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Original Research Article

Effects of dietary 1 alpha-hydroxycholecalciferol in calcium and phosphorous-deficient diets on growth performance, tibia related indices and immune responses in broiler chickens

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ABSTRACT

This experiment was conducted to investigate the effect of dietary 1α-hydroxycholecalciferol (1α-OH-D_3) in calcium (Ca)- and phosphorous (P)-deficient diets on growth performance, carcass characteristics, tibia related parameters, and immune responses of broiler chickens. A total of 280 one-day-old broiler chickens (Ross 308) were assigned to 20 floor pens and 4 dietary treatments with 5 replicates. Dietary treatments consisted of starter diets (starter diet of treatment A: 1% Ca, 0.73% total phosphorus [tP]; starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1α-OH-D_3), grower diets (grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1α-OH-D_3) and finisher diets (finisher diet of treatment A: 0.81% Ca, 0.64% tP; finisher diet of treatment B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1α-OH-D_3). Results showed that body weight gain (BWG) and feed intake (FI) of broilers in treatment B were similar to those of broilers in treatment A at the end of the trial ($P < 0.05$). Broilers in treatments C and D had lower BWG and FI than those in treatment A during the whole trial ($P < 0.05$). Feed conversion ratio, carcass traits and relative weight of lymphoid organs were not affected by dietary treatments ($P > 0.05$). Dietary treatments had no significant effect on antibody titers against Newcastle and Influenza disease viruses as well as sheep red blood cells. Dietary treatments had no significant effects on tibia ash and tibial dyschondroplasia score. Broilers fed Ca-P deficient diets had lower tibia Ca and P than those in treatment A ($P < 0.05$). In conclusion, results indicated that broilers fed Ca-P deficient diets supplemented with 5 μg/kg 1α-OH-D_3 failed to achieve the same tibia Ca and P values as broilers fed nonphytate phosphorus adequate diets.

1. Introduction

A major issue facing the poultry industry is maintaining bone quality while decreasing feed cost and phosphorus (P) excretion to the environment. In recent years, public pressure on poultry producers has increased to reduce excessive P wastage in the manure, which stimulated researchers into ways to increase the availability of dietary phytate phosphorus (PP) content. Several methods have been investigated to improve available PP utilization. Supplementations of low P diets with phytase have been shown to improve dietary P digestibility in sows (Torrallardona et al., 2012; Sands

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et al., 2001) and broilers (Jiang et al., 2013; Pieniazek et al., 2017; Woyengo et al., 2010; Ravindran et al., 2006). Consistently, Pillai et al. (2006) reported that inclusion of *Escherichia coli* phytase to P-deficient diets could improve growth performance, bone quality, and carcass yield in broiler chickens. Mitchell and Edwards (1996) reported the ability of 1,25-dihydroxycholecalciferol to enhance performance parameters and tibia related indices of young chickens by increasing dietary P absorption and retention.

Several trials have indicated affirmative efficacy of 25-OH-D_3 supplementation in broiler's diets on performance criteria (Fritts and Waldroup, 2003) and PP utilization (Zhang et al., 1997) which thereby makes it suitable to be included in poultry feed. It could be possible to use 1α-hydroxycholecalciferol (1α-OH-D_3) as an active vitamin D analog to be substituted for cholecalciferol in broiler feed. Edwards et al. (2002) reported that the 1α-OH-D_3 is approximately 8 times more effective than cholecalciferol. Landy and Toghyani (2014) indicated the ability of 1α-OH-D_3 to be replaced for cholecalciferol in broiler chickens. Han et al. (2009) reported that interaction between phytase and 1α-OH-D_3 in diets containing 2.9 g/kg nonphytate phosphorus (NPP) could improve tibia related parameters in broiler chicks, while it could not improve performance parameters. Han et al. (2015) reported that supplementation of 5 μg/kg 1α-OH-D_3 in diets containing 0.30% of NPP could improve growth performance and tibia mineralization of broiler chickens. Landy et al. (2015) reported that supplementation of broiler diets with 5 μg/kg 1α-OH-D_3 and 500 FTU/kg of phytase could not maximize growth performance and tibia parameters.

Kolb et al. (2000) and Van der Stede et al. (2000) reported that cholecalciferol and 1,25-dihydroxycholecalciferol have immunomodulatory effects. Bouillon et al. (2000) compared the efficacy of cholecalciferol and 1,25-$(OH)_2$-D_3 to treat cancer and skin disorders in mice. They reported that 1,25-$(OH)_2$-D_3 helped mice to treat cancer, skin, and immune related disorders. Vazquez et al. (2018) suggested that supplementation of 25-OH-D_3 to diet of broilers containing cholecalciferol could improve cellular immune responses.

Most of the studies on 1α-OH-D_3 only focused on the starter rearing period, and few experiments conducted on growing and finishing phases. Moreover, no study has evaluated the effect of 1α-OH-D_3 in P-deficient diets on the immunity of broiler chickens. Therefore, this experiment was conducted to investigate the effect of dietary 1α-OH-D_3 supplementation in Ca-P deficient diets on growth performance, carcass characteristics, immunity, and tibia related parameters in broiler chickens.

2. Materials and methods

2.1. Ethical matters

Broilers were raised in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures used in this study were approved by the Ethical Committee of Islamic Azad University, Isfahan branch, Iran (approval ref no. 2016-003).

2.2. Birds, diets, feeding, and management

Two hundred and eighty as-hatched chicks (Ross 308) were purchased from a local hatchery, weighed and randomly allocated to 20 pens (120 cm × 120 cm × 80 cm) with 14 chicks per pen. Dietary treatments were as follows: starter diets (starter diet of treatment A: 1% Ca, 0.73% total phosphorus [tP]; starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1α-OH-D_3 [Vitamin Derivatives Inc., Georgia, USA]; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1α-OH-D_3), grower diets (grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1α-OH-D_3) and finisher diets (finisher diet of treatment A: 0.81% Ca, 0.64% tP; finisher diet of treatment B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1α-OH-D_3) (Table 1). Broilers were fed the starter diets from 0 to 14 d (Table 2), grower diets from 15 to 28 d (Table 3), and finisher diets from 29 to 42 d (Table 4) according to Aviagen nutritional recommendation except for Ca and P. Broiler chickens had free access to mash feed and water throughout 6 weeks of trial. The lighting system consisted of 23 h of light from d 0 to 3, 20 h of light from d 4 to 14, and 18 h of light from d 15 to 42. The room temperature was controlled at 33 °C for the first week, and then gradually reduced by 3 °C per week to a final temperature of 23 °C.

2.3. Feed analyses

Feed samples were dried by oven at 100 °C for 16 h. Dry matter, tP, and Ca contents of each feed sample from the 4 experimental diets were measured. Calcium and tP contents of the feed were analyzed by the ICPOES method 2011.14 (AOAC, 1990).

2.4. Performance and carcass characteristics

At the end of trial, body weight and feed intake (FI) were recorded on a pen basis, for the determination of body weight gain (BWG) and average daily feed intake. Feed conversion ratio (FCR) was calculated accordingly. Mortality was recorded daily. On d 42 of experiment, 2 chickens per replicate were chosen based on the average weight of pens, weighed and slaughtered. Carcass yield was calculated by dividing eviscerated weight by live weight. Abdominal fat pad was removed, weighed, and calculated as a percentage of live weight.

Table 1
Dietary treatment in starter, grower, and finisher phases.

Treatments	1α-OH-D_3, μg/kg	Starter period (0 to 14 d)		Grower period (15 to 28 d)		Finisher period (29 to 42 d)	
		Ca, %	tP, %	Ca, %	tP, %	Ca, %	tP, %
A[1]	–	1.00	0.73	0.86	0.68	0.81	0.64
B[2]	5	0.85	0.64	0.73	0.59	0.68	0.56
C[2]	5	0.85	0.59	0.73	0.55	0.68	0.52
D[2]	5	0.85	0.54	0.73	0.50	0.68	0.48

tP = total phosphorus.
[1] Ca and tP adequate diets without 1α-OH-D_3.
[2] Ca-P deficient diets with 1α-OH-D_3.

Table 2
Ingredients, calculated and analyzed nutrient content of starter diets (g/kg, as fed basis).

Item	Treatments[1]			
	A	B	C	D
Ingredients				
Corn, 8% CP	542.3	556.2	558.0	561.3
Soybean meal, 44% CP	390.0	387.0	387.0	386.0
Soybean oil	22.4	18.3	17.6	16.8
Monocalcium phosphate	15.0	10.5	8.7	6.4
$CaCO_3$	17.3	15.0	15.7	16.6
NaCl	3.0	3.0	3.0	3.0
Trace mineral premix[2]	2.5	2.5	2.5	2.5
Vitamin premix[3]	2.5	2.5	2.5	2.5
DL-methionine	3.1	3.1	3.0	3.0
L-lysine	1.9	1.9	1.9	1.9
Calculated composition				
Metabolizable energy, kcal/kg	2,900	2,900	2,900	2,900
Crude protein	215	215	215	215
Calcium	10.0	8.5	8.5	8.5
Nonphytate phosphorus	4.8	3.8	3.3	2.8
Total phosphorus (tP)	7.3	6.4	5.9	5.4
Digestible methionine + cysteine	9.0	9.0	9.0	9.0
Digestible lysine	12.2	12.2	12.2	12.2
Analyzed nutrient content				
Calcium	9.4	8.8	8.0	9.0
tP	7.0	6.6	5.7	5.3

[1] Treatment A was Ca and tP adequate diets without 1α-OH-D_3, and treatments B, C, D were Ca-P deficient diets with 5 μg/kg of 1α-OH-D_3.
[2] Provided the following per kg of diet: Mg, 120 mg; Fe, 40 mg; Cu, 16 mg; Zn, 100 mg; Se, 0.3 mg; I, 1.25 mg.
[3] Provided the following per kg of diet: vitamin A, 11,000 IU; vitamin D_3, 5,000 IU; vitamin E, 7.5 IU; vitamin K, 3 mg; vitamin B_1, 3 mg; riboflavin, 8 mg; nicotinic acid, 60 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; biotin, 0.15 mg; folic acid, 2 mg; vitamin B_{12}, 0.016 mg; choline, 3 mg.

Table 3
Ingredients, calculated and analyzed nutrient content of grower diets (g/kg, as fed basis).

Item	Treatments[1]			
	A	B	C	D
Ingredients				
Corn, 8% CP	564.0	575.0	577.5	579.0
Soybean meal, 44% CP	368.0	366.0	365.0	365.0
Soybean oil	30.0	27.2	26.5	26.0
Monocalcium phosphate	12.9	9.1	7.1	5.2
$CaCO_3$	14.4	12.5	13.3	14.1
NaCl	3.0	3.0	3.0	3.0
Trace mineral premix[2]	2.5	2.5	2.5	2.5
Vitamin premix[3]	2.5	2.5	2.5	2.5
DL-methionine	2.2	2.2	2.2	2.2
L-lysine	0.4	0.4	0.4	0.4
Calculated composition				
Metabolizable energy, kcal/kg	3,000	3,000	3,000	3,000
Crude protein	207	207	207	207
Calcium	8.6	7.3	7.3	7.3
Nonphytate phosphorus	4.3	3.4	3.0	2.5
Total phosphorus (tP)	6.8	5.9	5.5	5.0
Digestible methionine + cysteine	8.0	8.0	8.0	8.0
Digestible lysine	10.5	10.5	10.5	10.5
Analyzed nutrient content				
Calcium	8.1	7.8	7.0	7.8
tP	7.0	5.7	5.6	5.2

[1] Treatment A was Ca and tP adequate diets without 1α-OH-D_3, and treatments B, C, D were Ca-P deficient diets with 5 μg/kg of 1α-OH-D_3.
[2] Provided the following per kilogram of diet: Mg, 120 mg; Fe, 40 mg; Cu, 16 mg; Zn, 100 mg; Se, 0.3 mg; I, 1.25 mg.
[3] Provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 5,000 IU; vitamin E, 5 IU; vitamin K, 3 mg; vitamin B_1, 2 mg; riboflavin, 6 mg; nicotinic acid, 60 mg; pantothenic acid, 15 mg; pyridoxine, 3 mg; biotin, 0.1 mg; folic acid, 1.75 mg; vitamin B_{12}, 0.016 mg; choline, 3 mg.

Table 4
Ingredients, calculated and analyzed nutrient content of finisher diets (g/kg, as fed basis).

Item	Treatments[1]			
	A	B	C	D
Ingredients				
Corn, 8% CP	613.7	624.0	627.2	628.8
Soybean meal, 44% CP	322	320	319	319
Soybean oil	29.0	26.0	25.0	24.5
Monocalcium phosphate	12.0	8.4	6.5	4.7
$CaCO_3$	13.8	12.1	12.8	13.5
NaCl	3	3	3	3
Trace mineral premix[2]	2.5	2.5	2.5	2.5
Vitamin premix[3]	2.5	2.5	2.5	2.5
DL-methionine	1.5	1.5	1.5	1.5
Calculated composition				
Metabolizable energy, kcal/kg	3,050	3,050	3,050	3,050
Crude protein	190	190	190	190
Calcium	8.1	6.8	6.8	6.8
Nonphytate phosphorus	4.0	3.2	2.8	2.4
Total phosphorus (tP)	6.4	5.6	5.3	4.8
Digestible methionine + cysteine	7	7	7	7
Digestible lysine	9.1	9.1	9.1	9.1
Analyzed nutrient content				
Calcium	8.6	6.5	6.9	6.3
tP	6.6	5.4	5.1	5.0

[1] Treatment A was Ca and tP adequate diets without 1α-OH-D_3, and treatments B, C, D were Ca-P deficient diets with 5 μg/kg of 1α-OH-D_3.
[2] Provided the following per kg of diet: Mg, 120 mg; Fe, 40 mg; Cu, 16 mg; Zn, 100 mg; Se, 0.3 mg; I, 1.25 mg.
[3] Provided the following per kg of diet: vitamin A, 9,000 IU; vitamin D_3, 5,000 IU; vitamin E, 5 IU; vitamin K, 2 mg; vitamin B_1, 2 mg; riboflavin, 5 mg; nicotinic acid, 40 mg; pantothenic acid, 15 mg; pyridoxine, 2 mg; biotin, 0.1 mg; folic acid, 1.5 mg; vitamin B_{12}, 0.016 mg; choline, 3 mg.

2.5. Immune responses

On d 9 of the experiment, broiler chickens from each pen ($n = 14$) were injected with a single dose (0.2 mL) of commercially vaccine against Newcastle (NDV) and avian influenza disease viruses (AIV; serotype H9N2) subcutaneously. Two male broilers from each pen were bled by a puncture of the brachial vein on d 19 of post-vaccination to collect serum. Serum samples were applied to hemagglutination inhibition in order to measure antibody titers against NDV and AIV and expressed as $\log_2$. On d 24 of trial, 1 mL of 1% sheep red blood cells (SRBC) was injected intravascularly to 2 broilers per pen. After 6 d, blood samples were taken and individual sera were tested for antibody production. Antibody titers were expressed as the $\log_2$ (Wegmann and Smithies, 1966). Lymphoid organs were sampled on d 42 of trial. In this respect 2 male broilers were randomly selected from each pen, slaughtered, and lymphoid organs (bursa of Fabricius and spleen) were removed, weighted, and calculated as a percentage of live body weight.

2.6. Tibia parameters

At the end of trial, 2 chickens per pen were selected based on the average weight of the pen and sacrificed by exsanguinations, and the left and right tibias were excised. The right tibia was evaluated for tibial dyschondroplasia (TD) as described by Edwards and Veltmann (1983). Tibia ash content was determined by removing the left tibia from broilers and ashing the bones on a dry fat-free basis (AOAC, 1995). Calcium and P contents of tibia ash were analyzed by the ICPOES method 2011.14 (AOAC, 1990).

2.7. Statistical analysis

Performance, tibia quality, and immune related parameters were analyzed via Analysis of Variance (ANOVA) using the General Linear

Model procedure of SAS (SAS Inst. Inc., Cary, NC). Means were deemed significance at $P \leq 0.05$ and compared using Tukey test.

3. Results

3.1. Growth performance and carcass yield

Data on the growth performance indices of broilers in starter, grower and finisher periods are summarized in Table 5. During the starter phase (0 to 14 d), broilers in treatment D had lower ($P < 0.05$) FI than those in treatments A, B, and C. Furthermore, broilers in treatment D had higher ($P < 0.05$) FCR than those in treatments A, B, and C. In the starter period, body weight gain was higher in treatment A than treatments B, C, and D.

In the grower phase, broilers in treatment D had lower ($P < 0.05$) BWG than those received diets of treatments A, B, and C. Moreover, dietary treatments failed to induce any significant effect on FCR, though broilers in treatment A had better FCR values than those in treatments B, C and D. Broilers in treatment A had significantly higher ($P < 0.05$) FI than those in treatments C, and D, but did not significantly differ from those in treatment B.

During the finisher phase, broilers in treatments A and B had significantly higher ($P < 0.05$) BWG than those in treatment D, but did not differ from treatment C. There was no significant difference in FCR among treatments in this period. Broilers in treatment D had significantly lower ($P < 0.05$) FI than those in treatments A and B, but did not differ from those in treatment C.

For broilers in treatment B, BWG, FI, and FCR were similar to those fed Ca-P adequate diets added with 1α-OH-D_3 (Table 6). At 42 d of age, broilers in treatment D, had significantly ($P < 0.05$) lower FI than those in treatments A, B, and C. Significant differences among treatments were observed in BWG of broilers during the entire trial. Broilers in treatments D and C had lower BWG than those in treatments A and B. Overall FCR values were better for treatments A, B and C than for treatment D whereas the results were not significantly different. There were no significant differences in the carcass yield and abdominal fat of broilers among treatments (Table 7).

3.2. Immune responses

There were no significant differences in the weight of lymphoid organs among treatments (Table 7). Dietary treatments had no significant effects on the antibody titers against AIV, NDV, and SRBC (Table 8).

Table 6
Effect of dietary1α-OH-D_3 in Ca-P deficient diets on performance of broilers in the whole experimental period.

Treatments[1]	FI, g/d	BWG, g/d	FCR, g/g
A	107.3[a]	59.3[a]	1.81
B	107.5[a]	59.1[a]	1.82
C	101.7[b]	56.2[b]	1.81
D	97.6[c]	51.9[c]	1.88
SEM	1.11	1.08	0.04
P-value	0.02	0.02	0.06

FI = feed intake; BWG = body weight gain; FCR = feed conversion ratio.
[a, b, c] Values in the same column not sharing a common superscript differ ($P < 0.05$).
[1] Starter diet of treatment A: 1% Ca, 0.73% total phosphorus [tP]; starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1α-OH-D_3. Grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1α-OH-D_3. Finisher diet of treatment A: 0.81% Ca, 0.64% tP; finisher diet of treatment B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1α-OH-D_3.

Table 7
Effect of dietary1α-OH-D_3 in Ca-P deficient diets on carcass yield, and relative weight of abdominal fat, and lymphoid organs of broilers at 42 d of age.

Treatments[1]	Relative organ weight, %			
	Carcass yield	Abdominal fat	Spleen	Bursa of Fabercius
A	71.3	1.21	0.138	0.106
B	70.5	1.01	0.124	0.101
C	71.3	1.05	0.111	0.072
D	71.9	0.92	0.117	0.088
SEM	0.88	0.152	0.011	0.014
P-value	0.09	0.08	0.23	0.06

[1] Starter diet of treatment A: 1% Ca, 0.73% total phosphorus [tP]; starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1α-OHD_3; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1α-OHD_3; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1α-OHD_3. Grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1α-OHD_3; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1α-OHD_3; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1α-OHD_3. Finisher diet of treatment A: 0.81% Ca, 0.64% tP; finisher diet of treatment B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1α-OHD_3; finisher diet of treatment C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1α-OHD_3; finisher diet of treatment D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1α-OHD_3.

3.3. Parameters of tibia

Effects of experimental diets on tibia parameters in broiler chickens are presented in Table 9. Tibia ash of broilers did not significantly differ among experimental treatments, whereas it

Table 5
Effect of dietary1α-OH-D_3 in Ca-P deficient diets on performance of broilers during starter, grower, and finisher periods.

Treatments[1]	Starter period (0 to 14 d)			Grower period (15 to 28 d)			Finisher period (29 to 42 d)		
	BWG, g/d	FI, g/d	FCR, g/g	BWG, g/d	FI, g/d	FCR, g/g	BWG, g/d	FI, g/d	FCR, g/g
A	23.8[a]	33.2[a]	1.39[b]	54.0[a]	93.6[a]	1.69	73.6[a]	149.8[a]	2.04
B	21.7[b]	32.0[a]	1.45[ab]	53.1[a]	90.8[ab]	1.71	74.6[a]	148.7[a]	2.01
C	22.5[ab]	32.0[a]	1.43[b]	52.5[a]	87.4[b]	1.78	70.7[ab]	143.0[ab]	2.02
D	19.5[c]	30.0[b]	1.53[a]	44.1[b]	81.6[c]	1.84	66.3[b]	138.2[b]	2.01
SEM	0.44	0.41	0.02	1.03	1.25	0.03	1.51	1.76	0.03
P-value	0.03	0.04	0.04	0.04	0.02	0.06	0.04	0.05	0.08

BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio.
[a, b, c] Values in the same column not sharing a common superscript differ at $P < 0.05$.
[1] Starter diet of treatment A: 1% Ca, 0.73% total phosphorus (tP); starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1α-OH-D_3. Grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1α-OH-D_3. Finisher diet of treatment A: 0.81% Ca, 0.64% tP; finisher diet of treatment B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1α-OH-D_3.

Table 8
Effect of dietary 1α-OH-D_3 in Ca-P deficient diets on antibody titers against Newcastle and Influenza viruses on d 28 of trial and sheep red blood cells (SRBC) on d 30 of trial.

Treatments[1]	Newcastle (Log_2)	Influenza (Log_2)	SRBC (Log_2)
A	5.15	5.75	9.87
B	5.35	5	9.45
C	5.75	5.25	9.75
D	5.25	5	9.75
SEM	0.32	0.35	0.41
P-value	0.09	0.35	0.27

[1] Starter diet of treatment A: 1% Ca, 0.73% total phosphorus [tP]; starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1α-OH-D_3. Grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1α-OH-D_3. Finisher diet of treatment A: 0.81% Ca, 0.64% tP; finisher diet of treatment B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1α-OH-D_3; finisher diet of treatment D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1α-OH-D_3.

Table 9
Effect of dietary 1α-OH-D_3 in Ca-P deficient diets on tibial parameters of broilers at 42 d of age.

Ttreatments[1]	Tibia ash, %	Phosphorus, %	Calcium, %	TD scores
A	48.7	25.3[a]	35.2[a]	1.10
B	48.9	23.2[b]	32.3[b]	0.80
C	47.3	22.5[c]	32.4[b]	1.20
D	47.2	22.1[c]	32.7[b]	1.40
SEM	0.98	0.33	0.44	0.35
P-value	0.07	0.02	0.04	0.26

TD = tibial dyschondroplasia.
[a, b, c] Values in the same column not sharing a common superscript differ ($P < 0.05$).
[1] Starter diet of treatment A: 1% Ca, 0.73% total phosphorus [tP]; starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1α-OH-D_3. Grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1α-OH-D_3; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1α-OH-D_3. Finisher diet of treatment A: 0.81% Ca, 0.64% tP; B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1α-OH-D_3; C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1α-OH-D_3; D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1α-OH-D_3.

tended to decrease in treatments C and D. Broilers in treatment A had significantly ($P < 0.05$) higher tibia Ca than those in treatments B, C, and D. Broilers in treatment D had significantly ($P < 0.05$) lower tibia P than those in treatments A and B but did not differ from treatment C. Dietary treatments failed to induce any marked effect on TD score, whereas it tended to enhance TD in treatments C and D ($P > 0.05$).

4. Discussion

4.1. Performance and carcass yield

Previous studies on 1α-OH-D_3 in broilers have focused on the starter period, in which 1α-OH-D_3 improved performance related parameters (Biehl and Baker, 1997; Edwards, 2002). The supplementation of 1α-OH-D_3 could not maximize BWG, FI, and FCR of broilers received Ca-P deficient diets during this experiment from d 1 to 42, whereas the results indicated that BWG, FI, and FCR were similar for broilers in treatment B compared with those fed the Ca-P adequate diet.

In agreement with our results, Han et al. (2009) reported that 1α-OH-D_3 could not improve performance of broiler chicks when NPP content in basal diets was up to 2.9 g/kg and vitamin D_3 was adequate. Edwards (2002) reported that supplementation of 1α-OH-D_3 in basal diets with tP of 7.0 g/kg and without cholecalciferol could increase performance of broilers. Landy and Toghyani (2018) reported the possibility of interaction between 1α-OH-D_3 and cholecalciferol. Therefore, the efficiency of 1α-OH-D_3 in diets with or without cholecalciferol should be investigated in broilers chickens. Ledwaba and Roberson (1769) evaluated the ability of 25-OH-D_3 to increase the digestion and absorption of dietary Ca and P, and their results indicated that dietary 25-OH-D_3 increased the digestion of PP at a lower concentration of dietary Ca. Probably, the Ca level applied in the current trial was not suitable to cause a beneficial effect on growth performance, since there are reports of significant improved performance criteria in broilers receiving diets supplemented with 0.4% Ca (Han et al., 2012) which is considerably lower level compared with the level used in our trial. It seems that dietary Ca levels reduce the efficacy of 1α-OH-D_3 in P-deficient diets in broiler chickens.

4.2. Immune responses

Dietary 1,25-dihydroxycholecalciferol and cholecalciferol have been proposed to exhibit immunomodulatory effects (Kolb et al., 2000; Van der Stede et al., 2000), so enhanced antibody titers were expected. However, in the current trial, dietary treatments did not induce any marked influence on the relative weights of lymphoid organs and homural immune responses. Results of a trial conducted by Chou et al. (2009) indicated that a supplementation of 25-OH-D_3 could enhance humoral immune responses in challenged broilers. Gomez-Verduzco et al. (2013) indicated that the supplementation of dietary high levels of cholecalciferol (2,000 IU/kg) in comparison with the levels recommended by NRC (1994) enhanced the antibody titer against NDV, and the supplementation of 25-OH-D_3 enhanced the cellular immunity of broiler chickens. Vazquez et al. (2018) reported that the supplementation of 25-OH-D_3 to diets containing cholecalciferol improved cellular immune responses in broiler chickens. There has been a dearth of information on the effect of 1α-OH-D_3 on immune responses in broiler chickens, thus further investigations are warranted.

4.3. Tibial parameters

In the present trial, the tibia ash of broilers did not differ among treatments, whereas it tended to decrease in birds fed dietary treatments C and D. Broilers in Ca-P deficient diets had significantly lower tibia Ca and P compared with those given Ca-P adequate diets. Driver et al. (2005) reported that broilers fed diets containing 1α-OH-D_3 and phytase had lower tibia ash than those fed normal P diet. However, Snow et al. (2004) reported that interaction between phytase and 1α-OH-D_3 had an affirmative influence on PP release in broilers from 1 to 21 d of age. In this experiment, we evaluated the efficacy of 1α-OH-D_3 alone, considering the interaction between 1α-OH-D_3 and phytase, and the efficiency of 1α-OH-D_3 either alone or in combination with phytase is worthy to be investigated in broilers chickens further.

In the current trial, we evaluated the efficacy of 1α-OH-D_3 in Ca-P deficient diets containing 5,000 IU cholecalciferol/kg of diets. Biehl and Baker (1997) reported that the supplementation of 1α-OH-D_3 in purified diets could improve the tibia ash only in broilers fed diets without cholecalciferol. Landy et al. (2015) reported that in Ca-P deficient diets and without vitamin D_3 supplementation, 1α-OH-D_3 improved tibia parameters in broiler chickens. However, when vitamin D_3 was enough, the tibia quality of broilers was not improved by dietary 1α-OH-D_3 supplementation. Atencio et al. (2005) reported that the supplementation of 25-OH-D_3 increased the hen-day egg production in broiler breeders but only at very low levels of dietary vitamin D_3 supplementation. Similarly, Edwards (2002) indicated that an interaction between cholecalciferol and

calcitriol exists in tibia ash. It seemed that 1α-OH-D_3 could not improve tibia parameters via high levels of cholecalciferol inclusion in the diet in our experiment.

Ledwaba and Roberson (1769) reported that dietary 25-OH-D_3 enhanced PP digestion at low levels of dietary Ca compared with diets containing high levels of Ca. Han et al. (2012) investigated the relationship between dietary Ca levels (0.40%, 0.60%, 0.80%, 1.00%, and 1.20% Ca) and 1α-OH-D_3 in P-deficient diets. Results indicated that 1α-OH-D_3 had the highest activity at a lower concentration of dietary Ca. It seemed that 1α-OH-D_3 could not improve tibia parameters in our experiment due to the applied dietary Ca levels.

In our study, dietary treatment failed to induce any marked effect on TD score, whereas it tended to enhance in birds in treatments C and D. In another trial, the supplementation of 1α-OH-D_3 as a replacement for cholecalciferol in broiler diets increased TD score (Landy and Toghyani, 2014). Edwards (1990) investigated effects of vitamin D analogs in order to inhibit TD in broiler chickens and reported that the supplementation of vitamin D analogs except 24R,25-$(OH)_2D_3$ could induce favorable influences on incidence and severity of TD compared with the control group. Edwards and Veltmann (1983) reported that diets containing high levels of Ca might prevent TD and the incidence of TD in 2-week-old chicks was only 13% when received with a diet containing 1.1% Ca and 0.55% available P, but was 39% in diets containing 0.8% Ca. The present study was in agreement with the results reported by Edwards and Veltmann (1983), when dietary Ca and P levels were decreased and 5 μg/kg of 1α-OH-D_3 was supplemented (treatments C and D), the average TD score was increased as a result of lower tibia Ca and P contents.

5. Conclusion

In conclusion, results indicated that broilers fed Ca-P deficient diets supplemented with 5 μg/kg of 1α-OH-D_3 were unable to achieve the same tibia Ca and P content as broilers fed Ca-P adequate diets without 5 μg/kg of 1α-OH-D_3. Considering the possibility of interaction between 1α-OH-D_3 and cholecalciferol, the efficiency of 1α-OH-D_3 in diets containing different levels of cholecalciferol should be investigated in broilers chickens. Furthermore, considering the interaction between 1α-OH-D_3 and phytase, the efficiency of 1α-OH-D_3 alone or in combination with phytase should be investigated in broilers chickens.

Conflict of interest

The authors declare that they have no competing interests.

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