Effects of Dietary Supplemental Vitamins and Periods of Administration on Growth Performance and Antibody Titre of Broiler Chickens Vaccinated against Newcastle Disease

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Abstract

This study investigated the effects of supplemental vitamins and varying administration periods on growth performance and antibody titre of broiler chickens vaccinated against Newcastle Disease (ND). A total of 300 unvaccinated against ND Arbor Acre day-old chicks were used for the study for 8 wk. Birds were brooded together on day 1 of age, and 30 chicks were selected randomly for evaluating the maternally derived antibody titre against ND. At 2 days of age, the remaining 270 chicks were divided based on weight equalization into 9 treatment groups and replicated thrice. The 9 treatments consisted of a factorial arrangement of 4 supplemental vitamins (A, C, E and combination of A, C, E) and 2 periods of administration (3 days pre- and post-ND vaccinations) with a control. The birds were managed intensively throughout the experimental period, ND vaccines were administered on the 5th (i/o) and 24th (Lasota) day of age, respectively. Supplemental combined vitamins A, C and E at 0.15, 16.67 and 3.03 mg/kg, respectively, resulted in higher (P < 0.05) final body weight of 1785.00 g/bird and better feed conversion ratio (FCR) of 2.89. Also, birds fed vitamin A supplemented diet 3 d pre-i/o vaccine had higher (p<0.05) serum antibody titre (75.20) against ND while higher (p<0.05) serum antibody titre (741.33) was also obtained in birds fed diet supplemented with vitamin E 3 d post-Lasota vaccination. Conclusively, broiler chickens diets can be supplemented with combined vitamins A, C, and E for better growth performance measured as final body weight and FCR, in addition, vitamins A (0.45mg/kg) and E (9.1mg/kg) dietary supplementation at 3 d pre-i/o and 3 d post-Lasota vaccines, respectively, can be adopted for improved antibody production.

Keywords: Growth performance, antibody titre, Newcastle disease, vitamins, broiler chickens

Introduction

Broiler chicken production is of high economic importance, due to its roles in the provision of valuable food product (meat) and a source of income for producers. The relatively short production cycle, better feed utilization and high biomass per unit of agricultural land makes broiler chickens attractive for increased poultry production (Ayo *et al.,* 1996). In spite of the enormous potentials of broiler chicken production in solving many socio-economic problems around the world, especially in the developing countries, it is still confronted with a major challenge of infectious diseases which has led to reduced production capacity. One of such diseases is Newcastle Disease (ND) which is a highly contagious viral disease that attacks many species of domestic and wild birds (Al-Garib *et al.*, 2003). The disease causes high economic losses due to increased mortality and stress, decreased egg production and hatchability (Alexander, 2003). The effective control measure against the Newcastle Disease virus is vaccination and the managerial techniques that synergize with its success. Vaccination is known to enhance the immune status against pathogenic agents and management techniques are used to enhance the effectiveness of vaccine in preventing immunological failure. A good example is the use of vitamins prior to vaccination as an anti-stress and immune response stimulant. Vitamins are essential components of a well-balanced diet and their major function involves metabolism and utilization of nutrients. According to Bourre and Galea (2006) vitamins act as co-factors in several metabolic functions in immune reaction and are needed for optimum health and normal physiological functions such as growth, development, maintenance and reproduction, whose deficiency in diets might cause impairment of immunity. Rama-Rao *et al.* (2004) stated that generally higher levels of dietary vitamins than the recommendation of NRC (1994) for preventing deficiency syndromes would increase the immune response and contributes to productive performance of poultry. This study therefore investigated the effects of different vitamins as dietary supplement in relation to periods of administration on growth performance and antibody titre of broiler chickens.

Materials and Methods

Experimental Location

The field experiment was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS) located within latitude 7° 13' N and longitude 3° 26' E (Google Earth, 2015), while the laboratory works were carried out at the Animal Products and Processing Laboratory of the Department of Animal Production and Health and Veterinary Medicine and Surgery Laboratory of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Experimental Birds and Management

A total of 300 unvaccinated against ND day-old Arbor Acre broiler chicks were purchased from a reputable hatchery in Abeokuta, Ogun State, Nigeria. These chicks were used for the 8-wk study. Prior to arrival of the chicks from the hatchery, the brooding equipment were cleaned and disinfected. The birds were intensively managed through brooding, growing and finishing phases with the provision of a straight diet containing 20.82% crude protein, 3.90% ether extract, 3.86% crude fibre, 0.25% lysine 0.25% methionine, 12.16 MJ/kg metabolizable energy and water was provided *ad-libitum*.

Experimental Procedures

The 300 day-old Arbor Acre chicks were brooded together in a single brooding unit on day 1 of age and 30 chicks were randomly selected from the total number and sacrificed for evaluating the maternally derived antibody titre against ND. Thereafter, on day 2 of age, the remaining 270 chicks were sub-divided by weight equalization into 9 treatment groups each in 3 replicates with 10 birds per replicate. The 9 treatment groups consisted of a factorial arrangement of 4 supplemental vitamins (A, C, E and combination of A, C, E) and two periods of administration (3 d pre- and post- ND vaccinations) and a control group. Birds were vaccinated with ND vaccine on day 5 of age (i/o, intra-ocular) and day 24 of age (Lasota, oral). Vitamins were administered as dietary supplement for 3 d pre- and post-ND vaccinations at the inclusion levels of 0.45 mg vitamin A/kg diet, 50 mg vitamin C/kg diet and 9.1 mg vitamin E/kg diet and 0.15, 16.67 and 3.0 mg/kg diet of combined vitamins A, C and E, respectively. Sera samples were collected on the 19^{th} and 38^{th} d of age for determination of antibody titre against ND vaccinations in the broiler chickens. Growth performance indices were also measured on weekly basis.

Growth Performance Indices

The birds were weighed at the start of the feeding trial and subsequently on weekly basis. The initial mean live weights were subtracted from the final mean live weight to determine the weight gained by the birds. The total weight gained was divided by the product of the number of birds per replicate and number of days of the study to obtain the daily weight gain. Feed offered to birds were measured weekly and residual feed were also measured to evaluate the feed intake. The feed intake was divided by the product of the number of birds per replicate and number of days of the study to obtain the daily feed intake. Feed conversion ratio was obtained by dividing daily feed intake and weight gain. The number of dead birds per replicate was also expressed as a percentage of the total number of birds alive to obtain the percentage mortality.

Serological Test - Collection of Serum Samples

On day 1 of age, 1 ml of blood were collected each from 30 Arbor Acre chicks selected at random for evaluating the maternally derived antibody titre, also on the 19^{th} and 38^{th} d of age, 2 ml of blood were collected each from 5 birds selected at random per replicate through jugular veno-puncture to determine antibody titre to ND vaccination. Blood samples were allowed to stand for 15 min for sera formation, after which sera were collected in ependof tubes and stored in a freezer until ready for use. Haemaglutination inhibition test was used to access the level of antibody titre against ND vaccinations (Allan and Gough, 1974). Lasota vaccine was used as the source of antigen.

Haemaglutination Test

Each micro-titre well on the first row (A1 to A12) of a micro-titre plate was filled with 50 µl of phosphate buffer saline (PBS) and 50 µl of diluted Lasota vaccine was added to the first well (A1) and double diluted serially to the eleventh well (A11) on the row. Fifty µl of 0.5% RBC suspension was added to each of the wells starting from the first (A1) to the last well (A12). This was then allowed to incubate for 30 min at room temperature. Then the wells were read while well A12 served as the negative control. Positive result was indicated by RBC evenly distributed at the bottom of the plate, and negative result was indicated by RBC clump together to form a button like shape. Antibody titre value was the inverse of serum dilution giving 100% haemaglutination (Allan and Gough, 1974).

Haemaglutination Inhibition Test (HI)

The 4HA unit (standard unit for HI test) of diluted Lasota vaccine was calculated by using the formula: $C_1V_1= C_2V_2$ where C_I = HA unit of the diluted Lasota vaccine, V_1= 1 ml (initial volume of diluted Lasota vaccine collected), C_2= 4 (HA standard unit), V_2 is volume of PBS to be added to make 4HA unit of diluted Lasota vaccine. Fifty microlitres of PBS were placed into the micro-titre wells across the rows and along the columns depending on the number of samples to be analyzed (wells 1-12). Each row was used for one serum sample (i.e., serum sample 1 for rows A1-A12). Fifty µl of each serum sample was added to the corresponding well 1 and double diluted serially to well 12 after which 50 µl of the diluted Lasota vaccine (4HA unit) was added to each well from well 1 to well 12. Fifty µl of 0.5% RBC suspension was also added to each well and allowed to incubate for 30 min. Results were read and titre value was the reciprocal of serum dilution with 100% haemaglutination (Allan and Gough, 1974). The result is negative, if RBC were evenly distributed at the bottom of the plate and positive if RBC clumped together to form a button like shape.

Data obtained were subjected to analysis of variance in a Completely Randomized Design and significanty ($p<0.05$) different means among variables were separated using Duncan's Multiple Range test using SAS (2010) package.

Results and Discussion

The supplemental vitamins influenced ($p<0.05$) final body weight and feed conversion ratio (Table 1). Higher ($p<0.05$) mean final body weight of 1821.71 and 1785.00 g were obtained in birds offered diets supplemented with vitamins A and the combined vitamins A, C and E, compared to 1593.33 g in birds fed the control diet. Furthermore, significantly better feed conversion ratio of 2.89 was obtained in birds fed diet supplemented with the three combined vitamins A, C and E. Growth performance indices were not affected ($p<0.05$) by the periods of supplemental vitamins administration. The higher final body weight recorded in birds fed diet supplemented with the combined vitamins A, C and E may be ascribed to the synergistic growth enhancing effects of the combined vitamins A, C and E as evident in the reduced feed intake with better feed conversion ratio. This is in agreement with the report of Sandal and Oyewole (2015) who stated that birds fed diet supplemented with combined vitamins A and C had a higher final body weight relative to birds fed diets supplemented singly with vitamins A and C, respectively. The effect of vitamin A dietary supplementation which elicited similar higher final body weight as the combined vitamins A, C and E supplementation contradicted the reports of Rajput *et al*. (2009) who observed no significant difference between birds offered water supplemented with vitamin A (8500 IU/litre) and the control. Dietary vitamin C supplementation also enhanced high final body weight similar to that of vitamin E. This implies that the dietary inclusion level of vitamin C (50 mg/kg) in this study is adequate to stimulate growth in broiler chickens. This observation agrees with earlier reports of Oruseibio and Alu (2006), Sabah *et al.* (2008) and Onu (2009) that weight gain was depressed in heat stressed birds fed diet without ascorbic acid supplementation.

Table 1: Main effects of supplemental vitamins and administration periods on growth performance of broiler chickens vaccinated against Newcastle disease.

Parameter	Control	Vitamins				Periods of Administration	
		A (0.45 mg/kg)	C (50 mg/kg)	E (9.1 mg/kg)	A+C+E (0.15+16.67 +3.03 mg/kg)	PRV	PSV
Initial weight (g/bird)	55.17±0.17	57.20±0.99	55.12±0.61	57.12±0.58	56.70±1.36	56.99±0.60	55.53±0.44
Final weight (g/bird)	1593.33 ±14.76[b]	1821.71 ±80.03[a]	1743.00 ±50.70[ab]	1741.37 ±58.21[ab]	1785.00 ±37.64[a]	1720.92 ±37.68	1752.91 ±37.49
Weight gain (g/bird/day)	27.99±0.29	31.28±1.36	30.07±0.90	31.31±1.78	31.17±0.64	29.78±0.62	30.95±0.84
Feed intake (g/bird/day)	92.51±3.79	96.84±5.80	90.92±3.34	97.75±6.36	89.84±1.83	92.30±2.41	94.85±3.18
Feed conversion ratio	3.31±0.14[a]	3.09±0.12[ab]	3.03±0.15[ab]	3.13±0.12[ab]	2.89±0.06[b]	3.11±0.08	3.07±0.08
Mortality (%)	16.67±5.58	16.67±4.94	6.67±3.33	18.33±6.01	6.67±2.11	12.00±2.96	14.00±3.21

[a,b] Means in the same row having different superscript are significantly different ($p<0.05$).
PRV: 3 days pre- Newcastle Disease vaccination administration of vitamins; PSV: 3 days post- Newcastle disease vaccination administration of vitamins

Higher ($p<0.05$) daily weight gain of 34.23 g was recorded in birds fed vitamins E supplemented diet for 3 d post-ND vaccination (Table 2). The interaction effect of vitamins and periods of administration with respect to the weight gain further indicated the positive influence of vitamins A, C and E dietary supplementation on growth rate in broiler chickens. This observation contradicted Ogbamgba *et al.* (2007) who stated that vitamins A, C and E supplementation did not have beneficial effects on growth in broiler chickens.

Table 2: Interactive effects of supplemental vitamins and administration periods on growth performance of broiler chickens vaccinated against Newcastle disease.

Vitamins	Control	A (0.45mg/kg)		C (50mg/kg)		E (9.1mg/kg)		A+C+E (0.15+16.67 +3.03 mg/kg)	
Periods Parameter		PRV	PSV	PRV	PSV	PRV	PSV	PRV	PSV
Initial weight (g/bird)	55.17 ±0.27	57.43 ±2.02	56.97 ±0.90	55.53 ±0.80	54.70 ±1.01	57.13 ±1.09	57.10 ±0.68	59.67 ±0.58	53.73 ±0.38
Final weight (g/bird)	1593.33 ±23.33	1830.82 ±106.12	1812.59 ±143.80	1753.70 ±79.18	1732.59 ±80.43	1658.20 ±88.20	1824.54 ±47.39	1768.52 ±77.54	1801.48 ±28.29
Weight gain (g/bird/day)	27.99 ±0.46^{b}	31.40 ±1.82^{ab}	31.15 ±2.44^{ab}	30.28 ±1.36^{ab}	29.85 ±1.46^{ab}	28.40 ±1.51^{b}	34.23 ±2.24^{a}	30.81 ±1.08^{ab}	31.52 ±0.85^{ab}
Feed intake (g/bird/day)	2.51 ±5.99	100.64 ±7.31	93.05 ±10.02	86.61 ±2.65	95.24 ±5.50	91.94 ±6.69	103.56 ±11.13	89.78 ±2.70	89.8 ±3.07
Feed Conversion Ratio	3.31 ±0.23	3.22 ±0.22	2.97 ±0.11	2.86 ±0.08	3.21 ±0.29	3.24 ±0.20	3.01 ±0.13	2.92 ±0.10	2.85 ±0.09
Mortality (%)	16.67 ±8.82	20.00 ±5.77	13.33 ±8.82	13.33 ±3.33	10.00 ±5.77	13.33 ±8.82	23.33 ±8.82	6.67 ±3.33	6.67 ±3.33

$^{abc:}$ Means on the same row having different superscripts are significantly ($p<0.05$) different.
PRV: 3 days pre- Newcastle disease vaccination administration of vitamins; PSV: 3 days post- Newcastle disease vaccination administration of vitamins.

The mean maternally derived antibody titre to Newcastle disease in 30 broiler chicks at day one of age was 6.33. There were no significant differences in the serum antibody titre of broiler chickens fed diets supplemented with the different supplemental vitamins at 14 d post-i/o (19 d of age) and Lasota (38 d of age) vaccines (Figure 1). The period of vitamins administration did not significantly influence the antibody titre of broiler chickens (Figure 2). Figure 3 shows the interaction effect of supplemental vitamins and period of administration on the serum antibody titre of broiler chickens vaccinated against Newcastle Disease. Higher ($p<0.05$) serum antibody titre of 75.20 against i/o vaccine was observed in birds fed diet supplemented with vitamin A 3 d pre-i/o vaccination, and 741.33 from dietary supplementation with vitamin E administered for 3 d post-Lasota vaccination. The non-significant influence observed in the main effects of supplemental vitamins and period of administration on the serum antibody titre of the broiler chickens may be attributed to the quantity of vitamins used as stated by Dalloul *et al.* (2003) that increasing vitamin A dietary density resulted in improvement in the immune system of broiler chickens. The improved serum antibody titre observed in the interaction effect of supplemental vitamins and period of administration implies that supplemental vitamins administration period enhances the efficiency of the immune system in antibody production against antigens even at low level of dietary vitamin supplementation. This result is similar to the reports of Kammon *et al.* (2012) who reported that supplementation of vitamin E resulted in marked improvements in humoral immunity and pathology of lymphoid organs. Vitamin C dietary supplementation resulted in positive influence on the immune system of the birds due to considerably high antibody titre against i/o and Lasota vaccines, relative to that obtained in birds fed the control diet. This agrees with the reports of Sanda and Oyewole (2015) who found that the supplementation of vitamin C at the rate of 2.5g /25kg feed resulted in high antibody titre of 2048 in broiler chickens. The increase in the antibody titre against i/o vaccine as a result of vitamin A supplementation may be explained from its significant roles in production of antibodies, haematopoiesis and its functions on T and B lymphocytes (Semba, 1999). This is similar to the findings of Safarizadeh and Zakeri (2013) who reported that vitamin A supplementation resulted in improvement in antibody production against ND vaccination in broiler chickens.

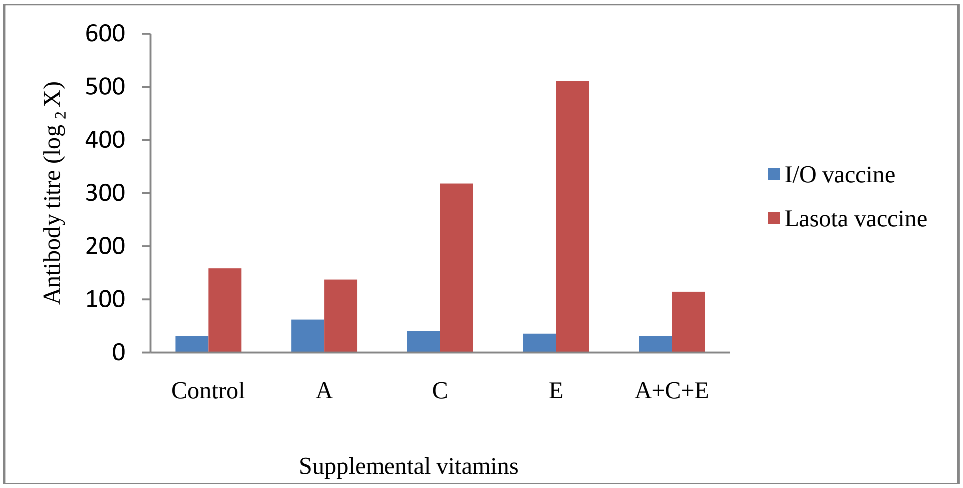

Figure 1: Main effect of supplemental vitamins on antibody titre of broiler chickens

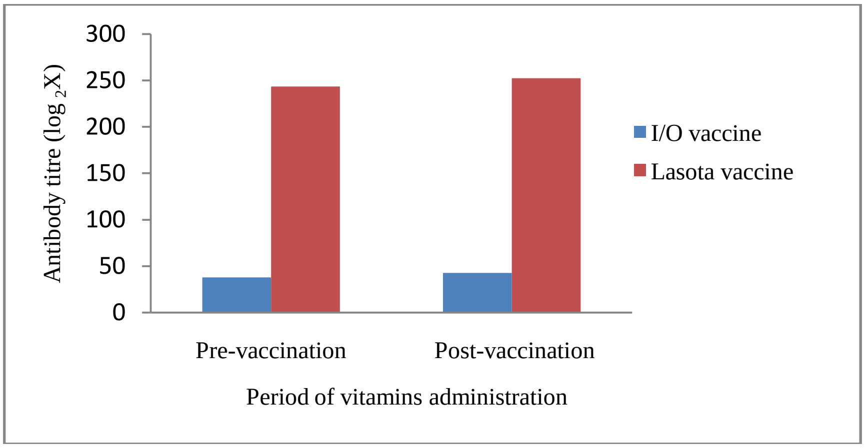

Figure 2: Main effects of period of vitamins administration on antibody titre of broiler chickens

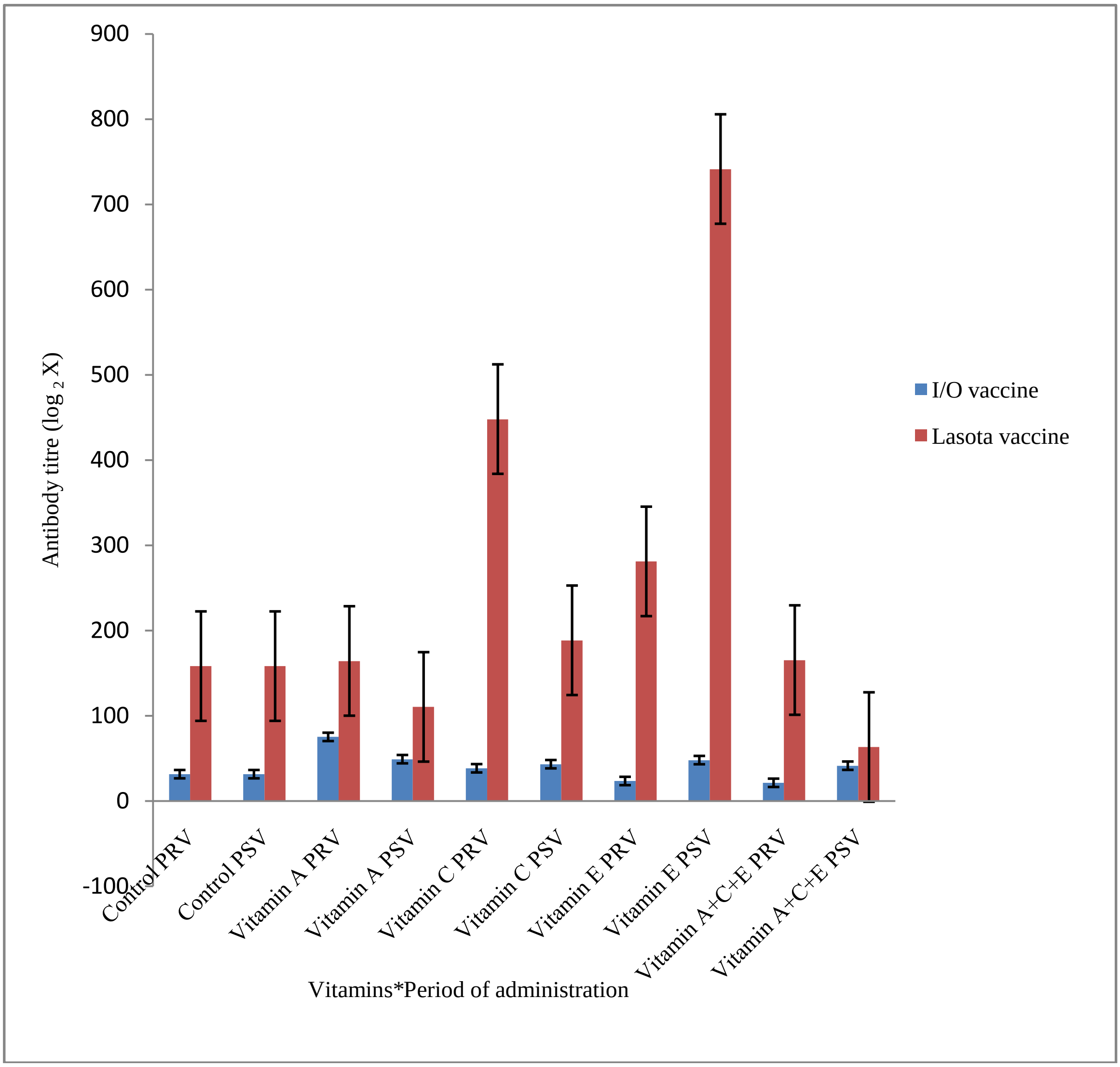

Figure 3: Effect of interaction between vitamins and period of administration on antibody titre of broiler chickens.

Conclusion

Broiler chickens fed diets supplemented with combined vitamins of A, C and E had higher final body weight with better feed conversion ratio. In addition, administration of vitamin A supplemented diet for 3 d pre-i/o vaccine and vitamin E supplemented diet for 3 d post-Lasota vaccine enhanced higher antibody titre against Newcastle disease. Therefore, for improved growth performance in terms of final body weight and FCR, supplementation of broiler chickens diet with combined vitamins A, C and E at the rates of 0.15, 16.67 and 3.03 mg/kg diet, respectively, can be adopted for 3 d pre or post Newcastle disease vaccinations. Antibody production against Newcastle Disease in broiler chickens can be enhanced by administering vitamin A at 0.45 mg/kg supplemented diet 3 d pre-i/o vaccination and vitamin E at 9.1 mg/kg supplemented diet 3 d post-Lasota vaccination.

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