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# Effect of Supplementing Plant Derived 1, 25 Dihydroxy Cholecalciferol on Performance and Bone Mineralization in Broiler Chicken Fed Suboptimal Concentrations of Calcium and Non Phytate Phosphorus

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**A B S T R A C T**

An experiment was conducted on 250 day old broiler chicks to investigate the effect of replacing cholecalciferol (CC) (1800 ICU/kg diet) (diet 1) with plant derived 1, 25 $(OH)_2$ $D_3$, (PD metabolite) at 25, 50, 75 and 100% (diet 2 to 5, respectively) on performance, bone mineralization and Calcium (Ca) and Phosphorus (P) retention in broiler chicken (0 to 42 d of age) fed diets containing suboptimal levels of Ca and non phytate P (NPP) (0.7% Ca and 0.35% NPP during starter and 0.7% Ca and 0.35% NPP during finisher). The chicks were randomly allotted to 50 replicates, which were in turn allotted to above 5 diets. Each diet was offered *ad libitum* and all birds were reared under uniform conditions. During starter, finisher phases and for all overall period body weight gains, feed intake and feed conversion ratio were comparable among the all dietary treatments. Serum Ca and inorganic P levels estimated from blood collected on $35^{th}$ d were comparable among the treatments. Bone weight, strength, ash percentage, Ca and P contents in bone ash were significantly ($P<0.05$) improved by replacing synthetic CC with PD metabolite but level of replacement (25, 50, 75 or 100%) had no significant effect. Similarly, P retention improved ($P<0.05$) with dietary incorporation of PD metabolite but was independent to level of replacement, while Ca retention was comparable among the dietary treatments. The results of the study indicated that supplementing plant derived 1, 25 $(OH)_2$ $D_3$ (45, 90, 135 or 180 ICU/kg) significantly improved bone weight, strength, ash percentage, Ca and P contents in broiler chicken fed on suboptimal levels of Ca and NPP.

## Introduction

Broiler chicken has been genetically improved to attain maximum weight in short duration, predisposing the birds to leg disorders and locomotion problem due to higher body mass and skeletal weight ratio (Ref). These problems in a way reduce farmers profit not only by affecting birds' performance but also by increasing the carcass condemnation in slaughter house.

Vitamin $D_3$ is important for maintenance of skeletal health due to its involvement in calcium (Ca) and phosphorus (P) metabolism. Commercially higher concentration of vitamin $D_3$ (cholecalciferol; CC) is added in broiler diets to prevent skeletal disorders. Several researchers suggested that dietary supplementation with high levels of CC to diet with suboptimal Ca and P could reduce

the incidences and severity of tibial dyschondroplasia and skeletal disorders in broilers (Driver *et al.,* 2006; Rama Rao *et al.,* 2006). Also, the suboptimal concentration of these minerals enhances the utilization of phytic phosphorus (PP) utilization in chicken (Biehl and Baker, 1997; Applegate *et al.,* 2003).

Physiologically vitamin $D_3$ metabolites (25 (OH) $D_3$; 1(OH) $D_3$; 1, 25 $(OH)_2$ $D_3$) are more effective than cholecalciferol because the efficiency of conversion of cholecalciferol to active metabolites [25 (OH) $D_3$ in liver and 1, 25 $(OH)_2$ $D_3$ in kidney] in biological system is not 100% (Yan and Waldroup, 2006; Goodgame *et al.,* 2011). Active metabolites may directly reach the target tissue by bypassing the hydroxylation steps in liver and kidney, hence exerts more potency than vitamin $D_3$ (Goodgame *et al.,* 2011).

Studies have indicated that 25 (OH) $D_3$ and 1, 25 $(OH)_2$ $D_3$ were superior to cholecalciferol as source of vitamin D in improving Ca and P utilization and tibial strength (Papesova *et al.,* 2008; Michalczuk *et al.,* 2010; Goodgame *et al.,* 2011). Even though the concept is well established, it is not penetrated to field level due to high cost of synthetic metabolites compared to vitamin $D_3$. Certain plants like *Solanum glaucophyllum* and *Cestrum dirunum* contains appreciable amounts of $D_3$ metabolites and could be used as source of vitamin $D_3$ and these plant derived compounds are less expensive than synthetic vitamin $D_3$ metabolite.

Moreover, the vitamin $D_3$ activity in PD metabolite was considered 10 times potent than CC (DeLuca, 1974). Thus, the present study was carried out to investigate the effect of supplementing plant derived vitamin $D_3$ metabolites at graded concentration in diet containing suboptimal levels of Ca and NPP on performance and bone mineralization in commercial broiler chicken.

## Materials and Methods

The plant derived 1,25 $(OH)_2$ $D_3$ (PD metabolite) and synthetic cholecalciferol (CC) used in the present investigation was procured from Indian Herbs Specialties Private Limited (India) and DSM India private Limited, respectively. Two hundred and fifty, day old commercial broiler male chicks were randomly allotted to 50 replicates with 5 chicks in each and reared under uniform management conditions from day one through 42 d of age. The replicates were randomly allotted to 5 treatments with 10 replicates in each. All replicates were housed in electrically heated battery cages having raised wire floors and fitted with feeder, water and a droppings tray underneath. Birds were immunized for Newcastle disease at 7$^{th}$ and 21$^{st}$ day of age with Lasota vaccine and infectious bursal disease at 14$^{th}$ day of age with Georgia strain vaccine. All replicates were offered the respective diets *ad libitum* and with clean and fresh drinking water.

A corn-soybean meal based basal diet (BD) was prepared for starter and finisher phases to meet the nutritional requirements (NRC, 1994) for commercial broiler chicken, except Ca and NPP which were kept at suboptimal levels *i.e.,* 0.7% Ca and 0.35% NPP during starter and 0.5% Ca and 0.25% NPP during finisher phase (Table 1). The control group was BD supplemented with (synthetic source, 1800 ICU/kg) and in experimental diets the synthetic CC and plant derived 1, 25 $(OH)_2$ $D_3$ were in ratio of 75:25 (1200:45 ICU/kg diet), 50:50 (900:90 ICU/kg diet), 25:75 (450:135 ICU/kg diet) and 0:100% (0:180 ICU/kg diet) *i.e.,* plant derived 1,25 $(OH)_2$ $D_3$ was replacing cholecalciferol at 25, 50 and 75% respectively. The appropriate quantity of plant derived vitamin $D_3$ metabolite was added considering its $D_3$ activity to be 10 times potent than cholecalciferol.

The body weight and feed intake of each bird was recorded at weekly intervals to arrive at the weight gain, feed intake and feed conversion ratio (FCR) (feed intake/ weight gain). On 35$^{th}$ day, blood samples were collected from one bird per replicate and serum was separated and stored at -20$^0$C for estimation of Ca (Gitelman, 1967) and P (Fiske and Subbarow, 1925). At 42 days of age, 1 bird from each replicate was randomly selected and slaughtered by cervical dislocation. Both the tibiae were freed from soft tissue. The dried (100$^o$C/3h) bone samples were defattened by extracting with petroleum ether for 48 h and its weight was measured. The breaking strength of the bone was measured using Universal testing machine (Kalpak Tech services**)** with 5 cm gauge length and 5cm/min load cell speed. After estimating the bone strength, both tibiae of each bird were ashed together (600±20$^0$C/6 h) in a microwave muffle furnace (BR, 6000521, Phoenix) to estimate the bone ash content and then mineral extract was prepared to determine the Ca (Atomic Absorption Spectrophotometer, Elmer Analyst 100) and P (Fiske and Subbarow, 1925) concentrations in bone ash.

After growth trial (42 d), 3 days metabolic trial involving total feces collection was conducted on one bird per replicate to determine the Ca and P retention. During metabolic trial, daily feed intake and faeces voided was recorded. Representative samples of feed offered, residue left and total faeces voided were analyzed for dry matter, Ca (AOAC, 2012) and total P concentrations (Fiske and Subbarow, 1925).

The data was subjected to one way analysis of variance (Snedecor and Cochran, 1994). The differences between the means were compared using Duncan's multiple range test (Duncan, 1955).

## Results and Discussion

### Growth performance

Dietary replacement of vitamin $D_3$ supplementation from synthetic source to plant derived 1, 25$(OH)_2$ $D_3$ at 25, 50, 75 or 100% level had no influence on body weight gain, feed intake and feed conversion ratio (FCR) and they were comparable with control group (vitamin $D_3$ solely from synthetic source) (Table 1). The lack of response among the birds on performance due to dietary replacement of synthetic CC with plant derived active 1, 25$(OH)_2$ $D_3$ (PD metabolite) might be due to the adequacy of Ca and NPP levels present in the BD and also due to the fact that the level of CC in the BD (1800 ICU/kg diet) might have supported the birds growth. Rama Rao *et al.,* (2006) observed no significant difference in broilers performance (weight gain and FCR) supplemented with suboptimal levels of Ca and P (0.5 and 0.25%, respectively) along with either 2400 or 3600 ICU CC/kg diet. Similarly, Garcia *et al.,* (2013) [25(OH) $D_3$ and 1,25$(OH)_2$ $D_3$] and Landy *et al.,* (2014) [1α(OH) $D_3$] observed no significant difference in broiler performance with dietary replacement of vitamin $D_3$ with their active metabolites.

### Bone variables, serum Ca and P concentrations and Ca and P retention

Bone strength, ash percentage and Ca content in bone ash significantly ($P<0.05$) improved with replacement of CC with PD metabolite (Table 2) but level of replacement (25, 50, 75 or 100%) had no influence on these parameters. Bone weight significantly ($P<0.05$) improved with replacement of CC at 25, 50 and 75% with PD metabolite compared to control (100% CC) while at 100% replacement, bone weight was statistically comparable with control and other dietary treatments (Table 2).

**Table.1** Effect of supplementing plant derived 1, 25 dihydroxy cholecalciferol on performance of broilers (0-6 weeks)

| Diet | Body weight gain (g) | | | Feed intake (g) | | | Feed conversion ratio (g intake/g gain) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Starter (0-3wk) | Finisher (4-6wk) | Total (0-6 wk) | Starter (0-3wk) | Finisher (4-6wk) | Total (0-6wk) | Starter (0-3wk) | Finisher (4-6wk) | Total (0-6 wk) |
| BD+100% CC (control) | 743.6 | 1516 | 2260 | 949.1 | 2678 | 3628 | 1.28 | 1.77 | 1.60 |
| BD+75% CC+25% PD-1,25$(OH)_2D_3$ | 721.0 | 1494 | 2215 | 909.4 | 2593 | 3503 | 1.26 | 1.74 | 1.58 |
| BD+50% CC+50% PD-1,25$(OH)_2D_3$ | 719.8 | 1524 | 2243 | 913.9 | 2766 | 3680 | 1.27 | 1.82 | 1.64 |
| BD+25% CC+75% PD-1,25$(OH)_2D_3$ | 748.2 | 1514 | 2263 | 947.6 | 2777 | 3725 | 1.27 | 1.83 | 1.65 |
| BD+100% PD-1,25$(OH)_2D_3$ | 743.0 | 1535 | 2278 | 944.6 | 2786 | 3731 | 1.27 | 1.82 | 1.64 |
| SEM | 7.39 | 12.40 | 16.20 | 8.65 | 30.30 | 35.44 | 0.006 | 0.015 | 0.012 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| P value | 0.624 | 0.890 | 0.792 | 0.404 | 0.199 | 0.232 | 0.965 | 0.215 | 0.224 |

Each pen is a replicate of 5 chicks, SEM: Standard Error Mean
CC: Synthetic cholecalciferol, PD-1,25$(OH)_2D_3$- Plant derived 1,25$(OH)_2D_3$

**Table.2** Effect of supplementing plant derived 1,25 dihydroxy cholecalciferol on serum Ca and P contents, bone variables and Ca and P retention in broilers

| Diet | Serum | | Bone variables | | | | | Retention | |
|---|---|---|---|---|---|---|---|---|---|
| | Ca (mg/dl) | P (mg/dl) | Weight (g) | Strength (N) | Ash (%) | Ca (%) | P (%) | Ca (%) | P (%) |
| BD+100% CC (control) | 9.29 | 4.41 | 4.77$^{b}$ | 49.54$^{b}$ | 47.75$^{b}$ | 33.70$^{b}$ | 16.75$^{b}$ | 54.51 | 42.84$^{b}$ |
| BD+75% CC+25% PD-1,25$(OH)_2D_3$ | 9.65 | 4.62 | 5.65$^{a}$ | 65.14$^{a}$ | 52.56$^{a}$ | 36.60$^{a}$ | 18.29$^{a}$ | 54.51 | 47.96$^{a}$ |
| BD+50% CC+50% PD-1,25$(OH)_2D_3$ | 9.48 | 4.73 | 5.68$^{a}$ | 67.46$^{a}$ | 50.34$^{a}$ | 36.20$^{a}$ | 17.50$^{ab}$ | 53.46 | 46.76$^{a}$ |
| BD+25% CC+75% PD-1,25$(OH)_2D_3$ | 9.78 | 4.71 | 5.73$^{a}$ | 69.24$^{a}$ | 51.34$^{a}$ | 36.50$^{a}$ | 18.35$^{a}$ | 55.87 | 48.75$^{a}$ |
| BD+100% PD-1,25$(OH)_2D_3$ | 9.53 | 4.27 | 5.34$^{ab}$ | 63.67$^{a}$ | 50.80$^{a}$ | 36.05$^{a}$ | 17.62$^{ab}$ | 54.57 | 48.75$^{a}$ |
| SEM | 0.42 | 0.12 | 0.12 | 2.22 | 0.42 | 0.29 | 0.15 | 0.47 | 0.54 |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| P value | 0.930 | 0.723 | 0.053 | 0.033 | 0.003 | 0.004 | 0.001 | 0.638 | 0.001 |

Each pen is a replicate of 5 chicks, SEM: Standard Error Mean

$^{ab}$ Means with different superscript in a row differ significantly: $P<0.05$, $P<0.01$

CC: Synthetic cholecalciferol, PD-1,25$(OH)_2D_3$: Plant derived 1,25$(OH)_2D_3$

Similarly, P content in bone ash improved with replacement of CC with PD metabolite but significant improvement was observed at 25 and 75% replacement (Table 2). Replacing CC with PD metabolite enhanced retention of P, but the improvement (P<0.05) was independent of level of inclusion. The Ca and P concentrations in serum and Ca retention were comparable among the dietary treatments (Table 2).

Rama Rao *et al.,* (2006) predicted the requirement of CC for maximum tibia ash as 3485 ICU/kg, which was higher than the requirement predicted for weight gain and FCR (3182 ICU/kg) in chicks fed diets containing suboptimal levels of Ca and P (0.5% Ca and 0.25% NPP). Similarly, Sun *et al.,* (2013) observed increased tibial ash percentage and strength in broiler with vitamin $D_3$ supplementation in dose dependent manner (200, 2000 and 4000 ICU/kg). Rama Rao *et al.,* (2007a and b) noticed increase (P<0.05) in tibial ash content with increase in the levels of CC from 200 to 3600 ICU/kg in broiler diet. Similarly, in the present study, dietary replacement of synthetic CC with PD metabolite significantly (P<0.05) increased bone ash, calcium and phosphorus content (Table 2). This could be due to higher bioavailability of 1, 25$(OH)_2$ $D_3$ compared to synthetic CC, as CC has to be converted into biological active form (1,25 $(OH)_2D_3$) in the body to produce action and this conversion in not 100% in biological system (Goodgame *et al.,* 2011). In concurrence to our results, Papesova *et al.,* (2008) observed significant (P<0.05) improvement in bone Ca and P contents in bone with partial replacement of vitamin $D_3$ with 25 (OH) $D_3$ in broilers. Similarly, Gómez-Verduzco *et al.,* (2013) observed improvement (P<0.05) in bone calcification of broiler chicken with supplementation of vitamin $D_3$ in combination of CC and 25 (OH) $D_3$. Supplementation of 0.25% *Cestrum dirunum* (Chennaiah *et al.,* 2007), 10 µg/kg of *Solanum glaucophyllum* (Bachmann *et al.,* 2013) or 5 µg/kg *Solanum glaucophyllum* (Cheng *et al.,* 2004) improved the bone variables in broilers compared to basal or control diet.

The P retention improved (P<0.05) with dietary substitution of synthetic CC with PD metabolites, irrespective of level of inclusion. The enhanced P utilization with PD 1, 25 $(OH)_2$ $D_3$ could be the effective stimulation of intestinal phytase activity (Hardy and Edwards, 1993; Applegate *et al.,* 2003) and its deposition in bones (Table 2). Similarly, Edwards (2002) observed significant (P<0.01) improvement in P retention in broiler chicken with 1, 25 $(OH)_2$ $D_3$ supplementation compared to addition of CC. Increased (P<0.05) Ca and P levels in the bone (mineralization), consequently increased (P<0.05) the bone weight and strength with substitution of CC with plant derived 1, 25 $(OH)_2$ $D_3$ with no effect of level of replacement.

The results of the study indicated that supplementing plant derived 1, 25 $(OH)_2$ $D_3$ as source of vitamin $D_3$ replacing synthetic CC to diets with suboptimal levels of Ca and NPP improved bone weight, strength, ash, Ca and P contents in bone ash, with no effect on performance in broilers, but replacing CC beyond 25% by PD 1, 25 $(OH)_2$ $D_3$ had no further improvement.

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