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Effect of Supplemental 25-Hydroxycholecalciferol on Live Performance, Bone Development, and Mineral Utilization of Broiler Chickens Fed Low Dietary Ca and P

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Abstract

The 25-hydroxycholecalciferol (25-OH-D_3) is a metabolite of vitamin D_3 which is required by the chicken for proper Ca and P metabolism and bone development. An experiment was conducted to investigate the effect of 25-OH-D_3 on growth performance, bone growth and mineralization, and apparent mineral utilization of broiler chicks fed low dietary Ca and P. A total of 250 1-d-old male broiler chicks (Ross × Ross) were randomly allotted to 5 dietary treatments with 10 replicate battery cages per treatment (5 birds/replicate cage). Five dietary treatments were: a corn-wheat-soybean meal based diet with adequate Ca and available P (avP) (1% and 0.5%) plus 200 IU/kg 25-OH-D_3 as positive control (PC); low Ca and avP (0.5% and 0.25%) plus 25-OH-D_3 200 IU/kg as negative control (NC); low Ca and avP plus 2,760 IU/kg 25-OH-D_3 (HY-D1); low Ca and avP plus 5,000 IU/kg 25-OH-D_3 (HY-D2); and low Ca and avP plus 10,000 IU/kg 25-OH-D_3 (HY-D3). Results showed that body weight gain was significantly ($P < 0.05$) increased in PC, HY-D1 and HY-D2 groups compared to NC and HY-D3 groups, but feed intake was significantly lower in NC than PC and HY-D2 groups of broiler chicks. In addition, feed conversion was significantly ($P < 0.05$) improved in NC and HY-D1 than PC; however, dietary supplementation of 25-OH-D_3 did not affect on livability of broiler chicks. The bone mineral density (BMD) and the bone mineral content (BMC) from both femur and tibia of broiler chicks were significantly ($P < 0.05$) higher in PC, HY-D2 and HY-D3 compared to NC and HY-D1 treatments; however, bone area from both femur and tibia of broiler chicks was significantly improved in only HY-D2 among the treatment groups. At d 14, dietary supplementation of 25-OH-D_3 did not affect on apparent P utilization, but higher apparent Ca utilization was found when birds were fed HY-D2. At d 21, the apparent Ca utilization was significantly higher in NC, HY-D2 and HY-D3 groups compared to PC, and the apparent P utilization was also greater in HY-D1, HY-D2 and HY-D3 groups compared to NC and PC. It is concluded that dietary supplementation of 25-OH-D_3 improves growth performance, BMD, BMC and area of the bone, and apparent mineral utilization of broiler chicks fed low Ca and P, where 5,000 IU/kg 25-OH-D_3 is the best supplementation level in this regard.

Keywords: *25-Hydroxycholecalciferol; Live Performance; Bone Mineralization; Ca and P Utilization; Broiler Chickens*

Introduction

Vitamin D is an important fat soluble vitamin which is required for the chicken for proper metabolism of Ca and P in the function of normal bony skeleton, hard beaks and claws, and strong eggshell for laying hens. The deficiency of this vitamin in chick's causes rickets, a severe weakness of the legs, low feathering, poor bone mineralization and reduced the structural integrity of adult chickens, and tibial

dyschondroplasia in commercial broiler chicks [1,2]. Tibial dyschondroplasia (TD) is a major leg problem in poultry, resulting from accumulation of chondrocytes along the growth plates, histologically distinguishable by the plug of avascular cartilage which forms beneath the growth plates, causing bone abnormalities and reducing skeletal integrity [3,4]. Due to genetic improvement, the commercial broilers are growing fast and producing a high carcass yield. As the production increases, energy rich grain based diets were fed to broilers in a confined environment and the incidence of bone disorders enhanced by imbalance between meat production and skeletal growth of the birds. Bone disorders are important welfare and economic issues in the broiler industry causes about 15% mortality in the flock and estimated losses $ 120 million/year [5-7]. In addition, low Ca:P or vitamin D deficient diets provided to the broiler chicks has been hypothesized as a consequence of TD that can partially remove by inducing the maturation of immature chondrocytes through feeding vitamin D_3 or some of its metabolites [8,9]. In addition to the vitamin D_3 status of bird, lack of chondrocyte differentiation is affected by the levels of Ca and P in the diets of broiler chickens [10].

On the other hand, mineral excretion especially the P excretion to the environment from the poultry barn is a challenge in order to maintain a clean environment. Inadequate utilization of dietary P will lead to increase the P load in the poultry manure. It has been reported that dietary supplementation of vitamin D_3 reduced the incidence of leg problem, increased the utilization of phytate phosphorus (PP), and retention of Ca and P [9,11]. Previous research with broilers [12] showed that the utilization of Ca, P and PP were improved when dietary vitamin D_3 increased from 1100 to 8800 IU/kg based on increased tibia ash from 29.1 to 34.2%. An important factor may influence the vitamin D_3 requirements of poultry is the Ca and P content and the ratio of Ca to P in the diets. The vitamin D_3 requirements would vary from 200 to 1600 IU/kg under normal to sub optimal Ca and P ratios [13]. A diet deficient in P fed to three weeks old broiler chickens showed that high levels of vitamin D_3 was required to produce optimum growth and bone ash content [14]. It has been reported that higher levels of vitamin D_3 or suboptimal levels of P in the diet enhanced the intestinal mucosal phytase activity [15]; however, the endogenous enzyme activity decreased when broiler chickens fed normal levels of dietary Ca [16]. Ledwaba and Roberson [8] reported that PP retention was improved when low Ca content diet was fed to the broilers, but it was not improved when Ca was fed at 0.85% or higher in the starter diets. However Bar., *et al*. [17] reported that dietary supplementation of 25-hydroxycholecalciferol (25-OH-D_3) restrained the effect of moderate dietary P restriction, but not of Ca restriction on growth performance and bone ash content in broiler chickens.

Recently 25-OH-D_3, a metabolite of vitamin D_3 has received more attention in feed industry due to higher bioavailability and potential benefit for bone mineralization in poultry [18,19]. The metabolite is formulated in a stable form which is safe and approved for use in the poultry feed industry. It is hypothesized that the supplementation of 25-OH-D_3 to broiler diets with low Ca and P may maintain the structural integrity and increase the mineral retention, resulting lower P loading through excreta to the environment. Bar., *et al*. [18] reported that the intestinal absorption of 25-OH-D_3 was better than vitamin D_3 and considered to be an efficient nutrient to vitamin D_3 deficient poultry diets. The current vitamin D_3 requirement for broiler chicks is 200 IU/kg [20]; however, commercial broiler diets are typically fortified with 10 to 20 times above the NRC values. Over recent years there has been a growing interest in the exact requirement of vitamin D for poultry and hence several metabolites of vitamin D have been the subject of more research. Moreover, the feeding of vitamin D metabolites leads to higher availability compared to vitamin D_3 [19], thereby reducing mineral excretion [21] and therefore, minimizing the environmental contamination. Biehl and Baker [21] reported that Ca and P utilization were increased in chicks fed 25-OH-D_3 compared to vitamin D_3. Fritts and Waldroup [22] demonstrated that 25-OH-D_3 was more metabolically potent on a per unit basis than vitamin D_3 for supporting body weight (BW) and tibia ash content in broiler chickens. In addition, vitamin D_3 metabolites were effective for reducing leg disorders and enhancing the utilization of pp and trace minerals when supplemented to poultry diets [23]. Therefore, a study was conducted to evaluate the effect of 25-OH-D_3 on growth performance, bone mineralization, and mineral utilization of broiler chicks fed low dietary Ca and avP.

Materials and Methods

Experimental design, birds and management

A total of 250 1-d-old male broiler chicks (Ross × Ross 308) were randomly allotted to 5 dietary treatments with 10 replicate pens per treatment (5 birds/replicate pen). Five dietary treatments were: a corn-wheat-soybean meal based diet with adequate Ca and avP

(1% and 0.5%) plus 25-OH-D_3 200 IU/kg as positive control (PC); low Ca and avP (0.5% and 0.25%) plus 25-OH-D_3 200 IU/kg as negative control (NC); low Ca and avP plus 25-OH-D_3 2760 IU/kg (HY-D1); low Ca and avP plus 25-OH-D_3 5000 IU/kg (HY-D 2); and low Ca and avP plus 25-OH-D_3 10000 IU/kg (HY-D 3). Birds were fed commercial starter diet for the first 4d and experimental diets were fed *ad libitum* from 5 to 21d of age. The levels of dicalcium phosphate, calcium carbonate and corn were adjusted to prepared two different levels of Ca: avP of the experimental diets (Table 1). All chicks were raised in the multistoried battery brooders situated in a windowless room with proper ventilation. The initial room temperature was 30°C, and reduced by 3°C each week until 21d of age. Birds were allowed free access to feed and water throughout the feeding period. Continuous lighting was provided throughout the experimental period. All procedures were approved by the Canadian Council on Animal Care (2012).

Ingredients	Treatments[1]				
	PC	NC	HY-D 1	HY-D 2	HY-D 3
Wheat	37.59	44.11	44.11	44.11	44.11
Corn	21.86	21.0	21.0	21.0	21.0
Soyabean meal	25.8	26.0	26.0	26.0	26.0
Rape seed (Black)	4.2	1.5	1.5	1.5	1.5
Canola oil	5.4	4.0	4.0	4.0	4.0
Calcium carbonate	1.25	0.7	0.7	0.7	0.7
Di-calcium phosphate	1.91	0.6	0.6	0.6	0.6
DL-Methionine	0.115	0.12	0.12	0.12	0.12
L-Lysine	0.035	0.07	0.07	0.07	0.07
Threonine	0.04	0.1	0.1	0.1	0.1
Mineral premix[2]	0.5	0.5	0.5	0.5	0.5
Vitamin premix[3]	1.0	1.0	1.0	1.0	1.0
Marker (Cr_2O_3)	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100
25-OH-Vit D_3	200 IU/kg	200 IU/kg	2760 IU/kg	5000 IU/kg	10000 IU/kg
Calculated composition					
CP, %	21.00	21.04	21.04	21.04	21.04
ME, Kcal/Kg	3095.22	3103.58	3103.58	3103.58	3103.58
Ca, %	1.00	0.50	0.50	0.50	0.50
Avail-P, %	0.50	0.25	0.25	0.25	0.25
Lysine, %	1.17	1.14	1.14	1.14	1.14
Methionine, %	0.50	0.502	0.502	0.502	0.502
Analyzed composition					
CP,%	21.53	21.91	22.55	22.17	22.36
Ca, %	1.154	0.580	0.615	0.609	0.564
P, %	0.86	0.45	0.53	0.54	0.52

Table 1: *Ingredients and composition of experimental diets (as-fed basis, %).*

[1]PC, positive control contained adequate Ca: P (1:0.5) plus HY-D 200 IU/kg; NC, negative control contained low Ca: P (0.5:0.25) plus HY-D 200 IU/kg; HY-D 1, low Ca: P plus HY-D (2760 IU/kg); HY-D2, low Ca: P plus HY-D (5000 IU/kg); HY-D3, low Ca: P plus HY-D (10000 IU/kg).

[2]Mineral premix supplied per kg of complete feed: manganese oxide, 70 mg; zinc oxide 80mg, ferrous sulfate, 80 mg, copper sulfate, 10 mg; sodium selenium, 0.3 mg; calcium iodate premix, 0.5 mg.

[3]Vitamin premix supplied per kilogram of complete feed: vitamin A, 8250 IU; vitamin E, 30 IU; vitamin B_{12}, 0.013mg; vitamin K_3, 2.0 mg; niacin, 23.6 mg; choline chloride, 1081 mg; folic acid, 4.0 mg; biotin, 0.25 mg; pyridoxine, 4.0 mg; thiamine,4.0 mg.

Growth performance

The BW and feed intake were measured weekly by pen. Feed conversion was calculated as the feed to gain ratio. The BW gain, feed intake, and feed conversion were adjusted for dead birds. The livability of bird was recorded daily and calculated as percentage within the pen.

Sample collection

On d 14 and d 21, excreta samples from each pen were collected, mixed, homogenized, dried in an oven and stored for Ca and avP analysis. At the termination of the feeding trial, one bird close to the mean BW were selected from each pen and killed by cervical dislocation. The left tibia and femur were dissected from the carcass and stored in refrigerator at 4°C for bone mineral analysis.

Determination of bone mineralization

Bone mineral density (BMD), bone mineral content (BMC) and area of the femur and tibia bone were measured using dual energy x-ray absorptiometry (pDEXA®, Bone Densitometer, Norland Medical System, Inc. WI, USA). Scanning was performed across each surface of the bone. All scans were obtained at a scan speed of 2.5 mm/s, with a voxel resolution of 0.07 × 0.07 × 0.50 mm.

Measurement of Ca, P, and Cr

One gram (g) of each diet and 0.5g of excreta samples were dried at 105°C and then placed into a muffle furnace at 600°C for overnight, and the resulting ash was dissolved in 1% HNO_3 and 5N HCl. Then the samples were boiled in a sonication bath at the temperature of 70°C for one hour and cooled at room temperature. For Cr analysis, the ash sample was dissolved in 85% ortho-phosphoric acid and 4.5% potassium bromate, boiled on a hot plate, and then cooled at room temperature. The digested samples were transferred into 100-mL volumetric flasks and diluted to volume using double-deionized water. The samples were shaken thoroughly by hand and filtered using Q5 filter paper (Whatman Ltd. Kent, UK). An inductively coupled plasma optical emission spectrometer (Varian ICP, VISTA MPX, CCD Simultaneous, USA) was employed for the analysis of Ca, P and Cr in the diets and feces according to AOAC [24].

Calculation of apparent minerals (P and Ca) utilization

The apparent P utilization (APU) was calculated according to the following equation [25]:

$$APU\ (\%) = 100 - [(Cr_I/Cr_0) \times (P_0/P_I) \times 100]$$

Where Cr_I is the chromium content in the dietary intake, Cr_0 is the chromium content in fecal output, P_0 is the P content in fecal output, and P_I is the P content in the dietary intake. Apparent Ca utilization was also calculated by using the above equation with some modification.

Statistical analysis

All data from the experiment were subjected to a one way ANOVA as a completely randomized design using the General Linear Models procedure of SAS (SAS Institute Inc., Cary, NC). Significant differences among the means were determined using Duncan's multiple-range test at $P < 0.05$.

Results

The BW gain was significantly ($P < 0.05$) increased in PC, HY-D1 and HY-D2 groups compared to NC and HY-D3 groups, but feed intake was significantly lower in NC than PC and HY-D2 groups of broiler chicks (Table 2). In addition, feed conversion was significantly ($P < 0.05$) improved in NC and HY-D1 than PC; however, dietary supplementation of 25-OH-D_3 did not affect on the livability of broiler chicks. The bone mineral density (BMD) and the bone mineral content (BMC) from both femur and tibia of broiler chicks were significantly ($P < 0.05$) higher in PC, HY-D2 and HY-D3 compared to NC and HY-D1 treatments; however, bone area from both femur and tibia of broiler chicks was

significantly improved in only HY-D2 among the treatment groups (Table 3). At d-14, dietary supplementation of 25-OH-D_3 did not affect on apparent P utilization, but higher apparent Ca utilization was found when birds were fed HY-D2 (Figure 1 and 2). At d 21, the apparent Ca utilization was significantly higher in NC, HY-D2 and HY-D3 groups compared to PC (Figure 3) and the apparent P utilization was also greater in HY-D1, HY-D2 and HY-D3 groups compared to NC and PC (Figure 4).

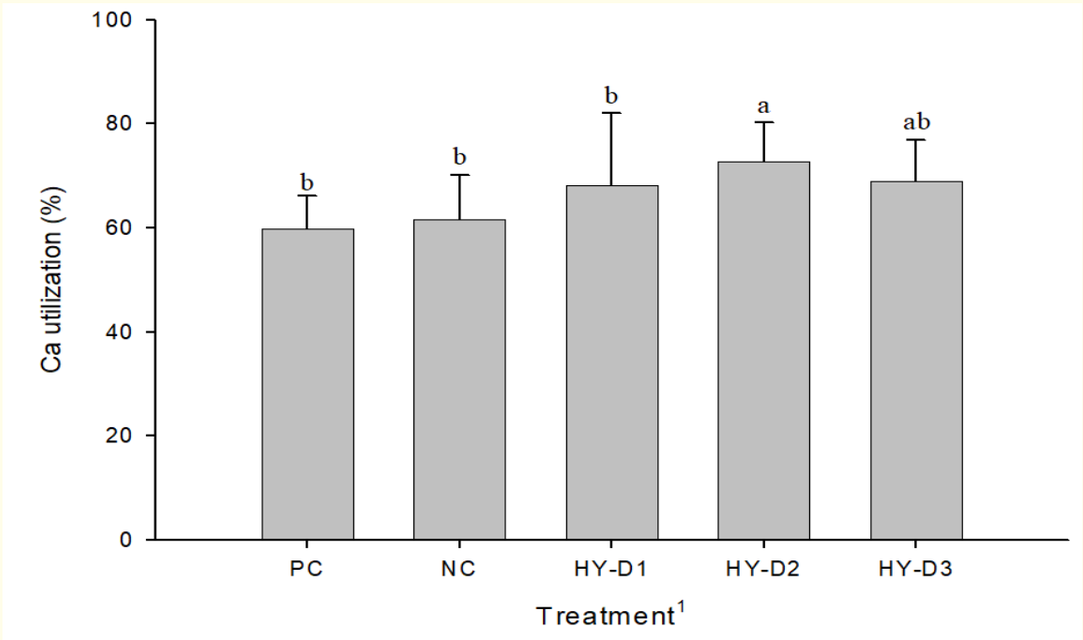

Figure 1: *Effect of 25 hydroxycholecalciferol on apparent Ca utilization of broiler chicks fed low Ca and P at 14 days of age. 1PC, positive control contained adequate Ca: P (1:0.5) plus HY-D 200 IU/kg; NC, negative control contained low Ca: P (0.5:0.25) plus HY-D 200 IU/kg; HY-D 1, low Ca: P plus HY-D (2760 IU/kg); HY-D 2, low Ca: P plus HY-D (5000 IU/kg); HY-D 3, low Ca: P plus HY-D (10000 IU/kg). Bars with different letters (a-b) differ significantly (P < 0.05, n = 10).*

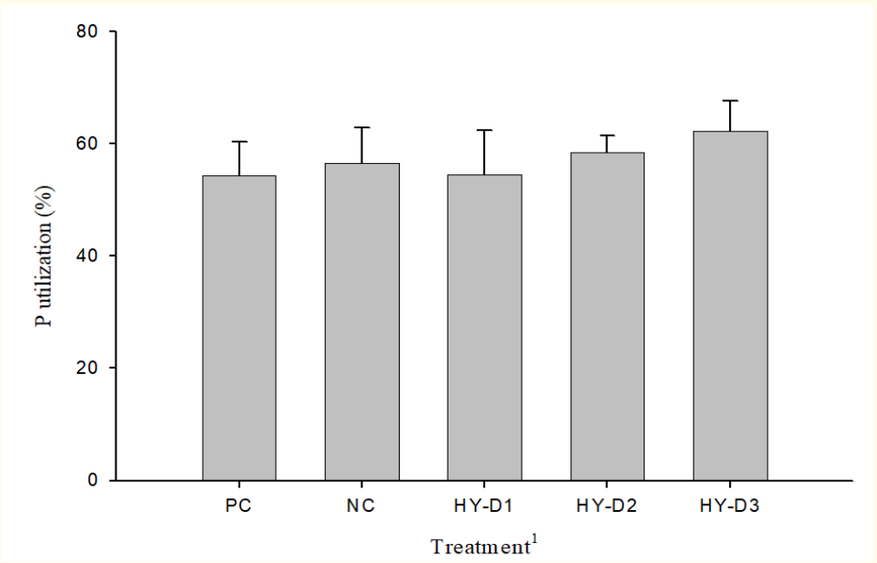

Figure 2: *Effect of 25 hydroxycholecalciferol on apparent P utilization of broiler chicks fed low Ca and P at 14 days of age. 1PC, positive control contained adequate Ca: P (1:0.5) plus HY-D 200 IU/kg; NC, negative control contained low Ca: P (0.5:0.25) plus HY-D 200 IU/kg; HY-D 1, low Ca: P plus HY-D (2760 IU/kg); HY-D 2, low Ca: P plus HY-D (5000 IU/kg); HY-D 3, low Ca: P plus HY-D (10000 IU/kg). (P < 0.05, n = 10).*

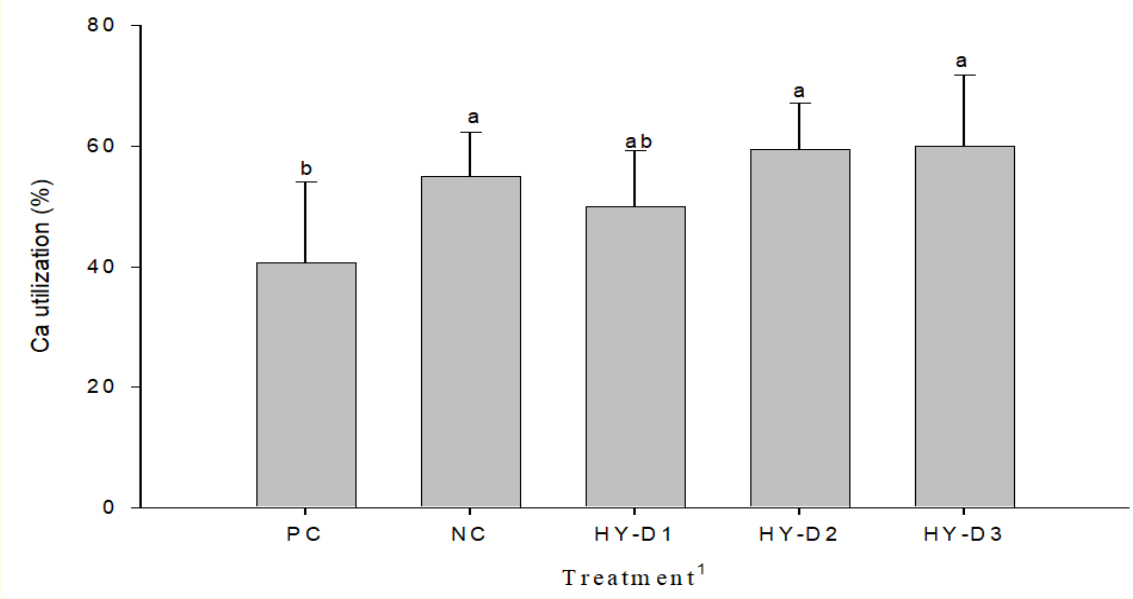

Figure 3: *Effect of 25 hydroxycholecalciferol on apparent Ca utilization of broiler chicks fed low Ca and P at 21 days of age. 1PC, positive control contained adequate Ca: P (1:0.5) plus HY-D 200 IU/kg; NC, negative control contained low Ca: P (0.5:0.25) plus HY-D 200 IU/kg; HY-D 1, low Ca: P plus HY-D (2760 IU/kg); HY-D 2, low Ca: P plus HY-D (5000 IU/kg); HY-D 3, low Ca: P plus HY-D (10000 IU/kg). Bars with different letters (a-b) differ significantly (P < 0.05, n = 10).*

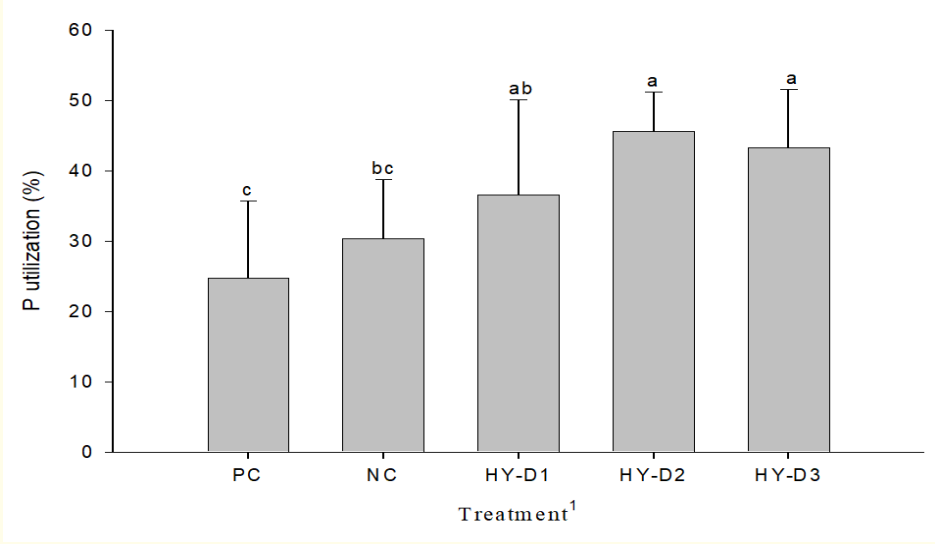

Figure 4: *Effect of 25 hydroxycholecalciferol on apparent P utilization of broiler chicks fed low Ca and P at 21 days of age. 1PC, positive control contained adequate Ca: P (1:0.5) plus HY-D 200 IU/kg; NC, negative control contained low Ca: P (0.5:0.25) plus HY-D3 200 IU/kg; HY-D3 1, low Ca: P plus HY-D (2760 IU/kg); HY-D 2, low Ca: P plus HY-D (5000 IU/kg); HY-D 3, low Ca: P plus HY-D (10000 IU/kg). Bars with different letters (a-c) differ significantly (P < 0.05, n = 10).*

Growth performance	Treatments[1]					SEM
	PC	NC	HY-D 1	HY-D 2	HY-D 3	
BW at d 4 (g/bird)	81.80	81.59	81.32	81.71	81.62	0.182
BW gain (g/bird)	733.1^{a}	656.6^{b}	712.9^{a}	720.3^{a}	642.1^{b}	8.829
Feed intake (g/bird)	944.4^{a}	720.8^{c}	799.4^{bc}	845.8^{b}	753.5^{bc}	17.87
Feed conversion (feed/gain)	1.288^{a}	1.100^{b}	1.123^{b}	1.175^{ab}	1.178^{ab}	0.020
Livability (%)	96.00	92.00	98.00	94.00	96.00	1.518

Table 2: *Effect of 25 hydroxycholecalciferol on growth performance of broiler chicks fed low dietary Ca and P.*

[1]PC, positive control contained adequate Ca: P (1:0.5) plus HY-D 200 IU/kg; NC, negative control contained low Ca: P (0.5:0.25) plus HY-D 200 IU/kg; HY-D 1, low Ca: P plus HY-D (2760 IU/kg); HY-D$_3$2, low Ca: P plus HY-D (5000 IU/kg); HY-D 3, low Ca: P plus HY-D (10000 IU/kg).

$^{a, b, c}$ Means with different superscripts within a column differ significantly (P < 0.05).

SEM: Standard Error of the Mean.

Treatments[1]	Femur			Tibia		
	BMD (g/sq.cm)	BMC (g)	Bone area (Sq. cm)	BMD (g/sq.cm)	BMC (g)	Bone area (Sq. cm)
PC	0.141^{a}	0.880^{a}	6.220^{b}	0.152^{a}	1.302^{a}	8.495^{b}
NC	0.108^{c}	0.662^{d}	6.093^{b}	0.114^{d}	0.946^{d}	8.300^{b}
HY-D 1	0.115^{c}	0.725^{cd}	6.271^{b}	0.119^{cd}	1.021^{cd}	8.504^{b}
HY-D 2	0.129^{b}	0.864^{ab}	6.679^{a}	0.135^{b}	1.242^{ab}	9.168^{a}
HY-D 3	0.125^{b}	0.788^{bc}	6.286^{b}	0.131^{bc}	1.116^{bc}	8.512^{b}
SEM	0.002	0.018	0.062	0.003	0.030	0.092

Table 3: *Effect of 25 hydroxycholecalciferol on bone mineral density (BMD), bone mineral content (BMC) and bone area of broiler chicks fed low dietary Ca and P.*

[1]PC, positive control contained adequate Ca: P (1:0.5) plus HY-D 200 IU/kg; NC, negative control contained low Ca: P (0.5:0.25) plus HY-D 200 IU/kg; HY-D 1, low Ca: P plus HY-D (2760 IU/kg); HY-D 2, low Ca: P plus HY-D (5000 IU/kg); HY-D 3, low Ca: P plus HY-D (10000 IU/kg).

$^{a, b, c, d}$ Means with different superscripts within a column differ significantly (P < 0.05).

SEM, standard error of the mean.

Discussion and Conclusion

Vitamin D$_3$ is required by the chicks for mineral utilization, skeletal development, and growth performance of broiler chicks; however, several metabolites of this nutrient have been reported to higher biological efficacy for optimum performance of birds [12,21]. The biologically active form of vitamin D$_3$ firstly takes place in the liver as 25-OH-D$_3$ and secondly in the kidney to produce 1, 25-OH-D$_3$ which are more bioavailable to the birds [26]. The intestinal absorption of these metabolites was higher in chicks and considered to be an efficient nutrient compared with vitamin D$_3$ [18]. It was reported that 25-OH-D$_3$ could be safely used in replacement of vitamin D$_3$ to the diets of broiler chicks with improved BW and feed efficiency [27,28]. In the current experiment, the dietary supplementation of 25-OH-D$_3$ significantly improved BW gain when birds were fed low dietary Ca and P; however, dietary 25-OH-D$_3$ lowered the feed intake of birds, resulting in an improvement of feed efficiency. This result was in agreement with those reported by Bar., *et al*. [17] and Angel., *et al* [29]. Bar., *et al*. [17] conducted three experiments in chicks fed 25-OH-D$_3$ under adequate Ca and P supplementation, and moderate dietary restriction of Ca and P. The author reported that in one out of the three experiments, 25-OH-D$_3$ increased BW gain and restrained the effect of moderate dietary P restriction, but not of Ca restriction on BW gain and bone ash content of broiler chicks. In addition, Fritts and Waldroup

[22] reported that dietary 25-OH-D_3 improved both BW gain and feed efficiency in broiler chickens. These findings could be explained by a higher bioavailability of Ca and P in chicks fed 25-OH-D_3 where the bone area and mineral utilization were also affected by dietary supplementation of 25-OH-D_3 activity in the present experiment [17]. It was shown that dietary 25-OH-D_3 was more metabolically potent than cholecalciferol for supporting BW and bone ash content of broiler chicks [22]. The author suggested that the use of the 25-OH-D_3 may allow for supplementation with lower levels or may provide with greater safety margin to the commercial broiler diets.

Mineral metabolism, vitamin D and its related compounds contribute to chicks quality in terms of the development of bone health and a sound skeletal structure. Especially the several metabolites of vitamin D play an important role in Ca and phosphate homeostasis in the body. The major function of these metabolites is to enhance serum Ca and phosphate concentrations by enhancing the dietary absorption of Ca and phosphate through the intestine, and stimulate the accumulation of these minerals in the bone, resulting strong the skeletal integrity of the animal [30-32]. Previous researches have shown that dietary supplementation of 25-OH-D_3 significantly reduced the incidence and severity of TD in broiler chickens [33,34]. By contrast, Roberson [35] reported that 25-hydroxycholecalciferol did not prevent TD in broiler chicks raised in battery brooders. It was assumed to be an interactive effect with total Ca and P level in the feed stuffs for these studies. However, BMD, BMC and total area of the bone are considered to reflect the exact status on bone health where the mineral matrix is the major component of the extracellular matrix of the bone. Recently, dual-energy X-ray absorptiometry (pDEXA) has shown a useful means to assess BMD, BMC and total bone area of poultry [36,37]. In the present experiment, BMD, BMC and area of the bone measured by pDEXA were significantly affected by dietary 25-OH-D_3 under adequate and restricted Ca and P levels in the broiler diets. Interestingly, dietary supplementation of 25-OH-D_3 seemed to be more effective at lower Ca and P levels in the diets which might be attributed to mineral homeostasis to maintain bone health of the birds [31]. However, higher concentration of 25-OH-D_3 did not affect on the total bone area of both femur and tibia of the broiler chicks fed adequate or low dietary Ca and P level in the present experiment except HY-D2. Therefore, the inclusion of up to 5, 000 IU/kg of 25-OH-D_3 is required in the conventional diets to improve the development of the bone and growth performance of young broiler chicks under low dietary Ca and P.

Ledwaba and Roberson [8] reported that increased tibia ash content and decreased incidence and severity of TD in broiler chicks fed increased levels of 25-OH-D_3, but this response was dependent upon the Ca level in the diet. As far our knowledge, few studies has used pDEXA to evaluate bone quality in broiler chicks fed 25-OH-D_3 under low dietary Ca and P; however, a recent study [38] reported that chicks fed vitamin D_3 had higher midshaft cortical BMC, bone thickness, bone area, and marrow area compared to the control chicks. Bar., *et al*. [17] conducted two feeding trials to evaluate the effects of 25-OH-D_3 under moderate Ca or P restriction, and found that dietary 25-OH-D_3 significantly increased the bone ash content in broiler chicks fed low P diet. Therefore, our results are in agreement with those of Bar., *et al*. [17] and Kim., *et al* [38]. However, restriction of both dietary Ca and P have been reported to increase circulating and intestinal 1,25-OH-D_3 [39,40] with consequent increases in the intestinal absorption of Ca and P, resulting strong bone integrity of birds [41-43]. Moreover, Ca and P homeostasis are maintained by the actions of vitamin D_3, parathyroid hormone (PTH) and calcitonin on the small intestine, kidneys and bone [44]. Low blood Ca level stimulates the parathyroid gland to secrete PTH which induces the kidney to produce more 1,25-OH-D_3, which in turn enhances the intestinal absorption of Ca and P, and P reabsorption from the kidney and bone [45].

Vitamin D_3 and its metabolites are crucial to Ca and P absorption and utilization and proper skeletal development, subsequently reduced mineral excretion and improved leg health in birds. An earlier study [46] indicated that the intestinal phytase and phosphatase activities increased when birds were fed increased levels of vitamin D_3 under low dietary P Mohammed., *et al*. [47] reported that the dietary cholecalciferol significantly increased phytate digestibility and the retention of Ca and P in chicks fed low Ca and P content diets. These results are in agreement with the present experiment where mineral utilization increased when birds were fed increased levels of dietary 25-OH-D_3 under low dietary Ca and P. A recent study on swine, O'Doherty., *et al*. [48] reported that dietary supplementation of 25-OH-D_3 increased Ca retention in animals fed low P diet, but P retention increased when the diet supplemented with phytase in addition to 25-OH-D_3. By contrast, Biehl., *et al*. [23] reported that 25-OH-D_3 did not affect the utilization of P in chicks fed diets adequate in vitamin D_3. In the present experiment; however, the P utilization measured at d 14 was not affected by dietary 25-OH-D_3 supplementation either adequate

or low Ca and P diets might be attributed to the PP content in the basal diets that are not fully digested by the chicks at early age [17,49]. But the increased Ca and P utilization at the later age of the birds fed low Ca and P compared to adequate Ca and P might be indicated a physiological response by the chicks to mitigate mineral deficiencies by up-regulated nutrient transfer and deposition [50]. In poultry, the metabolism of vitamin D, Ca, and P are greatly interlinked. The intestinal Ca and P absorption depend on many factors including age of the birds, PP and sources of vitamin D in the diets [51,52]. It is documented that vitamin D is required for the synthesis of Ca binding protein (CaBP) in the intestinal cells and this CaBP actively transport Ca across the intestinal epithelial wall to the plasma of the chicks [53]. In addition, the vitamin D metabolites may also facilitate an increase in Ca uptake and thus reduces the formation of a phytin complex. It has been shown that dietary supplementation of vitamin D significantly increased phytate digestibility and decreased the rickets in chicks fed low Ca and high phytate diets [52,54]. On the other hand, serum Ca and P content, bone ash, and bone strength are the main indicators of Ca and P status of birds. Though serum Ca and P level were not measured in the present experiment, but the bone mineral data are supported by the increased utilization of Ca and P in chicks fed dietary 25-OH-D_3 (Table 3). The present data also indicate that the negative effect of low dietary Ca and P on the availability of these minerals in chicks may be corrected by the increased vitamin D metabolites to escalate mineral absorption and retention, resulting reduced mineral excretion to the environment [21,47]. It is concluded that dietary supplementation of 25-OH-D_3 improves growth performance, BMD, BMC and area of the bone, and apparent total tract mineral utilization of broiler chicks fed low Ca and P, where 5,000 IU/kg 25-OH-D_3 is the best supplementation level in this regard.

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