International Journal of Agriculture and Forestry 2018, 8(6): 213-219
DOI: 10.5923/j.ijaf.20180806.03

# Performance and Blood Profile of Growing Pullets Fed Diets Supplemented with Cholecalciferol

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**Abstract** Nutritional additives in animal production are often targeted at further improving productivity. Supplementation beyond the conventional requirement of animals however, may make or mar animals' performance and well being. This study was conducted to assess the effects of dietary supplemental cholecalciferol on performance and blood profile of growing pullets. Bovan Brown pullets (n=128) aged 12 weeks and weighing 0.82±0.03 were allotted to four dietary treatments of four replicates and eight pullets per replicate in a completely randomised design. They were initially raised on an iso-caloric and iso-nitrogenous basal diet for the first three weeks and thereafter the diets were supplemented with 0 (T1), 1000 (T2), 2000 (T3) and 3000 IU/Kg $D_3$ (T4), respectively. At week 20, final weight (kg) (1.19, 1.16, 1.18 and 1.15), weight changes (kg) (0.36, 0.34, 0.35 and 0.33), feed intake (0.89, 0.89, 0.89 and 0.89) and feed conversion ratio (2.48, 2.72, 2.57 and 2.79) for pullets on T1, T2, T3 and T4, respectively were not significantly affected ($P>0.05$) by the treatments. Platelet was the only haematological parameter that was significantly increased ($P<0.05$) by vitamin $D_3$ supplementation. Platelets of pullets on 1000 IU $D_3$ (2.36) was significantly higher ($P<0.05$) than 1.55, 1.51 and 1.63 in pullets on 0, 2000 and 3000 IU $D_3$. Serum calcium and phosphorus were not significantly ($P>0.05$) affected by dietary $D_3$ supplementation. The ALP, however was significantly higher ($P<0.05$) at 3000 IU supplementation (36.21) compared to similar ($P>0.05$) values of 17.57, 15.04 and 16.35 in pullets on 0, 1000 and 2000 IU $D_3$ supplementations, respectively. The highest serum cholesterol (154.82), HDL (76.43) and LDL (69.59) levels were observed in pullets on supplemental 2000 IU $D_3$ compared to other treatments while triglyceride was lowered ($P<0.05$) with increasing level of $D_3$ supplementation from 82.58 in T1 to 45.59, 44.01 and 42.45 in those on 1000, 2000 and 3000 IU $D_3$, respectively. Supplemental vitamin $D_3$ in the diets of pullets had no gross effect on performance attributes and blood indices monitored.

**Keywords** Growing pullets, Supplemental cholecalciferol, Dietary vitamin, Serum cholesterol

## 1. Introduction

Nutritional additives are becoming a normal pre-requisite in the formulation of animal feeds as their inclusion in diets is associated with increased livestock production. Supplemental vitamins are among such additives of immense importance in livestock production. Vitamin requirements established decades ago do not take into account the modern genetically superior birds with increased growth, egg production and improved feed efficiency. Vitamin intake per unit of output is continually declining, as yearly decline for layers is around 1% per egg produced, while for broilers is 0.6-0.8% for body gain [1].

Cholecalciferol also known as vitamin $D_3$ plays a major role in livestock production because of its involvement in the maintenance of skeletal integrity. It can be synthesized in the skin, catalyzed by ultraviolet radiation, from 7-dihydrocholesterol present in the dermis and epidermis [2] or be supplied in the feed. Commercial chickens are usually maintained indoors, and do not receive enough solar radiation to convert 7-dihydrocholesterol in sufficient levels to supply their cholecalciferol requirements. This is importantly the reason why cholecalciferol is routinely added to poultry feed for the maintenance of egg production, eggshell formation and calcium homeostasis. After absorption of $D_3$ by the intestinal mucosa, it is transported to the liver, where it is hydroxylated in the carbon position 25, resulting in 25-hydroxycholecalciferol (25(OH) $D_3$). This metabolite is thereafter hydroxylated at carbon 1, in the kidney thus resulting in the active metabolite 1,25 dihydroxycholecalciferol (1, 25$(OH)_2D_3$) [3]. This active form stimulates bone calcium mobilization and inhibits urinary calcium excretion when hens are calcium deficient or need more calcium, through molecular mechanisms involving bone cells [4], vitamin D binding proteins [5] such

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Published online at http://journal.sapub.org/ijaf

as calbindin [6, 7] and ovocleidin-17 [8] as well as other hormones such as estrogen and thyroxin [9].

Most documentation on $D_3$ are associated with calcium homoestasis with avalanche of information in broiler and laying hens but little information in growing pullets. Also studies in poultry had hitherto laid emphases on egg production and bone integrity [10-16] with very little attention drawn to the effect on performance attributes and blood profile of chickens. Blood is an important index of physiological, pathological and nutritional status in animals [17, 18]. Aletor [19] and Egberongbe [20] observed that blood variables were most constantly affected by dietary factors. Since supplements were often added above the NRC [21] recommended dosage in feed, the safety of such additional supply in feed needed to be assessed in the blood. Also, the appropriate supplemental dosage in chicken's feed that would exert no detrimental consequence on productivity needs to be documented.

The study was therefore aimed at investigating the effect of supplemental cholecalciferol on performance attributes and blood profile of growing pullets.

# 2. Materials and Methods

### 2.1. Experimental Site

The study was carried out at the poultry unit of the Teaching and Research farm, University of Ibadan, Ibadan, Nigeria. The university is located in Ibadan in the tropical rain forest zone of Nigeria within latitude 7° 26 N and longitude 3° 54 E, with a mean altitude of 277 meters above sea level. Average temperature and relative humidity of the location is about 26.5°C and 55%, respectively.

### 2.2. Experimental Animals and Management

Bovan Brown pullets (n=128) aged 12 weeks and weighing 0.82±0.03 were purchased from a reputable farm and allotted to four dietary treatments of four replicates and eight pullets per replicate in a completely randomised design. Pullets were housed in a conventional 3-tier battery cage housing system. Each cubicle measured 50 x 45 x 40 $cm^3$ with a floor space of 450$cm^2$/ bird that accommodated four pullets. Pullets were initially raised on an iso-caloric and iso-nitrogenous basal diet for the first three weeks after which the diets were supplemented with 0 (T1), 1000 (T2), 2000 (T3) and 3000 IU/Kg $D_3$ (T4), respectively.

Details of the basal experimental diet have been previously documented and are shown in Table 1 [22, 23]. Routine management practices including vaccination and drug administration were adhered to while feed and water were offered to the pullets *ad libitum.* The duration of the experiment was eight weeks.

### 2.3. Experimental Design

The experiment was a completely randomized design and **the experimental model is as follows:**

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

*Where* $Y_{ij}$ = j$^{-th}$ observation of the i$^{th}$ treatment

$\mu$ = Overall population mean

$\alpha_i$ = Effect of i$^{th}$ level of cholecalciferol supplementation

$e_{ij}$ = Random error assumed to be independently and normally distributed with zero mean and variance $\sigma^2$.

**Table 1.** Gross composition (%) of basal diet fed to point of lay pullets

| Ingredient | % (Kg) |
|---|---|
| Maize | 50.80 |
| Soybean meal | 18.00 |
| Wheat offal | 27.85 |
| Oyster shell | 1.00 |
| Bone meal | 1.60 |
| Premix | 0.25 |
| Table Salt | 0.30 |
| DL-Methionine | 0.10 |
| L-lysine | 0.10 |
| **Calculated Nutrients** | |
| ME (Kcal/kg) | 2751.27 |
| Crude Protein (%) | 16.87 |
| Crude Fibre (%) | 4.55 |
| Methionine (%) | 0.37 |
| Lysine (%) | 0.98 |
| Calcium (%) | 1.07 |
| Available Phosphorus (%) | 0.42 |

Premix* - Vitamin A–10,000IU, Vitamin $D_3$–1800IU, Vitamin E–40mg, Vitamin K–1.43 mg, Vitamin B1–0.7mg, Vitamin B2–4mg, Vitamin B6–2.5mg, Vitamin B12–0.2mg, Niacin–10mg, Panthothenic–10,000mg, Folic acid –0.25mg, Biotin–100mg, Choline Chloride–300mg, Manganese–80mg, Zinc–60mg, Iron–40mg, Copper– 80mg, Iodine–0.8mg, Selenium–0.2mg, Cobalt–0.3mg, Antioxidant–100mg

### 2.4. Data Collection

#### 2.4.1. Performance Characteristics

The pullets were weighed at the inception of the trial and then subsequently on weekly basis. The pullets in each replicate were weighed individually and weight gain (kg) was calculated on a weekly basis by deducting the initial weight from the final weight. Feed intake was recorded weekly by subtracting the left over from the total feed served. Feed Conversion Ratio (FCR) was obtained by dividing feed intake (kg) with weight gain (kg).

#### 2.4.2. Blood Collection and Evaluation

At week 20, blood (5 mLs) was sampled from three pullets per replicate using needles and syringes from the jugular vein into heparinised and non heparinised bottles for haematology and serum analyses, respectively. Haematological parameters assessed were white blood cells (WBC), platelets, leukocyte differential count: lymphocytes, heterophils, monophils, eosinophils and basophils [24, 25] while blood samples for serum analysis were left to clot and the serum was separated immediately by centrifugation at

3500rpm for 10 minutes. The serum biochemical parameters assessed were alkaline phosphatase [26], Serum Ca and P [27]. The serum lipids examined were triglycerides (Trinder's enzymic method), total cholesterol [28] cholesterol profile (High density lipoprotein and low density lipoprotein) according to Friedwal *et al*. [29] and [30]. Samples were read using spectrophotometry at wave length specific for each parameter.

### 2.5. Statistical Analysis

Data were subjected to One-way ANOVA using General Linear Model of SAS [31] and means separated using NDMRT option of the software at $\alpha_{0.05}$.

## 3. Results

Performance of point of lay pullets (POL) fed diets supplemented with varying levels of cholecalciferol is shown in Table 2. Final weight (1.19, 1.16, 1.18 and 1.15)), weight change (0.36, 0.34, 0.35 and 0.33), feed intake (0.89, 0.89, 0.89 and 0.89) and FCR (2.48, 2.72, 2.57 and 2.79) of pullets on 0 IU, 1000 IU, 2000 IU and 3000 IU, respectively were not significantly influenced (P>0.05) by the addition of cholecalciferol in the diets of the chickens. Pullets on zero supplementation of cholecalciferol had relative improvement in the measured parameters though were not significantly different (P<0.05) from other treatments.

**Table 2.** Performance of point of lay pullets fed diets supplemented with varying inclusion levels of vitamin $D_3$

| | Supplemental Cholecalciferol (IU/kg) | | | | |
|---|---|---|---|---|---|
| **Parameters** | **0** | **1000** | **2000** | **3000** | **SEM** |
| Initial Weight (Kg) | 0.83 | 0.81 | 0.83 | 0.82 | 0.01 |
| Final Weight (Kg) | 1.19 | 1.16 | 1.18 | 1.15 | 0.01 |
| Weight Change (Kg) | 0.36 | 0.34 | 0.35 | 0.33 | 0.01 |
| Feed Intake (Kg) | 0.89 | 0.89 | 0.89 | 0.89 | 0.001 |
| Feed Conversion Ratio | 2.48 | 2.72 | 2.57 | 2.79 | 0.11 |

[abc]Mean with similar superscript along the same row are not significantly different (P>0.05); SEM: Standard Error of Mean

Haematology of point of lay pullets fed diets supplemented with cholecalciferol is shown in Table 3. Similar to the observation in performance parameters, all monitored parameters except platelet were not affected (P>0.05) by cholecalciferol supplementation. Platelets of pullets on 1000 IU $D_3$ (2.36) was significantly higher (P<0.05) than 1.55, 1.51 and 1.63 in pullets on 0, 2000 and 3000 IU $D_3$, respectively.

Serum indices of POL pullets fed supplemental cholecalciferol are shown in Table 4. Serum Ca and P were not significantly affected (P>0.05) by dietary $D_3$ supplementation. The ALP was however significant (P<0.05) at 3000 IU supplementation (36.21) compared to statistically similar (P>0.05) ALP values of 17.57, 15.04 and 16.35 in pullets on 0, 1000 and 2000 IU $D_3$, respectively.

**Table 3.** Haematological indices of point of lay pullets fed diets supplemented with cholecalciferol

| **Parameters** | **0** | **1000** | **2000** | **3000** | **SEM** |
|---|---|---|---|---|---|
| PCV (%) | 27.5 | 30.00 | 30.00 | 27.00 | 1.45 |
| Haemoglobin (g/dL) | 9.25 | 9.88 | 9.75 | 9.08 | 0.47 |
| RBC | 3.07 | 3.13 | 3.03 | 3.05 | 0.19 |
| WBC x $10^4$ | 1.75 | 1.38 | 1.76 | 1.96 | 0.01 |
| Platelet x $10^5$ | $1.55^b$ | $2.36^a$ | $1.51^b$ | $1.63^b$ | 0.01 |
| Lymphocytes (%) | 65.25 | 66.00 | 66.25 | 67.25 | 0.90 |
| Heterophil (%) | 26.00 | 25.50 | 27.00 | 26.50 | 0.93 |
| Heterophil/Lymphocyte | 0.40 | 0.39 | 0.41 | 0.40 | 0.02 |
| Monocytes (%) | 2.00 | 3.50 | 2.50 | 2.50 | 0.34 |
| Eosinophil (%) | 4.00 | 4.50 | 3.50 | 3.50 | 0.43 |
| Basophil (%) | 0.75 | 0.50 | 0.75 | 0.25 | 0.13 |

[abc]Mean with similar superscript along the same row are not significantly different (P>0.05); SEM: Standard Error of Mean

**Table 4.** Serum indices of point of lay pullets fed supplemental cholecalciferol

| **Parameters** | **0** | **1000** | **2000** | **3000** | **SEM** |
|---|---|---|---|---|---|
| Calcium | 8.35 | 8.12 | 7.75 | 8.27 | 0.62 |
| Phosphorus | 9.18 | 8.56 | 9.26 | 8.13 | 0.69 |
| Alkaline Phosphatase | $17.57^b$ | $15.04^b$ | $16.35^b$ | $36.21^a$ | 2.38 |
| Cholesterol | $123.03^b$ | $92.93^c$ | $154.82^a$ | $52.69^d$ | 10.07 |
| High Density Lipoprotein | $67.63^a$ | $53.61^b$ | $76.43^a$ | $32.79^c$ | 4.63 |
| Low Density Lipoprotein | $38.66^b$ | $23.83^c$ | $69.59^a$ | $16.48^c$ | 5.53 |
| Triglyceride | $82.58^a$ | $45.59^b$ | $44.01^b$ | $42.45^b$ | 5.02 |

[abc]Mean with similar superscript along the same row are not significantly different (P>0.05); SEM: Standard Error of Mean

Serum cholesterol level of POL on 2000 IU $D_3$ (154.82) was higher (P<0.05) than 123.03 (0 IU), 92.93 (1000 IU) and 52.69 (3000 IU). The high density lipoprotein of POL fed diets supplemented with 0 IU (67.63) and 2000 IU $D_3$ (76.43) were similar (P>0.05) but significantly different (P<0.05) from 53.61 and 32.79 in POL fed diets supplemented with 1000 IU and 3000 IU $D_3$, respectively. The low density lipoprotein in POL fed 2000 IU (69.59) was significantly higher than those in POL on other levels of $D_3$ supplementation although, pullets on 1000 IU and 3000 IU $D_3$ supplementation had similar (P>0.05) low density lipoprotein. Triglyceride in POL on 0 IU $D_3$ supplementation (82.58) was significantly higher (P<0.05) than 45.59, 44.01 and 42.45 in POL on 1000 IU, 2000 IU and 3000 IU $D_3$, respectively.

## 4. Discussion

Performance attributes monitored in this study were not affected by vitamin $D_3$ supplementation. Feed was equally consumed by the pullets on the different levels of $D_3$ indicating that dietary supplement of vitamin $D_3$ did not influence their intake of feed. These were evident in the

similarities observed in weight change, final weight and FCR. This observation implied that inclusion of supplemental $D_3$ in the diets of pullets did not have any beneficial nor harmful effect on pullets performance. Similar report was documented for feed intake and final weight in growing pullets fed diets supplemented with ascorbic acid [23]. Earlier reports [32-34] on dietary supplementation of vitamin $D_3$ in chickens equally revealed that performance traits were not affected when adequate levels of vitamin $D_3$ were supplemented in diets of pullets. This could be an indication that the vitamin $D_3$ requirement of the pullets must have been met and additional supplementation for the growing pullets did not exert any extra influence on their performance. However, other workers [35, 36] obtained significant increase in growth performance of chickens fed varying inclusion levels of vitamin $D_3$ supplementation. Han *et al.* [37] also documented significant increases in weight gain, feed intake and improved FCR in broiler chicken with increasing dietary levels of vitamin $D_3$ supplementation.

Blood has been shown as an important index of physiological, pathological and nutritional status in animals [17, 20]. Aletor [19, 20] indicated that blood variables most constantly affected by dietary factors include RBC, PCV and plasma protein. The PCV range (27.00 to 30.00%) observed in this study, was higher than 24.67 and 27.00% reported by Talebi *et al*. [38] and similar to 30.07% reported by Oyewale [39] but was lower than 35.55% documented for caged chickens [40]. The PCV below 21-35% has been reported in poultry to be associated with anaemia, a condition attributed to intake of poor quality protein [41, 42].

Haemoglobin, the iron-containing oxygen-transport protein and the oxygen carrying capacity (RBC) of the blood remains an important component in the normal physiological functioning of cells. The Hb (g/dL) of 9.08 to 9.88 reported in this study was within the normal range of 7 to 13 [42, 43] for poultry. Index of haemoglobin has been used as an indication of nutritional anaemia in animals [44]. It may be used as a pointer to nutrient utilisation in diets since it has the physiological function of transporting oxygen to tissues in animals for oxidation of ingested food in order to release energy for body functions and transport carbon dioxide out of the body of animals [45]. Isaac *et al.* [46] opined that red blood cell is involved in the transport of oxygen and carbon dioxide in the body and a reduced red blood cell implies a reduction in the level of oxygen that would be carried to the lungs. The RBC which was in the range of 3.03 to 3.15 in this study were higher than the average reported in different strains of broiler chickens [38] and in broiler chickens fed diets containing varying levels of bitter Kola [47].

Vitamin $D_3$ has been implicated in several immunological functions. Calcitriol has been shown to have immunoregulatory and immunomodulatory activity and directly affects all cells of the mononuclear lineage [48-51]. This metabolite inhibits growth of *Mycobacteria tuberculosis* in cultured human monocytes and macrophages [52, 53] and improves resistance to tuberculosis in a murine model [54]. Macrophages from vitamin $D_3$-deficient mice functioned abnormally, and their function could be restored both *in vitro* and *in vivo* by treatment with 1, 25 $(OH)_2$ $D_3$ [55]. The major functions of the white blood cell and its differential are to fight infections, defend the body by phagocytes against invasion by foreign organism. Low differential counts can precipitate haematological abnormalities [56] which could undermine birds' performance.

As observed from this study, the WBC counts did not vary across treatments showing that dietary supplements did not influence the immune function of the pullets. The variation in components of leukocyte is indicative of external factors alien to the body of the animals. Observed similar values of heterophil, lymphocyte and heterophil/lymphocyte across treatments in this study showed that the test pullets possessed similar prowess to curtail invading aliens. Heterophil:lymphocyte is commonly used as an indicator of stress in chickens [57]. The presence of stress (nutritional, environmental, diseases etc) could cause variation in the population of heterophils and lymphocyte in the blood thus leading to an imbalanced ratio of heterophils:lymphocytes [57]. However, the similarity in the H:L of pullets across the treatment could imply that the supplementation did not impose any form of stress capable of disrupting the normalcy of the pullets' immune system and as such the levels of supplementation in this trial were not in excess of dietary need so as to compromise their health. Although vitamin $D_3$ has been implicated to have regulatory roles in immune cell functions [48], experiments have illustrated the successful treatments of autoimmune diseases with vitamin $D_3$ in mice [58]. The deficiency of cholecalciferol has also been associated with a depression in cellular responses in young broiler chicks [59] while increment in supplemental vitamin $D_3$ was associated with a 70% enhancement of lymphocyte proliferation [60].

Platelets also known as thrombocytes are cells involved in blood clotting. Reduction in number of platelets has been suggested to infer that the process of clot formation (blood clotting) would be prolonged thus resulting in excessive loss of blood during injury [61]. As observed in this study, supplementation of vitamin $D_3$ at 1000 IU increased the potentials of the blood to clot in tested pullets, although supplementation beyond this level further lowered the clotting potential. Park *et al.* [62] had attributed increased platelet count to deficiency of vitamin $D_3$ in human subjects which clearly indicated that dietary vitamin $D_3$ has metabolic roles in platelet function *in vivo*.

Cholecalciferol had been implicated in the mobilisation of Ca and P by authors [3, 12, 15] contrary to findings in this study where supplementation of vitamin $D_3$ did not influence serum Ca and P. Han *et al.* [37] also had similar observation in broiler chickens. The authors noted that different levels of cholecalciferol did not influence serum Ca and P concentration. However, performance was reportedly improved by dietary supplementation of vitamin $D_3$. The ALP activities were only increased at supplementation of 3000 IU $D_3$ thus suggesting that the lower levels may not be

sufficient to trigger further activities in the enzymes for mobilisation of phosphates, especially for bone growth in preparation of the pullets for the onset of lay.

Although, cholecalciferol is closely involved in calcium metabolism, it is synthesised from 7-dehydrocholesterol which could possibly compensates for its deficiency by increasing cholesterol synthesis [63]. There was a gradual reduction in serum cholesterol with increasing levels of vitamin $D_3$. Serum cholesterol was highest at 2000 IU vitamin $D_3$ supplementation which however, was lowered with increasing supplemental levels. The HDL and LDL followed a similar trend as corresponding increases in the values of these parameters at 2000 IU $D_3$ was related to high serum cholesterol at the same level. Effect of cholecalciferol on serum cholesterol composition has been inconsistent [63] though, authors reported a reduction in blood cholesterol in rowers administered vitamin $D_3$ supplementation. Chow *et al*. [64] also reported cholesterol reduction in mouse given different levels of vitamin $D_3$ supplementation. Supplementation of dietary cholecalciferol led to a progressive reduction in triglyceride levels in the serum. This corroborated the reported [65] reduction of blood lipids as < 5% changes in levels of lipids was adduced to vitamin $D_3$ and calcium supplementation.

## 5. Conclusions

It was concluded in this study that dietary supplement of vitamin $D_3$ had no effects on performance attributes of growing pullets. Except for the serum lipids and platelets, the gross blood profile was unaffected. Supplemental cholecalciferol level of 2000 IU/kg may be beneficial to platelets and serum lipid compositions.

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