Immunological response of broiler chickens fed graded levels of vitamin C to Newcastle disease vaccinations

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SUMMARY

A feeding trial was carried out for a period of 56 days to investigate the effects of supplementing varying levels of dietary vitamin C in diet of broiler chickens on their performance characteristics and immunological response to Newcastle disease vaccinations. Two hundred (200) day-old broiler chicks (Arbor acre breed) were randomly divided into 4 dietary treatments and fed diets supplemented with 0, 100, 200 and 300 mg/kg of vitamin C. The treatments were replicated 5 times with 10 birds each in a completely randomized design. The birds were vaccinated with Newcastle disease vaccines using a stipulated vaccination regime. The results showed that supplementing vitamin C at varying supplementary rates significantly influenced the growth performance of broiler chickens with the final body weight and feed intake of experimental bird significantly different ($p < 0.05$) among treatments. The final body weight of birds (2230.0g) fed diet containing 100mg/kg of vitamin C was significantly different ($p < 0.05$) from those of birds (1950.0g) fed diet containing 300mg/kg of vitamin C. The feed intake of birds (5.08kg) fed 100mg/kg of vitamin C was significantly ($p < 0.05$) from that of birds fed the rest test diets. The antibody titre values were significantly different among treatment groups after vaccination with second NDV and birds on diet containing 100mg/kg of vitamin C had the highest titre of $\log_2 9$ which was significantly ($p < 0.05$) different from those on diet containing 300mg/kg of vitamin C with titre value of $\log_2 7$. It can be concluded that vitamin C at the 100mg/kg supplementary rate had the best effect on immune response and productive performance of broiler chickens.

Keywords**:** Body weight, broiler chickens, feed intake, immune response, supplementation, vitamin C

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INTRODUCTION

The immune system benefits greatly from adequate nutrition. Not only does the immune system benefit directly from adequate nutrition, it indirectly prepares the body for periods of stress by reducing the adverse effects and enhancing speedy recovery from such stressful periods. Therefore in many instances, proper nutrition lessens the immune suppression associated with the stress response in the body. In the case of domestic chickens, these facts are equally true (Butcher and Miles, 2002).

Broiler performance has improved enormously in recent decades. As a result, nutrition patterns have also changed. Based on the genetic progress of the birds, proper vitamin supplementation levels are needed. The Optimum Vitamin Nutrition (OVN) concept is a useful tool for supplying the correct amount of vitamins to broiler diets.

Besides production rates, other parameters are also presently evaluated to determine vitamin requirements, such as immunity, animal welfare, carcass characteristics, microbiological analysis, etc. Supplementation with higher levels than the minimum recommendations may/will result in higher production performance and carcass quality, better health and welfare of the birds (Fanatico, 2008). There are repercussions with decrease in the intake of vitamins and metabolism such as for vitamin A, E and C which play important roles in performance and immune function. Supplementation of these vitamins is helpful for maintaining performance and immune function of broiler chickens.

Poultry are able to synthesize vitamin C and thus it is assumed they do not require dietary sources of the vitamin. However, for newly hatched poultry there is a slow rate of ascorbic acid synthesis and this combined with encountered stress increases probability of vitamin C deficiency. The chick is subject to considerable stress conditions, including rapid growth, exposure to hot or cold temperatures, starvation, vaccination and disease conditions such as coccidiosis. Pardue and Williams (1990) reported that plasma ascorbic acid levels in poults were depressed significantly by cold stress, beak trimming, and injection at one and fourteen days of age. Supplemental vitamin C (150 mg per kg or 68.2 mg per lb diet) enhanced performance of broiler chicks exposed to multiple concurrent environmental stressors (McKee and Harrison, 1995).

Literature shows a large variation in vitamin levels used in commercial supplements for broilers. It is for this reason that there is great interest in new studies to determine the levels that will ensure the best immunological status, without interfering with the production performance of birds.

Newcastle disease is one of the most rampant viral diseases of poultry with a prevalence rate of 28.9% (Adene, 2004). Two of the major tools that can be used to provoke immunity in birds for both the prevention and controlling

of the spread of the disease are vaccination and good nutrition especially when adequate in micro-nutrients like vitamins.

Therefore this present study was designed to investigate the immune response to Newcastle disease vaccinations and growth performance of broiler chickens fed diets supplemented with varying levels of vitamin C.

MATERIAL AND METHODS

Experimental site

The approval to conduct this study was given by the Research Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure (FUTA) Nigeria. Thus, the feeding trial was conducted at the Poultry unit of the Teaching and Research Farm of FUTA, Nigeria. The laboratory analyses were carried out at the Microbiology Laboratory of the Department of Animal Production and Health, and the Department of Biochemistry, FUTA.

Experimental diets

Two basal diets were formulated based on the National Research Council (1994) table of feedstuffs that met the requirements of broiler chickens and used for the trial. The gross composition of the broiler starter and finisher are presented in Table 1. The starter diet was mixed in one batch and sub-divided into four equal portions. One portion served as the control (diet 1) and the other three portions were designated diets 2, 3 and 4 respectively. Thereafter dietary vitamin C was added to the experimental diets at 0, 100mg/kg, 200mg/kg and 300mg/kg respectively and thoroughly mixed. The finisher diets were also prepared using the same procedure as that of the starter diets.

Experimental layout and animal management

A batch of two hundred (200) day-old broiler chicks of the Marshal breed purchased from a reputable hatchery in Ibadan, Oyo State, Nigeria was used for the study. Brooding was done in a conventional manner with temperature ranging from 35^{o}C at day old to 29^{o}C at 3 weeks of age and then kept stable at 25^{o}C thereafter. The birds were randomly arranged in completely randomized design. Fifty (50) chicks of mixed sexes were randomly allotted to each of the 4 dietary treatments in ten chicks per replicate of five and each replicate contained equal number of sexes. The chicks were reared using the deep litter system with wood shavings as beddings. They were fed their respective experimental diets by feeding the starter diets from day 1 to 28 day and the finisher diets from day 29 to 56 day. The birds were given water and feed *ad libitum* and reared using common management practices for broiler chickens

as outlined by the Teaching and Research Farm of FUTA. The experimental chickens were vaccinated with Newcastle disease vaccines (NDV) - NDV intra-ocular (Hithner B1 strain) at 3 days old and NDV LaSota via the oral route at 21 days old.

Table 1: Gross composition of experimental diets

Ingredients %	Starter diet	Finisher diet
Maize	58.50	50.50
Soy bean meal	18.50	17.00
Wheat offal	-	13.00
Groundnut cake	12.50	11.00
Fish meal	4.00	1.00
Bone meal	2.75	2.75
Oyster shell	1.00	1.00
Methionine	0.15	0.15
Lysine	0.10	0.10
*Premix	0.20	0.20
Salt	0.30	0.30
Vegetable oil	2.00	3.00
Total	100	100
Calculated		
Crude protein (%)	23.09	19.90
ME (MJ/kg)	13.13	12.66
Calcium (%)	1.73	1.54
Available Phosphorus (%)	0.77	0.72
Lysine (%)	1.12	1.01
Methionine (%)	0.49	0.44

ME= metabolizable energy; *2.5kg of premix contains the following: Vitamin A (i.u)- 12,000,000; Vitamin D3 (i.u)- 2,500,000; Vitamin E (i.u)- 30,000; Vitamin K (mg)- 2,000; Vitamin B1 (mg)- 2,250, Vitamin B2 (mg)- 6,000; Vitamin B6 (mg)- 4,500; Vitamin B12 (mcg)- 15; Niacin (mg)- 40,000; Panthotenic acid (mg)- 15,000; Folic acid (mg)- 1,500; Biotin (mcg)- 50; Choline chloride (mg)- 300,000; Manganese (mg)- 80,000; Zinc (mg)- 50,000; Iron (mg)- 20,000; Copper (mg)- 5,000; Iodine (mg)- 1,000; Selenium (mg)- 200; Cobalt (mg)- 500; Anti-oxidant (mg)- 125,000.

Performance indices measurement:

The initial weight of birds was measured at day old and thereafter weight changes on a weekly basis over the trial period were measured as the difference between the initial weight and the final weight. The feed consumption was recorded per replicate and the feed conversion ratio calculated as a ratio of feed consumed to weight gain of birds per replicate.

*Blood and sera collection***:**

Samples of blood for the purpose of serum analysis were collected from 3 birds per replicate in each treatment group before the trial commenced via the

heart to determine baseline maternal antibody titre levels against Newcastle disease. The birds were sedated using chloroform before the bleeding exercise. Thereafter, in each treatment 20 birds (4 per replicate) were randomly selected and blood was collected 10 days after administering each of the ND vaccines through the jugular vein for serological analysis to determine the antibody titre values. At the end of the 8 weeks experimental period blood was also collected for haematological and serum protein biochemistry analysis from 20 birds in each treatment.

Laboratory analysis

Haemagglutination and Haemagglutination Inhibition Test (HA/HI Test):

Serum samples were analysed using beta (β) micro haemagglutination inhibition technique (Thayer and Beard, 1998) to determine the antibody titre levels as a measure of the immunological response elicited in the vaccinated experimental birds.

Haemagglutination (HA) Titration:

The aim of the HA titration was to determine the viability or the potency of the vaccine used. The Newcastle disease vaccine (LaSota strain) used as the antigen for the HA titration was locally produced by the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State. Clean, dry, micro-titre plates used were labelled as required, and 0.2ml of normal saline was dispensed into each of a pair of wells using a micro-pipette. A drop of the antigen was added into the first pair of wells and mixed thoroughly using a pair of inoculating loops and serial dilution was carried out. Finally 0.02ml of the guinea pig RBC indicator previously diluted with normal saline was added to each well. The plates were then incubated on the laboratory bench for about 30 minutes at room temperature. After precisely 30 minutes, the end point of the titre was determined as the pair of wells where haemagglutination was clearly observed.

Haemagglutination inhibition (HI) titration:

The beta haemagglutination inhibition technique was used and the stock antigen was diluted according to the HA titre obtained; thus for an antigen with a titre 1:256, the 4HAμ will be equal to 1:64 dilution of test stock. The micro-titre plates were labelled as required and 0.2ml of the test stock antigen was then dropped into each pair of the wells on a row of the micro-titre plates. After this, a drop of the serum sample was added into the first pair of wells, thoroughly mixed and serially diluted. Lastly a drop of the prepared guinea pig RBC indicator was added to each well. The micro-titre plates were incubated at room temperature on the bench for 30 minutes. The end point of the titre was

determined as the pair of wells where haemagglutination inhibition is clearly observed.

Haematological parameters:

The erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (HB) and white blood cell differentials were analysed. The Mean corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were calculated as described by Lamb (1981).

Serum protein biochemical analysis:

The protein content- albumin, globulin and the total protein of serum samples were estimated using diagnostic kits (Randox Laboratories Limited, UK test kits).

Economic analysis:

The economics and cost of producing the experimental diets were estimated based on the prevalent market prices for the ingredients as at the time of experiment and percentage cost reduction was evaluated. All other costs which were common to the experimentation were ignored. Thus, variables were calculated accordingly:

$$The\ cost\ of\ feed\ consumed\ = cost\ per\ kg\ of\ feed\ \times total\ feed\ consumed\ (kg) \ldots\ldots\ldots\ldots\ldots (1)$$

$$Feed\ cost\ per\ kg\ body\ weight\ = \frac{cost\ of\ feed\ consumed}{final\ body\ weight\ of\ bird\ fed\ the\ diet} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$\%Cost\ reduction\ per\ kg\ of\ diet\ = \frac{cost\ of\ control\ diet - cost\ of\ test\ diet}{cost\ of\ control\ diet} \times 100 \ldots\ldots\ldots\ldots\ldots (3)$$

$$Cost\ of\ feed\ per\ kg\ gain\ = \frac{cost\ of\ feed\ consumed}{weight\ gain\ of\ bird\ fed\ diet} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Statistical analysis

Data on immunological responses, haematological variables, and serum protein biochemistry were subjected to one-way analysis of variance (ANOVA). Where significant differences were found, the mean were separated using the Statistical Analysis System (SAS).

RESULTS

Immunological response to ND vaccinations

Table 2 shows the average titre values of broiler chickens fed vitamins C supplemented diets after vaccinations with NDV intra-occular and NDV LaSota. A random sampling of experimental birds for maternal antibodies gave an

average value of $log_2 4$. The antibody titre values after the first vaccination was not significantly ($p > 0.05$) different among the treatment groups. However, after vaccination with second NDV birds on diet 2 had the highest titre of $log_2 9$ which was significantly ($p < 0.05$) different from those on diet 4 with titre value of $log_2 7$.

Table 2: Average antibody titre value of experimental birds fed varying levels of Vitamin C after Newcastle disease vaccination

Treatment	Base line antibody titre	Anti body titre for the 1st NDV	Anti body titre for the 2nd NDV
Trt A	$Log_2 4$	$Log_2 6$	$Log_2 8^a$
Trt B	$Log_2 4$	$Log_2 7$	$Log_2 9^a$
Trt C	$Log_2 4$	$Log_2 7$	$Log_2 8^a$
Trt D	$Log_2 4$	$Log_2 6$	$Log_2 7^b$
Pooled mean	$Log_2 4$	$Log_2 6.25$	$Log_2 8.50$
Significance	-	NS	*

Means on the same column with different superscripts are statistically significant ($p<0.05$), * - Significant, ns - Not significant, NDV- Newcastle disease vaccination,

Table 3: Performance characteristics of broiler chickens fed Vitamin C supplemented diets (1-56 days)

Parameters	A	B	C	D	±SEM
Initial weight(g/bird)	44.93	44.98	45.80	45.87	0.89
Final body weight (g/bird)	1980.0^b	2230.0^a	2180.0^a	1950.0^b	0.58
TWG (g/bird)	1935.07^b	2185.02^a	2134.20^a	1904.13^b	0.54
TFI (kg/bird)	4.86^b	5.08^a	4.79^b	4.52^b	0.56
Weight gain (g/bird/day)	34.64^b	39.11^a	38.22^a	34.11^b	0.27
Feed intake(g/bird/day)	86.75^{ab}	90.70^a	85.55^{ab}	80.70^b	0.68
Feed conversion ratio	2.50^b	2.31^{ab}	2.24^a	2.31^{ab}	0.34

TWG- Total weight gain, TFI- Total feed intake

C1: treatment 1 (diet supplemented with 0.00mg/kg of vitamin C); C2: treatment 2 (diet supplemented with 100mg/kg of vitamin C); C3: treatment 3 (diet supplemented with 200mg/kg of vitamin C); C4: treatment 4 (diet supplemented with 300mg/kg of vitamin C).

Performance characteristics

Table 3 shows the performance characteristics of experimental birds over the period of feeding trial. The final weight of birds (2230.0g) fed diet supplemented with 100mg/kg of vitamin C was significantly ($p < 0.05$) different from weight of birds (1950.0g) fed diet containing 300mg/kg of vitamin C. The feed intake by birds (5.08kg) on diet having 100mg/kg of vitamin C was also the highest and it was significantly ($p < 0.05$) different from those of birds (4.52kg) fed diet containing 300mg/kg of vitamin C. The FCR was also significantly different among treatment groups. Birds on diet supplemented with 200mg/kg of vitamin C had the best FCR (2.24) which was significantly ($p <$

0.05) different from those on control diet (2.50) but not significantly ($p > 0.05$) different from those fed the rest test diets.

Haematological parameters

Table 4 shows the haematological parameters of broiler chickens fed with varying levels of vitamin C supplemented diets. Only lymphocyte values was significantly ($p < 0.05$) affected by the dietary treatments. The birds fed 100mg/kg vitamin C recorded the highest value (64.33%) and was only significantly different from that of birds (60.00%) fed diet containing 200mg/kg of vitamin C.

Table 4: Haematological parameters of broiler chickens fed vitamin C supplemented diets

Parameters	A	B	C	D	±SEM
ESR (mm/hr)	2.33	2.33	2.33	2.33	0.04
PCV (%)	27.67	27.00	27.33	27.33	0.11
RBC (x 10^6 /mm^3)	2.10	1.91	2.01	2.03	0.32
Hb (g/100ml)	9.23	9.00	9.10	9.10	0.08
MCHC (%)	33.35	33.33	33.29	33.29	0.13
MCH (pg)	4.38	4.70	4.52	4.46	0.09
MCV (μ^3)	1.31	1.41	1.35	1.34	0.21
Lymphocyte (%)	63.33^a	64.33^a	62.00^{ab}	60.00^b	0.31
Heterophils (%)	20.67	21.67	22.00	23.00	0.14
Monocytes (%)	12.35	12.34	12.57	12.43	0.19
Basophils (%)	2.33	2.33	2.33	2.67	0.06
Eosinophils (%)	1.61	1.57	1.58	1.60	0.08

ESR = Erythrocyte sedimentation rate, PCV = Packed cell volume, RBC= Red Blood cell
Hb = Haemoglobin, MCHC = Mean cell haemoglobin concentration, MCH = Mean cell Haemoglobin, MCV = Mean cell volume.
Treatment A: diet supplemented with 0.00mg/kg of vitamin C; Treatment B: diet supplemented with 100mg/kg of vitamin C; Treatment C: diet supplemented with 200mg/kg of vitamin C; Treatment D: diet supplemented with 300mg/kg of vitamin C.

Serum protein biochemical analysis

Table 5 shows that the total protein and globulin contents in serum of chickens fed vitamin C supplemented diets are significantly ($p < 0.05$) affected by the dietary treatments. The total protein of birds on diet 3 (5.73g/dl) was higher and significantly different ($p<0.05$) from the other groups, while those on diet 1(2.63g/dl) was the lowest. The albumin content value was also higher in birds fed diet 3(4.12g/dl) and significantly different from other groups.

Table 5: Serum protein biochemistry of broiler chickens fed with vitamin C supplemented diets

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Treatment A	2.63^{c}	1.57	1.06^{c}
Treatment B	4.03^{b}	2.04	1.99^{b}
Treatment C	5.73^{a}	1.61	4.12^{a}
Treatment D	3.74^{c}	1.46	2.29^{b}
Pooled means	4.03	1.67	2.36
±SEM	0.06	0.08	0.03
Significance	*	NS	*

Means on the same column with different superscripts are significantly different (p< 0.05),*-significant, NS - not significant

Treatment A: diet supplemented with 0.00mg/kg of vitamin C; Treatment B: diet supplemented with 100mg/kg of vitamin C; Treatment C: diet supplemented with 200mg/kg of vitamin C; Treatment D: diet supplemented with 300mg/kg of vitamin C.

Economic analysis

Table 6 shows the economic analysis of the cost of production of the experimental chickens. The cost of experimental feed of the varying diets was not significantly ($p > 0.05$) different, though cost of total feed intake by birds fed the various diets was significantly ($p < 0.05$) different among treatments. Birds given diet containing 100mg/kg of vitamin C had the highest cost ($3.17/kg), while those fed diet with 300mg/kg of vitamin C had the least cost ($2.82/kg).

Table 6: Comparative Estimates of the cost of production of birds fed vitamin C supplemented diets (1-56 days)

Parameters	A	B	C	D
Initial weight(g/bird)	45	45	46	46
Final weight (g/bird)	1980^{b}	2230^{a}	2180^{a}	1950^{b}
Total weight gain (g/bird)	1940^{ab}	2190^{a}	2140^{a}	1910^{b}
Total feed intake (kg/bird)	4.86^{a}	5.08^{a}	4.79^{ab}	4.52^{b}
Cost of feed consumed ($/kg)	3.03^{b}	3.17^{a}	3.00^{b}	2.82^{c}
Cost of experimental feed ($/kg)	0.62	0.62	0.63	0.63
Cost of feed/Kg gain	253^{a}	235^{b}	227^{b}	240^{ab}
% Cost reduction/kg diet	-	7.22	10.3	4.97

1$= ₦162 as at time of the trial.

Treatment A: diet supplemented with 0.00mg/kg of vitamin C; Treatment B: diet supplemented with 100mg/kg of vitamin C; Treatment C: diet supplemented with 200mg/kg of vitamin C; Treatment D:diet supplemented with 300mg/kg of vitamin C.

DISCUSSION

In the present study results showed that birds fed 100mg/kg of vitamin C elicited the highest antibody titre values after the ND vaccinations. This is supported by the work of Sahin and Kucuck, (2001) and Lohakare *et al*. (2005) which states that supplementing Vitamin C at the rate of 100ppm and 200ppm had been shown to improve the immunity of birds. Also vitamin C has been demonstrated to improve immune responsiveness (Nameghi *et al* 2007). Pardue and Thaxton (1986) and Null (2001) have indicated that ascorbic acid takes part in the synthesis of leukocytes, especially phagocytes and heterophils, which enhance immunity in broiler chickens and by so doing lower mortality rates in any disease outbreak.

The birds fed dietary vitamin C had those given 100mg/ kg of inclusion level performing better than birds in other groups on account of their greater final body weight and body weight gain. This is similar to the report of McKee and Harrison, (1995) that supplemental vitamin C (150 mg per kg or 68.2 mg per lb diet) enhanced performance of broiler chicks exposed to multiple concurrent environmental stressors.

Higher inclusion levels above 100mg/kg did not improve the performance of the broiler chickens. This supports the hypothesis of National Research Council (NRC, 1994) that there was no need for dietary supplementation of vitamin C in broiler chickens except for stressful conditions such as heat stress and other factors. Birds in the control group fed non- supplemented diets had a more efficient feed conversion ratio throughout the study period which further buttresses this fact. However, literature has shown that higher amounts of supplemental vitamin C have likewise proved beneficial to poultry. Several workers like Peebles and Brake (1984), Njoku (1986), Orban *et al*. (1993) and Zapata and Gernat, (1995) have proven this in their findings.

Lymphocyte counts of birds fed dietary vitamin C was also siginificant among the groups, though in earlier work done it was seen that vitamin C increased in chicken the number of CD 8 (+) and IgM (+) cells (Wu *et al*., 2000) and also increase bacterial killing by heterophils and lowered plasma corticosterone (McKee and Harrison, 1995). Satterlee *et al*., (1989) reported a smaller number of heterophils and a higher one of lymphocytes in the blood of broiler chickens fed vitamin C supplemented diets and examined 8 hours before slaughter, as compared with birds unsupplemented with vitamin C. Several other workers have reported that dietary vitamin C has effect on blood variables. Kontecka *et al.* (1997) reported a higher (by 0.56×1012/L) erythrocyte count in birds fed with 200 mg/kg of supplemented vitamin C in comparison with birds from the control group. According to Torgowski and Kontecka (1998), supplementing with vitamin C in the middle and towards the

end of the reproductive period in broiler chickens, a higher erythrocyte count and higher (on average by 0.99 mmol/L) haemoglobin level were observed.

This study also showed that dietary vitamin C significantly influenced serum proteins. Many workers (Donkoh, 1989; Kutlu and Forbes, 1993) have reported that vitamin C supplementation increases serum albumin concentrations.

Economically, the cost of production of the experimental feed with varying levels of vitamin C supplements was comparatively not significantly different as shown by the results. Differences were only observed in the cost of total feed consumed by the birds in varying dietary treatments which can be attributed to the fact that feed intake was significantly different among groups. Therefore the cost of the different experimental feed due to the varying levels of supplementary rates of the vitamins was negligible.

CONCLUSIONS

The supplementary rate of 100mg/kg of vitamin C in diets of broiler chickens used in this study brought about the best performance characteristics and immune response to Newcastle disease vaccinations. It is therefore recommended for use by livestock producers particularly feed millers and poultry farmers at this level of concentration with no overly excessive high cost which they may even incur in case of disease outbreaks.

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