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Effects of the Graded Levels of Vitamin D₃ on the Serum Protein, Electrolytes and Bone Mineralization of Broilers under Hot Humid Southern Nigeria

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Abstract

This study was carried out to determine the effects of graded levels of vitamin D₃ on the serum protein, electrolytes and bone mineralization of broilers under hot humid southern Nigeria. 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 formulated in which vitamin D₃ extra commercial feed grade vitamin D₃ 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 serum proteins and electrolyte characteristics of the birds. Bone mineralization was also determined using the tibia bone. There were no significant differences between the mineral levels of calcium and phosphorus in the tibia bone across all the treatments. There were however slight numerical increase in serum calcium values with increasing vitamin D₃ supplementation, while PO₄⁻ followed the reverse trend. In the tibia bone, calcium values decreased, while phosphorus values remained similar to control. It was concluded that with higher levels of vitamin D₃ in treatment D, calcium was readily mobilized from the bones and this correlated with the higher plasma calcium level observed in the same group.

Keywords: Broilers, vitamin D, cholecalciferol, serum electrolytes, bone mineralisation

Introduction

Today, all industrialized poultry feeds are fortified with vitamins. Research on adequate supplementation levels to achieve optimum feed utilization, health and growth rates, especially in broilers as slaughter age continues to shorten remains ongoing. Several factors affect vitamin supplementation such as breed, sex, management practices, developmental status of the birds, stress and diseases. The common commercial source of vitamin D activity for poultry nutrition is vitamin D₃ (Chou *et al.*, 2009). Synthetic forms of Vitamin D₃ such as Hy.D® that provides a more bioactive form of vitamin D₃ to the animals are available commercially.

Vitamin D₃ is best known for its role in calcium and phosphorus metabolism in skeletal development and maintenance as well as eggshell strength. Vitamin D₃ has also been recognized for cell growth and differentiation, its effect on the immune system and reproduction (Ward, 2003). In the production of commercial feed, vitamin D₃ could be lost from the feed through oxidation, especially under hot humid tropical environments during extended storage.

Various parameters have been used as indicators of bone mineralization including tibia ash, toe ash and radiography (Hall *et al.*, 2003). There is a direct correlation between increased bone ash, higher amounts of available calcium and phytate phosphorus in the diet and increased body weight gain. Poor mineralization has been associated with weak bones resulting in breakage during processing and lower meat grade, with leg abnormalities probably causing more economic losses than any other single abnormality in rapidly growing chicken (Atencio *et al.*, 2005), while tibia strength has been shown to be proportional to the level of vitamin D₃ in the feed (Whitehead *et al.*, 2004).

This study determined the effects of graded levels of vitamin D₃ on serum proteins, electrolytes and bone mineralisation of broiler chickens.

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 formulated in which vitamin D₃ extra commercial feed grade vitamin D₃ 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 D ₃	Vitamin D ₃ (UI/2.5 kg)	Vitamin D ₃ (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 Silvertown, 1986).

Serum samples were analysed for their total protein, albumen, calcium and phosphorus concentrations according to the method described by Reece (2009). Bone mineralization was determined by carefully removing the flesh from the left and right tibia bones of each bird. The weight of the bones were taken and recorded. There after the bones were dried in an oven and crushed for tibia ash determination according to AOAC (1990).

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 Discussions

The mean values of calcium (Ca²⁺), phosphate (PO₄⁻), total protein and albumin were are shown in table 3. There were no significant ($p > 0.05$) differences across the Ca²⁺, PO₄⁻, total protein and albumin values. There were however slight numerical increase in serum calcium values with increasing vitamin D₃ supplementation, while PO₄⁻ followed the reverse trend. Vitamin D₃ is essential in regulation of the calcium- phosphorus metabolism. It enhances the resorption of calcium ions in the intestinal tract and influences bone metabolism.

Table 3: Effect of graded levels of vitamin D₃ on serum proteins and electrolytes of broilers

Parameters	A	B	C	D
Ca ²⁺ (nmol/L)	2.10 ± 0.20	2.20 ± 0.05	2.23 ± 0.09	2.36 ± 0.03
PO ₄ ⁻ (nmol/L)	1.93 ± 0.22	1.90 ± 0.06	1.70 ± 0.06	1.63 ± 0.03
Total protein (g/L)	47.0 ± 4.00	38.67 ± 5.81	41.33 ± 2.40	43.64 ± 6.06
Albumin (g/l)	16.67±0.67	16.33 ± 1.20	16.67 ± 0.67	17.00 ± 2.08

Ca²⁺- Calcium ions; PO₄⁻- Phosphate ions

This regulation is done by its active metabolite, 1, 25-dihydroxycholecalciferol (calcitriol). Biosynthesis of calcitriol is dependent upon the availability of vitamin D₃ from the feed and its hydroxylation in both liver and kidneys. Green (2011) reported increased serum phosphorus concentrations with increasing vitamin D₃ supplementation. Rama *et al.* (2008) found serum phosphorus concentrations significantly increased when chicks were fed a supplemented diet of vitamin D₃. Increasing the levels of vitamin D₃ however did not affect the haematological and serum biochemical indices of the birds adversely. The values however remained within the normal range reported for broilers.

The effect of graded levels of vitamin D₃ on bone mineralization of broilers is presented in table 4. The mean values of the average tibia weight, calcium and phosphorus content of the tibia bones determined were not significantly ($p>0.05$) different. However, average tibia weight increased with increasing vitamin D₃ supplementation, while calcium values decreased and phosphorus values remained similar to control values especially in treatment C and D. Bone status is commonly used as an indicator of mineral adequacy in poultry diets, the major minerals being calcium and phosphorus (Onyango *et al.*, 2003). Vitamin D₃ functions to mobilize calcium from the bones, thus a diet deficient in vitamin D₃ would have high calcium levels in the bones. Tibia bone is sensitive to the amount of calcium and phosphorus as well as other ingredients in the diet (Hall *et al.*, 2003).

Table 4: Effect of the graded levels of vitamin d₃ on bone mineralization of broilers

Parameters	A	B	C	D
Average tibia weight (g)	23.10	23.35	28.50	24.55
Calcium (mg/kg)	315	215	230	220
Phosphorus (mg/kg)	30.94	32.28	30.94	30.94

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

Conclusion

It was concluded that with adequate levels of vitamin D₃ in treatment group D, calcium was readily mobilized from the bones and this could be correlated with the higher plasma calcium levels observed in treatment group D.

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