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Influence of dietary 1αlpha-hydroxycholecalciferol, individually or in combination with microbial phytase in calcium and phosphorus deficient diets on growth performance and tibia parameter of Japanese quails (*Coturnix japonica*)

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ABSTRACT. The effect of 1α-OH-D_3 in calcium-phosphorus (Ca-P) deficient diets on Japanese quail growth performance and tibia parameters was investigated. Eight-day-old (n = 160) newly hatched quails were weighed and randomly allocated to 20 groups, each with 4 replicate pens of 8 birds. Treatments were as follows: T_1, Ca-P-adequate; T_2, Ca-P-deficient; T_3, Ca-P-deficient + 500 FTU kg^{-1} of phytase (Ph); T_4, Ca-P-deficient diet + 5 µg kg^{-1} of 1α-OH-D_3; T_5, Ca-P-deficient + Ph + 5 µg kg^{-1} of 1α-OH-D_3. Results showed that quails fed Ca-P-adequate had significantly higher body weight compared with quails fed Ca-P-deficient, Ca-P deficient supplemented with 1α-OH-D_3 and Ca-P-deficient supplemented with 1α-OH-D_3 and phytase, but did not differ from Ca-P-deficient diet supplemented with phytase. Quails fed Ca-P deficient were unable to achieve FCR comparable to quails fed Ca-P-adequate ($p < 0.05$). The percentage of bone ash data indicated that quails fed Ca-P-adequate had higher tibia ash compared with other groups except for quails fed Ca-P deficient diet supplemented with combination of 1α-OH-D_3 and phytase. Quails fed Ca-P-adequate had higher tibia P compared with quails fed Ca-P-deficient. In conclusion, these results indicated that quails fed Ca-P-deficient supplemented with 5 µg kg^{-1} of 1α-OH-D_3 in combination of 500 FTU kg^{-1} of phytase were able to achieve the same tibia ash and Ca compared with quails fed Ca-P-adequate.

Keywords: Bone mineralization; bird; calcium and phosphorus deficiency; phytase; vitamin D derivative.

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Introduction

Approximately less than one-third of the phosphorus (P) in feedstuffs of plant origin is biologically accessible to poultry [National Research Council (NRC), 1994] because they do not have the digestive enzyme phytase to hydrolyze the phytate phosphorus (Nelson, McGillivray, Shieh, Wodzinski, & Ware, 1968). As a result, inorganic sources of P are incorporated to diets to meet P requirements of poultry (Selle & Ravindran, 2007). Growing pressure on farmers to decrease environmental pollution with poultry manure has stimulated research into ways to increase the accessibility of phytate phosphorus (PP) in feedstuffs (Landy & Toghyani, 2018). The addition of phytase enzyme is one such method to reduce environmental pollution (Edwards Junior, Shirley, Escoe, & Pesti, 2002; Jiang et al., 2013; Nelson, Shieh, Wodzinski, & Ware, 1971; Simons et al., 1990). Edwards Junior and Veltmann Juniorr (1983) indicated that enhancing dietary calcium (Ca) level will reduce PP usage by poultry due to the formation of insoluble Ca phytate. Similarly, Applegate, Angel, and Classen (2003) reported that intestinal mucosa phytase activity enhances and more PP is hydrolyzed at lower levels of dietary Ca compared with higher levels of Ca in broiler chickens.

Cholecalciferol (Vitamin D_3) is generally and widely used in poultry production. As an analog of vitamin D, 25-OH-D_3 is used as feed additive for the vitamin D requirement of poultry (Ghasemi et al., 2018). The metabolite 1α-hydroxycholecalciferol (1α-OH-D_3) is the derivative of cholecalciferol. Edwards Junior et al. (2002) reported that the 1 α- OH-D_3 is approximately five to eight times as effective as cholecalciferol in promoting growth and tibia quality. The results of a trial conducted by Landy and Toghyani (2014) indicated

the ability of 1α-OH-D_3 to substitute for cholecalciferol in broiler chickens. Snow, Baker, and Parsons (2004) investigated efficiency of 1α-OH-D_3 and phytase in dietary containing low level of non-phytate phosphorus (NPP), the results indicated that interaction between 1α-OH-D_3 and phytase had affirmative effects on PP release of young broilers. Driver et al. (2005) also reported that combination of phytase and 1α-OH-D_3 could improve the percentage of tibia ash in broilers fed low level of Ca and P. Furthermore, Han et al. (2012a) reported that 1α-OH-D_3 had higher activity at low levels of dietary Ca in comparison to high levels of Ca. Han et al. (2015) reported that the supplementation of 5 µg of 1alpha-OH-D_3 per kg of diet could improve growth performance and tibia mineralization when dietary non-phytate phosphorus levels reached 0.30% in 1- to 21-d-old broilers. Landy, Toghyani, Bahadoran, and Eghbalsaied (2015) reported that supplementation of 1α-OH-D_3 in Ca-P deficient diet could improve tibia ash, Ca and P of broilers.

Despite these findings, there has been a dearth of information on the possible effects of 1α-OH-D_3 on Japanese quails. The aim of the experiment reported here was to investigate the effects of dietary1α-OH-D_3, individually or in combination of microbial phytase on phytate P utilization in Ca-P deficient diet of growing Japanese quails.

Material and methods

Ethical approval

The birds were handled in compliance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Also, all procedures complied with the ethical guidelines of the Shahrekord University's Ethical Committee (approval ref No. 2017-03).

Animals and dietary treatments

Eight-day-old (*n* =160) as-hatched Japanese quails (*Coturnix coturnix japonica*) were individually weighed, selected, and divided into 20 groups of 8 chicks. The dietary treatments were as follows: T_1, Ca-P-adequate (0.80% Ca, 0.59% total phosphorus (tP)); T_2, Ca-P-deficient (0.60% Ca, 0.44% tP); T_3, Ca-P-deficient + 500 FTU kg^{-1} of Phyzyme XP 5000 unique bacterial phytase developed by Danisco Animal Nutrition; T_4, Ca-P-deficient + 5 µgvkg^{-1} of 1α-OH-D_3 (Vitamin Derivatives Inc., Georgia, USA); T_5, Ca-P-deficient + 500 FTU kg^{-1} of phytase + 5 µg kg^{-1} of 1α-OH-D_3. The basal diet was formulated to meet or exceed the nutritional requirements of Japanese quails (NRC, 1994) except for Ca and P (Table 1). Chicks were placed in wire cages and had free access to feed and water throughout the whole trial. The chick house was completely enclosed and light was on during d 1 to 3, which was reduced to 14 h on d 16 and kept constant thereafter. The ambient temperature was controlled at 37°C during the first d and then gradually reduced to 24° C by the end of third wk and then was maintained constant.

Feed analyses

Dry matter (DM), Ca and tP content of formulated experimental diets were assayed for each experimental diet. Feed, were dried by using Oven for DM determinations. Ca and tP contents of experimental diets were evaluated by the ICPOES method 2011.14 (Association Official Analytical Chemist [AOAC], 1990).

Performance parameters

Average body weights (BW, g) of pens were recorded at day 8 and at the end of trial. Feed intake of each group was determined at 42 d of age. Growth performance was evaluated in terms of BW, average daily feed intake (DFI, g), and feed conversion ratio (FCR, Feed (g): Gain (g)). These data, determined on pen basis, and then averaged by treatment.

Chemical analysis

At the end of trial, 8 male quails/treatment were selected, sacrificed, and the left tibia of each quail was removed for determination of percentage of bone ash on a fat-free dry-weight basis, according to AOAC (1995; method 22.10). The percentages of Ca and P in tibia ash were determined via the ICPOES method 2011.14 (AOAC, 1990).

Table 1. The ingredients, calculated and determined composition of experimental diets.

Item	Adequate	Deficient
Ingredient, g kg^{-1}		
Corn	546.99	547.25
Soybean meal (45% CP)	410	41
Soybean oil	8	8
DL-Methionine	0.50	0.50
L-Lysine	0.45	0.45
L-Threonine	1.45	1.45
Choline chloride 60%	1.80	1.80
Monocalcium phosphate 15% Ca, 2.5% P	9	2.42
Calcium carbonate	13.42	10.72
Salt	3.39	3.39
Trace mineral premix[1]	2.5	2.5
Vitamin premix[2]	2.5	2.5
Sand	0	9
Calculated composition, g kg^{-1}		
ME, kcal	2,800	2,800
CP	228.6	228.6
Ca	8.0	6.0
Phosphorus-total	5.9	4.4
Nonphytate P	3.0	1.5
Analyzed nutrient content, g kg^{-1}		
Calcium	8.2	6.3
Phosphorus-total	6.0	4.7

[1]Provided the following per kg of diet: Mg, 60 mg; Fe, 120 mg; Cu, 5 mg; Zn, 25 mg; I, 0.3 mg; Se, 0.2 mg. [2] Provided the following per kg of diet: vitamin A, 1,650 IU; vitamin D_3, 750 IU; vitamin E, 12 IU; vitamin K, 1 mg; Thiamin, 2 mg; riboflavin, 4 mg; vitamin B_{12}, 0.003 mg; pantothenic acid, 10 mg; nicotinic acid, 40 mg; folic acid, 1 mg; Biotin, 0.3 mg; Pyridoxine, 3 mg.

Statistical analysis

All of data were analyzed using the General Linear Model procedures of SAS (2012). Differences between means were tested using Tukey's HSD (Honestly Significant Difference). Values were considered statistically different at $p \leqslant 0.05$.

Results and discussion

Growth performance

Treatments failed to induce any significant effect on DFI (Table 2), though it tended to enhance in quails fed Ca-P-deficient diets supplemented with 1α-OH-D_3 ($p > 0.05$). At the end of trial, quails fed Ca-P-adequate diet had significantly higher BW (189 g) compared with quails fed Ca-P-deficient diet (163 g), Ca-P-deficient diet supplemented with 1α-OH-D_3 (168 g), and Ca-P-deficient diet supplemented with 1α-OH-D_3 and phytase (169 g), but did not differ from the quails fed Ca-P-deficient diet supplemented with phytase (174 g) that was intermediate. Supplementation of phytase to Ca-P-deficient diet could improve BW at 42 d of age, whereas the results were not statistically significant ($p > 0.05$). Quails that received the Ca-P-adequate diet during the trial were the most feed efficient ($p < 0.05$). The birds fed the Ca-P-deficient diets throughout the trial were unable to achieve FCR index comparable to the quails fed Ca-P-adequate diet, although it tended to improve in birds fed Ca-P-deficient diet supplemented with phytase and 1α-OH-D_3.

In the present trial supplementation of 1α-OH-D_3 and phytase to Ca-P-deficient diet couldn't maximize growth performance of quails, although, supplementation of Ca-P-deficient diet with phytase could improve BW of quails at 42 d of age. Similarly, Attia et al. (2012) indicated that addition of phytase to broilers diet enhanced BW and this may be due to the increment in the availability and absorption of nutrients. In agreement with our results, Edwards Junior et al. (2002) reported that supplementation of 1α-OH-D_3 to dietary containing 0.30% of NPP could not improve FCR and BW in 1- to 16-d-old broilers but later in another trial Snow et al. (2004) indicated that addition of 1α-OH-D_3 and phytase to dietary containing 0.13% of NPP could improve the growth of broilers, although the growth was lower than those fed adequate levels of NPP. Our data in the current trial suggest that interaction for growth performance indices between phytase and 1α-OH D_3 might exist at lower level of NPP.

Parameters of tibia

Dietary treatments did not have any significant effect on tibia weight, length and diameter (Table 3). At the end of trial, quails fed Ca-P-adequate diet had significantly higher tibia ash (32.4%) compared with quails fed Ca-P-deficient diet (25.2%), Ca-P-deficient diet supplemented with phytase (25.8%), Ca-P-deficient diet supplemented with 1α-OH-D_3 (25.8%), but did not differ from the quails fed Ca-P-deficient diet supplemented with phytase and 1α-OH-D_3 (31.1%). Treatments failed to induce any significant effect on tibia Ca. The birds fed Ca-P-deficient diet supplemented phytase and 1α-OH-D_3 were able to achieve the same tibia Ca (11.2%) as chicks fed Ca-P-adequate diet (11.4%). The birds fed Ca-P-deficient diet (10.26%), Ca-P-deficient diet supplemented with phytase (11.1%), and Ca-P-deficient diet supplemented with 1α-OH-D_3 (10.3%) had lower tibia Ca than those fed Ca-P-adequate (11.4%), whereas the differences were not statistically significant ($p > 0.05$). Quails fed Ca-P-adequate diet had significantly higher tibia P (5.55%) compared with quails fed Ca-P-deficient diet (4.40%), but did not differ from the quails fed Ca-P-deficient diet supplemented with phytase (4.97%), Ca-P-deficient diet supplemented with 1α-OH-D_3 (4.6%), and Ca-P-deficient diet supplemented with 1α-OH-D_3 and phytase (4.7%), that were intermediate.

In the current trial the dietary treatments had no effect on tibia weight, length and diameter. In contrast with obtained results in our trial, Han et al. (2012b) reported that addition of 5 or 10 μg kg^{-1} of 1α-OH-D_3 to Ca-P-deficient diet could increase tibia length and weight of broilers, but it had not any effects on tibia width. Our data in the present trial showed that addition of phytase or 1α-OH-D_3 alone to Ca-P deficient diet had not any effect on tibia ash but, addition of phytase and 1α-OH-D_3 could promote tibia ash of quails. In contrast with our results, Edwards Junior et al. (2002) indicated that supplementation of 1α-OH-D_3 to P deficient diet could improve tibia ash and P utilization of broilers but later in another experiment Driver, Pesti, Bakalli, and Edwards Junior (2005) reported that supplementation of phytase and 1α-OH-D_3 could improve the percentage of tibia ash in broilers. Snow et al. (2004) indicated that interaction between phytase and 1α-OH-D_3 had beneficial effects on PP release of young broilers. It seems that interaction exist between 1α-OH-D_3 and phytase on bone mineralization.

In the current trial 1α-OH-D_3 in combination with phytase could improve tibia ash and Ca. The 1α-OH-D_3 improves PP utilization by facilitating Ca absorption and reducing the restriction of Ca on endogenous phytase. Han et al. (2009) reported that 1α-OH-D_3 facilitate intestinal P absorption due to stimulating small intestinal NaPi-IIb cotransporter gene expression. In addition, these findings show that 1α-OH-D_3 can regulate NaPi-IIb cotransporter gene transcription, and increase absorption of phosphate.

Table 2. Effect of dietary Calcium (Ca) and phosphorus (tP), phytase (P) and 1α-OH-cholecalciferol supplementation (1α) on performance indices of quails at 42 d.

	Treatments		Performance parameters		
	Suppl[1]	Ca and tP	Feed intake,g d^{-1}	Body weight,g	Feed : gain,g g^{-1}
T_1	-	Adequate	15.2	189[a]	2.78[b]
T_2	-	Deficient	15.4	163[b]	3.70[a]
T_3	Ph	Deficient	15.4	174[ab]	3.63[a]
T_4	1α	Deficient	16.0	168[b]	3.67[a]
T_5	Ph+1α	Deficient	15.3	169[b]	3.46[a]
SEM			0.23	4.10	0.08

SEM = standard error of the mean. [a,b] Values in the same column not sharing a common superscript differ ($p < 0.05$). [1] Ph represent 500 phytase units kg^{-1}, 1 α represent 5 μg kg^{-1} of 1 α-OH cholecalciferol and P+1 α represent 500 phytase units kg^{-1} plus 5 μg kg^{-1} of 1 α-OH cholecalciferol.

Table 3. Effect of dietary Calcium (Ca) and phosphorus (P), phytase (P) and 1α-OH-cholecalciferol supplementation (1α) on tibia parameters of quails at 42 d.

	Treatments		Tibia parameters					
	Suppl[1]	Ca and tP	Weight, g	Length, cm	Diameter, cm	Tibia ash, %	Calcium, %	Phosphorus, %
T_1	-	Adequate	1.05	5.45	0.17	32.35[a]	11.42	5.55[a]
T_2	-	Deficient	1.05	5.42	0.18	25.2[b]	10.26	4.40[b]
T_3	Ph	Deficient	1.02	5.47	0.17	25.8[b]	11.09	4.97[ab]
T_4	1α	Deficient	1.05	5.42	0.17	25.8[b]	10.3	4.60[ab]
T_5	Ph+1α	Deficient	1.05	5.45	0.17	31.1[ab]	11.23	4.66[ab]
SEM			0.02	0.04	0.05	1.46	0.57	0.21

SEM = standard error of the mean. [a,b]Values in the same column not sharing a common superscript differ ($p < 0.05$). [1] Ph represent 500 phytase units kg^{-1}, 1 α represent 5 μg kg^{-1} of 1 α-OH cholecalciferol and P+1 α represent 500 phytase units kg^{-1} plus 5 μg kg^{-1} of 1 α-OH cholecalciferol.

Conclusion

In conclusion, in the current study quails fed Ca-P-deficient diet supplemented with 5 μg kg^{-1} of 1α-OH-D_3 in combination of 500 FTU kg^{-1} of phytase were able to achieve the same tibia ash and Ca compared with quails fed Ca-P-adequate when dietary nonphytate phosphorus and Ca levels were 0.15% and 0.6%, respectively, whereas addition of 1α-OH-D_3 alone or in combination with phytase could not maximize growth performance of quails.

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