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Effects of Ethanol Extract of *Momordica balsamina* and some Antioxidants on Antibody Production in Broilers Challenge with a Virulent Newcastle Disease Virus

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ABSTRACT

Newcastle disease (ND) possess a serious threat to the poultry industry as it has economy and ecological impact on pet, free living as well as domestic birds. The aim of the effects of some antioxidants and *M. balsamina* on antibody production in broilers challenged with ND Kudu II3 virus. Two hundred day-old broilers were procured from a hatchery in Ibadan. The chicks were brooded for 4weeks then divided into 8 groups of 20 chicks each. Each group was housed in a pen on deep litter system with a floor space of 1.14 cm^2. Birds in GI were housed outside the faculty. All the experimental groups except group 1 were challenged with NDV Kudu II3 virus strain and that group I was given 2 L of distilled water at 5 weeks of age. Birds in group 2 were challenged with NDV and not treated with any antioxidant but given 2 L of water at 5 weeks of age. Birds in group 3 were administered vitamin C (600 mg tablets in 2 L of water orally. Birds in group 4 were treated with Vitamin E (400 mg/2 L IU soft gel Capsules orally and challenged. Birds in group 5 were treated with selenium-vitamin E (1 ml/ 2 L) of water orally. Birds in group 6 were treated with ethanol extract of *M. balsamina* leaves (400 mg/ 2 L) of water orally. Birds in group 7 were administered ethanol extract of *M. balsamina* roots (200 mg/ 2 L) of water orally and birds in group 8 were

administered ethanol extract of *M. balsamina* leaves (400 mg + Vitamin C 600 mg) in 2 L of water orally. The birds were bled for whole blood and serum weekly at 3,4,5,6 and 7 weeks of age. The mean haemagglutination inhibition (HI) antibody titres at week 6 were 0.8 log_2 4.5 log_2 6.8 log_2 for groups 1, 2 and 4, respectively. The mean enzyme Linked Immunosorbent assay antibody titres at week 6 were 3.5, 3.7 and 3.8 for groups 1, 2 and 8, respectively. In conclusion, ethanol extract of *M. balsamina* has no site effect even when administered at high doses of up to 5,000 mg per bird. Ethanol extract of the roots of *M. balsamina* has ameliorative effect against NDV by reducing mortality rate. Vitamin C also had ameliorative properties against NDV by reducing the pathology induced, and reduced mortality rate. Combining vitamin C (synergistically) with ethanol extract of *M. balsamina* leaves was recommended for treatment of ND.

Keywords: Momordica balsanina, haemagglutination inhibition, antioxidants, Enzyme Link, Immunosorbent Assay, log, Cobb 500, Newcastle disease

INTRODUCTION

Background of the study

Newcastle disease was first recognized in Java, Indonesia and Newcastle – Upon – Tyne, England in 1926 and it was named Newcastle (Doyle, 1927) The disease is Known in certain regions as Ranikhet disease, Pseudo fowl pest, pseudo poultry plague, avian pest, avian distemper, avian pneumoencephalitis and Korean fowl plague (Roy, 2012). Latter, it was found in various part of the world (Ashraf and Shah, 2014). By 1944, ND has been recognized throughout the world. Since then, the disease of chickens and is widely spread throughout Nigeria with annual epidemics being recorded in highly susceptible poultry flocks (Adu *etal.*, 1986; Sa'idu *etal.*, 1994). Newcastle disease has been a devastating avian disease globally before the recent outbreaks of highly pathogenic avian Influenza in the 90s and 2000s and usually, it manifests as acute rapid spreading

contagious, with nervous and respiratory disease of birds of all ages (Doyle, 1927). The disease can cause up to 100% Mortality in susceptible populations during devastating outbreaks and sporadic losses throughout the year.

Poultry production is a very important part of the agricultural subsector in Nigeria. This is due to increase demand of animal protein in Nigeria as the population is growing (Salami *etal.*, 1989). However, one of the constraints to the development of the poultry Industry is outbreak of disease (salami *etal.*, 1989). Newcastle disease (ND) is a highly contagious viral disease of chickens and turkeys, other domestic poultry, various species of wild birds, and humans are susceptible to it, but in man it is mild and characterized by inflammation of one eye, seldom both (Aldous and Alexander, 2001, Knipe *etal.*, 2007). The first documented outbreak of ND in Nigeria occurred between December, 1952 and February, 1953 in and around Ibadan (Hill *etal.*, 1953). Since then, the disease became the most important viral disease of chickens, and it is widely spread throughout the country with annual epidemic being in highly susceptible poultry flocks (Adu *etal.*, 1986; Sai'du *etal.*, 1994), Halle *etal.*, 1999; Orajaka *etal.*, 1999). Many species of domestic, semi-domestic and wild birds are susceptible to ND (Arshad *etal.*, 1988; kaleta and Baldout, 1988; Alexander *etal.*, 1997; Aldous and Alexander, 2001). Newcastle disease virus was isolated from apparently healthy ducks in and around Jos Plateau State, Nigeria, but there is no report of clinical ND in the ducks (Majiyagbe and Nawathe, 1981; Echeonwu *etal.*, 1993.

Newcastle disease virus is a non- segmented single stranded RNA genome of negative polarity and replicates in the

cytoplasm of cells (Emmersion, 1999, Knipe *etal.*, 2007). The envelope contains lipids and surface glycoproteins (haemagglutinin- neuraminidase (HN) and fusion (F), which surround the virion (Alexander, 1988). The penetration of the host cell by NDV occurs by fusion and mediated by a protein of the external envelope called fusion protein (F protein) (Rott and Klenk, 1988). The protein is synthesized as a precursor (FO) and needs to be cleaved into smaller fragments in order to be active (FI and F2) (Roth and Klenk, 1988. The pathogenicity of the NDV is determined by the amino acid sequence at the cleavage site of F-protein (Roth and Klenk, 1988; Lee *etal.*, 2004). About 250 thousand of NDV grouped together measured an inch (Lee *etal.*, 2004). Nasal secretions, saliva and droppings of infected birds contain the virus within 1 or 2 days after they are exposed to the infection (Lee *etal.*, 2004).

There are four forms of ND which are caused by pathotype of different strains of the virus Doyle's (Viscerotropic Velogenic), Beach's (Neurotropic Velogenic), Baudette's (mesogenic) and Hitchner's (Lentogenic) forms. In the Doyle's form all ages are susceptible. Morbidity rate may reach up to 100% and mortality is very high, usually 90% (Dobson, 1939). In the Beach's form (Neurotropic Velogenic), Morbidity is also high and mortality is variable. About 10% mortality in adult is very common though may be higher by the mesogenic strain (Elam, 1993). Among immature chickens, mortality is as high as 90%. Mortality in the adult in the case of the Beaudette's form (mesogenic) is rare and morbidity is variable (Elam, 1993). The mortality rate in young birds especially when complicated with other infections, the disease can reach 30% (Elam, 1993).

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Statement of the Research Problems

Newcastle disease belongs to one of the important World Organization for Animal Health listed avian diseases (OIE, 2009). ND is one of the most devastating disease in the poultry industry worldwide and poses a serious threat as it causes economic loses to farmers, and leads to embargo on trade and ecological impact on pet, free living and domestic birds (OIE, 2009)

Justification of the Study

Newcastle disease also caused great losses in Nigerian poultry industry within the last several years and still poses a threat to the poultry farmers. So there is need for improvement in the poultry industry which should incorporate emphasis on the prevention and control of diseases that cause economic losses (Akwor *et al.*, 2009). *Momordica. balsamina* was reported to be effective for control of ND by researchers and local farmers whose effectiveness on ND would be confirmed. Local poultry farmers have better access to *M. balsamina* than NDV vaccines that requires: proper storage, transportation, skills for reconstitution and administration (Mgbojikwe *et al.*, 2002).

Aim of the Study

The aim of the study was to evaluate the effects of some antioxidants and *M. balsamina* on antibody production in experimentally infected broilers following challenged with a virulent Newcastle disease virus.

Objectives of the Study

The objectives of the study were to determine the ameliorative effect of some antioxidants and *M. balsamina* leaves and roots on:

i. Vitamin C, E and selenium-vitamin E on antibody production in experimentally infected broiler challenged with VNDV.
ii. Ethanol extract of M. *balsamina* leaves on antibody production in experimentally infected broilers challenged with VNDV.
iii. Ethanol extract of *M. balsamina* roots on antibody production in experimentally infected broilers challenged with VNDV.
iv. Combination of ethanol extract of M. *balsamina* and Vit.C on antibody production in experimentally infected broilers challenged with VNDV.

Research Questions

i. Can vitamin C, E and selenium-vitamin E affect antibody production in experimentally infected broiler challenged with VNDV?
ii. Can ethanol extract of *M balsamina* leaves affect the antibody production in experimentally infected broilers challenged with VNDV?
iii. Can ethanol extract of *M. balsamina* roots affect antibody production in experimentally infected broilers challenged with VNDV?
iv. Can combination of ethanol extract of *M. balsamina* and Vit.C affect antibody production in experimentally infected broilers challenged with VNDV?

MATERIALS AND METHODS

Ethical Clearance

Ethical clearance was obtained from the ethical committee on animal use and welfare, Ahmadu Bello University, Zaria, with the reference number ABUCAUC/2018/038

Newcastle disease virus

Virulent NDV strain (Kudu II3 strain) was obtained from the National Veterinary Research Institute (NVRI), Vom, plateau

State, Nigeria ($10^{8.5}$EID). The chicks were inoculated with 0.1 ml/bird reconstituted challenge virus after dilution of 1:9 ratio according to the source recommendations Echeonwu *etal.*, 1993.

Broilers

A total of 200 days old Cobb 500 breed of broilers were procured from a hatchery in Ibadan without ND vaccination and transported to Zaria. The chicks were brooded for 4 weeks before they were divided into 8 groups of 20 chicks each. Twenty broilers were assigned to each group but separated under deep litter system with a floor space of 1.14 cm^2/bird at one week of age.

Table 3.1 Experimental Design for Evaluation of antibody production at Five weeks Broilers challenged with a Virulent Newcastle Disease Kudu II3 Virus.

Group N=20	Treatment	Week of Treatment
1	Negative control 2 litres of water orally	Distilled water daily from week 5 to 8
2	Virus (0.1ml 1/n) + (positive control) continuous administration of 2 litres of water oral route	Distilled water daily from week 5 to 8
3	Virus (0.1ml 1/n) + continuous administration of vitamin C 600mg tablets in 2litres of water oral route	Distilled water daily from week 5 to 8
4	Virus (0.1ml 1/n) + continuous administration of vitamin E 400IU soft gels in 2 litres of water oral route	Distilled water daily from week 5 to 8
5	Virus (0.1ml 1/n) + continuous administration of selenium – vitamin E (1ml/L in 2litres oral route	Distilled water daily from week 5 to 8
6	Virus (0.1ml 1/n) + continuous administration of plant leaves extract	Distilled water daily from week 5 to 8

	(400mg/ml) in 2litres of water oral route.	
7	Virus (0.1ml 1/n) + continuous administration of plant root extract (200mg/ml) in 2litres of water oral route	Distilled water daily from week 5 to 8
8	Virus (0.1ml 1/n) + continuous administration of plant leave extract (400mg/ml) in 2 litres of water oral route + vitamin C (600mg tablets)	Distilled water daily from week 5 to 8

Key: Plant leaves = M. balsamina leaves, plant roots= M. balsamina roots, vit C, Se – vit E= selenium – vitamin E, vit E=vitamin E, plant leaves + vit C = M. balsamina leaves + vitamin C, 1/ = intra nasal route, H_2O = Water.

Feed

Commercial broiler starter feed, Grand cereal U.A.C, Nigeria.

Drugs

On arrival, vitalyte (Anupco company cruckatt, Road lady lane lud esdtate, U/C England).

Vaccine

Infectious bursal vaccine (Gumboro) was sourced from NVRI, Vom, Plateau State, Nigeria and it was used to vaccinate birds through water at the age of 2 weeks against infectious bursal disease with the dose of 200 doses/2 L of water through free chlorinated drinking water.

Enzyme – Linked Immunosorbent assay Kits

The ELISA test kits were obtained from IDVET, 310rue Louis Pasteur – France. The ELISA test was carried out according to manufacturer's recommendations at the Department of public

Health and Preventive Medicine, Faculty of Veterinary Medicine, ABU, Zaria, Nigeria,

Antigen

Newcastle disease virus laSota was sourced from NVRI, Vom and was used as the antigen for the haemagglutination inhibition test as well as haemaglutination test.

Selenium – Vitamin E

MIAVIT (Selenium – VitaminE) GmbH, Robert Bosch – Strabe 3, D-49632 Essen (Oklb), Distributed by Agriculture STD plus Nig. LTD, No. 36, 7up road Oluyole, off ring road Ibadan Nigeria was obtained from a veterinary pharmaceutical shop. The dose was 1ml/L, through water.

Vitamin C

Pure vitamin C (Michelle laboratory LTD, Nigeria) was sourced from an Agrovet shop at Samaru Zaria, Nigeria. The dose was 600 mg/2 L through water.

Tween 80 (Surfactant)

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 ethanol extract of *M. balsamina* leaves of 1 kg and roots 200 g.

Management

Housing: The chicks were housed in the research poultry pen of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Samuru, Zaria, Nigeria with

a floor space of 1.14 cm^2/bird under deep litter system, for one week of age.

Heat: Heat was provided using 200 W.bulbs (6) with a range of temperature of 36 – 37°C (Demerchi *etal.*, 2008).

METHODS

Challenge birds

At the age of the five weeks, the birds were challenged with 0.1 ml of Kudu II3 strain of Newcastle disease virus (!$0^{8.5}$ EID_{52}) intranasally Echeonwu *etal.*, 1993).

Vaccination procedure for infectious Bursa Disease (IBD)

A vial of IBD vaccine was sourced from National Veterinary Research Institute, Vom, Jos, South Local Government Area, Plateau State, Nigeria containing 200 doses of IBD Vaccine. It was diluted into 2 L of free Chlorinated water by aspirate 2 ml of saline water to dissolve the freeze dried vaccine. This was done after fasting or with hold water for 2 h. The reconstituted vaccine was given immediately and should be taken within an h. the leftover should be buried with the empty vial to avoid spreading of the virus since it is air born. Vaccination has to be done when the birds are two weeks of age.

Plant Collection

Momordica balsamina leaves and roots were collected from Zaria environ and taken to Department of Botany, Faculty of Life Sciences, ABU, Zaria for Identification. The plant leaves and roots were washed and shade dried and pounded to fine powder using mortar and pestle and stored in dry containers until used.

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Extraction of Plant

A 1.0 kg of the fine powdered plant leaves were soaked in 7 litres of 70% ethanol and extracted by shaking the container/mixture for 6 h on wrist action shaker. Two hundred grams (200.0 g) of powdered plant root parts were processed using the same protocol as the leaves. The preparations were left to stand for 24 h. After filtration through whatman's paper, samples were concentrated to dryness on a water bath at 40°C for 1 – 2 weeks and packaged in water proof polythene bags and stored in the refrigerator at – 4°C until used (Atawodi, 2005).

Determination of the median lethal dose (LD_{50})

A total of 12 broilers of 5 weeks old were used for the experiment. The birds were divided into two groups (A = 9 birds and B = 3 birds). Group A was further divided into subgroups of 3 broilers each. The birds in subgroups A1, A2, and A3 were orally treated with *M. balsamina* 10,100 and 1,000 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 1,600, 2,900, and 5,000 mg/kg body weights were administered to the groups, respectively. The procedure was the same for *M. balsamina* roots. The LD_{50} of the leaves and roots were calculated based on Lorke's Method, (1983).

Determination of Newcastle disease Virus antigen titre

Newcastle disease virus LaSota was sourced from NVRI, Vom and used as the antigen for the haemagglutination test as described by Allan and Gough, (1974).

Haemagglutination test

Procedure to prepare 4HA units antigen. The test was conducted according to OIE, (2009).

Haemagglutination inhibition test

Sera collected from broilers were tested for NDV specific antibody by the HI as described by Allan and Gough (1974) and OIE (2009). The HI, test was performed using beta technique (Constant Virus and Varing Sera) against 4 HA units of the virus computed from the HA titration.

Determination of antibody titre

The antibody titre was determined using enzyme – linked immunosorbent assay (ELISA). The ELISA Kit (ID Vet, France) was used according to the manufacturer's instructions.

Data analysis

Data from HI & ELISA parameters were reduced to mean and standard error (deviation) and one way ANOVA was applied using statistical package for social services (SPSS) version 20, (2015) to analyse the difference between groups to establish the level of significance. Value of $P \leq 0.05$ were considered significant. Results were presented in tables, figures and plates.

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RESULT

Plates 1-4 showing: *M. balsamina* leaves, fruits and seeds, group 6, 7 and 8 showing mortality and survival rates.

Plate 1: Photograph showing *Momordica balsamina* leaves, fruits and seeds used for the experiment 2019

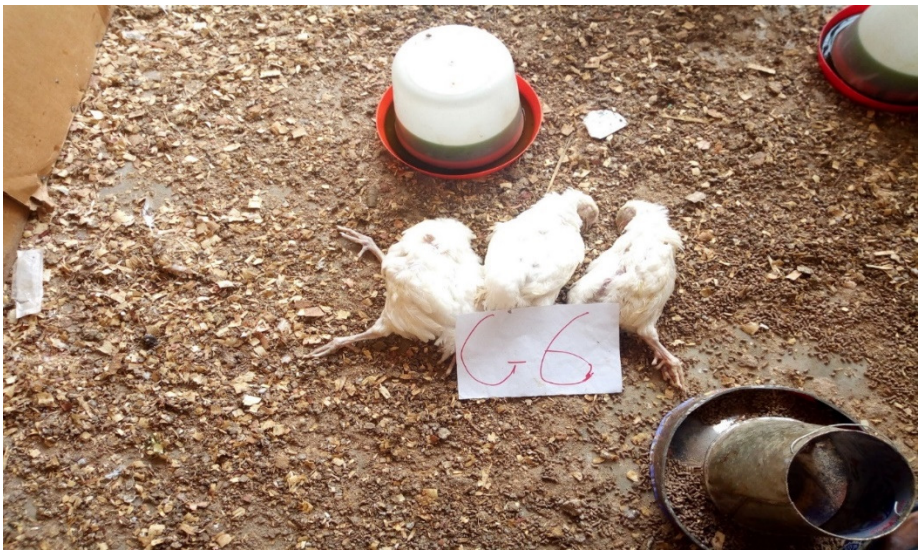

Plate 2: Photograph showing high mortality in group 6 of broilers challenged with ND Kudu 113 virus and administered M.balsamina leave extract 2019

Plate 3: Photograph showing recovered broilers of group 7 challenged with ND Kudu 113 virus and administered *M. balsamina* roots ethanol extract 2019

Plate 2: Photograph showing recovered broilers of group 8 challenged with ND Kudu 113 virus and administered *M. balsamina* leaves ethanol extract plus vitamin C (synergy) 2019

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ANTIBODY RESPONSE TO NEWCASTLE DISEASE VIRUS.
Haemagglutination inhibition antibody titres of broilers challenged at 5 weeks old with Newcastle disease Kudu II3 virus. *At week 4* not challenge the average HI titres for groups 1, 2, 4 and 8 were 0.1, 0.09, 0.1 and 0.09, respectively. At weeks 5 not challenge the average HI titres for groups 1, 2, 4 and 8 were 0.1, 0.09, 0.1 and 0.09, respectively. After challenge at week 6 the average HI titres for groups 1, 2, 4, and 8 were 1.5, 4.1, 6.5 and 4.0, respectively. At week 7 after challenge the average HI titres for groups 1,2,4 and 8 were 2.5, 4.1, 2.0 and 1.8, respectively while at week 8 after challenge the average HI titre for groups 1,2,4 and 8 were 4.4, 6.0, 4.3 and 4.0, respectively (**Figure 4.4**).

Enzyme linked Immunosorbent assay of broilers challenged at 5 weeks old with Newcastle disease Kudu II3 virus.

Before inoculation at week 4, the average ELISA titres for groups 1, 2, 4 and 8 were 2.2, 2.5, 2.3, and 2.4, respectively. At week 5 before challenge the average ELISA titre for group 1, 2, 4, and 8 were 2.4, 2.5, 2.6 and 2.6, respectively. At week 6 after challenge, the average ELISA titre for groups 1, 2, 4 and 8 were 3.5, 3.6, 3.7 and 3.8, respectively. At week 7 after challenge due to latent period of Virus (1week), the average ELISA titre for groups 1, 2, 4 and 8 were 3.6, 3.5, 4.0 and 3,0, respectively (**Figure 4.5**).

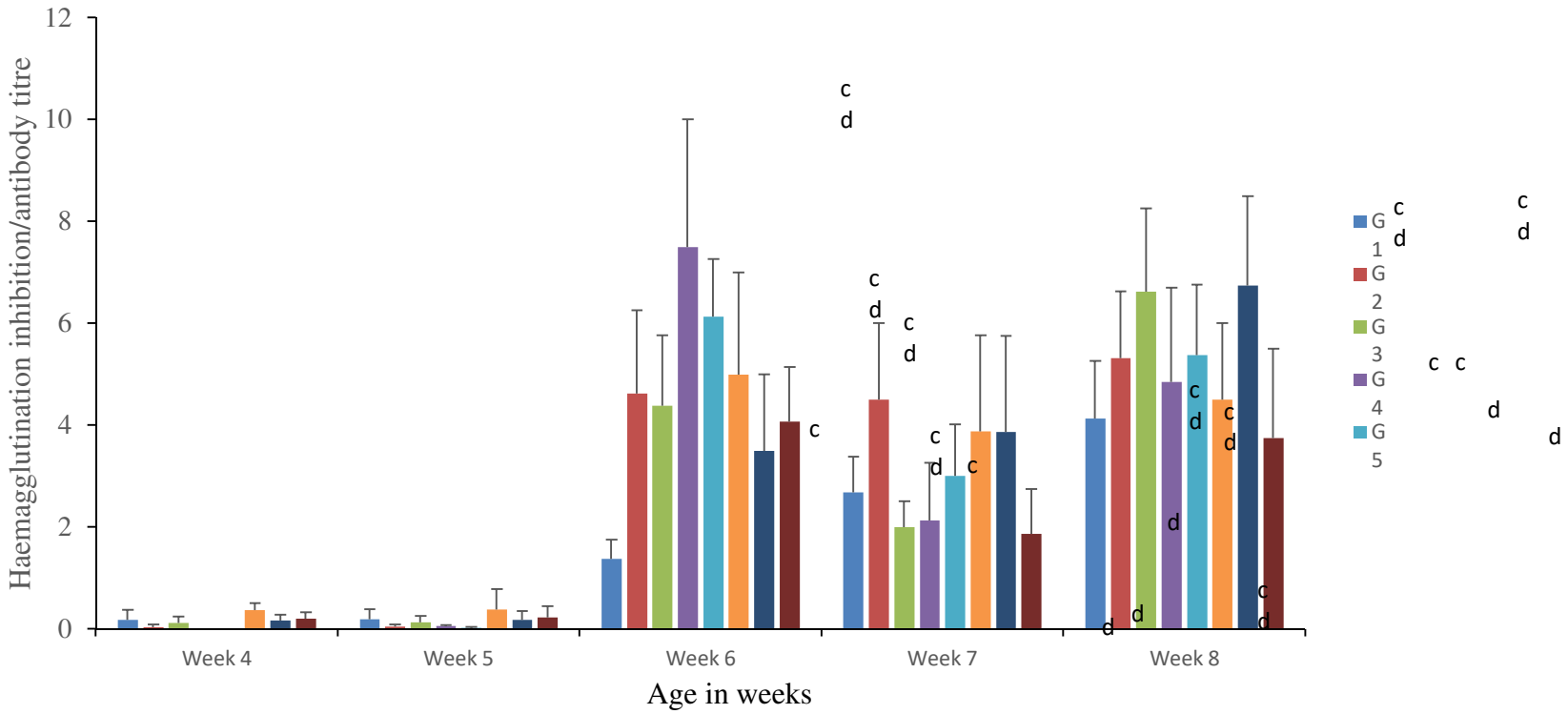

Figure 4:4 Haemagglutination inhibition antibody titre of broilers challenged at 5 weeks old with Newcastle disease Kudu 113 virus.

Keys: c = statistical significant difference with negative control, d = statistical significance difference with positive control, G1= negative control, G2 = Postive control, G3 = vitamin C, G4 = Vitamin E, G5 = Selenium-Vitamin E, G6 = *Momordica balsamina* leaves, G7 = *Momordica balsamina* roots, G8 = *Momordica balsamina* leaves + Vitamin C, n = 20

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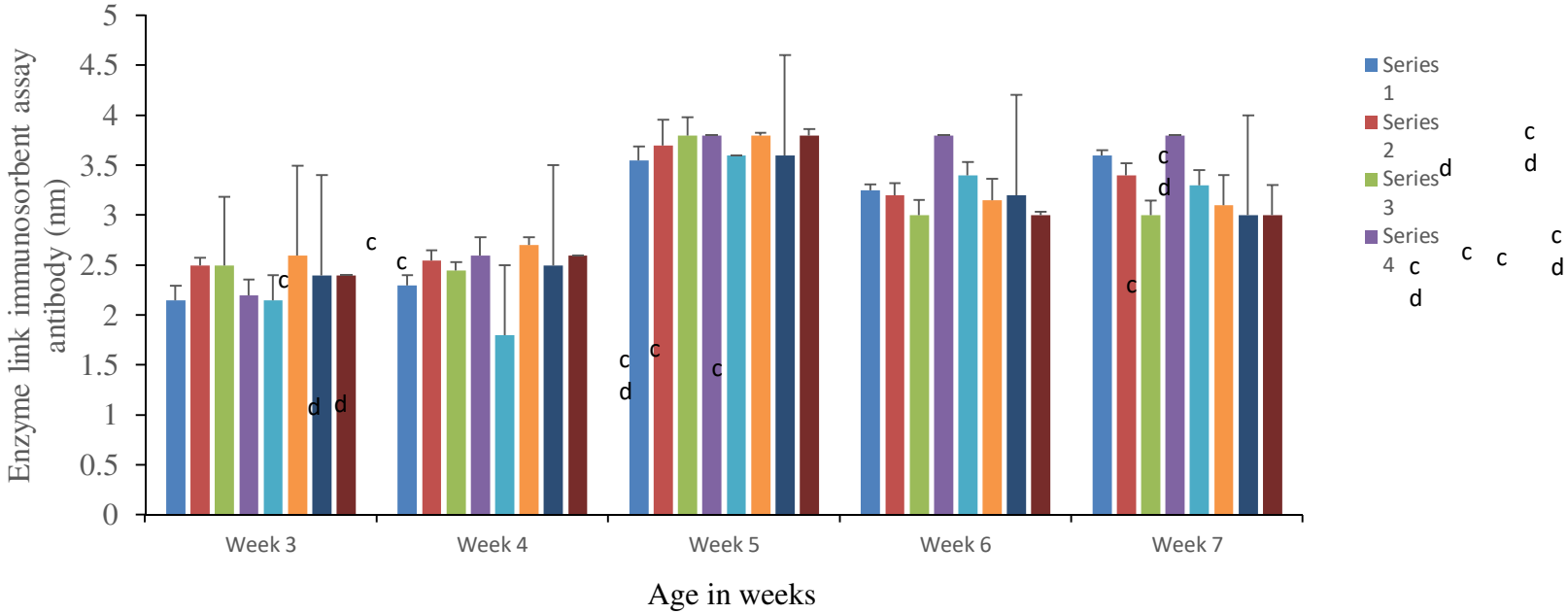

Figure 4.5: Mean enzyme-linked immunosorbent assay antibody titre of broilers challenged at 5 weeks old with Newcastle disease Kudu 113 virus

Keys: c = statistical significant difference with negative control, d = statistical significance difference with positive control, G1= negative control, G2 = Postive control, G3 = vitamin C, G4 = Vitamin E, G5 = Selenium-Vitamin E, G6 = *Momordica balsamina* leaves, G7 = *Momordica balsamina* roots, G8 = *Momordica balsamina* leaves + Vitamin C, n = 20

DISCUSION

Haemagglutination inhibition (HI) antibody titre level have a cut-off point of 4 log_2 as the protective antibody titre level as recorded by Alexander *et' al.*, (1995). Group 1 had the lowest mean HI antibody titre of 2.8 log_2 which was low response meaning that the group was not protected because it is not vaccinated or challenged. Group 3 of week 8 and 6 had the highest mean HI antibody titre of 3.5 log_2 which shows that ethanol extract of *M. balsamina* leaves and vitamin C modulated HI antibody production. It is important to note that the HI antibody titres in all the experimental groups were below the protective level of 4.0 log_2 probably due to latent period of the virus when immediately challenged. This was similar to the report of Allan *etal.*, (1978). Despite the low HI titre, the morbidity and mortality rates were not recorded in the treated group 1, but in the treated challenged groups 2 was 0.8 log_2. There was no significant statistical difference ($p \leq 0.05$) in the HI titres of all the treated groups. There was an increased (1.8 – 3.5 log_2) in all the HI titres in the groups except group 1 which was 1.8 log_2 , although the titres after challenged were above protective level in all the groups (4.0 log_2).

The low HI titres observed in this work may be attributed to first dose of infectious bursal disease virus vaccine given via water at second week of age since the study revealed a cut-off point of 3.5 log_2 which was below the protective antibody titre level. It could also be attributed to lack of vaccination at day-old and the environmental factors such as high temperature, humidity and secondary infection where the birds were kept. The study agreed with the work of Baba *et. al.*, (1995) who had a cut-off point of 3.0 log_2 indicating low protection as seen in week 4 and 5. Low antibody titres showing low responses.

There were increased in mean antibody titre in week 6-8, especially group 2, 3, 5 and 6. The histogram of this study showed a bell shape which had week 5 with low response. Week 7 and 8 with high response, while week 6 showed uniformity of protection. These findings agreed with the work of wakamatsu, (2006), who reported that for any NDV serum isolate, there is always a concomitant increase in mean antibody titre post infection.

In this study, the ELISA mean antibody titre of 3.8 for group 4 was higher than the cut-off point 3.0 indicated protection for these birds at week 7 and 8. This was probably due to the ELISA has higher sensitivity and multiplexing for serum samples and also agreed with the work of wakamatsu, (2006) who reported that for any NDV isolate, there is always a concomitant increase in mean antibody titre post infection as stated above. Enzyme linked immunosorbent assay had 4.0 mm. The histogram of study showed that week 4 and 5 had normal antibody titres probably due to maternal derived antibody (MDA) response but when challenged at week 6 antibody titre increased, then slightly decreased again except group 4 as the birds were recovering. There was a further increased in antibody titre in group 4 at week 8, followed by groups 1 & 2, then appeared to have higher ELISA level thangroups 5 and 8.

CONCLUSION

Haemagglutination inhibition antibody titre response was highest in broilers at week 6 (8.0 mm) due to effect in pathology which is less by the NDV treated with vitamin C in group 3.

RECOMMENDATIONS

From this work, it was recommended that;

- Farmers should make use of ethanol extract of *M. balsamina* roots (200 mg/2 L of water) for treatment of ND).
- Farmers should use the combination of ethanol extract of *M. balsamina* leaves (400 mg/2 L) plus vitamin C (600 mg/2 L of water) for treatment of Newcastle disease.
- Farmers should make use of vitamin C (600 mg/2 L of water) to treat birds against Newcastle disease.
- Ethanol extract of *M. balsamina* roots subjected to proximal analysis to determine the phytochemical constituents.
- Further studies are needed to know the real medicinal properties by extraction of the roots of ethanol extract of *M. balsamina* plant.

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