immune system cannot be overemphasized in defense mechanism, the humoral immune system plays a significant role in defense prevention. Antibodies in the serum will confine infection to the respiratory mucosa (Al-Garib *et al.*, 2003). Therefore, Ab titres of less than 4 log2 might not be protective. A good correlation exists between level of HI Ab titre and degree of protection (Kouwenhoven, 1993; OIE, 2009).

CHAPTER SIX

CONCLUSIONS AND RECOMMONDATIONS

6.1 Conclusions

From this work it was concluded that:

i. *M. balsamina* was found to be non toxic to 4-week- old pullets which dose up to 5 000 mg/kg body weight.

ii. *M. balsamina* fruits contain bioactive and nutritional substances.

iii. *M. balsamina* fruit was not effective in the control of Newcastle disease in pullets because 88.1 % mortality rate was recorded but selenium was effective with 37.1 % mortality.

6.2 Recommendations

From this work it was recommended that:

i. Further research is needed on other parts of the plant.

ii. Determine the effective benefit of *M. balsamina* for the control of Newcastle disease.

iii. Selenium (Se) has good potentials for the control of Newcastle disease in pullets.

iv. *Momordica balsamina* fruit ethanol extract and selenium could be tried in other avian species.

v. Further research on biochemical substances.

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APPENDICES

Appendix I: Mean antibody titre of pullets at day old treated with *M. balsamina* and Challenged with Newcastle disease virus 113 for group I-VI

GROUP	Mean Ab titre ±S.E.M
1	2.2 ± 0.49
II	3.14 ± 0.34
III	3.17 ± 0.54
IV	2.0 ± 0.45
V	2.33 ± 0.33
VI	2.43 ± 0.37

No Significant difference mean antibody titre between the groups p = 0.2626

Appendix II: Newcastle disease means antibody titre of pullets at four weeks of age following treatment with *M. balsamina* before challenge with Newcastle disease virus 113 for group I-VI

GROUP	Mean Ab titre ± S.E.M.
I	0.00 ± 0.00
II	3.00 ± 0.58
III	3.50 ± 0.50
IV	2.75 ± 1.18
V	2.00 ± 0.00
VI	3.00 ± 1.00

There was no significant difference mean antibody titre between the group p = 0.1

Appendix III: Mean antibody titre of pullets at five weeks treated with *M. balsamina* and challenged with Newcastle disease virus 113 for group I-VI

GROUP	Mean Ab titre ± S.E.M.
1	10.00 ± 0.00
II	4.00 ± 1.16
III	4.60 ± 0.68
IV	8.29 ±0.18
V	1.00 ± 0.00
VI	5.17 ± 0.91

There was significant difference mean antibody titre between the groups especially GP1 & GP II $P < 0.01$, GP1 & GP III, GP1 & GP V, GP1 & GP VI, GP II & GP IV, GP III & GP IV , GP IV & GP VI

Appendix IV: Newcastle disease means antibody titre of pullets at six weeks of age following treatment with *M. balsamina* and challenged with Newcastle disease virus 113 for group I-VI

GROUP	Mean b titre ±S.E.M.
1	5.38 ± 1.39
II	9.56 ± 0.24
III	9.50 ± 0.31
IV	6.22 ± 0.78
V	8.13 ±0.81
VI	6.00 ± 0.00

There was significant difference mean antibody titre between the groups especially: Gp 1 & II $P < 0.01$ 1 & III, II & III, III & IV

Appendix V: Newcastle disease means antibody titre of pullets at seven weeks of age following treatment with *M. balsamina* and challenge with Newcastle disease virus 113 for group I-VI

GROUP	Mean Ab titre ± S.E.M.
I	7.56 ± 1.25
II	9.50 ± 0.34
III	9.00 ±0.21
IV	10.00 ± 0.00
V	9.25 ± 0.75
VI	8.50 ± 1.50

There was no significant difference mean antibody titre between the groups p = 0.238

Appendix VI: Clinical signs of five weeks old pullets pretreated with *M.balsamina* at five weeks of age before challenged with Newcastle disease virus 113 (group I)

Clinical signs	Days post challenge									
	3	4	5	6	7	8	9	10	11	12
Ruffled feathers	-	+	-	+	+	+	+	+	-	-
Seclusion	-	-	-	-	+	-	-	-	-	-
Dullness	-	+	+	+	+	+	+	+	-	-
Inactive	-	+	+	+	+	+	+	-	+	-
Anorexia	-	+	+	+	+	+	+	+	+	-
Weakness	-	-	+	+	+	+	+	+	+	-
Coughing	-	-	-	+	-	-	-	-	+	-
Sneezing	-	-	-	+	-	-	-	-	+	-
Greenish white diarrhoea	-	-	-	-	-	-	-	-	+	-
Torticolis	-	-	+	+	-	-	-	-	-	-
Salivation	-	-	+	-	+	-	-	-	-	-
Leg paralysis	-	-	-	+	-	-	+	-	-	-
Mortality	-	-	5	9	8	1	7	-	-	-

KEY: +Present–Absent clinical signs

Appendix VII: Clinical signs of four weeks old pullets post treated with *M.Balsamina* at four weeks of age after challenged with Newcastle disease virus113 (group II)

Clinical signs	Days post challenge										
	3	4	5	6	7	8	9	10	11	12	13
Seclusion	-	+	+	-	-	+	+	+	+	-	-
Inactive	-	+	+	-	-	-	+	+	+	+	-
Emaciation	-	+	+	-	-	-	-	-	-	-	-
Dullness	-	-	-	+	+	+	+	+	+	+	-
Ruffled feathers	+	+	+	+	+	-	-	-	-	-	-
Anorexia	-	+	+	+	+	+	+	+	-	-	-
Weakness	-	-	-	+	+	+	+	+	+	+	-
Coughing	-	-	-	-	+	-	-	-	-	-	-
Sneezing	-	-	-	-	+	-	-	-	-	-	-
Rales	-	-	+	-	-	+	+	+	+	-	-
Mortality	-	-	-	1	2	7	5	1	-	-	-

KEY: +Present–Absent clinical signs

Appendix VIII: Clinical signs of four weeks old pullets treated with lamivudine at 4 weeks of age after challenged with Newcastle disease virus113 (group III)

Clinical signs	Days post challenge						
	3	4	5	6	7	8	9
Seclusion	-	+	+	-	-	-	-
Inactive	-	+	+	+	-	+	-
Emaciation	-	+	-	-	-	-	-
Dullness	-	-	+	+	-	+	-
Ruffled feathers	-	-	+	+	+	+	-
Anorexia	-	+	+	+	-	+	-
Weakness	-	-	+	+	+	+	-
Coughing	-	-	-	-	+	-	-
Sneezing	-	-	-	-	+	-	-
Greenish white diarrhoea	-	-	-	-	+	+	-
Rales	-	-	-	+	-	+	-
Mortality	-	-	-	12	13	3	-

KEY: +Present–Absent of clinical signs

Appendix IX: Clinical signs of four weeks old pullets treated with selenium at four weeks of age after challenged with a virulent Newcastle disease virus 113 (group IV)

Clinical signs	Days post challenge												
	3	4	5	6	7	8	9	10	11	12	13	14	
Seclusion	-	+	-	-	-	-	-	-	-	-	+	-	
Inactive	-	+	+	-	-	-	-	-	-	-	+	-	
Emaciation	-	+	+	-	-	-	-	+	-	-	+	-	
Dullness	-	+	-	-	+	+	-	+	-	+	-	-	
Ruffled feathers	-	-	+	-	+	+	-	+	-	+	+	-	
Anorexia	-	+	+	-	+	+	-	+	-	+	+	-	
Weakness	-	-	+	-	+	+	-	+	-	+	+	-	
Coughing	-	-	-	-	+	-	-	-	-	-	-	-	
Sneezing	-	-	-	+	-	-	-	-	-	-	-	-	
Greenish white diarrhoea	-	-	-	-	+	-	-	-	-	+	-	-	
Rales	-	-	-	-	-	+	-	+	-	-	-	-	
Paralysis of the feet	-	+	-	-	-	-	-	-	-	-	-	-	
Mortality	-	-	-	4	3	2	-	-	-	2	-	-	

KEY: +Present–Absent of clinical signs

Appendix X: Clinical signs of four weeks old pullets treated with water at four weeks of age after challenged with Newcastle disease virus113 (group VI)

Clinical signs	Days post challenge												
	3	4	5	6	7	8	9	10	11	12	13	14	15
Seclusion	-	+	-	-	-	-	-	-	-	-	-	-	-
Inactive	-	+	-	-	-	+	-	-	-	-	-	-	-
Emaciation	-	+	-	+	-	-	-	-	-	-	-	-	-
Dullness	-	-	-	-	+	+	-	-	-	-	-	-	-
Ruffled feathers	-	+	+	+	+	+	-	-	-	-	-	-	-
Anorexia	-	+	+	+	+	+	-	-	-	-	-	-	-
Weakness	-	+	+	+	+	+	-	-	-	-	-	-	-
Green/white diarrhoea	-	-	-	-	+	-	-	-	-	-	-	-	-
Rales	-	-	-	+	-	-	-	-	-	-	-	-	-
Mortality	-	-	1	3	9	9	7	-	-	-	-	-	-

KEY: +Present–Absent of clinical signs

Appendix XI: Gross lesions of four weeks old pullets pretreated with *M. balsamina* at four weeks of age before challenged with Newcastle disease virus113 (group I)

Gross lesions	Days post challenge					
	22	23	24	25	26	27
Congestion	L,Lu,TH,Kid,	L,Lu,Th,Sp,Lu,Th	Lu,Kid, L,Sp,H, Cae	L,Lu,Sp, kd,Th	L,Lu,Sp, Kid	L,Sp,H
Haemo	Tr, Cae, I,	Th, tr,Cae,Sk,	Tr,I,Cae,Pr,Th	I,Pr,Cac, Tr	I,Th,Pr,cae,Tr,	I,Cae,T, Pr
Haemo/ bands			Pr			
Necrosis	Tr					
Mucoid	Cae, L	Tr,Cae, L	Tr	Tr		Tr
Severely / haemo	Pr	Pr				
Cloudy	A,	A,	A,	A,	A,	A,
Emaciation	Sk,	Sk,				
Blood clot in thorax & heart	Thor, H	Thor Lu,				
Pus	Pr					
Dist/gall / bladder		Gal				

Key: L=liver, Lu=lungs, Th=thymus, Kid=kidney, Sp=spleen, H=heart, Tr=trachea, Cae=caecaltonsil, Sk=skeletal Muscle, Pr=proventriculus, A=Air sacs, Thor=thorax, Gal= gall bladder, Empty spaces=absent, Haemo= haemorrhages
Ven=ventricle

Appendix XII: Gross lesions of four weeks old pullets post treated with *M. balsamina* at four weeks of age and challenged with Newcastle disease virus113 (group II)

Gross lesion	Days post challenge					
	16	17	18	19	20	21
Congestion	Sk,Lu	L,Kid,Sk ,Lu,Th	L,Sp,Th, Kid	L,Kid,Lu	L,Sp	L,Lu,
Haemo	Pr,I,C ae,Tr	Tr,I,Pr,C Cae,	Sp,I,Cae,t Tr,Sk	Tr,L,Pr,Th	Th, Tr,Cae,Pr	Pr,L,
Mucoid	Tr	Tr	Cae,Tr	Tr,L	Tr,cae	Cae,Tr
Cloudy	A					
Emaciation			SK			
Dist/ gall blad				I		
Pale			Gal			
Enlargement				Sp		
Green/ ingest				Ven	Ven	

Key: L=liver, Lu, =lungs, Th=thymus, Kid=kidney, Sp=spleen, H= heart, Tr=trachea, Cae=caecal tonsil, Sk=skeleton Muscle, Pr= proventriculus, A=Air sacs, Thora=thorax, Gal= gall bladder

Appendix XIII: Gross lesions of four weeks old pullets post treated with lamivudine at Four weeks of age after challenged with Newcastle disease virus113 (group III)

Gross lesion	Days post challenge				
	16	17	18	19	20
Congestion	Lu,Kid	L,Kid,I,Sk,Lu	L		
Haemo	Pr,Cae, Tr,thor, L	Tr,I,Cae	Sp,kid,Th,Tr, Sk,Cae,pr,I		
Haemo/bands		Pr			
Mucoid	Tr	Tr	Tr, Cae		
Cloud		A			Sk
Emaciation					
Pus				L	
Dist/gall/blad		Gal	Gal		
Pale		Kid		Ven	ven

Key: Lu=lungs, Kid=kidney, L=Liver, I=intestine, Sk=skeletal muscle, Tr=trachea, Pr= proventriculus, Cae=caecal tonsil, Th=thym A=Air Sac, Gal= gall bladder, Haemo= haemorrhages, Ingest= ingester, Dist=gallbladder

Appendix XIV: Gross lesions of four weeks old pullets post treated with selenium at four weeks of age after challenged with Newcastle disease virus113 (group IV)

Gross lesions	Days post challenge					
	16	17	18	19	20	21
Congestion	L,Lu, TH,Sp,	L,,Lu,Sp, kid,Sk	L	L,Sp,,L Lu	L,Sp,Kid ,Lu,th	L,Lu,Sp,Kd ,Th
Haemo	Pr,Du, Je,Cae	Tr,I,Cae	Tr,SK,Cae,I,p ,Sp,kid	Pr,I,Ca, Th	I,Cae,Tr, Pr,SK	I,Cae,Sk,Tr
Haemo/ bands		Pr				
Necrosis	Tr					
Mucoid		Tr	Tr, Pr	I	I,Tr	I
Sellerely/haemo		Pr				
Cloudy	A	A	A		Sk	
Emaciation			SK		Sk	
Pus			Pr	I		

Key: L=liver, Lu, =lungs, Kid=kidneys, Cae=caecal tonsil, Sp=spleen, Th=thymus, Sk=skeletal muscles, Pr=proventriculus, Duo=duodenum, Je=jejunum, Tr=trachea, I=intestine, A=Air Sac, Haemo= haemorrhages, Ingest= ingester, Dist=distended gall bladder

Appendix XV: Gross lesions of four weeks old pullets treated with water at four weeks of age weeks of age and not challenged with Newcastle disease virus113 (group V)

Gross lesion	Days post challenge			
	16	**17**	**18**	**19**
Haemo	Due,C	I,Cae	Sp,Th,Pr,I,Sk,Tr,Kid,Cae	Cae, Tr,
Haemo/ bands	Pr			
Necrosis	Duo,Je	Sp		
Mucoid	Tr	Tr	Cae,I,Tr	
Sellerely/haemon		Pr		
Enlargement		Kid		
Cloudy		A		
Emaciation			Sk	

Key: Sk=skeletal muscle, Lu=lungs, L=liver, Sp=spleen, Duo=duodenum, Cae=caecal tonsil, I=intestine, Pr= proventriculus, Kid=kidney, Je=jejunum, A=Air Sac, Haemo= haemorrhages, Ingest= ingester, Dist=distended gall bladder, Tr=trachea

Appendix XVI: Two ripped yellow *M. balsamina* fruit harvested from National Veterinary Research Institute Vom, Jos South Local Government Area of Plateau State.

Appendix XVII: Two sliced *M. balsamina* fruit pulp from National Veterinary Research Institute (NVRI) Vom, Jos South Local Government Area of Plateau State.

Appendix XVIII: Newcastle disease infected pullets (NDV kudu 113 strain) post challenge and of treatment with *M. balsamina* arrow shewed sick birds (Group II).

Key
Arrow = All alive

Appendix XIX: Dead and sick Newcastle disease infected pullets (NDV kudu 113 strain). Blue arrow showed dead pullets and white indicated sick birds pullets (group IV).

Key
White arrow =Alive
Blue arrow =Dead

Appendix XX: Newcastle disease infected pullets (NDV kudu 113) post challenge and after treatment with selenium (arrow showed toe paralysis (Group IV).

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Appendix XXI: Newcastle disease infected pullets (NDV-kudu 113 strain) post challenge and after treatment with selenium (arrow showed torticollis (Group IV).

Key
A =Alive but neck paralysis

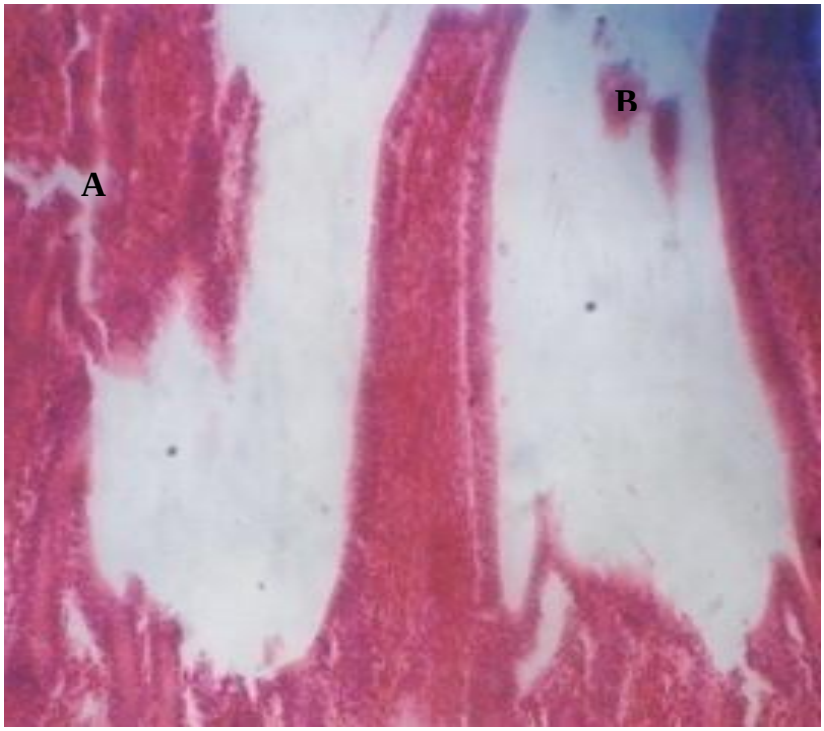
Appendix XXII: Newcastle disease infected pullets (NDV-kudu 113 strain) post challenge after treatment with water only (Group VI).

Appendix XXIII: Post mortem lesions/ Gross lesion of Newcastle disease infected /dead pullets after treatment with lamivudine (Group III) (Blue arrow showed greenish diarrhea at the vent and orange arrow showed haemorrhage of the liver.

Key

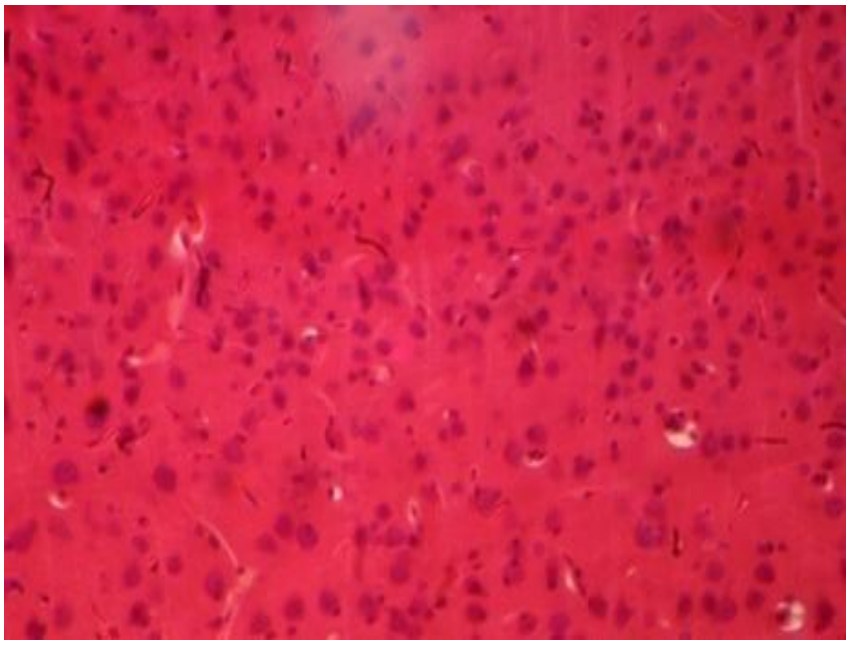
Orange arrow =liver haemorrhages
Blue arrow = greenish diarrhoea

Appendix XXIV: Newcastle disease infected dead pullets after treatment with *M. balsamina* (Group I) (A showed broken of the intestinal lining and B showed pieces of the intestinal villi hanging without attachment (× 200).

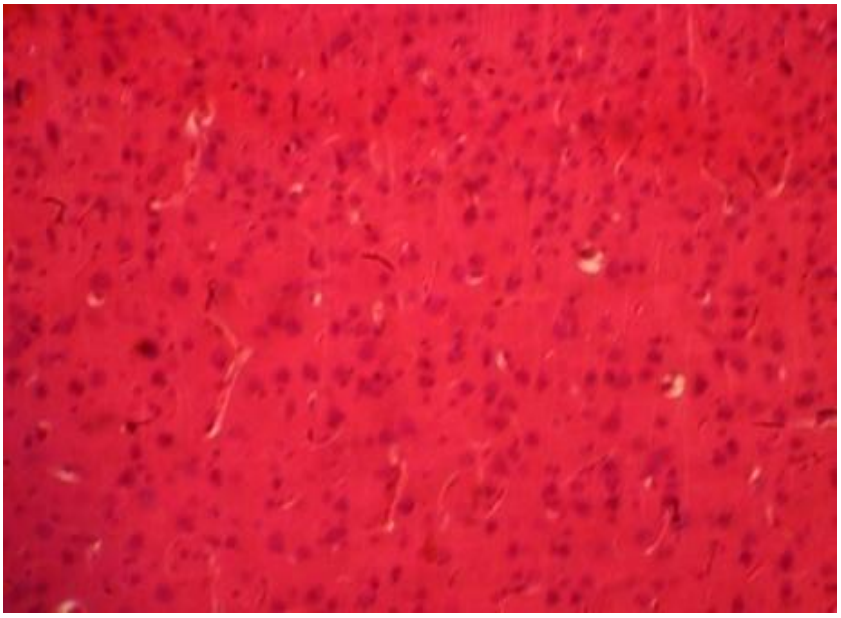


Key
A = Pieces of intestinal villi
B = broken intestinal lining

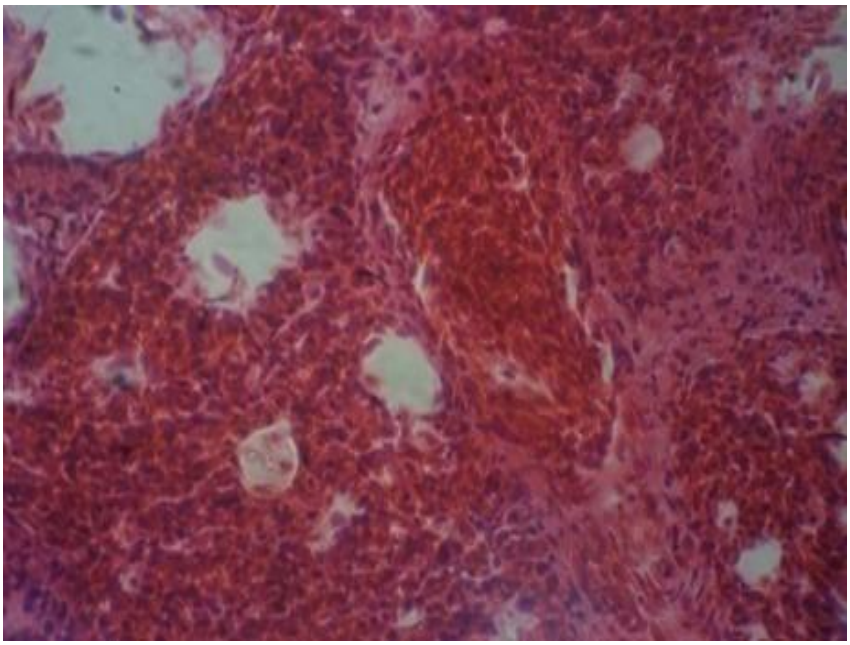
Appendix XXV: Plates X :(a) Liver showing congestion for group 1 when pretreated with *M. balsamina* before challenge with Newcastle disease virus kudu 113 strain (x400)



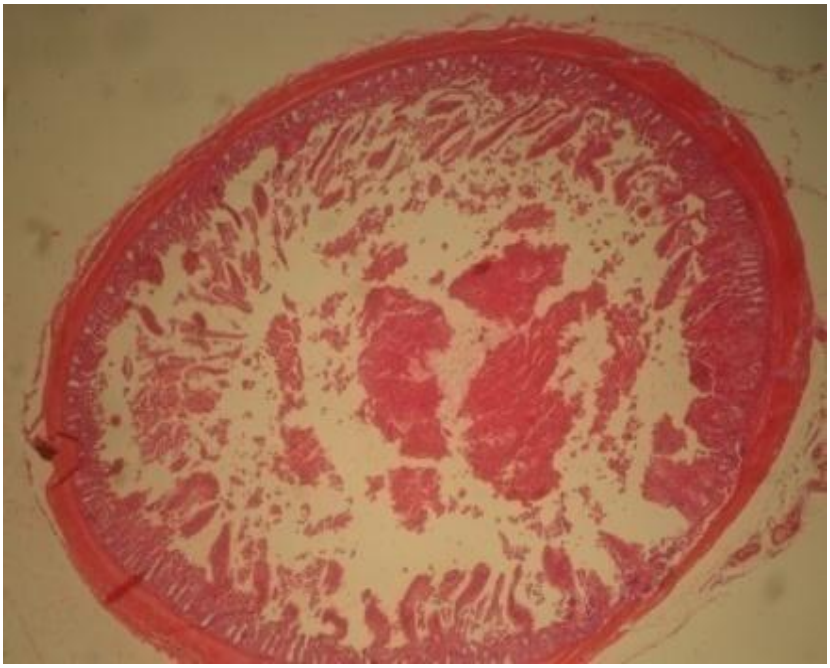
Appendix XXVI: Liver highly congested for group III when treated with lamivudine after challenged with Newcastle disease virus kudu 113 strain (x800)



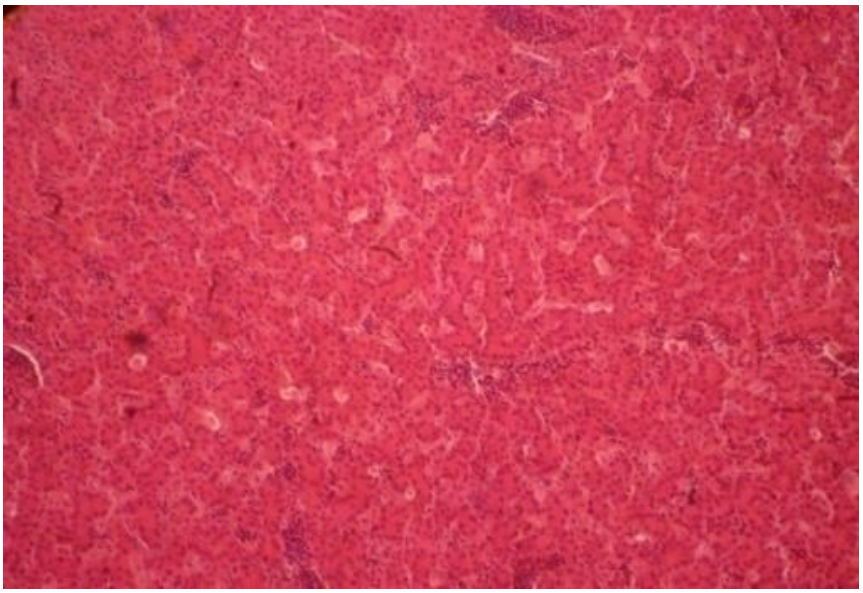
Appendix XXVII: Pulmonary congestion of the lungs of pullets (group I) pretreated with *M. balsamina* before challenge with Newcastle disease virus (NDV-kudu 113 strain) *(*x1120)



Appendix XXVIII: Desquamation, sloughing and severe enteritis of the intestinal mucosa of pullets (group II) treated with *M. balsamina* after challenged with Newcastle disease virus (NDV- kudu 113 strain) (x200).



Appendix XXIX: Liver congestion and vacuolation for group III when treated with lamivudine after challenged with Newcastle disease virus (NDV-kudu 113 strain) (x200)



Appendix XXX: Enteritis and intestinal villi broken of pullets treated with selenium after challenged with Newcastle disease virus (NDV-kudu 113 strain) (x200).