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Research article

The effects of 1αlpha-hydroxycholecalciferol supplementation on performance and tibia parameter of broiler chickens

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Abstract

This experiment was conducted to examine the effects of 1α-OH-D_3 either with or without cholecalciferol as well as two levels of dietary calcium (Ca) and phosphorus (P) on broiler chickens performance and tibia parameters. A total of 192 one day old mixed sex broiler chicks (Ross 308) were allocated to four treatments, each with 4 replicates. The dietary treatments were as follow: T_1: Ca-P-adequate + phytase; T_2: Ca-P-adequate + phytase + 1α-OHD_3; T_3: Ca-P-deficient + phytase + 1α-OHD_3; T_4: Ca-P-deficient diet without cholecalciferol + phytase + 1α-OHD_3. The feed conversion ratio (FCR) was significantly improved in starter period and decreased in grower period in broiler fed Ca-P deficient diet without vitamin D_3 when compared with the same diet with vitamin D_3. The broilers fed the deficient diet with vitamin D_3 throughout the starter period had lower body weight and FCR and ate more compared with broilers fed the adequate diet plus1α-OHD_3 ($P>0.05$). Chicks fed the deficient diet with vitamin D_3 were unable to achieve the same tibia ash, Ca and P values as chicks fed the deficient diet without vitamin D_3 ($P<0.01$). These values for chicks fed the deficient diet without vitamin D_3 were not significantly different from chicks fed adequate diets. In conclusion, the results indicate that Ca-and P-deficient diet without vitamin D_3 supplemented with 5 µg/kg of 1α-OH D_3 and 500 FTU/kg of phytase appear to be adequate for broilers' bone mineralization, however, there are some variables that may limit growth performance of broilers.

Keywords: Bone mineralization; cholecalciferol; phosphorus deficiency; phytase

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Introduction

Phytate phosphorus (PP) is utilized inefficiently by chickens and other monogastric animals because they do not possess the digestive enzyme phytase to significantly hydrolyze the phytate molecule. Growing pressure on poultry producers to reduce the amount of phosphorus entering the environment with poultry manure has stimulated research into ways to enhance the availability of PP. One such method to lower P excretion is to render available the P in phytate by the addition of microbial phytase (Nelson et al., 1971; Simons et al., 1990; Jiang et al., 2013).

Vitamin D_3 is generally used in poultry nutrition. As an analogue of vitamin D, 25-OH D_3 is authorized for use in feed for poultry nutrition. It is possible that

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1alpha-hydroxycholecalciferol (1alpha-OHD_3) become a feed additive in future. Landy and Toghyani (2012) described the ability of 1alpha-OHD_3 to substitute for Vitamin D_3 in broiler chickens. The 1 α- OHD_3 has approximately eight times as effective as cholecalciferol (Edwards et al., 2002). Han et al. (2009) reported that the addition of 1alpha-OHD_3 could improve growth performance, tibia development, and meat quality in 1 to 21day old broilers by increasing the absorption and retention of P. Driver et al. (2005) also reported that addition of 5 µg/kg of 1α-OH D_3 and 1,000 U/kg of phytase improved growth and tibia ash in broilers fed with Ca and P deficient diets, but later in another study Han et al. (2009) found that 1α-OH D_3 decreased body weight gain (BWG) and feed intake (FI) in starter broilers when 1α-OH D_3 and phytase were added together. Edwards et al. (2002) reported that in basal diet with total P of 7.0 g/kg and without vitamin D_3, 1α-OH D_3 improved BW of young broilers, while when vitamin D_3 was enough growth of broilers was not improved (Biehl et al., 1997c; Han et al., 2009). Furthermore, Han et al. (2012b) observed the highest activity of 1α-OH D_3 at lower concentration of dietary Ca, and its efficacy responded negatively to dietary Ca level.

The present study was designed to evaluate the effects of 1α-OH-D_3 either with or without Cholecalciferol microbial phytase as well as different levels of dietary Ca and P on broiler chickens performance and tibia parameters.

Materials and Methods

Animals and dietary treatments

On the day of hatch 192 mixed sex broiler chickens (Ross-308) were individually weighed and randomly and equally allocated to each of the 4 treatment groups, each with 4 replicate pens of 12 chicks. There were 4 dietary treatments, where each treatment consisted of a starter diet from 0 to 14 day, a grower diet from 14 to 28 day, and finisher diet from 28 to 42 day. All diets (Table 1) contained 5000 IU/kg of vitamin D_3 except for regimen 4 that fed diet without vitamin D_3. All diets were treated with 500 FTU/kg of Phyzyme XP 5000 phytase (Danisco Animal Nutrition) and 0 or 5 µg/kg of 1*α*-OHD_3 (Vitamin Derivatives Inc., Georgia, USA). The dietary treatments were as follows: Starter diets (T_1: 0.90% Ca, 0.616% tP + phytase; T_2: 0.90% Ca, 0.616% tP + phytase + 1α-OHD_3; T_3: 0.80% Ca, 0.56% tP + phytase + 1α-OHD_3; T_4: 0.80% Ca, 0.56% tP + phytase + 1α-OHD_3) Grower diets (T_1: 0.75% Ca, 0.551% tP + phytase; T_2: 0.75% Ca, 0.551% tP + phytase + 1α-OHD_3; T_3: 0.65% Ca, 0.50% tP + phytase + 1α-OHD_3; T_4: 0.65% Ca, 0.50% tP + phytase + 1α-OHD_3) and Finisher diets (T_1: 0.695% Ca, 0.508% tP + phytase; T_2: 0.695% Ca, 0.508% tP + phytase + 1α-OHD_3; T_3: 0.595% Ca, 0.458% tP + phytase + 1α-OHD_3; T_4: 0.595% Ca, 0.458% tP + phytase + 1α-OHD_3). Chicks were raised on floor pens (120 × 120 × 80 cm) for 6 weeks and had free access to feed and water throughout the entire experimental period. The broiler house was completely enclosed and lighting was provided by incandescent bulbs to prevent birds from being exposed to ultraviolet radiation and synthesizing their own vitamin D. The lighting program consisted of a period of 23 h light and 1 h of darkness. The experimental house temperature was controlled at 32^{o}C during the first week and then gradually reduced by 3^{o}C per wk to finally fixed at 22^{o}C.

Performance parameters

Average pen weights of broilers were recorded at the start of the study, 14, 28, and 42 day of age. Daily weight gain (DWG) and daily feed intake (DFI) were recorded in different periods and feed conversion ratio (FCR) was calculated. Mortality was recorded as it occurred.

Chemical analysis

At 42 d of age, 2 birds per pen were chosen, based on the average weight of the group and killed by cervical dislocation, and the left tibia from each bird was collected for bone ash analysis on a dry fat-free basis (method 22.10; AOAC, 1995). Calcium and P contents of tibia ash were analyzed by the ICPOES method 2011.14 (AOAC, 1990).

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). Means were compared using the LSD method. Statements of statistical significance are based on $P<0.05$. Contrast comparisons (group 1 vs. group 2, group 2 vs. group 3, and group 3 vs. group 4) were carried out as well.

Results

Growth performance

Data on performance indices are summarized in Table 2. The body weight (BW), DFI, and FCR were higher for chickens fed the Ca-and P-adequate diet plus 1α-OHD_3 than those fed the same adequate diet without 1α-OHD_3 when compared at the end of starter period, whereas the results were not statistically significant ($P>0.05$). The BW of chicks fed the Ca and P deficient diet without vitamin D_3 was similar to chicks fed the adequate diet plus 1α-OHD_3 at the end of starter period. Broilers that received the deficient diet without vitamin D_3 during the starter period were the most feed efficient ($P<0.05$). The broilers fed the deficient diet with vitamin D_3 throughout the starter period had lower BW and FCR index and ate more compared with broilers fed the adequate diet plus1α-OHD, whereas the results were not statistically significant (P>0.05).

The BW, DFI, and FCR of chicks receiving adequate diet plus 1α-OHD_3 were lower than chicks fed the same adequate diet without 1α-OHD_3, when compared at the end of grower period, whereas differences were not statistically significant (P>0.05). The broilers fed the deficient diets throughout grower and finisher periods were unable to achieve BW responses comparable to broilers fed adequate diets, although differences were not statistically significant (P>0.05). Treatments failed to induce any marked effect on DFI, when compared during the grower and finisher periods, whereas it tended to decrease in broilers fed deficient diets or adequate plus 1α-OHD_3 (P>0.05). No significant differences (P>0.05) were found between treatments for FCR index throughout grower and finisher periods.

Contrast comparison of obtained data from performance indices are summarized in Table 3. Considering the contrast comparisons of performance indices, there were no significant differences between treatments groups except for FCR, which gave statistical significant for T_3. vs. T_4 in starter and grower periods. Addition of vitamin D_3 to the Ca and P-deficient diet significantly decreased FCR index in starter period (P = 0.021), and it improved FCR index in grower period (P = 0.010).

Parameters of tibia

Data on tibia quality are summarized in Table 4. No significant differences (P>0.05) were found between treatments for tibia diameter. The broilers fed Ca-P deficient diet with vitamin D_3 and 1α-OHD_3 were unable to achieve the same tibia weight as chicks fed Ca-P adequate diet with or without 1α-OHD_3. Addition of 1α-OHD_3 to the Ca and P- adequate diet significantly decreased tibia length, and broilers fed Ca-P adequate diet plus 1α-OHD_3 were unable to achieve the same tibia length as chicks fed Ca-P deficient diets. Chicks fed the deficient diet with vitamin D_3 were unable to achieve the same tibia ash, Ca and P values as chicks fed the deficient diet without vitamin D_3 (P<0.05). Furthermore, these values for chicks fed the deficient diet without vitamin D_3 were not significantly different (P>0.05) from that of chicks fed the adequate diets. No significant differences (P>0.05) were found between broilers fed adequate diet plus 1α-OHD_3 and the same adequate diet without 1α-OHD_3 for any of tibia variables measured, whereas it tended to decrease in broilers fed adequate diet plus 1α-OHD_3 (P>0.05).

Contrast comparison of obtained data from tibia parameters are summarized in Table 5. No significant differences (P>0.05) were found between treatment groups for tibia diameter. The broilers fed Ca-P deficient diet with vitamin D_3 and 1α-OHD_3 were unable to achieve the same tibia weight as chicks fed Ca-P adequate diet with vitamin D_3 and 1α-OHD_3 (T_2. vs. T_3). Considering the contrast comparisons of the tibia length there were significant differences between T_1 vs. T_2 and T_2 vs. T_3. Addition of 1α-OHD_3 to the Ca and P adequate diet significantly decreased tibia length (P = 0.041), and broilers fed Ca-P adequate diet plus 1α-OHD_3 were unable to achieve the same tibia length as chicks fed Ca-P deficient diet with vitamin D_3 and 1α-OHD_3 (P = 0.039). Addition of vitamin D_3 to the Ca

Table 1: The ingredient and calculated composition of experimental diets

Ingredient, g/kg	Starter		Grower		Finisher	
	Adequate	Deficient	Adequate	Deficient	Adequate	Deficient
Corn	545.5	545.5	576.6	577.7	626.97	626.97
Soybean meal	397	397	365	365	320	320
Soybean oil	11.7	11.7	20	20	16.6	16.6
Monocalcium phosphate	11	8.8	8.7	6.5	7.5	5.3
$CaCO_3$	16	14.3	13.6	11.9	12.9	11.2
Sand	5	8.9	5.2	8	5.8	9.7
NaCl	3.2	3.2	3.2	3.2	3.2	3.2
Trace mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix[2]	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	3	3	2.2	2.2	1.84	1.84
L-Lysine	2.1	2.1	0.5	0.5	0.19	0.19
L-Threonine	0.5	0.5	-	-	-	-
Calculated composition						
ME, kcal	2,850	2,850	2,950	2,950	2,976	2,976
CP, %	22.7	22.7	21.2	21.2	19.53	19.53
Calcium, %	0.9	0.8	0.75	0.65	0.695	0.695
Phosphorus-total, %	0.616	0.566	0.551	0.5	0.508	0.458
Nonphytate P, %	0.382	0.331	0.325	0.274	0.293	0.242

[1] Provided the following per kg of diet: Mg, 56 mg; Fe, 40 mg; Cu, 16 mg; Zn, 100 mg; Co, 125 mg; I, 1.25 mg; [2] Provided the following per kg of diet: vitamin A, 11,000 IU; vitamin D_3, 5000 IU; vitamin E, 75 IU; vitamin K, 3 mg; riboflavin, 8 mg; vitamin B_{12}, 0.016 mg; pantothenic acid, 15 mg; nicotinic acid, 60 mg; folic acid, 2 mg; choline, 3 mg.

Table 2: Effect of dietary with or without vitamin D_3, phytase (P) and 1α-OH-cholecalciferol supplementation (1α) on body weight (BW), feed intake (FI), and feed conversion ratios (FCR) of broilers at different ages

	Treatments				Starter period (0 to 14 d)			Grower period (15 to 28 d)			Finisher period (29 to 42 d)		
	Suppl[c]	D_3[d]	Ca	tP	BW (g)	FI (g)	FCR (g/g)	BW (g)	FI (g)	FCR (g/g)	BW (g)	FI (g)	FCR (g/g)
T_1	P	+	Adequate		240	20.9	1.48[ab]	1050	91.1	1.57	1905	155	2.60
T_2	P+1α	+	Adequate		270	22.0	1.41[ab]	983	85.4	1.71	1866	139	2.24
T_3	P+1α	+	Deficient		223	24.0	1.86[a]	966	81.7	1.54	1750	141	1.99
T_4	P+1α	-	Deficient		270	18.7	1.14[b]	916	86.7	1.87	1733	129	2.26
SEM[e]					12	2.58	0.149	36	2.79	0.135	47	7.6	0.16

[a,b] Values in the same column not sharing a common superscript differ (P<0.05); [c] P represent 500 phytase units/kg and P+1α represent 500 phytase units/kg plus 5 µg/kg of 1 α-OH cholecalciferol; [d]represent dietary containing 5000 IU/kg D_3 and – represent dietary without vit D_3; [e]Standard error of mean.

Table 3: Contrast comparisons of data obtained from performance parameters at different ages

Treatment Comparison[a]	Starter period (0 to 14 d)			Grower period (15 to 28 d)			Finisher period (29 to 42 d)		
	BW (g)	FI (g)	FCR (g/g)	BW (g)	FI (g)	FCR (g/g)	BW (g)	FI (g)	FCR (g/g)
	Probability								
T_1 vs. T_2	0.423[n.s]	0.337[n.s]	0.745[n.s]	0.526[n.s]	0.422[n.s]	0.604[n.s]	0.644[n.s]	0.421[n.s]	0.368[n.s]
T_2. vs. T_3	0.233[n.s]	0.368[n.s]	0.167[n.s]	0.879[n.s]	0.561[n.s]	0.534[n.s]	0.421[n.s]	0.910[n.s]	0.527[n.s]
T_3. vs. T_4	0.089[n.s]	0.202[n.s]	0.021[*]	0.468[n.s]	0.461[n.s]	0.010[**]	0.893[n.s]	0.525[n.s]	0.566[n.s]

[a]Linear contrast comparison between treatments indicated; [n.s] not significant; [*](P≤0.05); [**](P≤0.01).

Table 4: Effect of dietary with or without vitamin D_3, phytase (P) and 1α-OH-cholecalciferol supplementation (1α) on tibia parameters of broilers at 42 d

	Treatments				Tibia parameters					
	Suppl[c]	D_3[d]	Ca	tP	Weight (g)	Length (cm)	Diameter (cm)	Tibia ash (%)	Calcium (%)	Phosphorus (%)
T_1	P	+	Adequate		5.40[a]	9.48[a]	0.61	44.29[a]	16.36[a]	8.13[a]
T_2	P+1α	+	Adequate		5.00[a]	9.22[b]	0.61	43.42[a]	16.01[a]	7.95[a]
T_3	P+1α	+	Deficient		4.19[b]	9.58[a]	0.56	39.97[b]	14.77[b]	7.41[b]
T_4	P+1α	-	Deficient		4.68[ab]	9.62[a]	0.55	44.63[a]	16.20[a]	8.20[a]
SEM[e]					0.24	0.09	0.04	0.52	0.23	0.11

[a,b] Values in the same column not sharing a common superscript differ (P<0.05); [c] P represent 500 phytase units/kg and P+1 α represent 500 phytase units/kg plus 5 µg/kg of 1 α-OH cholecalciferol; [d]+ represent dietary containing 5000 IU/kg D_3 and – represent dietary without vit D_3; [e]Standard error of mean.

Table 5: Contrast comparisons of data obtained from tibia parameters

Treatment Comparison[a]	Tibia parameters					
	Weight (g)	Length (cm)	Diameter (cm)	Tibia ash (%)	Calcium (%)	Phosphorus (%)
	Probability					
T_1 vs. T_2	0.283 [n.s]	0.041[*]	0.883 [n.s]	0.295 [n.s]	0.314 [n.s]	0.341 [n.s]
T_2. vs. T_3	0.022[*]	0.039[*]	0.199 [n.s]	0.001[**]	0.001[**]	0.007[**]
T_3. vs. T_4	0.236 [n.s]	0.807 [n.s]	0.815 [n.s]	0.000[**]	0.000[**]	0.000[**]

[e]Linear contrast comparison between treatments indicated; [n.s] not significant; [*](P≤0.05); [**](P≤0.01).

and P- deficient diet with 1α-OHD_3 decreased tibia ash, Ca and P values (P<0.01). Effect of adequate and inadequate Ca-P with vitamin D_3 and 1α-OHD_3 (T_2. vs. T_3) on tibia parameters gave P<0.01. The broilers fed Ca-P deficient diet with vitamin D_3 and 1α-OHD_3 were unable to achieve the same tibia ash, Ca and P values as chicks fed Ca-P adequate diet with vitamin D_3 and 1α-OHD_3 (T_2. vs. T_3).

Discussion

In the current work, addition of 1α-OHD_3 and phytase to the Ca and P-deficient diet with or without vitamin D_3 could not maximize growth performance of broiler chicks, however, broilers fed deficient diet without vitamin D_3 supplemented with 1α-OHD_3 were able to achieve the same tibia ash, Ca and P values as chicks fed the adequate diets. Similarly, Edwards (2002) observed the beneficial influence of 1α-OH D_3 on tibia ash and P utilization of broilers, although it did not improve BW and FCR in 1 to 16 day old broilers when the dietary non-phytate phosphorus (NPP) level was as little as 0. 3% but later in another study Driver et al. (2005) reported that supplementation of Ca and P deficient diets with 5 µg/kg of 1α-OH D_3 and 1,000 FTU/kg of phytase improves growth and tibia ash in broilers. Han et al. (2009) and Shirley (2003) found that addition of 1α-OH D_3 and phytase had negative effects on BW and FI of broilers during starter period. In another study, Snow et al. (2004) indicated that interaction between 1α-OH D3 and phytase had affirmative effects on BW and phytate phosphorus release in starter broilers, although, NPP of basal diet was low (0.13%) and growth was lower than those fed adequate P. Attia et al. (2012) reported that phytase supplementation increased BW and this may be due to the improvement in the availability and absorption of nutrients. The result of our trial suggest that interaction for performance indices between 1α-OH D_3 and phytase might exist at lower level of Ca and P.

In the current study, addition of 1α-OH D_3 to the Ca and P adequate diet had not any significant effect on performance criteria and tibia parameters, which indicated that 1α-OH D_3 could not improve growth performance and tibia parameters of broilers when broilers fed normal Ca and P dietary and vitamin D_3 was abundant. Unfortunately, most studies focus only on Ca-P deficient diets, and to date, there has been dearth of information on the effect of 1α-OH D_3 on performance and tibia variables when Ca and P are normal and vitamin D_3 is abundant.

Previous studies on 1α-OH D_3 have been based on adequate dietary vitamin D_3 (Biehl and Baker, 1997a&b; Snow et al., 2004; Driver et al., 2005) so the present trial was designed to evaluate the effects of 1α-OH-D_3 either with or without vitamin D_3 on bone mineralization of broilers. Edwards (2002) reported that an interaction between vitamin D_3 and 1, 25-(OH)2 D_3 exists in tibia ash. Han et al. (2012a) investigated the effect of 1α-OH D_3 on bone mineralization in broilers fed Ca and P-deficient diet without D_3, result of their trial showed that the 1α-OH D_3 could improve tibia ash, Ca, P and breaking strength. In addition, it seems that an interaction between vitamin D_3 and 1α-OH D_3 exists in bone mineralization.

Han et al. (2012b) reported that supplementation of 1α-OH D_3 to the broilers diet enhanced serum Pi concentration. Research has shown that 1α-OH D_3 metabolized quickly to 1, 25-$(OH)_2D_3$ in intestinal membrane of chicks (Holick et al., 1976; Edelstein et al., 1978). The active 1,25-$(OH)_2D_3$ facilitated intestinal NaPi-IIb mRNA expression in suckling rats but had no effect in mature rats (Katai et al., 1999). NaPi-IIb, sodium-dependent phosphate cotransporter, is expressed in the small intestinal epithelium, where it is considered to be the major sodium-Pi co-transporter. Based on previous research, 1α-OH D_3 may facilitate intestinal phosphate absorption by stimulating the NaPi-IIb gene expression. In addition, these findings show that 1α-OH D_3 can regulate NaPi-IIb cotransporter gene transcription, and increase phosphate absorption.

Conclusion

In conclusion, in the current study addition of 1α-OH D_3 to the Ca and P adequate diet could not improve growth performance and tibia parameters. Also contrast comparison of data revealed that an interaction between vitamin D_3 and 1, 25-(OH)2 D_3 exist on bone mineralization. The results indicate that Ca and P deficient diet without vitamin D_3 supplemented with 5 μg/kg of 1α-OH D_3 and 500 FTU/kg of phytase appeared to be adequate for broilers' bone mineralization. However, supplementing broilers diet with 5 μg/kg of 1α-OH D_3 and 500 FTU/kg of phytase could not maximize growth performance and there are some variables that may limit performance of broilers. Further studies are required to determine the Ca and P levels necessary to maximize growth with levels of phytase and 1α-OHD_3.

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