

Effects of Vitamins A and C on Performance, Immune Response and Haematology of Broilers Vaccinated Against Newcastle Disease

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Abstract: The effects of vitamins A and C on performance, immune response and haematology of broilers vaccinated against Newcastle disease was evaluated with 200 3 weeks old broiler chicks. The birds were randomly assigned to 4 dietary treatment groups namely; T1, T2, T3 and T4 with 25 birds per replicate and 50 birds in each group. During the starter and finisher phases, Diet T1 was fortified with 0.33mg of vitamin A per 25kg of feed, T2 was fortified per 25kg feed with 2.5g of vitamin C, while T3 was fortified with 0.33mg vitamin A + 2.5g vitamin C in 25kg feed while T4 served as the control (vitamin supplementation). The birds were vaccinated against Newcastle disease virus using Newcastle disease vaccine B1 strain (I/O) at first week of life and LaSota at the 3rd week in drinking water. Performance indices indicated significant ($p < 0.05$) difference in final weight (FW), daily feed intake (DFI), daily weight gain (DWG) and feed conversion ratio (FCR) among the treatments. FW, DFI, DWG and FCR ranged from 1170.00-1390g, 88.41-95.20g, 24.97-30.29g and 2.63-3.53, respectively. The effect of vitamins supplementation on the haematology of broilers showed that there were no significant ($p > 0.05$) differences in the PCV, Hb, WBC, RBC, MCH, MCHC, and MCV of the different groups. The PCV ranged from 36.86-39.06%. The HI titres of the experimental birds were significantly ($p < 0.05$) different and above normal protective titre at weeks 5, 6, 7 and 8. The carcass characteristics of birds fed the experimental diets indicated dressed weight from each treatment group was significantly ($p > 0.05$) influenced by the treatments. All the other parameters namely; head, wing, drumstick, thigh, legs, neck, heart, gizzard and kidney were not significantly ($p > 0.05$) influenced by the treatments. The observed results may indicate that the diets promoted uniform growth of these parts despite differences in dressed weight. In conclusion, vitamins supplementation of broiler diets did not improve performance, though, the combination of vitamins A and C improved FCR. More so, vitamin supplementation enhanced the immunity of ND Vaccinated birds when exposed to Newcastle disease.

Keywords: Newcastle disease, vaccination, immunity, haematology

1. INTRODUCTION

Broiler production is seen as one of the ways of enhancing food security for the rapidly increasing population of Nigeria. The short production cycle, high feed efficiency and high biomass per unit of agricultural land of broilers are particularly attractive for the Nigeria production system [4]. However, compared to other domestic animals, broilers are more susceptible to changing environmental condition [17], as well as diseases. Broiler production in Nigeria is often faced with frequent outbreaks of Newcastle disease (ND) which is endemic in the country. Newcastle disease is an acute, mild to severe highly infectious disease of poultry [3]. According to [8], Newcastle disease is very important because it is associated with high flock mortality and loss of edible and breeding eggs. At present, the control of the disease which is common between the months of November and March without vaccination is inconceivable hence vaccination is the major measure of controlling ND [21; 22]. However, outbreaks have been reported even in vaccinated populations [10].

According to [7], vitamins act as co-factors in several metabolic functions in immune reaction and are required for optimum health and normal physiological functions such as growth, development, maintenance or reproduction, whose deficiency in the diet might cause impairment of immunity. Rama-Rao *et al.* [19] remarked that generally higher levels of vitamins than the current recommendation for preventing deficiency syndromes would increase the immune response of poultry. However, compared to other domestic animals, broiler chickens are more susceptible to changing environmental conditions [17]. In particular, high ambient temperatures depress feed intake, weight

gain and increase mortality rates among broilers [4]. A possible approach to counteracting the negative effect of heat stress among chickens is the supplementation of birds with vitamins A and C in their feed [5]. Vitamin A is necessary for increasing the immunity of vaccinated birds through the production of antibodies and its deficiency would reduce immune response and increase susceptibility to infection [13]. Micronutrients like vitamins A and C have been shown to play vital role in host immune response [12]. Many vitamins are lost during processing, storage or when exposed to sunlight. So even if some quantities of these vitamins are added to the feeds by the commercial feed millers, they might have been lost ever before the poultry have access to the feed. It may therefore become necessary for the farmers to add these vitamins to the feeds on the farm at levels recommended for immunity. This work was undertaken to evaluate the performance and immune responses of Newcastle disease vaccinated (LaSota) broilers fed diets supplemented with vitamins A and C.

2. MATERIALS AND METHODS

This study was conducted at the poultry unit of the Teaching and Research Farm of the Department of Animal Production, Kogi State University, Anyigba, Kogi State, which lies on Latitude $7^{\circ} 15'$ and $7^{\circ} 29'$ N of the equator and Longitude $7^{\circ} 11'$ and $7^{\circ} 32'$ E of the Greenwich Meridian [11].

2.1. Birds and Management

A total of 200 day-old broiler chicks were purchased and brooded for 3 weeks. At 3 weeks of age, the birds were randomly assigned to 4 dietary treatment groups namely; T1, T2, T3 and T4 with 25 birds per replicate and 50 birds in each group. At the starter phase, 25kg broiler starter mash containing 22% crude protein, 7.9% ether extract, 4.5% crude fibre, 2.0% calcium, 0.8% available phosphorus, 0.56% methionine, 1.2% lysine, and 2900kcal/kg metabolizable energy was fed. At week 5, broiler finisher mash which contained 21% crude protein, 6.8% ether extract, 3% crude fibre, 2% calcium, 0.7% available phosphorus, 0.5% methionine, 1.2% lysine, and 2980kcal/kg metabolizable energy was fed. During the starter and finisher phases, Diet T1 was fortified with 0.33mg of vitamin A per 25kg of feed, T2 was fortified per 25kg feed with 2.5g of vitamin C, while T3 was fortified with 0.33mg vitamin A + 2.5g vitamin C in 25kg feed while T4 served as the control, i.e. no vitamin supplementation. The birds were vaccinated against Newcastle disease virus using Newcastle disease vaccine B1 strain (I/O) at first week of life and LaSota at the 3rd week in drinking water while Gumboro vaccine was also administered to the birds in their drinking water at the 2nd and 4th weeks of age. Coccidiostat was given to the birds to prevent an outbreak of coccidiosis in the flock during the course of the research.

2.2. Performance Parameters

The birds were weighed at the start of the feeding trial and subsequently on weekly basis using a top loading weighing scale. The mean initial live weights were subtracted from the mean live weights to determine the weight gained by the birds [19]. The weight gain was divided by the number of day for the feeding trial to obtain daily weight gain. Feed offered to the birds were weighed weekly and leftovers were also weighed to determine the feed intake of the birds [19]. Feed intake was divided by the product of the number of bird and the number of day of the feeding trial to obtain daily feed intake. Feed conversion ratio (FCR) was taken as daily feed intake divided by daily weight gain.

2.3. Blood Collection

Ten broilers from each group were marked and their blood collected via the jugular vein at 5, 6, 7 and 8 weeks of age. Sera was separated each time and stored at -20°C prior to laboratory evaluation. Sera obtained were subjected to Haemagglutination Inhibition (HI) test at the Veterinary Medicine Laboratory of the Veterinary Teaching Hospital, University of Nigeria (UNN), Nsukka, Nigeria.

2.4. Challenge Infection

Six broilers from each group were picked at 6 weeks of age and isolated for experimental infection. The inoculum, a Virulent Viscerotropic Newcastle Disease Virus (VVNDV) was obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria, with a lethal dose-fifty (LD_{50}) of $10^{8.7}$ per ml. Each broiler was inoculated intramuscularly with 0.2ml of the virulent NDV and observed for 20 days for clinical signs of ND as described by [9].

2.5. Haematological Parameters

At four and eight weeks of age, three birds from each treatment were selected for blood collection and 1ml of blood sample was collected from each bird for haematological evaluation using wing web vein puncture method. Blood samples for haematology were collected into bottles containing ethylene

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diamine tetraacetic acid (EDTA an anticoagulant) and the samples were taken immediately to the laboratory for analysis. Parameters that were evaluated for include packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV). The analysis was done at the biochemical laboratory of Grimmad Catholic Hospital, Anyigba, Kogi State, Nigeria using ABACUS Junior Analyzer.

2.6. Carcass Analysis

Carcass evaluation was done when the birds were 8 weeks old. The birds were fasted for overnight prior to carcass evaluation. A bird was selected from each replicate such that its weight was similar to the average weight of the replicate from which it was selected. Each bird was marked and bled by severing the head from the neck with a sharp knife. Bleed weight was recorded, as well as dressed weight. The carcass was eviscerated and was cut into head, neck, wings, breast, back, thigh, legs, heart, kidney, and gizzard. The weight of each cut was taken and recorded using sensitive weighing scale and then converted to percentage of the dressed weight.

2.7. Data Analysis

All data obtained were subjected to Analysis of Variance (ANOVA), and significantly different means were separated using the MINITAB Statistical Software [15].

3. RESULTS AND DISCUSSION

3.1. Performance of Broilers

The performance of broilers fed the experimental diets is presented in Table 1. There was significant ($p < 0.05$) difference in final weight (FW), daily feed intake (DFI), daily weight gain (DWG) and feed conversion ratio (FCR) among the treatments. FW, DF1, DWG and FCR ranged from 1170.00-1390g, 88.41-95.20g, 24.97-30.29g and 2.63-3.53, respectively. The control birds were better in all the performance parameters measured except for FCR. The values obtained for daily weight gain may indicate that the vitamin supplementation did not contribute to high weight gains of the broilers. This observation supports the findings of [18] that vitamins A, C and E supplementation did not have beneficial effect on the growth of broilers. DFI of the experimental birds appear adequate but the observation is different from that of [6] who reported that feed intake of broilers was not affected by the supplementation of their diets with vitamins. The pattern of the observed FCR showed that birds on T3 (vitamins A+C) best utilized the feed while those on T2 (vitamin C) least.

Table1. Performance of broilers fed diets supplemented with vitamins

Parameters	T1(vit A)	T2(vit C)	T3(vit A+C)	T4(control)	SEM
Final weight (g)	1298.00 ^b	1170.00 ^c	1334.00 ^a	1390.00 ^a	30.55
Initial weight (g)	318.00	296.00	308.00	330.00	22.00ns
Daily weight gain (g)	27.90 ^b	24.97 ^c	29.32 ^{ab}	30.29 ^a	0.87
Daily feed intake (g)	92.74	88.41	90.23	95.20	7.58ns
Feed conversion ratio	3.32 ^b	3.53 ^b	2.63 ^a	3.15 ^b	0.22

a, b, c: Means on the same row with different superscripts are significantly different at $p < 0.05$, SEM : Standard error of mean, ns: not significant at $p > 0.05$

3.2. Haematological Indices of Broiler

The effect of vitamins supplementation on the haematology of broilers is presented in Table 2. The result showed that there were no significant ($p > 0.05$) differences in the PCV, Hb, WBC, RBC, MCH, MCHC, and MCV of the different groups. The PCV (36.86-39.06%) value range observed is within the range of 24.9 to 45.2% reported for healthy birds [16]. These values showed that the birds received sufficient vitamins, minerals and protein for haematopoiesis, Similar values of Hb, RBC, MCH, MCHC, and MCV observed among the treatment groups may suggest that the birds received adequate nutrients from the diets for haematopoiesis, and they were not anaemic, besides being in good health. Similar WBC (5.76×10^9 to 9.10×10^9 l) values indicate similar immune status of the birds to fight infection. The observed values were within the range $5.00-15.00 \times 10^3$ mm reported by [14] for birds.

Table2. haematology of broilers fed diets supplemented with vitamins

Parameters	T1(vit A)	T2(vit C)	T3(vit A+C)	T4(control)	SEM
PCV(%)	37.37	39.06	37.99	36.86	3.10ns
RBC($\times 10^{12}$ /l)	5.60	6.02	7.36	8.71	2.68ns
Hb(g/l)	187.00	197.00	188.33	198.67	22.05ns
WBC($\times 10^9$ /l)	9.10	6.69	6.50	5.76	2.68ns
MCV(fl)	73.33	93.33	76.67	78.33	16.08ns
MCH(pg)	18.96	23.50	23.43	17.50	4.33ns
MCHC(g/l)	198.33	219.33	250.67	247.00	47.13ns

SEM : Standard error of mean, ns: not significant at $p > 0.05$, PCV: Packed cell volume, RBC: red blood cell count, WBC: white blood cell count, Hb: haemoglobin, MCHC: mean corpuscular haemoglobin concentration, MCH: mean corpuscular haemoglobin, MCV: mean corpuscular volume

The HI titres of the experimental birds are shown in Table 3. Observed values among the groups were significantly ($p < 0.05$) at weeks 5, 6, 7 and 8. At weeks 5, 6, 7 and 8 of life, the HI titres of the experimental birds were high and above the normal protective titre of 32.00. Immunity of T1 (800.00) was the highest for week 5, while for week 6, T2 (250.67) had the highest value. During week 7 the immunity of T1 as revealed by the HI titre was the highest. HI titres in week 8 showed that broilers on vitamin C (T2) had the highest (2048.00) titre. The observed values showed that birds on diets supplemented with vitamins had consistently higher HI titres than the control birds. In addition broilers supplemented with vitamin C (T2) had both high and long lasting HI titres even at the 8th week with HI titre of 2048, i.e., they sustained higher immunity longer than others. Moreover, after inoculating the broilers in the different groups with the virulent NDV, only birds in the control group had paralysis of limbs, wings and neck, and with 50% mortality observed later (Table 4). On the contrary, broilers on vitamin supplemented feeds were very active and showed no clinical signs of ND (Table 4). The high immunity to NDV in birds on vitamin supplemented diets agrees with the findings of [23; 1] who observed a positive correlation between antibody titre and vitamin C supplementation.

Table3. haemagglutination inhibition (hi) titres of Newcastle vaccinated broilers fed vitamin fortified diets

Age (weeks)	Mean HI Titres				SEM
	T1(vit A)	T2(vit C)	T3(vit A+C)	T4(control)	
5	800.00 ^a	624.00 ^c	757.30 ^b	421.30 ^d	57.23
6	61.33 ^b	250.67 ^a	53.33 ^c	48.00 ^d	47.23
7	1024.00 ^a	608.00 ^b	1088.00 ^a	148.70 ^d	119.80
8	602.70 ^c	2048.00 ^a	1055.30 ^b	170.00 ^d	59.10

a b c d: Means on the same row with different superscripts are significantly different ($p < 0.05$)

SEM: standard error of mean

Table4. Virulent newcastle disease virus challenge infection of broilers fed diets fortified with vitamin a and c

Days post infection	T1(vit A)	T2(vit C)	T3(vit A+C)	T4(control)
4	-	-	-	Loss of appetite
5	-	-	-	Paralysis of the limbs and wings
8	-	-	-	Torticollis and mortality
11	-	-	-	Mortality

The carcass characteristics of birds fed the experimental diets are presented in Table 5. Dressed weight from each treatment group was significantly influenced by the treatments ($p > 0.05$), with T3 (vitamins A + C) having the highest weight. All the other parameters namely; head, wing, drumstick, thigh, legs, neck, heart, gizzard and kidney were not significantly ($p > 0.05$) influenced by the treatments. The observed results may indicate that the diets promoted uniform growth of these parts despite differences in dressed weight.

Table5. Carcass characteristics of broilers fed vitamin supplemented diets

Parameters	T1(vit A)	T2(vit C)	T3(vit A+C)	T4(control)	SEM
Dressed weight (g)	1275.00 ^b	1320.00 ^b	1820.00 ^a	1700.00 ^a	136.10
Head %	3.96	3.82	2.58	4.10	1.00ns
Wing %	11.87	11.28	10.98	10.74	0.75ns
Drumstick %	13.85	12.33	10.98	11.80	0.88ns
Thigh %	13.85	13.48	10.98	12.36	0.72ns

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Back %	20.44	22.56	24.68	23.38	1.74ns
Breast %	24.81	24.32	21.98	26.38	1.24ns
Legs %	7.92	5.55	6.03	6.68	1.30ns
Neck %	6.49	6.60	7.43	7.24	1.42ns
Heart %	0.97	0.97	1.23	1.18	0.20ns
Gizzard%	2.20	2.85	4.38	2.95	0.49ns
Kidney%	0.79	0.76	0.60	0.59	0.08ns

SEM: Standard error of mean, ns: not significant at $p > 0.05$

In conclusion, vitamins supplementation of broiler diets did not improve daily weight gain as well as live weight, though the combination of vitamins A and C improved FCR. More so, vitamin supplementation enhanced the immunity of ND Vaccinated birds when exposed to Newcastle disease. It is therefore recommended that broiler feeds be supplemented with vitamins A and C at levels recommended for immunity.

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