Evaluation of dietary vitamin E supplementation on performance characteristics and immuno-competence of broiler chickens

O.B. Faluyi†, J.O. Agbede, I.A. Adebayo, M. Adegbenro

Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria

SUMMARY

A 56 day feeding trial was carried out to investigate the effects of graded levels of dietary vitamin E on broiler chickens using growth performance and immune response to Newcastle disease vaccinations as response criteria. One hundred and sixty (160) 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 E. The treatments were replicated 4 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 revealed that of the growth performance indices measured only the total feed intake (TFI) was significantly influenced by the dietary treatments with birds fed diet containing 300mg/kg of vitamin E having the highest feed consumption (4.18kg/bird) and those fed the control diet the lowest (3.48kg/bird). The antibody titre values were significantly ($p<0.05$) different among dietary treatments after the 2nd ND vaccination and birds fed diet supplemented with 100mg/kg vitamin E had the highest titre ($\log_2 10$), while those fed the control and 300mg/kg of vitamin E diets had the least titre ($\log_2 8$). Of the haematological indices measured, only the lymphocytes were significantly ($p<0.05$) influenced with birds fed 100mg/kg vitamin E supplemented diet having the highest value (67.33%) and lowest (61.33%) in birds fed 300mg/kg vitamin E supplemented diet. It was more profitable to supplement vitamin E at 200mg/kg with % cost reduction/kg diet of 11.49 compared with 9.25 and 8.91 observed in diets with 100 and 300mg/kg vitamin E supplementation respectively. It can therefore be concluded that while the FBW and TWG numerically improved with vitamin E supplementation in diets, the immune response of the birds to vitamin E activity varied with age

† Corresponding author: faluyi2005@yahoo.com

of birds and that cost benefit of supplementing vitamin E in broiler chicken diets might be highest when supplemented at 200mg/kg.

Keywords**:** Broiler chickens, Newcastle disease vaccinations, vitamin E, performance criteria

INTRODUCTION

In field conditions, the chicken is exposed to a variety of stress factors and disease pathogens, which may adversely influence the immune system. Thus, suboptimal immune response is often observed when chickens are infected with infectious bursal disease, Newcastle disease, malabsorption syndrome, Reovirus infections, Adenovirus infections, Marek's disease, chicken anaemia agent, mycotoxins, coccidiosis, stress factors and others. The plague of poultry disease is a major problem and one of the main endemic viral diseases of birds in Nigeria is Newcastle disease in the fore front of Gomboro and Marek's diseases (Adene, 2004). However, certain nutrients are capable of modulating the function of the immune system through a variety of mechanisms (Berczi and Sabbadim, 1998). Until recently most of the studies overlooked the potential role of vitamins in optimizing immune response in the chicks, particularly in response to infections from bacteria and viruses.

In commercial poultry, a number of therapeutic substances are being used in combating various pathogens. The ultimate success of therapeutics is based not only on the direct effect on a pathogen, but also efficiency of the immune response. An effective disease prevention program may be provided by proper vaccination and suitable supplementation with a given vitamin to provide optimum immune response. Therefore, vitamin supplementation may be used as an adjunct to both therapeutic and prophylactic treatment.

Of all vitamins, vitamin E (tocopherol) appears to have most significant effect on immune system (Tizard, 1987). It is known that vitamin E improves the phagocytic cell action, antibody production, the activity of the T helper cells, the responsiveness to mitogens and passive immune transfer (Hussain et al., 2004). It also plays an important role in the stimulation of the immune system against certain diseases and stressors (El-Boushy, 1988). As a fat soluble intracellular antioxidant, a primary function of vitamin E is a protective effect on cell membranes. Deficiency of vitamin E is also attributed to cause impaired immune function in chickens (Marsh et al., 1981).

Thus, this present study investigated the effect of supplementing vitamin E at varying concentrations on performance indices, haematological parameters and immune response to Newcastle disease vaccinations in broiler chickens under tropical conditions. Also, the economics of feeding the broiler chickens on the different diets for 8 weeks of trial was studied.

MATERIAL AND METHODS

Experimental site and materials

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.

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.

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 E 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 one hundred and sixty (160) day-old broiler chicks of the Arbor acre 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. Forty (40) chicks of mixed sexes were randomly allotted to each of the 4 dietary treatments in ten chicks per replicate of four 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. Blood and serum samples were collected from experimental birds via the jugular vein 10 days after each ND vaccination for haematological and serological analysis.

Response criteria

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. At the end of 8 weeks feeding trial, 3 birds per replicate were randomly selected, weighed and slaughtered for carcass and organ measurements.

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.

Haematological indices:

The packed cell volume (PCV) was estimated by spinning about 70µl of each blood sample in heparinised capillary tubes in a hematocrit microcentriffuge for 5 minutes, and the erythrocyte sedimentation rate (ESR), red blood cell count (RBC) and white blood cell differentials were determined as described by Lamb (1981). The haemoglobin concentration (Hb) was estimated using the cyano-methemoglobin concentration method. The Mean corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were also calculated as described by Lamb (1981).

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

All data collected during the study were compared by analysis of variance (ANOVA) and significant differences among treatment groups were determined by Duncan's Multiple Range Test (DMRT) using Statistical Analysis System (2008).

RESULTS

Table 2 shows that the final body weight (FBW) and total weight gain (TWG) of birds fed the control diet (2.20 kg/ bird and 2.15 kg/bird respectively) were not significantly ($P>0.05$) different from those fed the vitamin E supplemented diets. However, the FBW and TWG increased as the level of vitamin E increased from 0mg/kg to 300mg/kg in the diets. The total feed intake (TFI) of birds fed control diet was the lowest (3.48 kg/bird) but was similar ($P>0.05$) to those of birds fed diet supplemented with 100mg/kg vitamin E and significantly ($P<0.05$) lower than those fed the rest test diets.

Both the TFI and average daily feed intake increased as the levels of vitamin E supplementation increased in the diets. While the FCR increased with increased vitamin E supplementation in the diets, birds fed the control (1.85), 100mg (1.87) and 200mg/kg (1.81) vitamin E supplemented diet had similar (P>0.05) FCR.

Table 2: Performance characteristics of broiler chickens fed Vitamin E supplemented diets

Parameters	E1	E2	E3	E4	±SEM
Initial weight (g/bird)	45.53	48.80	45.87	44.07	0.56
Final body weight (kg/bird)	2.20	2.25	2.35	2.44	0.31
TWG (kg/bird)	2.15	2.20	2.31	2.40	0.18
TFI(kg/bird)	3.48^{b}	3.99^{ab}	4.13^{a}	4.18^{a}	0.45
Average daily weight gain (g/bird/day)	38.57	39.46	41.25	42.85	0.47
Average daily feed intake (g/bird/day)	62.14^{b}	71.25^{a}	73.75^{a}	74.64^{a}	0.44
Feed conversion ratio	1.85^{b}	1.87^{b}	1.81^{b}	1.71^{a}	0.81

TWG-Total weight gain, TFI- Total feed intake, SEM- Standard error of mean
E1: Diet 1 (diet supplemented with 0.00mg/kg of vitamin E); E2: Diet 2 (diet supplemented with 100mg/kg of vitamin E); E3: Diet 3 (diet supplemented with 200mg/kg of vitamin E); and E4: Diet 4 (diet supplemented with 300mg/kg of vitamin E).

Table 3: Average antibody titre values of chickens fed vitamin E supplemented diets after Newcastle disease vaccinations

Treatments	Baseline antibody titre values	Anti body titre for the 1st NDV	Anti body titre for the 2nd NDV
E1	$Log_2 4$	$Log_2 5$	$Log_2 8^{b}$
E2	$Log_2 4$	$Log_2 6$	$Log_2 10^{a}$
E3	$Log_2 4$	$Log_2 7$	$Log_2 9^{b}$
E4	$Log_2 4$	$Log_2 6$	$Log_2 8^{b}$
Pooled means	$Log_2 4$	$Log_2 6$	$Log_2 8$
Significance	Ns	Ns	*

Means on the same column with different superscripts are statistically significant ($p<0.05$)
* - Significant, Ns = Not significant; NDV-Newcastle disease vaccination
E1: Diet 1 (diet supplemented with 0.00mg/kg of vitamin E); E2: Diet 2 (diet supplemented with 100mg/kg of vitamin E); E3: Diet 3 (diet supplemented with 200mg/kg of vitamin E); and E4: Diet 4 (diet supplemented with 300mg/kg of vitamin E)

Table 3 shows that the antibody titre values after 1st ND vaccination of broiler chickens fed the control diet and vitamin E supplemented diets was not significantly (P>0.05) different among treatment groups with birds on the control diet having the lowest value of $log_2 5$ and those on diet supplemented with 200mg/kg vitamin E having the highest value of $log_2 7$. After the 2nd

vaccination significant ($p < 0.05$) difference was observed among treatment groups. Titre values of birds fed diet supplemented with 100mg/kg vitamin E ($log_2 10$) was significantly ($p < 0.05$) higher than those fed the control, 200mg and 300mg/kg vitamin E supplemented diets.

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

Parameters	E1	E2	E3	E4	±SEM
ESR (mm/hr)	2.33	2.00	2.00	2.33	0.05
PCV (%)	28.68	29.67	28.67	28.33	0.12
RBC (x 10^6 /mm^3)	2.29	2.48	2.27	2.17	0.31
Hb (g/100ml)	9.47	9.90	9.57	9.43	0.06
MCHC (%)	33.38	33.36	33.37	33.28	0.13
MCH (pg)	4.16	4.00	4.20	4.33	0.08
MCV (μ^3)	1.24	1.21	1.26	1.30	0.03
Lymphocyte (%)	61.53^b	67.33^a	62.67^b	61.33^b	0.29
Heterophils (%)	21.43	22.33	20.67	21.00	0.14
Monocytes (%)	12.33	12.33	12.67	12.33	0.18
Basophils (%)	2.00	2.33	2.33	2.33	0.02
Eosinophils (%)	1.67	1.67	1.58	1.70	0.07

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.
E1: Diet 1 (diet supplemented with 0.00mg/kg of vitamin E); E2: Diet 2 (diet supplemented with 100mg/kg of vitamin E); E3: Diet 3 (diet supplemented with 200mg/kg of vitamin E); and E4: Diet 4 (diet supplemented with 300mg/kg of vitamin E)

The haematological parameters of broiler chickens fed with varying levels of vitamin E supplemented diets are as presented in Table 4. Only the lymphocyte values were significantly ($p<0.05$) different among treatment groups. Birds fed on diet supplemented with 100mg/kg vitamin E had the highest value (67.33%) and was significantly different from the other groups (61.33-62.67 ± 0.29%). However, birds fed on diet with 100mg/kg of vitamin E supplementation had consistently highest PCV: 29.67%, RBC: 2.48 (×10^6/mm^3), Hb: 9.90g/100ml and heterophils: 22.33% when compared with those fed the rest test diets.

Table 5 shows that the cost of feed consumed ($/kg) increased with vitamin E supplementation in the diets from 2.17 $/kg in control diet to 2.67 $/kg in diet supplemented with 300mg/kg vitamin E. The cost of feed/kg gain was significantly ($p<0.05$) influenced by the dietary treatment and highest in diet supplemented with 200mg/kg vitamin E (1.19$/kg) and lowest (1.06$/kg) in control diet. Also, the % cost reduction/kg diet was highest (11.49) in diet supplemented with 200mg/kg vitamin E and lowest (8.91) in diet with 300mg /kg vitamin E.

Table 5: Comparative estimates of the cost of production of broiler chickens fed vitamin E supplemented diets (1-56 days)

Parameters	E1	E2	E3	E4
Total number of birds	40	40	40	40
Supplementation rate of Vitamin E (mg/kg)	-	100	200	300
Initial weight(g/bird)	45.53	48.80	45.87	44.07
Final body weight (kg/bird)	2.20	2.25	2.35	2.44
Total weight gain (kg/bird)	2.16	2.21	2.31	2.40
Total feed intake (kg/bird)	3.48^{b}	3.99^{ab}	4.13^{a}	4.18^{a}
Cost of feed consumed ($/kg)	2.17^{c}	2.51^{b}	2.62^{a}	2.67^{a}
Cost of experimental feed ($/kg)	0.62	0.63	0.63	0.64
Cost of feed/kg gain	1.06^{c}	1.16^{b}	1.19^{a}	1.16^{b}
% Cost reduction/kg diet	-	9.25	11.49	8.91

1$= ₦162.30 as at time of the trial.

E1: Diet 1 (diet supplemented with 0.00mg/kg of vitamin E); E2: Diet 2 (diet supplemented with 100mg/kg of vitamin E); E3: Diet 3 (diet supplemented with 200mg/kg of vitamin E); E4: Diet 4 (diet supplemented with 300mg/kg of vitamin E).

DISCUSSION

This present findings on the final body weight and weight gain of birds fed vitamin E supplemented diets were similar to earlier report made by Siegel et al (2001) that greater body weight (BW) gain of laying pullets was observed when fed on a 300 mg vitamin E/kg supplemented diet than those fed on 100 mg/kg. Also, in another study using broiler chickens BW gain was not significantly affected by vitamin E supplementation (Bartov and Frigg, 1992). Sahin et al. (2002) concluded that a 250 mg per kg of diet of vitamin E provides an optimal performance in broiler chicks thereby causing maximal economic benefit. High levels of supplemental vitamin E to broiler flocks with subclinical infectious bursal disease (IBD) have proved beneficial (McIlroy, 1993).

In addition, Kennedy et al (1992) examined the productivity of broiler flocks fed diets containing dietary vitamin E and reported that at the highest level of 180mg/kg of vitamin E supplementation, productivity was 8.4% greater as a result of improvements in both feed conversion efficiency and higher average weight gain which is supported by the present study in which birds fed 300mg/kg vitamin E supplemented diet had 10.12% TWG over those fed the control diet and other test diets (2.22-6.35%) with highest feed utilization ability of 7.5% over those fed the control diet. This suggests that this inclusion rate could be the likely or most suitable concentration level required to wield influence on the physiological mechanism necessary for enhanced productivity in broiler chickens. Similarly, Sahin and Kucuk (2001) found that dietary vitamin

E inclusions resulted in a greater performance in Japanese quails. Chung and Boren (1999) also recommended a lower vitamin E at 240 mg per kg (109.1 mg per lb) in broiler starter diets than the 300mg/kg as observed in this current study to achieve optimum health, production and processing performance.

The lymphocyte counts of birds fed vitamin E diets were significantly influenced by the varying diets and the lymphocyte count was highest in birds fed 100mg/kg of vitamin E. Since lymphocytes are responsible for antibody production, the trend of the immunological response to ND vaccinations observed in this study could be attributed to the lymphocyte count of birds in the different dietary treatments, in that, the antibody titre values were highest in birds with the highest lymphocyte values. This finding is consistent with the earlier report by Erf and Bottje (1996) who examined dietary vitamin E effects on lymphocyte subpopulations in broiler chickens. Dietary vitamin E has been reported to have significant influence on haematological variables (Veulterinor, 1995) which is contrary to the findings observed in this study as only lymphocyte values were significantly influenced by the dietary treatments. These differences might be attributed to many factors such as breed effect and the differences in the potency of the vitamin E used in the two studies. However, the ESR: 2.00-2.33mm/hr; PCV: 28.33-29.67%; RBC: 2.17-2.48×10^6 /mm^3 and Hb: 9.43-9.90/100ml observed in this study compared favourably with those reported by Ogbe et al. (2003) and Agbede et al. (2011).

Studies by Miles et al. (1994), McIlroy et al. (1993) and Gore and Qureshi (1997) concluded that the increased performance in the vitamin E supplemented flocks was due to the increased immune-competence and increased disease resistance. The results of this present study showed that the supplementary rate of vitamin E required for maximum growth performance is higher than that required for optimal immune response because it was seen that low supplementary rate at 100mg/kg gave the best immunological response to ND vaccinations while the higher rate of 300mg/kg resulted in high body weight gain. The group fed 100mg/kg of vitamin E elicited the highest antibody titre values to ND vaccines and this is consistent with reports by Leshchinsky and Klasing (2001) that moderate levels of supplemental vitamin E modulate the immune system more than high levels (200-300 mg/kg).

The increased immunity in broiler chickens fed 100mg/kg of vitamin E supplemented diet as compared to those fed the control diet recorded in this present study agrees with the work of Kammon et al. (2012) that supplementation of vitamin E resulted in marked improvements in humoral immunity and pathology of lymphoid organs. Several other similar reports have been recorded by many workers. An instance is Hussain et al. (2004) who reported that addition of vitamin E and selenium to poultry ration was found to significantly increase antibody titre values against ND Virus at 10 days after

infection. Studies by Tengerdy and Nockels (1975) showed that Vitamin E supplementation improved humoral response while Swain and John (2000) reported significantly high antibody levels against Newcastle disease virus. In another study Meydani and Tengerdy (1993) reported that many parameters of the immune system including resistance to infection, specific antibody production and number of antibody producing cells are altered by supplementing diets that are deficient or marginal in vitamin E. But Latshaw (1991) indicated that high levels of vitamin E (greater than 10 times the required level) were immunostimulatory in chicks.

The effect of vitamin E on breeding birds and progeny had also been considered. Haq et al. (1996) fed broiler breeders a diet containing 300 IU per kg (136.4 IU per lb) of vitamin E and other antioxidants were fed as well, only vitamin E appeared to improve the immune status of the chicks hatched when measured by seven-day post-hatch antibody titers. All of these findings together with the result of this present study points to the fact that Vitamin E supplementation is of benefit to immune status in poultry birds. The protective effects of vitamin E on animal health may be involved or related with its role in reduction of glucocorticoids, which are known to be immunosuppressive (Golub and Gershwin, 1985).

However, contrary studies have shown that vitamin E supplementation had not improved resistance against disease or helped immune competence. For instance Qureshi et al. (1993) who reported that broilers fed 100 or 250 IU of Vitamin E in their diet gave no significant influence on the rate of antibody production.

Furthermore, in this study the cost of the different experimental diets was not influenced by the varying supplementary rates of vitamin E, however cost of feed consumed by broiler chickens increased as the vitamin E supplementation in diets increased. Cost of feed consumed was significantly higher in birds fed 300mg/kg vitamin E than those fed the control diet and this could be adjured to the fact that total feed intake was highest for broiler chickens fed 300mg/kg vitamin E supplemented diets which inadvertently led to higher FBW, TWG and better feed utilization. However the % cost reduction was highest (11.49) in birds fed diet supplemented with 200mg/kg vitamin E which tend to suggest that though TWG increased with increased vitamin E supplementation, more save could be obtained in supplementing the diet with 200mg/kg vitamin E.

CONCLUSIONS

It can be concluded that judging from this current study vitamin E supplementation in broiler chicken diets had significant influence on feed

intake which led to numerical improvements in final body weight and that it will be more economical to supplement at 200mg/kg as this led to the highest % cost reduction. Vitamin E supplementation also increased antibody titres at the 200mg/kg supplementary rate in first ND vaccination and 100mg/kg at second ND vaccination which seemed to imply that the optimal immune response to Newcastle disease vaccinations might likely not be attained beyond these levels respectively at different vaccination time in broiler chickens.

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