



# Investigation of different levels of cholecalciferol and its metabolite in calcium and phosphorus deficient diets on growth performance, tibia bone ash and development of tibial dyschondroplasia in broilers

Nasir Landy<sup>\*</sup>, Farshid Kheiri, Mostafa Faghani and Ramin Bahadoran

Department of Animal Science, Shahrekord Branch, Islamic Azad University, Shahrekord 8813733395, Iran. <sup>\*</sup>Author for correspondence.  
E-mail: n\_landy1984@yahoo.com

**ABSTRACT.** This experiment was conducted to examine the effects of 1- $\alpha$ (OH) $D_3$  alone or in combination with different levels of cholecalciferol on performance, and tibia parameters of one-d-old male broilers fed a tibial dyschondroplasia (TD)-inducing diet. A total of three hundred male broilers were randomly allocated to 5 treatment groups with 4 replicates. The dietary treatments consisted of TD inducing diet, TD inducing diet supplemented with 5  $\mu$ g per kg of 1- $\alpha$ (OH) $D_3$ ; TD inducing diet supplemented with 5  $\mu$ g per kg of 1- $\alpha$ (OH) $D_3$  and 1,500; 3,000 or 5,000 IU cholecalciferol  $kg^{-1}$  of diet. At 42 d of age, broiler chickens fed diets containing 1- $\alpha$ (OH) $D_3$  and 1,500 IU cholecalciferol  $kg^{-1}$  of diet had higher body weight ( $p < 0.05$ ). In the complete experimental period the best FCR and the highest daily weight gain were obtained in broilers supplemented with 1- $\alpha$ (OH) $D_3$  and 1,500 IU cholecalciferol  $kg^{-1}$  of diet. Broilers supplemented with 1- $\alpha$ (OH) $D_3$  and 1,500 IU cholecalciferol  $kg^{-1}$  of diet had significantly lower incidence and severity of TD in comparison with other groups. In conclusion, the results indicated that the supplementation of 1- $\alpha$ (OH) $D_3$  in combination of 1,500 IU cholecalciferol  $kg^{-1}$  of diet could maximize tibia bone ash, performance and prevent TD in broilers fed TD inducing diet.

**Keywords:** bone ash; chick; phosphorus deficiency; 1- $\alpha$ (OH) $D_3$ ; vitamin  $D_3$ .

Received on July 19, 2019.  
Accepted on May 22, 2020.

## Introduction

Avian tibial dyschondroplasia (TD), a cartilage abnormality, which commonly occurs in broilers, layers, turkeys, and ducks is very sensitive to calcium (Ca)-to-phosphorus (P) ratio (Rennie, Whitehead & Thorp, 1993), and vitamin D metabolism (Ledwaba & Roberson, 2003). The major perturbation leading to TD is disruption of normal growth plate chondrocyte maturation (Farquharson & Jefferies, 2000). Whitehead, McCormack, McTeir and Fleming (2004) indicated that the supplementation of high levels of cholecalciferol could prevent TD in broiler chickens. Edwards (1990) indicated that the supplementation of a Ca-deficient diet containing 27.5 mg  $kg^{-1}$  cholecalciferol with 10 mg  $kg^{-1}$  1,25-dihydroxycholecalciferol enhanced tibia ash and reduced the incidence and severity of TD. Similarly, several previous studies indicated that the supplementation of vitamin D metabolites to broiler diet reduced the incidence and severity of TD (Roberson & Edwards, 1996; Elliot & Edwards, 1997; Nääs et al., 2012; Atencio, Pesti, & Edwards, 2005).

According to Edwards, Shirley, Escoe, and Pesti (2002), the relative biological value of one-alpha-hydroxy-cholecalciferol (1- $\alpha$ (OH) $D_3$ ), a metabolite of cholecalciferol is eight times as effective as cholecalciferol. Landy and Toghyani (2014) investigated the effect of feeding 5  $\mu$ g  $kg^{-1}$  of 1- $\alpha$ (OH) $D_3$  as a substitution for 5,000 IU cholecalciferol  $kg^{-1}$ . The results showed that 1- $\alpha$ (OH) $D_3$  can be used as a substitute for cholecalciferol. Several studies investigated the efficacy of 1- $\alpha$ (OH) $D_3$  alone or in combination of phytase where the results showed a positive interaction between 1- $\alpha$ (OH) $D_3$  and phytase (Snow, Baker, & Parsons, 2004; Driver, Pesti, Bakalli, & Edwards, 2005; Kheiri, Poshtvar, Jalali Haji Abadi, & Landy, 2019). Ghasemi, Toghyani, & Landy (2018) investigated the efficacy of 1- $\alpha$ (OH) $D_3$  alone in Ca-P deficient diets, and the results indicated that 1- $\alpha$ (OH) $D_3$  couldn't improve tibia parameters of broilers. It has been documented that 1- $\alpha$ (OH) $D_3$  was more effective in phytate phosphorus (PP) digestion in broilers fed low Ca diets (Ledwaba &

Roberson, 2003; Han et al., 2012). Landy, Toghyani, Bahadoran and Eghbalsaied (2015) reported the ability of  $1\text{-}\alpha(\text{OH})\text{D}_3$  in Ca-P deficient diets without cholecalciferol to improve tibia quality of broilers, although when cholecalciferol was adequate, tibia parameters were not improved by  $1\text{-}\alpha(\text{OH})\text{D}_3$  supplementation. Similarly, Landy and Toghyani (2018) reported that the supplementation of cholecalciferol to Ca-P deficient diets containing  $1\text{-}\alpha(\text{OH})\text{D}_3$  reduced tibia bone ash of broilers, however it could be reduced the incidence and severity of TD.

To differentiate the effect of cholecalciferol supplementation when  $1\text{-}\alpha(\text{OH})\text{D}_3$  incorporated to the diets deficient in Ca and P, the null hypothesis of no effect was used. The alternative hypothesis is that there is an effect of cholecalciferol supplementation on growth performance, and tibia quality of male broiler chickens. The current study was conducted to examine the effects of  $1\text{-}\alpha(\text{OH})\text{D}_3$  alone or in combination with different inclusion rate of cholecalciferol on growth performance, tibia bone ash, and development of tibial dyschondroplasia in broilers.

## Material and methods

### Ethical approval

Experimental procedures were conducted in accordance with the Ethical Committee of the Shahrekord University, Islamic Azad University, Shahrekord branch, Iran (License number 2017-02/18).

### Animals and dietary treatments

This experiment was conducted to evaluate the effects of  $1\text{-}\alpha(\text{OH})\text{D}_3$  individually or in combination with different levels of cholecalciferol (0, 1,500, 3,000 and 5,000 IU cholecalciferol  $\text{kg}^{-1}$  of diet) in TD inducing diets on growth performance, incidence and severity of TD of broilers. On d of hatch, a total of 300 male broiler chickens (Ross 308) were individually weighed ( $34 \pm 1$ ) and randomly allocated to 5 treatment groups, 4 replicates per pen with 15 male broiler chickens per replicate. Feed and water were provided *ad libitum*. Starter (1 to 14 d), grower (15 to 28 d) and finisher (29 to 42 d) diets were formulated to meet nutrient requirement of Ross 308 strain (Aviagen, 2014), except for Ca, P, Cl and cholecalciferol. The basal diet was low in Ca and P and high in chlorine (Cl) and the vitamin premix that included in the diets did not contain cholecalciferol (TD inducing diet). The 5 treatment groups were supplemented with 500 FTU phytase  $\text{kg}^{-1}$  of diets (Phyzyme XP 5000, Danisco Animal Nutrition). The dietary treatments consisted of basal diet (Table 1), basal diet supplemented with 5  $\mu\text{g}$  per kg of  $1\text{-}\alpha(\text{OH})\text{D}_3$  (Vitamin Derivatives Inc., Georgia; USA); basal diet supplemented with 5  $\mu\text{g}$  per kg of  $1\text{-}\alpha(\text{OH})\text{D}_3$  and 1,500; 3,000 or 5,000 IU cholecalciferol  $\text{kg}^{-1}$  of diet (BASF GmbH., Düsseldorf, Germany). The male broiler chickens were raised in cages (120 × 120 × 80 cm) with wood shaving-lined floors and room temperature was set via wire-floored battery brooders in moderate temperature rooms (33°C during first week, and gradually reduced by 3°C in the second and third weeks, and finally fixed at 22°C. The broiler house was windowless and lighting was presented by incandescent bulbs for 23h each day.

### Feed analyses

The dietary treatments were formulated after analyzing of feed ingredient (monocalcium phosphate,  $\text{CaCO}_3$ , Soybean meal, and corn), for Ca and tP contents according to standard Association Official Analytical Chemist (AOAC, 1995) procedures. Dietary Ca and tP concentration were determined by the ICPOES method 2011.14 (AOAC, 1990).

### Growth performance

The average body weights of broilers from each cage was determined and recorded at d 1, 14, 28 and at the end of the experiment (42 d) to determine average daily weight gain (DWG). Feed intake data of male broiler chickens were collected at the end of the experiment to determine daily feed intake (DFI). Mortalities recorded daily to correct DFI: DWG (FCR).

### Chemical analysis

At the conclusion of experiment, 2 male broiler chickens/cage (8 birds per treatment) were killed, right tibias were separated and pooled for the determination of tibia bone ash according to the method (22.10)

described by AOAC (1995). The left tibia was removed and evaluated for TD by making a longitudinal cut across the tibia. Each bird was scored for incidence and severity of TD as described by Edwards and Veltmann (1983).

**Table 1.** Composition of the basal diets in different growth periods.

Ingredient (g kg <sup>-1</sup> )	Starter	Grower	Finisher
Corn	575.32	570.49	605.89
Soybean meal, 45% CP	380	362	325
Soybean oil	10	36	40
DL-Methionine	3.15	3.09	3
L-Lysine	1.74	1.59	1.55
L-Threonine	1.06	0.92	0.81
Choline Chloride 60%	1.2	1.3	1.15
Monocalcium Phosphate (15 Ca, 22.5 P)	10.4	8.55	7.3
CaCO <sub>3</sub>	10.47	10.38	9.6
NaCl	3.66	3.68	3.7
Trace mineral premix <sup>2</sup>	1.25	1.25	1.25
Vitamin premix <sup>3</sup>	1.25	1.25	1.25
Calculated composition			
Metabolizable energy (kcal kg <sup>-1</sup> )	2,870	3,083	3,120
Crude protein (g kg <sup>-1</sup> )	220	212.4	196.0
Lysine (g kg <sup>-1</sup> )	13.7	12.3	11.6
Methionine + cysteine (g kg <sup>-1</sup> )	10.3	9.54	9.08
Threonine (g kg <sup>-1</sup> )	9.2	8.9	8.2
Calcium (g kg <sup>-1</sup> )	7.0	6.7	6.1
Phosphorus-total (g kg <sup>-1</sup> )	6.1	5.5	5.1
Nonphytate P (g kg <sup>-1</sup> )	3.3	2.8	2.5
Sodium (g kg <sup>-1</sup> )	1.6	1.6	1.6
Chlorine (g kg <sup>-1</sup> )	3.2	3.0	3.2
Analyzed Content			
Calcium (g kg <sup>-1</sup> )	7.2	6.8	6.2
Phosphorus-total (g kg <sup>-1</sup> )	6.2	5.8	5.3

<sup>1</sup>The dietary treatments consisted of basal diet, basal diet + 5 µg per kg of 1-α(OH)D<sub>3</sub>; basal diet supplemented with 5 µg per kg of 1-α(OH)D<sub>3</sub> and 1,500, 3,000 or 5,000 IU cholecalciferol kg<sup>-1</sup> of diet. <sup>2</sup>Provided the following per kg of diet: Mg, 96 mg; Fe, 16 mg; Cu, 12.8 mg; Zn, 88 mg; Se, 0.24 mg; I, 1.0 mg.

<sup>3</sup>Provided the following per kg of diet: vitamin A, 9,600 IU; vitamin E, 64 IU; vitamin K, 2.56 mg; Thiamin, 2.56 mg; Riboflavin, 6.8 mg; vitamin B<sub>6</sub>, 3.4; vitamin B<sub>12</sub>, 0.013 mg; pantothenic acid, 16 mg; nicotinic acid, 52 mg; folic acid, 2 mg; Biotin, 0.24 mg.

### Statistical analysis

The experimental design was completely randomized, and all obtained data were subjected to ANOVA using the General Linear Model procedures of Statistical Analysis Systems (SAS, 2012) according to the following model:

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

where  $\mu$  is the overall mean,  $T_i$  is the effect of experimental treatments and  $e_{ij}$  is the random residual error. Orthogonal comparisons were performed to determine the linear and quadratic effects of the cholecalciferol levels or 1-α(OH)D<sub>3</sub> on growth performance and tibia quality of broilers. Means were compared using the Tukey's test at 5% significance.

## Results and discussion

### Growth performance

Performance data of broilers during starter, grower, and finisher periods are presented in Table 2. Treatments failed to induce any significant effects on performance criteria in starter and grower periods ( $p > 0.05$ ). At 42 d of age the BW was increased in the broilers supplemented with 1-α(OH)D<sub>3</sub> and 1,500 IU cholecalciferol kg<sup>-1</sup> of diet; although it was decreased by increasing the level of cholecalciferol (linearly and quadratically affected). During the finisher period DFI was linearly and quadratically affected by addition of different levels of cholecalciferol ( $p < 0.0001$ ). As indicated in Table 2 DFI was decreased by addition of 1-α(OH)D<sub>3</sub> and 1-α(OH)D<sub>3</sub> and different concentrations of cholecalciferol. During the finisher period FCR was affected by the dietary treatments (linear and quadratic effects). The best FCR obtained in the group supplemented with 1-α(OH)D<sub>3</sub> and 1,500 IU cholecalciferol kg<sup>-1</sup> of diet.

**Table 2.** Effects of dietary 1- $\alpha$ (OH)D<sub>3</sub> individually or in combination with different levels of cholecalciferol on body weight (BW), daily feed intake (DFI), feed: gain ratio (FCR) and daily weight gain (DWG) of broilers in starter, grower and finisher periods.

Variables	Dietary treatments <sup>1</sup>					SEM <sup>1</sup>	p value	
	Basal	Basal + 1- $\alpha$ (OH)D <sub>3</sub>	Basal+ 1- $\alpha$ (OH)D <sub>3</sub> + 1,500 IU D <sub>3</sub>	Basal+ 1- $\alpha$ (OH)D <sub>3</sub> + 3,000 IU D <sub>3</sub>	E Basal+ 1- $\alpha$ (OH)D <sub>3</sub> + 5,000 IU D <sub>3</sub>		Linear	Quadratic
Body weight (g)	411	421	434	447	431	14.0	0.33	0.30
Daily feed intake (g d <sup>-1</sup> )	38.9	40.7	40.0	39.0	39.3	1.6	0.96	0.68
Feed:gain (g:g)	1.43	1.47	1.40	1.33	39.3	0.06	0.58	0.78
Body weight (g)	1,070	1,070	999	1,038	1,032	21.6	0.09	0.33
Daily feed intake (g d <sup>-1</sup> )	94.3	94.2	90.9	90.7	90.6	2.0	0.18	0.61
Feed:gain (g:g)	2.0	2.0	2.2	2.1	2.1	0.07	0.13	0.15
Body weight (g)	1,874 <sup>c</sup>	2,029 <sup>b</sup>	2,223 <sup>a</sup>	2,202 <sup>a</sup>	2,006 <sup>b</sup>	8.7	< 0.0001	< 0.0001
Daily feed intake (g d <sup>-1</sup> )	167 <sup>a</sup>	147 <sup>ab</sup>	142 <sup>b</sup>	142 <sup>b</sup>	141 <sup>b</sup>	4.6	< 0.0001	< 0.0001
Feed:gain (g:g)	2.90 <sup>a</sup>	2.15 <sup>b</sup>	1.62 <sup>d</sup>	1.71 <sup>cd</sup>	2.03 <sup>bc</sup>	0.06	< 0.0001	< 0.0001

<sup>a,d</sup>Values in the same row not sharing a common superscript differ (p < 0.05). <sup>1</sup>Standard error of mean.

Performance data of broilers throughout the entire experimental period are presented in Table 3. At 42 d of age, broilers fed 1- $\alpha$ (OH)D<sub>3</sub> in combination with 1,500 (2,223 g) or 3,000 (2,202) IU cholecalciferol kg<sup>-1</sup> of diet had significantly higher BW compared with broilers fed basal diet (1,874 g), 1- $\alpha$ (OH)D<sub>3</sub> alone (2,029 g), and 1- $\alpha$ (OH)D<sub>3</sub> in combination with 5,000 (2,006 g) IU cholecalciferol kg<sup>-1</sup> of diet. Throughout the entire experimental period broiler chickens fed basal diet had the highest DFI and the lowest final BW. Broiler chickens fed diet supplemented with 1- $\alpha$ (OH)D<sub>3</sub> in combination with different levels of cholecalciferol had significantly lower DFI than those fed basal diet or basal diet supplemented with 1- $\alpha$ (OH)D<sub>3</sub> alone. Broiler chickens fed basal diet supplemented with 1- $\alpha$ (OH)D<sub>3</sub> in combination with 1,500 (1.73) and 3,000 (1.74) IU cholecalciferol kg<sup>-1</sup> of diet had better FCR values compared with broilers fed basal diet (2.26), basal diet supplemented with 1- $\alpha$ (OH)D<sub>3</sub> alone (1.96), and basal diet supplemented with 1- $\alpha$ (OH)D<sub>3</sub> in combination with 5,000 (1.91) IU cholecalciferol kg<sup>-1</sup> of diet (p < 0.01).

**Table 3.** Effects of dietary 1- $\alpha$ (OH)D<sub>3</sub> individually or in combination with different levels of cholecalciferol on body weight (BW), daily feed intake (DFI), feed: gain ratio (FCR) and daily weight gain (DWG) of broilers throughout the entire experimental period.

Variables	Dietary treatments <sup>1</sup>					SEM <sup>1</sup>	p value	
	Basal	Basal + 1- $\alpha$ (OH)D <sub>3</sub>	Basal+ 1- $\alpha$ (OH)D <sub>3</sub> + 1,500 IU D <sub>3</sub>	Basal+ 1- $\alpha$ (OH)D <sub>3</sub> + 3,000 IU D <sub>3</sub>	Basal+ 1- $\alpha$ (OH)D <sub>3</sub> + 5,000 IU D <sub>3</sub>		Linear	Quadratic
Daily feed intake (g d <sup>-1</sup> )	99.15 <sup>a</sup>	93.21 <sup>b</sup>	90.27 <sup>c</sup>	89.85 <sup>c</sup>	89.70 <sup>c</sup>	0.30	< 0.0001	< 0.0001
Feed:gain (g:g)	2.26 <sup>a</sup>	1.96 <sup>b</sup>	1.73 <sup>d</sup>	1.74 <sup>d</sup>	1.91 <sup>c</sup>	0.006	< 0.0001	< 0.0001
Daily weight gain (g d <sup>-1</sup> )	43.79 <sup>c</sup>	47.49 <sup>b</sup>	52.10 <sup>a</sup>	51.57 <sup>a</sup>	46.92 <sup>b</sup>	0.18	< 0.0001	< 0.0001

<sup>a,d</sup>Values in the same row not sharing a common superscript differ (p < 0.05). <sup>1</sup>Standard error of mean.

As indicated in Table 4, broiler chickens fed basal diet had the lowest tibia ash, and the highest incidence and severity of TD that was resulted from Ca-P and cholecalciferol deficiency. Similarly, results of studies indicated that Ca and P deficiencies could increase skeletal abnormalities, such as rickets and TD, which lead to lameness and enhanced morbidity (Williams, Waddington, Solomon, & Farquharson, 2000; Edwards & Veltman, 1983). Since cholecalciferol is needed for Ca absorption and normal bone mineralization, in the current study cholecalciferol deficiency in broiler chickens fed basal diet massively intensified Ca-P deficiency which resulted in high incidence and severity of TD and poor growth rate as a result of morbidity.

In the present study, broiler chickens fed basal diet supplemented with 1- $\alpha$ (OH)D<sub>3</sub> in combination with 1,500 or 3,000 IU cholecalciferol kg<sup>-1</sup> of diet had better FCR, and higher BW than those fed basal diet supplemented with 1- $\alpha$ (OH)D<sub>3</sub> alone or basal diet supplemented with 1- $\alpha$ (OH)D<sub>3</sub> and 5,000 IU cholecalciferol kg<sup>-1</sup> of diet. Han et al. (2009) investigated the effects of 1- $\alpha$ (OH)D<sub>3</sub> in broiler diet contained adequate level of cholecalciferol, the results indicated that the supplementation of 5  $\mu$ g 1- $\alpha$ (OH)D<sub>3</sub> kg<sup>-1</sup> of diet in combination with 5  $\mu$ g cholecalciferol kg<sup>-1</sup> of diet had negative effects on BW and DFI of broilers. Biehl, Emmert and Baker (1997) reported that the supplementation of 1- $\alpha$ (OH)D<sub>3</sub> to broilers diets contained adequate levels of cholecalciferol couldn't improve BW of broilers, whereas the supplementation of 1- $\alpha$ (OH)D<sub>3</sub> to broilers diet that not supplemented with cholecalciferol could improve BW of 1- to 16-d-old broilers (Edwards et al., 2002). Atencio et al. (2005) reported that addition of 25-OHD<sub>3</sub> to broiler breeder diet containing very low levels of cholecalciferol could enhance hen-day egg production. Similarly, results of another experiment of

ours indicated that the supplementation of 5,000 IU cholecalciferol  $\text{kg}^{-1}$  of diet to broiler diet containing 5  $\mu\text{g}$   $1\alpha\text{-OHD}_3$   $\text{kg}^{-1}$  of diet reduced feed efficiency of broilers during the starter period (Landy et al., 2015). Reddy and Tserng (1989) reported that  $1\alpha\text{-OHD}_3$  can become toxic in high dosage, resulting in lower absorption of Ca and P. Since deficiency of Ca and P can impair broiler performance; thus it seem that in the present study supplementation of  $1\alpha\text{-OHD}_3$  become toxic when inclusion rate of cholecalciferol was higher than 1,500 IU  $\text{kg}^{-1}$  of diet, resulting in reduction of growth performance.

### Tibial dyschondroplasia

Tibia related parameters of broilers are presented in Table 4. Dietary supplementation of  $1\alpha\text{-OHD}_3$  alone or in combination with different levels of cholecalciferol linearly and quadratically ( $p < 0.01$ ) affected tibia bone ash, incidence and severity of TD. Broilers fed TD inducing diet had lower tibia bone ash (32.3%) compared with those fed TD inducing diet supplemented with  $1\alpha\text{-OHD}_3$  alone (38.2%), or with combination of 1,500 (38.1%), 3,000 (36.5%) and 5,000 (36.4%) IU cholecalciferol  $\text{kg}^{-1}$  of diet. Supplementation of the diet with  $1\alpha\text{-OHD}_3$  alone (57.1%) or with combination of 1,500 (50.0%), 3,000 (71.4%) and 5,000 (66.7%) IU cholecalciferol  $\text{kg}^{-1}$  of diet significantly lowered the incidence of TD compared with those fed TD inducing diet (100%). Supplementation of 3,000 (71.4%) or 5,000 (66.7%) IU cholecalciferol  $\text{kg}^{-1}$  of diet significantly increased the incidence of TD compared with those fed basal diet supplemented with 1,500 (50.0%) IU cholecalciferol  $\text{kg}^{-1}$  of diet. Supplementation of the diet with  $1\alpha\text{-OHD}_3$  alone or in combination with different levels of cholecalciferol lowered the percent of 3-scored birds. The addition of  $1\alpha\text{-OHD}_3$  alone (0.71) or in combination with 1,500 (0.75), 3,000 (1.43), and 5,000 (1.00) IU cholecalciferol  $\text{kg}^{-1}$  of diet significantly lowered the TD score than those fed basal diet (3.00).

**Table 4.** Effects of dietary  $1\alpha\text{-OHD}_3$  individually or in combination with different levels of cholecalciferol on the development of tibial dyschondroplasia.

Variables	Dietary treatments					SEM <sup>1</sup>	<i>p</i> value	
	Basal	Basal + $1\alpha\text{-OHD}_3$	Basal+ $1\alpha\text{-OHD}_3$ + 1,500 IU D <sub>3</sub>	Basal+ $1\alpha\text{-OHD}_3$ + 3,000 IU D <sub>3</sub>	Basal+ $1\alpha\text{-OHD}_3$ + 5,000 IU D <sub>3</sub>		Linear	Quadratic
Tibia ash (%)	32.3 <sup>b</sup>	38.2 <sup>a</sup>	38.1 <sup>a</sup>	36.5 <sup>a</sup>	36.4 <sup>a</sup>	0.64	0.003	< 0.0001
Incidence (%)	100.0 <sup>a</sup>	57.1 <sup>c</sup>	50.0 <sup>c</sup>	71.4 <sup>b</sup>	66.7 <sup>b</sup>	1.83	< 0.0001	< 0.0001
Score	3.00 <sup>a</sup>	0.71 <sup>b</sup>	0.75 <sup>b</sup>	1.50 <sup>b</sup>	1.00 <sup>b</sup>	0.29	< 0.001	< 0.0004
3 Scores (%)	100 <sup>a</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	0.00	< 0.0001	< 0.0001

<sup>a-c</sup>Values in the same column not sharing a common superscript differ ( $p < 0.05$ ). <sup>1</sup>Standard error of mean.

In the present trial addition of  $1\alpha\text{-OHD}_3$  alone to TD inducing diet could increase tibia bon ash. Supplementation of 1,500 IU cholecalciferol to TD inducing diet containing  $1\alpha\text{-OHD}_3$  had no effect on tibia bone ash, although the supplementation of higher levels of cholecalciferol had little influence on tibia bone ash. The tibia ash tended to decrease in broilers fed diets containing 3,000 or 5,000 IU cholecalciferol  $\text{kg}^{-1}$  of diet compared with those fed 1,500 IU cholecalciferol  $\text{kg}^{-1}$  of diet. As reported by Garcia et al. (2013)  $1\alpha\text{-OHD}_3$  become toxic in high dosage resulting in reduction the absorption of Ca and P, thus reduction in tibia ash of broilers supplemented with  $1\alpha\text{-OHD}_3$  and 3,000 or 5,000 IU cholecalciferol  $\text{kg}^{-1}$  of diet may be due to toxic effect of using combination of  $1\alpha\text{-OHD}_3$  and 3,000 or 5,000 IU cholecalciferol  $\text{kg}^{-1}$  of diet. Drewe, Dietsch and Keck (1988) observed an increase in plasma calcitriol of chickens when cholecalciferol deficient diets were supplemented with calcitriol, later in another study by Rennie et al. (1993) failed to monitor any marked effect of calcitriol supplementation on plasma calcitriol of broilers when calcitriol was supplemented to a diet that was cholecalciferol sufficient. Results of the experiment done by Edwards (2002) indicated that an interaction between calcitriol and cholecalciferol exists in bone mineralization. Similarly, Landy et al. (2015) investigated the efficiency of  $1\alpha\text{-OHD}_3$  alone or in combination of 5,000 IU cholecalciferol  $\text{kg}^{-1}$  of diet, the results indicated that the supplementation of  $1\alpha\text{-OHD}_3$  in combination of 5,000 IU cholecalciferol reduced tibia ash, Ca and P compared with those fed  $1\alpha\text{-OHD}_3$  alone. In the present trial we investigated the effects of  $1\alpha\text{-OHD}_3$  in Ca-P deficient diet alone or in combination of different levels of cholecalciferol, because of possible interactions between cholecalciferol and  $1\alpha\text{-OHD}_3$ . Our results indicated that an interaction between cholecalciferol and  $1\alpha\text{-OHD}_3$  exists on bone mineralization. Edwards (1989, 1990) reported that the supplementation of Ca deficient diet containing 27.5 mg  $\text{kg}^{-1}$  cholecalciferol with 10 mg  $\text{kg}^{-1}$  calcitriol enhanced tibia bone ash and reduced the incidence and severity of TD.

## Conclusion

In conclusion, the results indicated that the supplementation of 1- $\alpha$ (OH) $D_3$  alone to the TD inducing diet could maximize tibia bone ash and prevents incidence and severity of TD, but in couldn't maximize performance criteria. Performance parameters were maximized in broiler chickens fed dietary containing 1- $\alpha$ (OH) $D_3$  in combination with 1,500 IU cholecalciferol  $kg^{-1}$  of diet. Supplementation of 3,000 and 5,000 IU cholecalciferol  $kg^{-1}$  of diet in compare with 1,500 IU cholecalciferol  $kg^{-1}$  of diet enhanced incidence of TD. Furthermore, supplementation of higher levels of cholecalciferol (5,000 IU  $kg^{-1}$  of diet) decrease growth performance of broiler chickens, it seems that there are some variables that may affect growth performance and bone quality when higher dosage of cholecalciferol supplemented to diets.

## Acknowledgements

This project was supported by the Department of Animal Science of Islamic Azad University, Shahrekord Branch, Iran (Grant No. 2017/011).

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