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The effects of 1*α*(OH)D_3 individually or in combination with phytase, and different levels of cholecalciferol on performance, tibia criteria, and plasma minerals of Japanese quails

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ABSTRACT. The aim of studywas to compare efficacy of 1-α(OH)D_3 alone or in combination with phytase and 1-α(OH)D_3 in combination of phytase and different concentration of cholecalciferol on performance, tibia parameters, andplasma minerals of quails fed Ca-P deficient diet. A total of 280 mixed sex 5-d-old quails were allocated to 7 treatments with 5 replicates. The vitamin supplement which incorporated to basal diet did not contain cholecalciferol. The dietary treatments were as follows: Ca-P deficient diet (basal diet); basal diet + 500 FTU phytase/kg of diet; basal diet + phytase + 5 µg of 1-α(OH)D_3 kg^{-1} of diet;basal diet + phytase + 5 µg of 1-α(OH)D_3 and 250, 500, 750 and 1,000 IU of cholecalciferol $kg^{-1}$of diet. The highest final body weight and the best feed conversion ratioobtained in the group supplemented with 1,000 IU cholecalciferol kg^{-1} of diet ($p < 0.05$). Supplementation of 1-α(OH)D_3 alone or in combination with phytase and phytase and different concentration of cholecalciferol could improve tibia parameters ($p < 0.05$). In conclusion, supplementation of 1-α(OH)D_3 alone to Ca-P deficient diet could maximize tibia mineralization, whereas it couldn't maximize performance, performance criteria were maximized by supplementation of 1,000 IU cholecalciferol kg^{-1} of diet.

Keywords: bone mineralization; Ca and P deficiency; cholecalciferol; phytase; 1-α(OH)D_3; serum minerals.

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Introduction

Commonly, vitamin D_3 has been consumed in birds such as chickens, turkeys, ducks, and geese nutrition as a forebear for calcitriol. In recent years however, vitamin D metabolites such as 25 hydroxycholecalciferol (25-OHD_3) has been successfully consumed in bird's nutrition.After the successful commercialization processof 25-OHD_3, there has been more fondness from poultry nutritionist to test the possibility of supplementing1-alpha-hydroxycholecalciferol (1-α(OH)D_3) as a precursor for calcitriol (Ghasemi, Toghyani, & Landy, 2018; Landy & Toghyani 2014).Edwards Junior, Shirley, Escoe, and Pesti, (2002) evaluated the possibility of using 1-α(OH)D_3 as a replacement for cholecalciferol in broiler chicken nutrition; the results indicated that the activity of 1-α(OH)D_3 is nearlyeight times as effective as cholecalciferol. Similarly, Landy and Toghyani, (2014) reported that substitution of 5,000 IU cholecalciferol kg^{-1} of diet with 5 µg of 1-α(OH)D_3 kg^{-1} of diet resulted in better performance and tibia quality of broiler chickens.Snow, Baker, and Parsons, (2004) investigated efficacy of 1-α(OH)D_3 alone or in combination with phytase on phytate phosphorus utilization in broiler chicks fed a corn-soybean meal diet; the results indicated that a positive interaction exist between 1-α(OH)D_3 and phytase.Kheiri, Poshtvar, Jalali Haji Abadi, and Landy, (2019) compared efficacy of 1-α(OH)D_3 alone or in combination of microbial phytase on broiler quails performance and quality of tibia; the result indicated positive interaction between 1-α(OH)D_3 and phytase on bone mineralization. Edwards Junior et al. (2002) reported that supplementation of 1-α(OH)D_3 to broiler chicken's diet which didn't contain cholecalciferol resulted in better performance. Han et al. (2009) reported that addition of 1-α(OH)D3 to broiler chickens diet containing adequate level of cholecalciferol had negative effects on growth performance. Similarly, Landy, Toghyani, Bahadoran, and Eghbalsaied (2015) reported that supplementation of 5,000 IU cholecalciferol kg^{-1} to broiler chicken's diet containing 500 FTU phytase and

5 µg of 1-α(OH)D_3 kg^{-1} of diet had negative effects on bone mineralization. Landy and Toghyani (2018), reported that supplementation of 1-α(OH)D_3 in broiler chicken's diet which didn't supplemented with cholecalciferol increased tibia ash. Kheiri and Landy, (2019) reported that supplementation of 1-α(OH)D_3 alone in broiler chicken's diet deficient in Calcium (Ca) and phosphorus (P) increased bone mineralization whereas for maximizing performance it's necessary to supplement 1-α(OH)D_3 in combination of 1,500 IU cholecalciferol kg^{-1} of diet.

Despite these findings, there are a few trials about efficacy of 1-α(OH)D_3 on broilerquails,and most studies focused on broiler chickens.The aim of the current experiment was to compare efficacy of 1-α(OH)D_3 alone or in combination with microbial phytase, and 1-α(OH)D_3 in combination of microbial phytase and different inclusion rate of cholecalciferol on growth performance, tibia quality, plasma total mean alkaline phosphatase (ALP) enzyme activity and minerals of broiler quails fed diets deficient in Ca and P.

Materials and methods

Animals and dietary treatments

The present study was carried out to determine efficacy of 1-α(OH)D_3 individually or in combination of microbial phytase and 1-α(OH)D_3 in combination of microbial phytase and different inclusion rate of cholecalciferol in dietary containing inadequate levels of Ca and P on growth performance, tibia quality, plasma total mean ALP enzyme activity and minerals of broiler quails. The birds were reared under optimal environmental status and all processes including sampling and killing of the birds were done in agreement with the ethical guidelines of the Shahrekord University's Ethical Committee, Islamic Azad University, Shahrekord branch, Iran. A total of 280 mixed sex 5-d-old broiler quails (Coturnix japonica) were individually weighed and randomly allocated to 7 treatments with 5 replicates containing 8 quails. Water and feed were provided ad libitum and feed was presented in mash form during the experiment (5 to 37 d). A basal diet (Table 1) was formulated in accordance with National Research Council [NRC] (1994) recommendations except for Ca and P which were provided in inadequate levels and cholecalciferol as vitamin supplement which incorporated to basal diet did not contain cholecalciferol. The microbial phytase was provided at the inclusion rate of 500 FTU/kg of diet and was prepared commercially (ImEx Gulf, Inc., Texas, USA). The 1-α(OH)D_3 consumed in the current trial was commercially provided (Vitamin Derivatives Inc., GA, US) and incorporated to treatments in the inclusion rate of 5 µg kg^{-1} of diet. The dietary treatments were as follows: Ca-P deficient diet (basal diet); basal diet + 500 FTU microbial phytase kg^{-1} of diet; basal diet + phytase + 5 µg of 1-α(OH)D_3 kg^{-1} of diet; basal diet + phytase + 5 µg of 1-α(OH)D_3 and 250 IU of cholecalciferol $kg^{-1}$of diet;basal diet + phytase + 5 µg of 1-α(OH)D_3 and 500 IU of cholecalciferol $kg^{-1}$of diet; basal diet + phytase + 5 µg of 1-α(OH)D_3 and 750 IU of cholecalciferol $kg^{-1}$of diet; basal diet + phytase + 5 µg of 1-α(OH)D_3 and 1,000 IU of cholecalciferol $kg^{-1}$of diet**.** The birds were reared on a lighting program including a period of 23h light, and 1h darkness d^{-1}. The broiler house temperature was kept at 37°C, on the first day of experiment and gradually reduced to 24°C in the initial of fourth week and then fixed on it.

Feed analyses

Before formulize the basal diet, soybean meal, corn and monocalcium phosphate, were analyzed for P (method 965.17 (Association Official Analytical Chemist [AOAC], 1990) and Ca (method 968.08 (AOAC, 1995)), also the percentage of Ca was measured in calcium carbonate. Basal diet was formulated thereafter and was dried at 55°C for 72h in an oven and ground through a 1-mm sieve, then used to measure dry matter (DM) (method 934.01 (AOAC, 1990)), Ca (method 968.08 (AOAC, 1995)) total P (method 965.17 (AOAC, 1990)).

Growth performance

Mean body weight (BW) and feed intake were measured per cage at 37 d of age, to determine daily weight gain, and daily feed intake (DFI). Mortality was recorded as it happened to accurate daily weight gain (DWG) and feed conversion ratio (FCR).

Table 1. The ingredient, calculated and measured composition of basal diet.

Ingredients, g kg^{-1}	Basal diet
Corn	509.75
Soybean meal (45% CP)	450.00
Soybean oil	19.30
DL-Met	0.20
L-Thr	0.90
Choline chloride (45% choline)	1.45
MCP[1] (15% Ca and 22.5% P)	0.60
$CaCO_3$	12.30
NaCl	2.10
$NaHCO_3$	1.40
Trace mineral premix[2]	1.00
Vitamin premix[3]	1.00
Calculated composition	
ME[4], kcal kg^{-1}	2,900
CP[5], g kg^{-1}	240.00
Total Lys, g kg^{-1}	13.08
Total sulfur amino acids, g kg^{-1}	7.50
Total Thr, g kg^{-1}	10.20
Ca, g kg^{-1}	6.00
Total P, g kg^{-1}	3.88
Nonphytate P, g kg^{-1}	1.50
Na, g kg^{-1}	1.50
Cl, g kg^{-1}	1.80
Analyzed Content	
Ca, g kg^{-1}	6.30
Total P, g kg^{-1}	3.92

[1]Mono calcium phosphate.[2]Provided the following per kilogram of diet: Mg, 60 mg;Fe, 120 mg;Cu, 5 mg;Zn, 25 mg; Se,0.2 mg;I, 0.3 mg.[3] Provided the following per kilogram of diet:vitamin A, 1,650 IU;vitamin E, 12 IU; vitamin K, 1.0 mg; thiamin, 2.0 mg; riboflavin, 4.0 mg;vitamin B_6, 3.0;vitamin B_{12}, 0.003 mg;pantothenic acid, 10 mg; nicotinic acid, 40 mg;folic acid, 1 mg; biotin, 0.30 mg.[4] Metabolizable energy.[5]Crude protein.

Chemical analysis

At termination of the experiment, 2 birds/cage were sacrificed by cervical displacement to separate the left tibia to measure the percentage of ash on a dry fat-free basis (method 22.10 (AOAC, 1995)). The percentage of Ca and P were assayed by the inductively coupled plasma optical emission spectroscopy (method 2011.14 (AOAC, 1990)).

Plasma ALP and minerals

At termination of the experiment 2 birds per replicate were bled. Blood samples were taken by puncture of the brachial vein into heparinized tubes to determine plasma ALP enzyme activity, Ca and P concentrations. Serum Ca, P and ALP enzyme activity were measured by using commercial kits (Parsazmun, Tehran, Iran).

Statistical analysis

ANOVA was carried out to analyze treatments effects on growth performance, tibia quality, plasma total mean ALP enzyme activity and minerals by using the general linear model procedures of SAS (SAS/STAT Version 9.2, SAS Institute Inc., Cary, NC), in a completely randomized design. Differences between means were separated by Tukey's test at 5% probability.

Results and discussion

Growth performance

The effects of dietary 1-α$(OH)D_3$ supplementation alone or in combination with microbial phytase, and different concentration of cholecalciferol on BW, FI, and FCR of broiler quails are presented in Table 2.At 37 d of age, the highest BW obtained in the group supplemented with 1,000 IU cholecalciferol kg^{-1} of diet in compare with those fed basal diet and basal diet supplemented with 1-α$(OH)D_3$ alone ($p < 0.05$), but didn't statistically differ from other groups ($p > 0.05$). Broiler quails fed diets containing 500 IU cholecalciferol kg^{-1} of diet (20.8 g) had significantly ($p < 0.05$) higher DFI in compare with those fed basal diet (9.1 g), basal diet

supplemented with 1-α(OH)D_3 alone (18.7 g), basal diet supplemented with 1-α(OH)D_3 and phytase (17.0 g), basal diet supplemented with 1-α(OH)D_3,phytase and 250 IU cholecalciferol kg^{-1} of diet (18.4 g), basal diet supplemented with 1-α(OH)D_3,phytase and 750 IU cholecalciferol kg^{-1} of diet (19.3 g) and basal diet supplemented with 1-α(OH)D_3,phytase and 1,000 IU cholecalciferol kg^{-1} of diet (19.2 g). The worst FCR obtained in the group fed basal diet, and supplementation of 1-α(OH)D_3 alone, 1-α(OH)D_3 and phytase, and 1-α(OH)D_3, phytase and different levels of cholecalciferol significantly ($p < 0.05$) improved it. The best FCR obtained in the group supplemented with 1-α(OH)D_3,phytase and 1,000 IU cholecalciferol kg^{-1} of diet ($p < 0.05$).

Table 2. Effects of dietary 1-α(OH)D_3 alone or in combination with microbial phytase, and different component of cholecalciferol on body weight (BW), feed intake (FI), and feed conversion ratios (FCR) of broiler quails at 37 d of age.

Item	Whole experiment (5 to 37 d)		
	BW (g)	FI (g)	FCR (g g^{-1})
Basal	64.3^{c}	9.1^{e}	5.30^{a}
Basal + 1-α(OH)D_3	195.6^{b}	18.7^{bc}	3.21^{b}
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase	196.0^{ab}	17.0^{d}	2.93^{b}
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 250 IU D_3	210.6^{ab}	18.4^{c}	2.92^{b}
Basal + 1-α(OH)D_3 + 500 FTU $kg^{-1}$of phytase + 500 IU D_3	207.6^{ab}	20.8^{a}	3.34^{b}
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 750 IU D_3	208.3^{ab}	19.3^{b}	3.09^{b}
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 1,000 IU D_3	219.0^{a}	19.2^{b}	2.90^{b}
SEM[1]	4.80	0.15	0.12
p-value	0.001	0.001	0.001

^{a,e} Values in the same column not sharing a common superscript differ ($p< 0.05$).[1] Standard error of the mean.

Han et al. (2013) investigated efficacy of different inclusion rate of cholecalciferol (0, 5, 10, 25, 50, 100, 250, 500, and 1000 mg kg^{-1}) and 1-α(OH)D_3 (0, 2.5, and 5 mg kg^{-1}) on performance criteria and tibia quality of broiler chickens fed diet deficient in Ca and P. The lowest BW was seen in the group which were not supplemented with 1-α(OH)D_3or cholecalciferol, broiler chickens positively responded to cholecalciferol supplementation. Atencio, Pesti, and Edwards (2005) investigated efficacy of supplementing 25-OHD_3 in dietary which contain very low content of cholecalciferol on hen-day egg production in broiler breeders; the results indicated that broiler breeder positively responded to cholecalciferol supplementation. Similarly, Kheiri and Landy (2019) reported that supplementation of broiler chickens' diet containing 1-α(OH)D_3 with1,500 IU cholecalciferol kg^{-1} of diet resulted in higher performance, though supplementation of higher dosage (3,000 or 5,000 IU D_3 kg^{-1} of diet) reduced performance criteria. Landy et al. (2015) investigated efficacy of adding 5,000 IU cholecalciferol kg^{-1} of diet (recommended dosage by Aviagen, 2014) in broiler chickens fed diets deficient in Ca-P and containing 5 µg of 1-α(OH)D_3 $kg^{-1}$of diet in different growth periods on performance criteria; in starter period broilers negatively responded to cholecalciferol supplementation, though in grower and finisher phases cholecalciferol supplementation had not any significant effects on performance indices. Han et al. (2009) reported that addition of cholecalciferol in broiler chickens' diet containing 5 µg 1-α(OH)D_3 kg^{-1} of diet could not improve performance criteria. In contrast with the mentioned result in broiler chickens trials, in the current study increasing inclusion rate of cholecalciferol in Ca-P deficiet diet containing 5 µg 1-α(OH)D_3 kg^{-1} of diet resulted in better performance as an increment in final BW and improvement in FCR were obtained.

Tibia quality

The effects of experimental treatments on tibia criteria of broiler quails at 37 d of age are presented in Table 3. The lowest tibia weight obtained in the group supplemented with basal diet and addition of 1-α(OH)D_3 alone, 1-α(OH)D_3 and phytase, and 1-α(OH)D_3, phytase and different levels of cholecalciferol significantly ($p < 0.05$) increased it. Addition of phytase and different concentrations of cholecalciferol to basal diet supplemented with 1-α(OH)D_3 couldn't significantly affect tibia weight in compare with those fed diets supplemented with 1-α(OH)D_3 alone ($p > 0.05$). The lowest tibia length obtained in the group fed basal diet ($p < 0.05$). Broiler quails fed basal diet supplemented with 1-α(OH)D_3 alone had significantly higher tibia length in compare with those fed basal diet supplemented with 1-α(OH)D_3,phytase and 750 IU cholecalciferol kg^{-1} of diet ($p < 0.05$), but it didn't statistically differ from those fed basal diet supplemented with 1-α(OH)D_3, and phytase, and basal diet supplemented with 1-α(OH)D_3,phytase and 250, 500, and 1,000 IU cholecalciferol kg^{-1} of diet ($p > 0.05$). The lowest tibia diameter obtained in the group fed basal diet ($p < 0.05$). Broiler quails fed basal diet had significantly (p

< 0.05) lower tibia ash, Ca and P. Addition of phytase and different concentrations of cholecalciferol to basal diet supplemented with 1-α(OH)D_3 alone couldn't significantly ($p > 0.05$) affect tibia ash, Ca and P.

Table 3. Effects of dietary 1-α(OH)D_3 alone or in combination with microbial phytase, and different component of cholecalciferol on tibia criteria of broiler quails at 37 d of age.

Item	Tibia quality					
	Weight (g)	Length (cm)	Diameter (cm)	Tibia ash (%)	Calcium (%)	Phosphorus (%)
Basal	0.41[b]	3.93[c]	1.79[b]	30.9[b]	9.03[b]	4.64[b]
Basal + 1-α(OH)D_3	0.80[a]	5.43[a]	2.40[a]	47.0[a]	16.40[a]	8.64[a]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase	0.78[a]	5.35[ab]	2.49[a]	44.2[a]	15.86[a]	8.09[a]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 250 IU D_3	0.88[a]	5.39[ab]	2.60[a]	45.7[a]	16.66[a]	8.21[a]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 500 IU D_3	0.80[a]	5.35[ab]	2.43[a]	46.5[a]	17.57[a]	8.61[a]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 750 IU D_3	0.77[a]	5.16[b]	2.54[a]	45.1[a]	17.10[a]	8.36[a]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 1,000 IU D_3	0.81[a]	5.35[ab]	2.57[a]	44.9[a]	16.72[a]	8.31[a]
SEM[1]	0.03	0.60	0.07	1.77	0.62	0.28
p-value	0.001	0.001	0.001	0.001	0.001	0.001

[a,c] Values in the same column not sharing a common superscript differ ($p < 0.05$). [1] Standard error of the mean.

In the present trial, addition of 1-α(OH)D_3 alone could maximize tibia ash, whereas addition of phytase or phytase plus different concentrations of cholecalciferol had not any significant effects on tibia ash, though it tended to decrease. Similarly, Kheiri and Landy (2019) reported that inclusion of 1-α(OH)D_3 alone in broiler chicken's diet could maximize tibia ash, Ca and P, whereas addition of different concentrations of cholecalciferol in diets containing 1-α(OH)D_3 reduced tibia ash, Ca and P. In agreement with our findings, Landy et al. (2015) compared efficacy of supplementing Ca-P deficient diet with 5 µg of 1-α(OH)D_3 kg^{-1} of diet alone with combination of 5,000 IU cholecalciferol and 5 µg of 1-α(OH)D_3 kg^{-1} of diet in broiler chickens; the result indicated that combination of cholecalciferol and 1-α(OH)D_3 had negative effect on bone mineralization. Drewe, Dietsch, and Keck (1988) reported that supplementation of dietary inadequate in cholecalciferol with calcitriol resulted in an increment in plasma calcitriol. According to researchers' report an interaction between cholecalciferol and calcitriol exists in bone mineralization (Edwards Junior et al. 2002).

Plasma ALP and minerals

The effects of experimental treatments on plasma ALP, Ca and P are presented in Table 4. The highest plasma total mean ALP enzyme activity obtained in the group fed basal diet ($p < 0.05$). Broiler quails fed basal diet supplemented with 1-α(OH)D_3,phytase and 250, and 1,000 IU cholecalciferol kg^{-1} of diet had lower plasma total mean ALP enzyme activity in compare with those fed basal diet supplemented with 1-α(OH)D_3 alone, basal diet supplemented with 1-α(OH)D_3 and phytae, and basal diet supplemented with 1-α(OH)D_3,phytase and 500, and 750 IU cholecalciferol kg^{-1} of diet, though the results were not statistically significant ($p > 0.05$). The lowest plasma Ca obtained in the group fed basal diet ($p < 0.05$). The plasma Ca of broiler quails fed basal diet supplemented with 1-α(OH)D_3 alone and basal diet supplemented with 1-α(OH)D_3,phytase and 1,000 IU cholecalciferol kg^{-1} of diet was higher than those fed basal diet supplemented with 1-α(OH)D_3 and phytase and basal diet supplemented with 1-α(OH)D_3,phytase and 250, 500, and 750 IU cholecalciferol kg^{-1} of diet , though the results were not statistically significant ($p > 0.05$).

Supplementation of 1-α(OH)D_3 and phytase, and 1-α(OH)D_3,phytase and 500, and 750 IU cholecalciferol kg^{-1} of diet to basal diet significantly enhanced ($p < 0.05$) plasma P concentration. ALP enzyme activity is an index for the assessment of vitamin D insufficiency (Misra, Pacaud, Petryk, Collett-Solberg, & Kappy, 2008). Accordingly, cases without high plasma ALP enzyme activity may be excluded from a distinction of vitamin D insufficiency. Thus in the current trial addition of 1-α(OH)D_3 alone could decrease elevated plasma ALP enzyme activity as an indicator of vitamin D deficiency and consequently increase absorption of Ca and P.

Table 4. Effects of dietary 1-α(OH)D_3 alone or in combination with microbial phytase, and different component of cholecalciferol on plasma alkaline phosphatase (ALP), calcium (Ca), and phosphorous (P) of broiler quails.

Item	Plasma parameters		
	ALP (U L^{-1})	Ca (mg dL^{-1})	P (mg dL^{-1})
Basal	19544[a]	6.63[b]	5.26[c]
Basal + 1-α(OH)D_3	4202[b]	9.45[a]	7.16[abc]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase	3540[b]	8.72[a]	7.28[ab]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 250 IU D_3	2653[b]	8.05[a]	5.63[bc]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 500 IU D_3	3550[b]	8.34[a]	7.73[a]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 750 IU D_3	3219[b]	8.54[a]	7.66[a]
Basal + 1-α(OH)D_3 + 500 FTU kg^{-1} of phytase + 1,000 IU D_3	2750[b]	9.22[a]	7.00[abc]
SEM[1]	1197	1.21	0.45
p-value	0.001	0.001	0.001

Conclusion

In conclusion, supplementation of 1-α(OH)D_3alone to basal diet containing low levels of Ca and P maximizes tibia mineralization, whereas supplementation of 1-α(OH)D_3,phytase, and 1,000 IU cholecalciferol kg^{-1} of diet maximizes performance criteria, body weight and feed conversion ratio.

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