EFFECT OF DIETARY INCLUSION OF *GARCINIA KOLA* DRIED SEED POWDER ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF NEWCASTLE DISEASE VACCINATED BROILER CHICKS

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ABSTRACT

The effect of dietary inclusion of bitter kola (Garcinia kola) on the performance, organ weight and immune response to Newcastle disease vaccine in broiler chicks was assessed. Forty broiler chicks of five weeks old were randomly assigned into four groups (Groups A, B, C and D) of 12 birds each with the inclusion of sun dried ground bitter kola as a dietary additive at inclusion rate of 0, 5, 10 and 20 g/kg diet in groups A, B, C and D respectively. Birds were weighed weekly and vaccinated at week two with NCDV. Blood samples were collected from six birds in all the groups at weekly interval for PCV determination and serology. Serum samples were assayed for HI antibody using the HA/HI method. Results showed no significant difference (p>0.05) in PCV between groups B, C and D compared to the control A, There was no significant difference (p>0.05) in the weight gain and weight of the visceral organ. On day 14 post vaccination, the result of HI titres showed an increase in the humoral immune response of chicks in the control group when compared to groups B and C. There was a significant increase (p<0.05) in the humoral immune response in group D on day 14 compared with the control group. It can be concluded that bitter kola is well tolerated in broilers and better protected against NCD with bitter kola inclusion in their diet showing higher in the humoral immune response at 20 g/kg diet bitter kola inclusion.

Keywords: *Garcinia kola,* visceral organs, Newcastle disease, Broilers, Immune response

INTRODUCTION

Poultry production occupies an important position in the livestock industry. It is the quickest source of meat and its management involves the least hazardous process in relation to other livestock enterprises (FAO, 2013). Today's poultry sub-sector of animal production has evolved from a backyard business to a commercially oriented enterprise. This is attributed to growth in the technology (poultry genetics and poultry nutrition) of poultry production, increased demand and consumption of birds and eggs (FAO, 2013). However, a number of constraints to poultry production have been observed. Among these are parasites, pathogenic diseases and high cost of feed (Onwubuya *et al.*, 2015). In response to disease constraints, antibiotics have been in use to control diseases. They are also included in feed for apparently healthy birds as a protective measure, a practice observed to improve poultry

performance. However, these antibiotics inclusions as feed additives have been banned by European Union (EU) due to its potential to produce resistant strains of bacteria especially those that are of zoonotic importance (Mulder, 2016). Majority of the rising antimicrobial resistance problem in medicine is due to the overuse and misuse of antimicrobials (Michael *et al*., 2014).

There have been reported cases of Newcastle Disease vaccine failures in poultry industry when the vaccine administered failed to confer immunity on the birds against Newcastle disease. This has resulted in high morbidity and mortality of chickens and invariably increased cost of production (Marangon and Capua, 2006). The occurrence of vaccine failure has been linked to poor administration of vaccine in poultry, stressful condition (extreme temperature, poor nutrition) as well as inability of the vaccine to induce sufficient immune response (Hafez, 2005). Due to these effects, there is need to seek natural ways to boost the immune system of animals and enhance immune response to vaccine administration. Globally, there has been a growing interest in identifying and characterizing natural compounds with immunomodulatory activities and their possible clinical uses (Wen *et al*., 2012; Zhao *et al*., 2020; Mandić *et al*., 2021). These compounds which appear to stimulate immune response are being sought as adjuncts for vaccines (O'Hagan *et al*., 2001). Over the years, many plants have been identified to produce a vast number of secondary metabolites that have immunomodulatory potentials among which is *Garcinia kola* Heckel (Clusiaceae) (Dixon, 2001; Nworu *et al.,* 2008a,b). There is also the need for the use of alternative natural products to improve performance of broiler chickens. Plant parts have been a source of herbal medicine which has been shown to be effective to about 80 % of population as primary health care (Akinyemi*,* 2000). One of such plant is *G. kola* a member of the Clusiaceae species found throughout West and Central Africa. Every part of *G. kola* is an important component in traditional herbal medicine worldwide (Dalziel, 1937).

This plant has been referred to as a 'wonder plant' because every part of it has been found to be medicinal. *G. kola* has been found to possess significant antimicrobial properties. The *in-vitro* antimicrobial activities of crude extract of *G. kola* against some bacterial isolates showed that it has great effect on both Gram positive and Gram negative organisms (Adeboye *et al.,* 2008).

Bitter kola is generally safe to eat (WebMD, 2021). The phytochemical compounds isolated from *G. kola* include tannins, saponins, alkaloids, cardiac glycosides. Other phytochemical compounds isolated from *G. kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybi-flavonols. Two new chromanols; garcionic and garcinal, together with tocotrienol have also been isolated (Ebana *et al*., 1991). Bitter kola was reported to contain 0.58 % crude protein, 0.10 % crude fibre, 3 % ether, 5 % crude ash, 72.72 % nitrogen free elements (Ibekwe and Orok, 2010) and 6.27 % total sugar in addition to 8.46 % moisture (Dah-Nouvlessounon *et al.,* 2015). Kolaflavonone a compound in *G. Kola* have been reported to have antihepatotoxic effects linked to its membrane stabilizing effects which interferes with hepatic drug metabolism (Adaramoye and Akinloye, 2000). *G. kola* contains some antinutritional factors like alkaloids, saponins, oxalates, phytic acids and tannins in small quantities (Dah-Nouvlessounon *et al.,* 2015).

Although, *G. Kola* seed powder has been documented to have adaptogenic properties which can modulate the immune system and also has an anti-stress properties (Ofokansi *et al.,* 2008) so many studies have reported the medicinal effects of this plant seed, but there is still no scientific data on its effectiveness as adjuvant to vaccine in improving immune response, especially in poultry.

The objective of this study therefore is to investigate the effect of dried *G. kola* seed powder used as dietary additives on the growth performance and the immune response of broiler chicks vaccinated against Newcastle disease virus (NDV). The study also assessed the tolerance levels in the broilers using relative weight of the visceral organs such as liver, bursa, proventriculus, gizzard and spleen.

MATERIALS AND METHODS

Preparation of *Garcinia kola* Seed: The fresh seeds of *G. kola* were purchased from Ibagwa market in Igboeze South LGA, Enugu State, Nigeria. The fresh seeds were identified (Iwu and Igboko, 1982) and authenticated by a plant taxonomist in the Department of Plant Science and Biotechnology, where voucher specimen (PCG/UNN/0020) was deposited in the departmental herbarium. The outer covering of the seed was removed and the seeds were crushed using mortar and pestle and air dried to a constant weight. The dried seed were ground into fine powdered using an electric blender prior to mixing with the feed.

Toxicity and Phytochemical Assay of *Garcinia kola:* The toxicity profile and phytochemical compounds in *G. kola* was adopted from previous studies (Ebana *et al*., 1991; Adaramoye and Akinloye, 2000; Ibekwe and Orok, 2010; Dah-Nouvlessounon *et al.,* 2015).

Ethics: Animal studies were guided by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria Nsukka (FVM-UNN-IACUC-2019-0852) and the animals were used in accordance with the regulations and guidelines of this committee.

Experimental Animals: The study was carried out at the Poultry Unit of the Department of Animal Health and Production, University of Nigeria, Nsukka. Approval for the study was granted by the Faculty Ethics Committee on the use of animals. A total number of 48 day-old chicks of mixed sexes were procured from Agrited Hatcheries, Ibadan, Nigeria. On arrival, the birds were assigned into a complete randomized design (CRD) of four treatment groups (A – D), replicated thrice with each replicate having four birds. The chicks were housed in a pre-cleaned and disinfected pens and fed broiler super starter with 22 % crude protein and 2900 metabolizable energy (Top Feeds Limited, Nigeria) supplemented with graded doses of *G. Kola* powder. The birds were vaccinated against Newcastle disease virus with NDCV (Izovac ND, IZO S.r.l, 99/A 25124, Brescia, Italy). 100 doses administered at 0.05 ml/drop intra-occularly at week 2 of age and no other medication was given throughout the experimental period.

Experimental Design: Chicks in group A had no *G. kola* inclusion in their diets and served as control group. Chicks in group B received 5 g/kg of *G. kola* seed powder in their feed. Birds in group C had 10 g/kg of *G. kola* in feed, while birds in group D received 20 g/kg of *G. kola* in feed. Water and feed were supplied *ad libitum* during the whole period of the study.

Experimental Diet: The starter feed was purchased, measured and divided into four equal parts; each of the feed was mixed with approximate quantities of the prepared *G. kola* at 0, 5, 10 and 20 g/kg feed for groups A, B, C and D respectively. The actual concentration were made by measuring out the *G. kola* powder using Mettler sensitive digital weighing balance and mixing the seed powder with appropriate quantities of feed. The quantities of *G. kola* used were progressively to determine the point at which *G. kola* could have detrimental effects on the animal.

Determination of Changes in Live Weight: All birds from various groups were weighed using a weighing balance individually weekly between day zero and day 35 after which the group mean live weights was calculated by subtracting the initial weight (W1) of the animal from the current weight of the animal W2: (W2 — W1) (Kiczorowska *et al*., 2016).

Determination of Relative Organ Weight: On the 35thday of the study, one bird randomly selected per replicate was sacrificed and internal organs including the spleen, bursa of fabrocious, liver, proventriculus and gizzard were harvested and their weights determined to the nearest 0.01 g using a digital weighing balance (Mettler digital balance, Mettler Resources Company, Fulham road, West London). The relative organ weight for each organ was determined by using the formula:

Relative organ weight = organ weight/live weight x 100. Thereafter the mean relative weights of these organs were calculated per group.

Blood Collection: 5 ml of blood was collected from two birds per replicate every week from week two of age till the end of the study. 1 ml was emptied into sample bottle containing EDTA for the determination of packed cell volume (PCV). The remaining 4 ml were put in non-anticoagulant containing bottles to cloth. After the sera were harvested and stored in a freezer for the serological determinations.

Determination of PCV: The PCV of the birds was determined by the microhaematocrit method using a Haematospin 1400 centrifuge and a Hawksley microhaematocrit reader (Hawksley and Sons Limited, West Sussex, United Kingdom) as described by Thrall and Weiser (2002).

Serology: The sera were collected on weeks 2, 3 and 4 and were used to determine the antibody titres of birds in the various groups using haemagglutination test (HA)/ haemagglutination inhibition (HI) test as described by OIE (2012). The antibody geometric mean titre (GMT) was calculated using the tube and table described by Villegas and Purchase (1989).

Data Analysis: Statistical analysis was done using SPSS program version 16.0 (SPSS, 2005). Data from the study was analyzed using ANOVA and means were separated using Duncan's New Multiple Range Test. Differences in the means less than probability values of 0.05 ($p<0.05$) were considered significant. The results were presented as means ± standard error of mean. The statistical analysis of the HI data is presented as geometric mean titres (GMT) of the groups for the period of study.

RESULTS

Toxicity and phytochemical composition of Bitter Kola: The results of the phytochemical composition showed that *G. kola* contain 4.66 % alkaloids, 5.83 % saponins, 0.74 % oxalates, 1.09 % phytic acids and 3.54 % tannins. Bitter kola was reported to be non-toxic and contain 0.58 % crude protein, 6.27 % total sugar, 0.10 % crude fibre, in addition to 8.46 % moisture, 3 % ether, 5% crude ash and 72.72 % nitrogen free extract (Ibekwe and Orok, 2010; Dah-Nouvlessounon *et al.,* 2015).

Weight Gain: There was no significant difference ($p>0.05$) in the weight gain of the broiler chicks fed dietary supplementary dried ground bitter kola seed (Table 1).

Relative Organ Weight: The result of the mean relative organ weight showed that there was no significant difference ($p>0.05$) in the mean liver weight of all the four groups (Table 2). The mean weight of bursa in group C was slightly numerically higher compared to other groups A, B and D but there was no significant difference ($p>0.05$), in the bursa weight of all the four groups.

Packed Cell Volume: The mean PCV of the various groups at different weeks indicated that there was no significant difference ($p>0.05$) in the mean PCV between the groups.

Immune Response: On day 14 post vaccination, HI titres increased the humoral immune response of chicks in the control group (A) (GMT = 108.80 ± 39.97) as against groups B and C that had only slight increase (GMT = 22.40 ± 10.55) and (GMT = 28.80 ± 10.31) respectively (Table 4). Chicks in group D had significantly higher ($p<0.05$) humoral immune response (GMT = 339.20 ± 106.90) when compared with the control group (GMT = 108.80 ± 39.97).

DISCUSSION

There had been so much work carried out by some researchers using *G. kola*. The high value of saponin in *G. kola* as a result of the presence of glycosides attached to carbohydrate had been reported to be the cause of the characteristic bitter taste and foaming properties of bitter kola (Kumar, 1992).

Table 1: Mean body weight (g/bird) of birds fed different levels of *Garcinia kola*

Days	Group A (0 g/kg)	Group B (5 g/kg)	Group C (10 g/kg)	Group D (20 g/kg)
0	40.00 ± 3.22	39.75 ± 0.06	40.00 ± 1.12	39.84 ± 0.99
7	147.26 ± 9.95	142.64 ± 16.87	149.44 ± 10.40	156.04 ± 9.70
14	261.60 ± 17.28	260.30 ± 17.52	300.40 ± 26.02	320.60 ± 28.85
21	503.60 ± 39.76	470.00 ± 40.93	526.12 ± 52.52	529.60 ± 40.50
28	740.92 ± 69.40	651.58 ± 74.5	779.80 ± 61.98	846.68 ± 74.08
35	1004.26 ± 125.14	911.48 ± 101.34	1121.22 ± 71.49	1126.14 ± 102.42

Table 2: The relative organ weight of birds fed different levels of *Garcinia kola*

Organs	Group A (0 g/kg)	Group B (5 g/kg)	Group C (10 g/kg)	Group D (20 g/kg)
Liver	3.33 ± 0.15	3.22 ± 0.12	3.40 ± 0.00	3.50 ± 0.23
Gizzard	3.36 ± 0.32	3.40 ± 0.23	3.80 ± 0.21	3.83 ± 0.42
Proventriculus	0.67 ± 0.03	0.68 ± 0.03	0.63 ± 0.03	0.77 ± 0.08
Bursa	0.27 ± 0.07	0.23 ± 0.07	0.40 ± 0.06	0.23 ± 0.03
Spleen	0.13 ± 0.33	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03

Table 3: The mean packed cell volumes of birds fed different levels of *Garcinia kola*

Weeks	Group A (0 g/kg)	Group B (5 g/kg)	Group C (10 g/kg)	Group D (20 g/kg)
3	22.40 ± 1.03	25.60 ± 2.06	26.40 ± 0.87	22.80 ± 0.97
4	24.80 ± 1.82	24.20 ± 1.39	27.80 ± 1.02	24.80 ± 1.77
5	26.20 ± 1.74	23.40 ± 0.87	23.40 ± 0.68	24.60 ± 1.29

Table 4: Haemagglutination inhibition (HI) antibody titres (GMT) in all the groups for a period of four weeks

Weeks	Group A (0 g/kg)	Group B (5 g/kg)	Group C (10 g/kg)	Group D (20 g/kg)
1	22.4 ± 3.92	22.4 ± 3.92	22.4 ± 3.92	22.4 ± 3.92
2	10.4 ± 5.46^{b}	8.8 ± 1.96^{a}	12.0 ± 2.31^{c}	18.0 ± 5.03^{d}
3	11.2 ± 5.28^{a}	13.0 ± 6.40^{a}	26.0 ± 12.81^{b}	28.8 ± 3.92^{b}
4	108.8 ± 39.97^{b}	22.4 ± 10.55^{a}	28.8 ± 10.31^{a}	339.2 ± 106.9^{c}

GMT= Geometric mean titre with different superscripts across the group are significantly different ($p<0.05$)

The antinutritional effects of saponins have been studied, and they cause red blood cells haemolysis in animals (Johnson *et al.,* 1986). *G. kola* has the highest content of alkaloids and phytic acid. Tannins are water soluble phenolic compounds with reported antinutritional activities and they form a complex with proteins, making them less digestible by inhibiting the digestive enzymes (Kumar and Singh, 1984). In poultry, dietary tannins have been shown to impart beneficial effects when included in the poultry feed (Huang *et al.*, 2018). Although the use of tannins in monogastric animals' feed has been discouraged over the years because of the antinutrient contents (Butler, 1992), recent studies have revealed that if tannins are used with caution, they can be of benefit to monogastric animals (Brus *et al.*, 2019).

The low values of oxalates and phytic acid is preferable as excess oxalates and phytates binds with minerals like calcium and magnesium and interfere with their metabolism and can led to muscular weakness and paralysis in animal (Soetan and Oyewole, 2009).

The results of this study showed that broilers chicks fed dried bitter kola dietary inclusion had better weight gain. Previous studies demonstrated that bitter kola possesses antimicrobial and growth promoting effect (Adedeji *et al.*, 2006a,b). Enhanced growth performance has been reported in poultry at 20-40g/kg feed by Adedeji *et al.* (2006a; 2008). In this study the broiler had marked numerical not significant increase in body weight in the treated groups. The trend in weight gain and final body weight was D > C > A > B. Also, the increased growth rate observed in the present

study may be as a result of the antimicrobial activities of *G. kola* and growth promoting effect of *G. kola* (Adedeji *et al*., 2008). Weight gains obtained for groups B and C fed *G. kola* dry powdered seed were comparable with that of the control group.

Weight gain for group B birds fed 5 g/kg feed of dry powdered seed *G. kola* inclusion in the diet was the lowest. The reason for the variation is unclear and may be due to management error in that group.

The result showed that the PCV for all groups throughout the experiment were all within the normal range for experimental birds (Mituka and Rawnsley, 1987). It also showed that the *G. kola* has no toxic effects on the haematopoietic system.

The relative organ weight result showed that there was no significant difference in organ weight in all the groups. Conversely, Esiegwu and Udedibie (2009) reported that the inclusion of *G. kola* in diets lead to increased size of the liver in the chicken fed 2.5, 5.0 and 7.5 % *G. kola* seed meal inclusions. The counter result maybe as a result of different concentrations and preparation procedures. Bitter kola seed powder on the basis of the above can be said to be well tolerated by the broilers at the concentrations used in this study.

The effects of *G. kola* on the immune system from the results of the study showed that the administration of dried *G. kola* power as feed additive caused an elevation of humoral immune response to NDV at the highest concentration when compared to the control group. The modulatory activity of *G. kola* extracts on antibody synthesis improved humoral immunity of the animals. This protection was mediated through the various actions of the immunoglobulin. This actions include opsonization, direct neutralization of antigen, agglutination of antigen and activation of complement system that cause lyses and death of antigenic cells (Nworu *et al*., 2008b)

There was decreased in HI tires from week 1 to 3 in birds in groups B and C that received 5 and 10 g/kg inclusion of bitter kola in their diets which later increased in week 4. This agreed with the findings of Onah *et al.* (2018) who reported suppression of humoral immune response at a concentration of 250 mg/kg body weight of mice vaccinated against Hepatitis B virus. This was different from what was observed in groups A (control) and D, where HI titres started increasing by week 3 and reached a peak of very high titre at week 4. The dried *G. Kola* at lower concentrations may have caused increased complement lysing of the vaccine virus through the membrane attack complex (MAC) as reported by Nworu *et al.* (2008b), who observed an increased activation of the components of complement proteases when albino rats were challenged with sheep red blood cell and treated with *G. kola* methanolic extract. Vaccine viruses may have been wiped out too early by this increased complement lytic activity creating a lesser opportunity for the vaccine virus to trigger a higher immune response.

Birds in group D which received the highest concentration of *G. kola* in feed (20 g/kg diet) recorded a tremendous increase when compared with birds in group A. Higher concentrations of *G. kola* was reported to inhibit lytic activity of complement system (Nworu *et al*., 2008b), thus this inhibition may have provided enough time for more vaccine virus to be recognized by the immune system so as to trigger high antibody production. Apart from the formation of membrane attack complex which lyses and destroys invading cells, complement fragments that are liberated into the surrounding fluid have a number of other effects. These include chemotaxis, opsonisation and the stimulation of histamine release from mast cells and basophils (Guyton and Hall, 2005). This work provided evidence that *G. kola* can elevate antibody response to NDV only at certain concentrations since its stimulatory or inhibitory effect on complement system is dependent on the concentrations of the extract applied.

Conclusion: Information generated from this study indicated that broilers performed well when dried bitter kola was added to their diets with a progressive increase in weight gain and it is well tolerated following its effect on visceral organs and haematopoietic system. It also enhances immune responses in the broiler chick

with increasing concentration of *G. kola at* 20 g/kg diet by increasing the immune response of chicken against NDV; therefore, *G. kola* can be used as feed supplement as well as immune adjuvant in chicken feed. Further studies should be carried out to explain the mechanism of action by which *G. kola* act as growth promoter and immune adjuvant.

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