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Effects of The Graded Levels of Cholecalciferol (Vitamin D_3) on the Haematological Characteristics and serum enzymes of broilers in the hot humid southern Nigeria

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Abstract
This study was carried out to determine the effects of graded levels of cholecalciferol (Vitamin D_3) on the haematological characteristics and serum enzymes of broiler chickens. Two hundred and forty day old Marshall Broiler chicks were divided into four groups (A - D) of 60 birds each, and each group replicated thrice, with 30 birds per replicate in a completely randomized design (CRD). Broiler starter and finisher diets were formulate in which vitamin D_3 extra commercial feed grade vitamin D_3 was added at the levels of 0.0% (control) 40, 80 and 100% (manufacturers' formulation), and offered to the corresponding groups, B – D respectively during 56 days of feeding (28 days each for starter and finisher). At 56 days, three birds from each treatment group were randomly selected and blood samples collected to determine the haematological and serum enzyme characteristics of the birds. Haematological parameters such as Packed Cell Volume (PCV), haemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) counts and serum enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) remained within the normal range for broiler chickens and were not significantly ($p>0.05$) different across D_3 treatment levels. It was concluded that the inclusion of 100% vitamin D_3 in the broiler vitamin, trace mineral premix as formulated by the manufacturers for broiler ration is adequate.

Keywords: Broilers, vitamin D_3, cholecalciferol, haematology, serum enzyme

Introduction

Broilers are fast growing chickens noted for high feed conversion and efficiency. Therefore broiler diets are formulated to provide all of the birds' nutrient requirements if optimum growth and production are to be achieved. Such nutrients include vitamins such as A, D, E K, C and the B vitamins. Vitamin. The most important and biologically active vitamin D is cholecalciferol or vitamin D_3 produced by irradiation of 7-dehydrocholesterol (Fritts and Waldroup, 2003). In the production of commercial feed, vitamin D_3 could be lost from the feed through oxidation, especially under hot humid tropical environments during extended storage. (McDowell, 2000; (Al-Nasser *et al*., 1986) and may therefore not meet the minimum requirement as listed in NRC (1994) for broiler diets. but this recommendation is based on old studies, performed under controlled environment.

Blood parameters have been shown to be major indices of physiological, pathological and nutritional status of an organism. Thus their values could be used to interpret the metabolic stage of an animal as well as quality of feed (Nworgu *et al.*, 2007). Serum ALP, AST and ALT levels could serve as indicators of functionality of vitamin D_3 in the body since increase in ALP, for example, have been shown to reflect vitamin D_3 deficiency (Campbell and Cole, 1986).

This study thus seeks to determine the effects of graded levels of vitamin D_3 on broilers on the haematological characteristics and serum enzymes of broilers.

Materials and Methods

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Rivers State University of Science and Technology, Port Harcourt. The farm is situated on latitude 4°48'N and longitude 6°58'E in the hot humid Rivers State, Nigeria.

A total of 240 day old Marshal Breed of Broilers were used for the experiment. They were weighed to obtain the initial body weight and thereafter, randomly divided into four groups (A – D) of 60 birds each, and each group replicated thrice, with 30 birds per replicate in a completely randomized design (CRD). Broiler starter and finisher diets were formulate in which vitamin D_3 extra commercial feed grade vitamin D_3 was added at the levels of 0.0% (control) 40, 80 and 100% (manufacturers' formulation), and offered to the corresponding groups, B – D respectively (Table 1) in standard broiler pens during 56 days of feeding (28 days each for starter and finisher

feeding). The experimental diets as shown in table 2 were offered with water *ad libitum*. Other routine management practices such as vaccinations against Newcastle disease at the 3rd and 6th week, and Gumboro at the 2nd and 4th week respectively as well as prophylactic coccidiosis treatments were carried out.

Table 1. Test vitamin premix with graded levels of vitamin D3 inclusions

Dietary treatment	% inclusion of Vitamin D3	Vitamin D3 (UI/2.5 kg)	Vitamin D3 (UI/kg)
A (Control)	0	-	-
B	40	800,000	320,000
C	80	1,600.000	640,000
D	100	2,000,000	800,000

The Company's standard vitamin/trace mineral premix = 2,000,000 IU/2.5 kg Equivalent to 800,000 IU/kg

Table 2: Composition of the experimental diets

Ingredients Gross composition	Broiler Starter (%)	Broiler Finisher (%)
Maize	55.00	55.00
Palm kernel cake	10.30	14.30
Soya bean meal	28.00	25.00
Fish meal	3.00	2.00
Bone meal	3.00	3.00
Methionine	0.10	0.10
Lysine	0.10	0.10
Salt	0.25	0.25
Vitamin/trace mineral premix	0.25*	0.25**
Total	100.00	100.00

At the end of the feeding trial on the 56th day, a bird from each replicate were randomly selected and bled from the jugular vein such that two sets of blood samples, 2 ml each were collected, one in EDTA treated bottles to prevent clotting and the other in clean Bijour bottles for serum production (Baker and Silverton, 1986). Hematological analyses to deter mine Packed Cell Volume (PCV), haemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) counts according to the methods of Baker and Silverton, 1986). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated from the RBC, Hb, and PCV values.

Serum samples were analysed for their aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), according to the method described by Reece (2009). Data obtained were subjected to analysis of variance (ANOVA) (SAS, 2001) while significant differences where they existed were determined using the Duncan's Multiple Range Test (DMRT) (1955).

Results and Discussion

The effect of the graded levels of Vitamin D_3 on the haematological characteristics of broilers is presented in Table 3. There were no significant ($p>0.05$) differences in all the parameters evaluated, although the values numerically increased with increasing vitamin D_3 inclusion. The values were also within the normal values expected for broilers (Reece, 2009).

Table 3: Effects of vitamin D_3 on the haematological characteristics of broilers

Parameters	Treatments			
	A	B	C	D
PCV (%)	28.33 ± 1.15	28.0 ± 2.0	29.66 ± 0.67	30.0 ± 1.0
Hb (g/dl)	9.30 ± 0.51	9.45 ± 0.75	9.87 ± 0.22	9.87 ± 0.30
RBCx12g/l	3.10 ± 0.15	3.15 ± 0.25	3.27 ± 0.07	3.27 ± 0.09
WBC x10g/l		Uncountable		
MCH (%)	30.20 ± 3.40	30.20 ± 3.00	30.00 ± 3.14	29.98 ± 3.33
MCHC (%)	33.27 ± 0.44	32.90 ± 0.37	33.73 ± 0.32	32.82 ± 0.30
MCV (fl)	90.80 ± 7.67	91.81 ± 8.00	88.94 ± 9.57	91.38 ± 11.11

a, b – means within the same row were not significantly ($p>0.05$) different.

PCV= Packed cell volume MCH= Mean corpuscular haemoglobin

Hb= Haemoglobin
RBC= Red blood cell
WBC=White blood cell
MCHC= Mean corpuscular haemoglobin concentration
MCV= Mean corpuscular volume

The effect of graded levels of vitamin D_3 on the serum enzymes (Table 4) showed that significant ($p>0.05$) differences did not exist across treatments. ALP value increases when there is a deficiency of vitamin D_3, however this study showed a depression with higher vitamin D_3 levels (Campbell and Cole, 1986).

Table 4. Effect of graded levels of vitamin D_3 on the serum enzymes of broilers

Parameters	Treatments			
	A	B	C	D
AST (IU/L)	15.33±1.76	17.00 ± 4.04	14.0 ± 1.15	12.00 ± 0.58
ALT (IU/L)	10.33±1.67	10.67 ± 3.17	8.00 ± 00	7.67 ± 0.33
ALP (IU/L)	243.00±9.00	234.33±41.35	229.60 ± 20.15	181.33 ± 12.02

a, b – means within the same row were not significantly ($p>0.05$) different.
AST- Aspartate aminotransferase
ALT- Alkaline aminotransferase
ALP- Alkaline phosphatase

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