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HUMORAL ANTIBODY RESPONSE AND HAEMATOLOGIC ALTERATIONS ASSOCIATED WITH DIETARY SUPLEMENTATION OF VITAMINS E AND C IN VACCINATED BROILER CHICKENS CHALLENGED WITH A NIGERIAN VELOGENIC STRAIN OF NEWCASTLE DISEASE VIRUS (KUDU 113)

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ABSTRACT

The study evaluated the effects of dietary supplementation with graded doses of Vitamins C and E on humoral antibody response, clinical signs, body weight and haematologic changes in broiler chicken challenged with a velogenic strain of Newcastle disease virus virus KUDU 113. Two hundred and forty day old chicks were purchased and vaccinated against infectious bursal disease and Newcastle disease viruses on days 10 and 21 respectively. Thereafter they were subdivided into 8 (A - H) groups of 30 birds each: A (unchallenged+ no vitamin supplementation); B (challenged with Kudu 113 + no vitamin supplementation); C (challenged with Kudu 113+ 200 mg/kg vitamin C); D (challenged with Kudu 113+ 400 mg/kg vitamin C); E (challenged with Kudu 113+ 800 mg/kg vitamin C); F (challenged with Kudu 113+ 16.75 mg/kg vitamin E); G (challenged with Kudu 113+ 33.50 mg/kg vitamin E) and H (challenged with Kudu 113+ 67 mg/kg vitamin E). Vitamins C and E were included in the daily diet of the broiler chickens from 7 days before challenge with the velogenic Newcastle Disease virus and continued post infection until the end of the experiment. Blood samples were collected from all the groups, for haemagglutination inhibition titres and haematology on days 0, 3, 7, 10, 14 and 21 post infection. Body weight was taken on days 0, 3, 7, 14 and 21 post infection. Clinical signs and mortality were recorded. Increases in Antibody titres against Newcastle disease virus was observed in both the Newcastle disease virus challenged + vitamin supplemented and unsupplemented groups. Weight gain was improved in the supplemented + challenged group (especially in those groups supplemented with 80 mg/kg of vitamin C and 67 mg/kg of vitamin E) when compared with the challenged unsupplemented group. The erythrocytic parameters returned to normal by day 14 post infection in the supplemented groups when compared with the non-supplemented group. Leucocytosis was more evident in the supplemented group

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compared with the unsupplemented group. It was concluded that feed supplementation with vitamins C and E, particularly at 800 mg/kg and 67 mg/kg respectively, had significant effect on mortality, weight gain, erythrocytic profile and antibody response in broiler chickens experimentally infected with velogenic Newcastle disease virus.

Keywords: Vitamin supplementation, Newcastle disease, Broiler chickens, Antibody response, Mortality, Haematology.

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INTRODUCTION

Newcastle disease (ND) is a highly devastating viral disease of poultry characterized by pathological alterations in the digestive, reproductive, respiratory, nervous and lymphoid systems of the host. The disease results to severe economic losses in the poultry industry throughout the world [1]. Newcastle Disease Virus is an avian virus that belongs to the genus Orthoavulavirus in the subfamily Avulavirus and family paramyxoviridae [2]. Based on the pathogenicity of the infection, Newcastle Disease virus is categorized as asymptomatic, lentogenic, mesogenic, and velogenic strains of the virus. [3]. The mesogenic and velogenic strains are responsible for the heavy mortality and systemic infections observed in poultry. Although there has been significant improvement in the diagnosis and vaccination of poultry against ND, constant outbreaks are still recorded in both vaccinated and unvaccinated flocks [1].

Dietary supplementation with vitamins in poultry is a promising approach to ameliorate the mortalities associated with ND to reduce economic losses and ensure food security especially in developing countries of the world [4]. Vitamin supplementation is a major basis of disease control in the poultry industry. Vitamins E and C are well-known immune stimulants and immune system regulators and have always been subjects of interest in the control of many diseases including ND. Vitamin E is a natural antioxidant for cellular membranes and plays an important role in regulating the production of prostaglandins and leukotenes [5] thus, minimizing the damage resulting from cytotoxic actions in organisms while improving the phagocytic activity of macrophages in young birds [6]. Similarly, vitamin C is necessary for various biosyntheses as well as for regulation of diverse reactions, such as secretion of corticosterones, regulation of body temperature, and activation of the immune system [7].

Previous studies have shown that the use of vitamins in poultry improves performance and immune responses to Newcastle disease in poultry [8]. However, in spite of these advancements, outbreaks still occur in the poultry industry in Nigeria especially with the circulation of an NDV KUDU 113 strain; a velogenic strain of Newcastle disease virus first isolated from ducks in Kuru, Jos, Plateau State, Nigeria [9]. This strain is responsible for heavy mortalities in poultry, even among vaccinated flocks and results to huge economic losses especially in rural poultry. Consequently, there is need to re-evaluate the use of vitamins C and E in graded doses, in order to determine their effects on clinical signs, immune response and hematological profile of birds infected with Newcastle disease virus.

MATERIALS AND METHODS

Ethical consent

Ethical approval for this study was given by the Faculty of Veterinary Medicine Committee on Medical and Scientific Research Ethics, University of Nigeria, Nsukka, Nigeria.

Experimental Design

Two hundred and forty day old broiler chickens obtained from a commercial hatchery in Nigeria Sand housed in the Experimental Animals House of the Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria were used for this study. The chickens were routinely vaccinated with Gumboro and ND (using LaSota strain) vaccines on the 10 and 21 days of age respectively. The vaccines were

procured from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The velogenic NDV isolate, KUDU 113 (duck/Nigeria/Plateau/Kudu/113/1991) was used for the study [10]. The birds were provided with feed and water *ad libitum.*

The experimental chickens were divided into eight (A , B, C1, C2, C3, E1, E2 and E3) groups of thirty bids each: group A (non-supplemented and unchallenged chicken), group B (non-supplemented and NDV challenged chicken, group C1 (NDV challenged and supplemented with 200 mg/kg of Vitamin C), group C2 (NDV challenged and supplemented with 400 mg/kg of Vitamin C), group C3 (NDV challenged and supplemented with 800 mg/kg of Vitamin C), group E1 (NDV challenged and supplemented with 16.75 mg/kg of Vitamin E), group E2 (NDV challenged and supplemented with 33.5 mg/kg of Vitamin E), group E3 (NDV challenged and supplemented with 67 mg/kg of Vitamin E). The vitamins C and E were respectively procured from Enhalor Biotechnology Company (Beijing, China) and BASF group (Ludwigshafen, Germany). The vitamin supplements were included in the daily feed diet of the broiler chickens from day 35 of age until the end of the experiment. Groups B, C1, C2, C3, E1, E2 and E3 were challenged with 0.1ml of KUDU 113 containing $10^{8.46}$ 50% egg lethal dose (ELD 50) seven days after the start of vitamin inclusion in their diet.

Sample collection

Blood samples were collected from the jugular vein of five birds per treatment group on days 0, 3, 7, 10, 14, and 21 post infection (PI) into both heparin tubes and in plain sample tubes that were immediately transferred to the laboratory. Blood in heparin tubes were used for hematological determinations, while the blood in plain tubes were allowed to clot and the serum routinely harvested and stored in at −80 ◦C until use.

Clinical signs

The experimental birds were observed twice daily for clinical signs of NDV infection from day 0-21 post challenge with the KUDU 113 with the incubation period and mortality rates recorded.

Body weight measurements

Ten of the experimental birds in each of the groups were tagged and live body weight taken on days 0, 3, 7, 14 and 21PI.

Serology

Serum samples from birds from each group were assayed for antibody to NDV on days 0, 3, 7, 10.14 and 21 post infection (PI) with the viral inoculum using the Haemagglutination Inhibition (HI) tests [11].

Haematology

Haematological analyses were carried out immediately after blood collection on the heparinized blood samples. Packed cell volume (PCV) was determined by the microhaematocrit method, while haemoglobin concentration (HBC) was determined by the cyanomethaemoglobin method [11,12]. Red blood cell (RBC) and total white blood cell (WBC) counts were carried out by the haemocytometer method. Erythrocytic indices were calculated using the standard formula.

Statistical analysis

Data generated from body weight, HI titres, haematology were summarized as Means ± standard errors of means and subjected to one-way analysis of variance. Variant means were separated by Duncan multiple range test at 5% level of probability.

RESULTS

Clinical signs

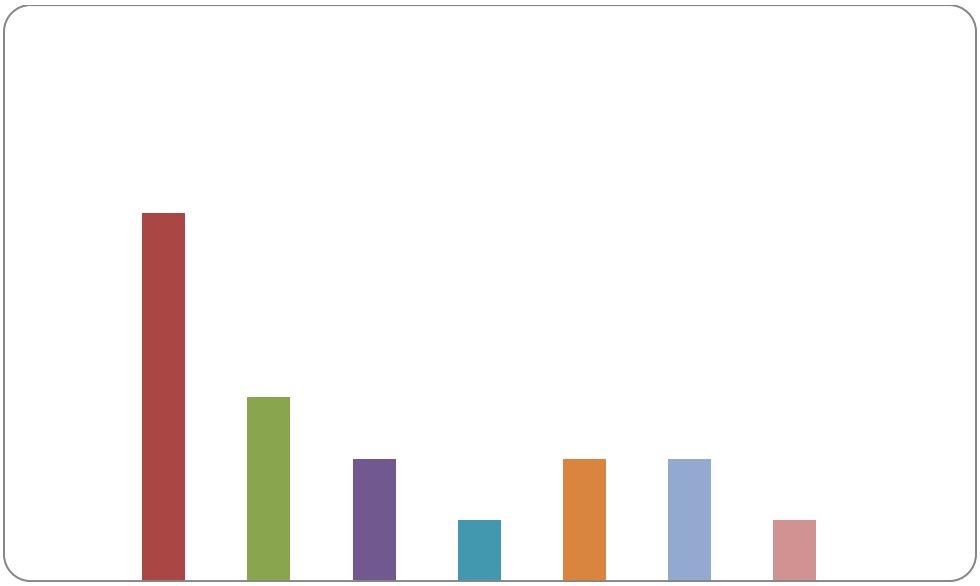
Clinical signs were first observed in group B (infected + no vitamin supplement) on day 3 post infection. The observed signs included ruffled feathers, lethargy and greenish white diarrhoea stained vent. Mild

torticollis was evident by day 6 PI. These clinical manifestations were consistent features observed in this group (Group B) until day 14PI. Clinical signs were first observed in the groups C1 and C2 on day 4 PI, in groups E1 and E2 on day 5 PI, while in groups C3 and E3 they were first observed on day 6 PI. Clinical signs observed in the infected and vitamin supplemented groups were mild compared with those of the unsupplemented group B. Mortalities were first observed in group B by day 4 PI followed by group C1 (on day 5 PI), groups C2, E1 and E2 (on day 6 PI) and groups C3 and E3 (on day 7 PI). Peak mortalities were observed in groups B and C1 on days 5 and 7 respectively post infection. The overall mortality rates were 25%, 10%, 6.6%, 6.6%, 6.6%, 3.3%, 3.3% and 0% for groups B, C1, C2, E1, E2, C3 and E3 respectively. No mortality was recorded in the control group A (uninfected+ non supplemented) (Fig. 1).

Body weight

There was a non-significant ($p>0.05$) decrease in mean body weight of the broiler chicken in the infected/vitamin supplemented (Groups C1, C2, C3, E1, E2 and E3) and in the infected/non supplemented with vitamins (Group B) on day 3 PI. However, the mean body weight of broiler chickens in group B was lower than those of the other groups between 7 and 21 days PI (Table 1). By day 7 PI, there was a gradual increase in the mean body weight of all groups in the NDV challenged+ supplemented with either vitamin C (Groups C1, C2 and C3) and/or E (Groups E1, E2 and E3). The observed increase in body weight was more evident in C3 and E3 when compared with the other NDV challenged + vitamin supplemented groups.

Fig. 1. The Mortality rate of broilers chickens challenged with KUDU 113 virus and fed diets supplemented with graded doses of Vitamin C and E.



*A = Uninfected Chicken /unsupplemented with vitamin, B = NDV infected Chicken/ unsupplemented with Vitamin , C1 = NDV Infected Chicken supplemented with 200 mg/kg of Vitamin C, C2 = NDV Infected Chicken supplemented with 400 mg/kg of Vitamin C, C3 = NDV Infected Chicken supplemented with 800 mg/kg of Vitamin C, E1 = NDV Infected Chicken supplemented with 16.75 mg/kg of Vitamin E, E2 = NDV Infected Chicken supplemented with 33.5 mg/kg of Vitamin E, E3 = NDV Infected Chicken supplemented with 67.5 mg/kg of Vitamin E.

Antibody response

There was a non-significant increase ($p>0.05$) in the mean HI titre against Newcastle disease in the broiler chickens challenged with NDV + supplemented with and without vitamin C or E (B, C1, C2, C3, E1, E2

and E3) when compared with the unchallenged + non supplemented group A from day 3 to 21 PI. All through the study, Group B had the highest mean HI titre followed by groups E3 and C3 then E2, C2, E1 and C1. Group A (control) had the lowest mean titre. Groups E3 and C3 had higher mean antibody titres than C1, C2, E1 or E2 (Table 2).

Table 1. Mean weight of broilers chickens challenged with KUDU 113 virus and fed diets supplemented with graded doses of vitamins C and E.

Days PI	A	B	CI	C2	C3	E1	E2	E3
0	1143.4± 65.4^{a}	1080.2± 67.^{a}	1113.2 ± 60.5^{a}	1144.0 ± 57.2^{a}	1112.4± 54.8^{a}	1113.2± 60.5^{a}	1154.2± 60.5^{a}	1055.0 ± 59.8^{a}
3	1340.6± 34.7^{b}	960.0 ± 46.8^{a}	1010.0± 42.5^{a}	1014.0 ± 38.6^{a}	1018.0± 33.5^{a}	1006 ± 39.7^{a}	1010.0 ± 54.6^{a}	1011.0± 59.6^{a}
7	1820.6± 47.2^{d}	940.0± 58.7^{a}	1166.0± 63.5^{b}	1200.0± 47.5^{b}	1490.4± 41.7^{c}	1320.0± 127.^{abc}	1220.0± 80.0^{b}	1500.0± 68.9^{c}
14	1999.6± 191.2^{bc}	1460.0± 50.7^{a}	1596± 55.1^{a}	1732.6± 51.6^{ab}	2166.0 ± 42.6^{c}	1900.0± 58.1^{bc}	1970.0± 88.1^{bc}	2080.0 ± 75.8^{bc}
21	2959.6± 145.9^{d}	1660.0± 41.5^{a}	2166.0± 43.9^{b}	2233.0± 59.5^{b}	2384.0± 59.2^{bc}	2300.0± 113.1^{b}	2265.0 ± 84.3^{b}	2558.0± 72.6^{c}

^{abcd}Values with different superscripts in the same row are significantly different (p<0.05).

*A = Uninfected Chicken /unsupplemented withVitamin, B = NDV infected Chicken/ unsupplemented with Vitamin , C1= NDV Infected Chicken supplemented with 200 mg/kg of Vitamin C, C2 = NDV Infected Chicken supplemented with 400mg/kg of Vitamin C, C3 = NDV Infected Chicken supplemented with 800 mg/kg of Vitamin C, E1 = NDV Infected Chicken supplemented with 16.75 mg/kg of Vitamin E, E2 = NDV Infected Chicken supplemented with 33.3 mg/kg of Vitamin E, E3 = NDV Infected Chicken supplemented with 67.5 mg/kg of Vitamin E.

Haematology

The packed cell volume of chickens supplemented and unsupplemented (Group B) with vitamins decreased non significantly in all the groups on day 3 PI. However, on days 7 PI and 14PI there was a significant difference ($p<0.05$) in the PCV values of group B when compared with group A and E3. The PCV of group E3 was highest by day 14 PI when a gradual return to normal level was observed in the vitamins C and E supplemented groups (Table 3). A non significant decrease was also observed in the RBC and HB values of both supplemented and unsupplemented broiler chicken when compared with the control from days 3 - 14 PI. However, a faster return to normal was observed in the vitamin E and C supplemented group than the unsupplemented group B (Table 4and 5). The WBC count of broiler supplemented with graded doses of vitamins C and E did not differ significantly ($p>0.05$) from that of the unsupplemented group B. However, a gradual increase was observed in the WBC count of all the groups infected with KUDU 113 with and without supplementation between days 3 and 14 PI. The increase was more notable in the group supplemented with graded doses of vitamins C and E (Table 6)

Table 2. Mean Haemagglutination inhibition titre of broiler chickens challenged with KUDU 113 virus fed diets supplemented with graded doses of Vitamins C and E.

Days PI	A	B	CI	C2	C3	E1	E2	E3
0	25.30± 7.50[a]	22.30 ± 5.30[a]	21.30± 5.30[a]	22.76± 7.10[a]	23.00± 8.10[a]	21.60 ± 5.30[a]	22.00± 6.00[a]	24.30± 7.50[a]
3	37.36± 14.10[a]	149.30± 56.40[a]	74.74± 28.20[a]	117.30± 69.90[a]	149.30± 56.70[a]	58.74± 35.00[a]	74.72± 28.20[a]	106.71± 21.30[a]
7	74.74± 28.20[a]	1365.3± 341.30[a]	469.34± 279.00[a]	597.30± 225.80[a]	843.34± 170.90[a]	768.04± 256.00[a]	853.30± 170.90[a]	1109.3± 519.00[a]
10	117.30± 69.90[a]	1594.7± 451.50[a]	1174 .7± 451.50[a]	1282.2± 170.70[a]	1494..2 ±451.50 [a]	938.70± 559.60[a]	1066.7± 554.70[a]	1194.70± 451.50[a]
14	341.30± 43.70[a]	1706.7± 341.30[a]	1193.3± 451.80[a]	1370.7± 597.30[a]	1536.0± 512.00[a]	1066.7± 554.70[a]	1194.7± 451.50[a]	1536.0± 513.90[a]
21	74.70± 28.20[a]	2048.0± 0.00[a]	1066.7± 554.70[a]	1450.74± 597.30[a]	1536.0± 512.00[a]	1194.7± 451.50[a]	1365.3± 341.30[a]	1706.70± 341.30[a]

*values with different superscripts in the rows indicate significant difference ($p<0.05$).

*A - Uninfected Chicken/unsupplemented withVitamin, B- NDV infected Chicken/ non-supplemented with Vitamin, C1- NDV Infected Chicken supplemented with 200mg/kg of Vitamin C, C2- NDV Infected Chicken supplemented with 400mg/kg of Vitamin C, C3 - NDV Infected Chicken supplemented with 800mg/kg of Vitamin C, E1- NDV Infected Chicken supplemented with 16.75mg/kg of Vitamin E, E2- NDV Infected Chicken supplemented with 33.3mg/kg of Vitamin E, E3- NDV Infected Chicken supplemented with 67.5mg/kg of Vitamin E.

Table 3. Mean packed cell volume (%) of broiler chickens challenged with Kudu 113 virus fed diets supplemented with graded doses of vitamins C and E.

Days PI	A	B	CI	C2	C3	E1	E2	E3
0	27.60± 1.20[a]	27.60± 3.00[a]	28.04± 1.60[a]	27.70± 1.70[a]	28.04±0 .60[a]	27.04±1 .50[a]	27.54±1 .00[a]	28.04±1 .50a
3	26.31± 0.80[a]	26.76± 2.30[a]	27.34± 0.90[a]	26.70± 1.30[a]	27.20±2 .30[a]	27.00±1 .20[a]	27.04±1 .50[a]	27.30±1 .20[a]
7	29.30± 1.20-[b]	23.04± 1.20[a]	22.04± 1.50[a]	22.70± 1.20[a]	24.70±0 .90[a]	23.70±1 .80[a]	25.70±1 .80[ab]	26.00±2 .10[b]
10	27.36± 0.90[c]	15.70± 0.90[b]	19.34± 0.90[ab]	21.00± 2.30[b]	21.74±1 .50[b]	19.24±1 .20[ab]	21.00±1 .70[b]	23.07±1 .20[b]
14	28.70± 1.50[b]	21.04± 0.60[a]	25.30± 0.70[ab]	26.70± 1.20[b]	27.00±3 .10[b]	24.30±1 .90[ab]	25.70±0 .70[ab]	29.00±0 .60[b]
21	29.30± 0.90[a]	24.30± 1.20[a]	26.76± 2.80[a]	27.00± 1.20[a]	27.70±1 .50[a]	25.30±2 .70[a]	28.20±1 .50[a]	30.13±0 .90[a]

[ab]Values with different superscripts in the rows indicate significant difference ($p<0.05$).

*A = Uninfected Chicken /unsupplemented with Vitamin, B = NDV infected Chicken unsupplemented with Vitamin , C1 = NDV Infected Chicken supplemented with 200 mg/kg of Vitamin C, C2 = NDV Infected Chicken supplemented with 400 mg/kg of Vitamin C, C3 = NDV Infected Chicken supplemented with 800 mg/kg of Vitamin C, E1 = NDV Infected Chicken supplemented with 16.75 mg/kg of Vitamin E,

E2 = NDV Infected Chicken supplemented with 33.3 mg/kg of Vitamin E, E3 = NDV Infected Chicken supplemented with 67.5 mg/kg of Vitamin E.

Table 4. Mean red blood cell counts (10^6/µl) of broiler chickens challenged with Kudu 113 virus fed diets supplemented with graded doses of vitamins C and E.

Days PI	A	B	CI	C2	C3	E1	E2	E3
0	2.75± 0.10^{a}	2.56± 0.10^{a}	2.60± 0.90^{a}	2.60± 0.10^{a}	2.50± 0.10^{a}	2.50± 0.20^{a}	2.70± 0.10^{a}	2.65± 0.10^{a}
3	2.70± 0.20^{a}	2.45± 0.20^{a}	2.60± 0.10^{a}	2.60± 0.20^{a}	2.50± 0.20^{a}	2.55± 0.20^{a}	2.65± 0.10^{a}	2.67± 0.02^{a}
7	2.60± 0.60^{b}	1.41± 0.20^{a}	2.05± 0.20^{ab}	2.22± 0.40^{ab}	2.10± 0.10^{ab}	2.20± 0.10^{ab}	2.35± 0.20^{ab}	2.40± 0.30^{ab}
10	2.70± 0.10^{a}	2.00± 0.20^{a}	2.40± 0.10^{a}	2.40± 0.20^{a}	2.60± 0.20^{a}	2.30± 0.10^{a}	2.40± 0.20^{a}	2.50± 0.10^{a}
14	2.81± 0.10^{a}	2.20± 0.10^{a}	2.50± 0.10^{a}	2.60± 0.20^{a}	2.67± 0.10^{a}	2.40± 0.02^{a}	2.55± 0.20^{a}	2.68± 0.20^{a}
21	2.76± 0.10^{a}	2.40± 0.10^{a}	2.62± 0.20^{a}	2.65± 0.20^{a}	2.71± 0.20^{a}	2.55± 0.10^{a}	2.64± 0.10^{a}	2.70± 0.30^{a}

^{ab}Values with different superscripts in the rows indicate significant difference ($p<0.05$).

*A = Uinfected Chicken/ unsupplemented with Vitamin, B = NDV infected Chicken unsupplemented with Vitamin , C1 = NDV Infected Chicken supplemented with 200 mg/kg of Vitamin C, C2 = NDV Infected Chicken supplemented with 400 mg/kg of Vitamin C, C3 = NDV Infected Chicken supplemented with 800 mg/kg of Vitamin C, E1 = NDV Infected Chicken supplemented with 16.75 mg/kg of Vitamin E, E2 = NDV Infected Chicken supplemented with 33.3 mg/kg of Vitamin E, E3 = NDV Infected Chicken supplemented with 67.5 mg/kg of Vitamin E.

Table 5. Mean haemoglobin concentration (g/dl) broiler chickens challenged with KUDU 113 virus fed diets supplemented with graded doses of Vitamin C and E.

Days PI	A	B	CI	C2	C3	E1	E2	E3
0	8.50 ± 0.70^{a}	8.90 ± 0.19^{a}	9.50 ± 0.10^{a}	8.70 ± 0.30^{a}	8.50 ± 0.50^{a}	8.90 ± 0.50^{a}	9.00 ± 0.50^{a}	8.5± 0.60^{a}
3	9.50± 0.20^{a}	8.32± 0.40^{a}	8.80± 0.20^{a}	8.60 ± 0.10^{a}	8.40± 0.10^{a}	8.40± 0.50^{a}	8.80± 0.50^{a}	8.47± 0.80^{a}
7	9.14 ± 0.30^{a}	6.70 ± 0.50^{a}	7.30 ± 0.70^{a}	7.40 ± 0.80^{a}	7.50 ± 0.40^{a}	7.20 ± 0.60^{a}	7.45 ± 0.20^{a}	7.80 ± 0.40
10	9.20 ± 0.10^{a}	5.40± 0.90^{b}	7.15 ± 0.30^{a}	7.30± 0.50^{a}	7.45 ± 0.60^{a}	7.10 ± 0.40^{a}	7.30 ± 0.60^{a}	7.62 ± 0.10^{a}
14	9.40 ± 0.30^{a}	6.40± 0.90^{a}	8.50 ± 0.20^{a}	8.50± 0.40^{a}	8.80± 0.30^{a}	8.30± 0.42^{a}	8.53 ± 0.60^{a}	8.92 ± 0.30^{a}
21	9.25 ± 0.10^{a}	7.50± 0.20^{a}	8.61 ± 0.70^{a}	8.84 ± 0.40^{a}	8.92 ± 0.50^{a}	8.60 ± 0.30^{a}	8.67± 0.40^{a}	9.02 ± 0.65^{a}

^{ab}Values with different superscripts in the rows indicate significant difference ($p<0.05$).

*A = Uninfected Chicken/ unsupplemented withVitamin, B = NDV infected Chicken unsupplemented with Vitamin , C1 = NDV Infected Chicken supplemented with 200 mg/kg of Vitamin C, C2 = NDV Infected Chicken supplemented with 400 mg/kg of Vitamin C, C3 = NDV Infected Chicken supplemented with 800 mg/kg of Vitamin C, E1 = NDV Infected Chicken supplemented with 16.75 mg/kg of Vitamin E,

E2 = NDV Infected Chicken supplemented with 33.3 mg/kg of Vitamin E, E3 = NDV Infected Chicken supplemented with 67.5 mg/kg of Vitamin E.

Table 6. The mean white blood cell counts (10^3/µL) OF broilers chickens challenged with KUDU 113 virus fed diets supplemented with graded doses of vitamin C and E.

Days PI	A	B	CI	C2	C3	E1	E2	E3
0	4.30 ± 0.20[a]	4.10 ± 0.20[a]	4.00 ± 0.00[a]	4.30 ± 0.20[a]	4.01 ± 0.20[a]	4.00 ± 0.20[a]	3.90 ± 0.20[a]	4.6± 0.10[a]
3	4.30± 0.30[a]	5.52± 0.30[a]	4.50± 0.50[a]	4.80 ± 0.50[a]	5.20± 0.30[a]	4.70± 0.40[a]	4.80± 0.40[a]	5.17± 0.10[a]
7	5.30 ± 0.30[a]	6.40 ± 0.60[a]	5.90 ± 0.50[a]	6.00 ± 0.40[a]	6.10 ± 0.40[a]	5.20 ± 0.60[a]	5.45 ± 0.50[a]	6.40 ± 0.60
10	5.10 ± 0.30[a]	6.52± 0.80[b]	6.10 ± 0.10[a]	6.20± 0.50[a]	7.75 ± 0.60[a]	6.25 ± 0.60[a]	6.90 ± 0.60[a]	7.20 ± 0.50[a]
14	4.84 ± 0.30[b]	6.70± 0.90[a]	8.54 ± 0.60[a]	8.61± 0.90[a]	10.20± 0.40[a]	8.60± 0.42[a]	8.33 ± 0.60[a]	11.52 ± 0.30[a]
21	4.90± 0.20[b]	7.50± 0.50[a]	9.61 ± 0.60[a]	9.84 ± 0.40[a]	11.72 ± 0.50[a]	9.60 ± 0.30[a]	9.97± 0.40[a]	11.92 ± 0.65[a]

[ab]Values with different superscripts in the rows indicate significant difference ($p<0.05$).

*A = Uninfected Chicken/ unsupplemented with Vitamin, B- NDV infected Chicken/ unsupplemented with Vitamin , C1 = NDV Infected Chicken supplemented with 200 mg/kg of Vitamin C, C2 = NDV Infected Chicken supplemented with 400 mg/kg of Vitamin C, C3 = NDV Infected Chicken supplemented with 800mg/kg of Vitamin C, E1 = NDV Infected Chicken supplemented with 16.75 mg/kg of Vitamin E, E2 = NDV Infected Chicken supplemented with 33.3 mg/kg of Vitamin E, E3 = NDV Infected Chicken supplemented with 67.5 mg/kg of Vitamin E.

DISCUSSION

The incubation period of 3 days observed in group B, (the NDV challenged + unsupplemented chickens) in this study is in agreement with the observation by earlier researchers in vaccinated chicken infected with KUDU 113 [13,14]. The incubation period of 4-6 days PI noted in the vitamins C and E supplemented groups suggests that vitamins E and C supplementation, as used in this study may have prolonged the time of onset of clinical signs in the supplemented groups.

However, the mode of this action is not immediately clear.

A notable reduction in mortality was observed in groups C3 (supplemented with 800 mg/kg of vitamin C) and E 3 (supplemented with 67.5 mg/kg of vitamin E) compared with C1, C2 E1 and E2. This reduction in mortality suggests that vitamin supplementation at the highest doses as used in groups C3 and E3 was most effective in reducing the pathologies al alterations and consequent mortality associated with Newcastle disease in broiler chickens.

Newcastle disease has been reported to significantly affect body weight of infected birds [17,13,14]. In the present study, there were reductions in body weight of the infected birds although this loss in weight was higher and significant in those infected groups that did not receive either vitamin C or E supplementation in their diet. An earlier report [17] suggested that loss in weight is a common occurrence in viraemic diseases and is caused by the reduction in feed and water consumption by the infected birds. However, between days 7 and 21 PI a gradual increase in the mean body weight was observed in both the infected but supplemented and unsupplemented groups although the increase in weight was more notable in the supplemented group compared to the unsupplemented group [18,19,20,8]. The highest mean body weight was observed in those groups supplemented with 800 mg/kg of vitamin C and 67.5 mg/kg of vitamin E on

days 14 and 21 PI, indicating that at increased doses of vitamin E or C, there was probably an enhanced additive effect of the vitamins [21]. This observation supports earlier studies that suggested that supplementation of vitamins C and E improved broiler performance [22,23,24].

The relatively higher (p>0.05) antibody titres recorded for both the supplemented and unsupplemented NDV challenged groups in comparison to the unchallenged control group may be attributed to the fact thatvirus multiplication in infected chickens enhanced antigenic stimulation of the immune system and consequent antibody response [17]. Vitamin C is known to possess antioxidant properties that protect immature lymphocytes from damage by free radicals thereby enhancing immune response while vitamin E enhances the production antibodies [8].

The relative decrease (p>0.05) in the erythrocytic parameters observed in both the infected/unsupplemented and infected/supplemented groups in this study had earlier been reported in previous studies [25,14] which suggested that vaccination prevented the destruction of red blood cells in NDV infections. Furthermore, the immunemodulatory activities of vitamins C and E supplementation may also have prevented the destruction of RBC and thus initiated a quick return of the erythrocytic parameters to normalcy in the infected supplemented group unlike in the infected/unsupplemented group B. The return of the parameters to normalcy was faster in the C3 and E3 groups than the other supplemented groups; Suggesting that higher dosages of Vitamins C and E initiated a faster recovery of these parameters in infected birds. The high WBC count observed in both supplemented and un-supplemeted groups may be due to higher heterophile count in birds with NDV infection due to the marked reactivity of the white cells of poultry species to NDV [12]. This relates to the magnitudes or severity of the inflammatory process in KUDU 113 infection.

In conclusion, therefore, the results of this study suggest that the supplementation of broiler diet with vitamins C and E at 800 mg/kg and 67.5 mg/kg, respectively in feed at the time of vaccination against Newcastle disease improved the clinical outcome and humoral antibody response, ameliorated NCD-induced weight loss and reduced mortality associated with the disease in broiler chickens. We recommend further investigation of feed supplementation with vitamins C and E in Newcastle disease of chickens.

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COMPETING INTEREST
The authors declare that they have no conflict of interest.

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