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Optimal Dietary Levels of 1α-Hydroxycholecalciferol in Broiler Chickens from 1 to 42 Days of Age

Xue Yang[1,2], Ning Zhang[1,2], Xiaona Wang[1,2], Hongxia Qu[2], Jinliang Zhang[2], Yongfeng Yan[2], Yeonghsiang Cheng[3] and Jincheng Han[2]

[1] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, Henan, China
[2] Department of Animal Science, College of Life Science, Shangqiu Normal University, Shangqiu, Henan, China
[3] Department of Biotechnology and Animal Science, National Ilan University, Taiwan

1α-Hydroxycholecalciferol (1α-OH-D_3) is an active vitamin D derivative. In this study, three experiments were conducted to evaluate the optimal dietary levels of 1α-OH-D_3 in broiler chickens from 1 to 42 days of age. 1α-OH-D_3 levels used were 0, 1.25, 2.5, 5, and 10 μg/kg in experiment 1, 0.625, 1.25, 2.5, 5, 7.5, and 10 μg/kg in experiment 2, and 2, 2.5, 3, 3.5, 4, 4.5, and 5 μg/kg in experiment 3. In experiment 1, the addition of 0 to 10 μg/kg of 1α-OH-D_3 quadratically improved growth performance, tibia development, and mRNA expression levels of nuclear vitamin D receptor (nVDR), membrane vitamin D receptor (mVDR), and type IIb sodium-phosphate cotransporter (NaPi-IIb) in the duodenum of broiler chickens from 1 to 12 days of age. Body weight gain (BWG), the weight and ash weight of the tibia, and mRNA expression levels of mVDR and NaPi-IIb of broilers fed with 0 and 10 μg/kg of 1α-OH-D_3 were lower than those of birds fed with 2.5 μg/kg of 1α-OH-D_3. In experiment 2, 1α-OH-D_3 levels were quadratically related to BWG and to weight and ash weight of the femur and the tibia of broiler chickens at 42 days of age. The highest values of growth performance and bone mineralization were recorded in broilers fed with 2.5 to 5 μg/kg of 1α-OH-D_3. In experiment 3, there was no difference observed in BWG and the weight and ash weight of the femur and the tibia of the 42-day-old broilers fed with 2 to 5 μg/kg of 1α-OH-D_3. These data suggest that the optimal dietary levels of 1α-OH-D_3 were 2 to 5 μg/kg for broiler chickens from 1 to 42 days of age.

Key words: 1α-hydroxycholecalciferol, broiler chicken, growth, bone, vitamin D receptor, type IIb sodium-phosphate cotransporter

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Introduction

Vitamin D is an essential nutrient that regulates calcium (Ca) and phosphorus (P) absorption and retention in poultry diets. The vitamin D products widely used in feeds in China include cholecalciferol (vitamin D_3) and 25-hydroxycholecalciferol (25-OH-D_3). 1α-Hydroxycholecalciferol (1α-OH-D_3) is a new vitamin D. Its relative biological value was higher than that of vitamin D_3 and 25-OH-D_3 (Edwards *et al*., 2002; Han *et al*., 2017). The addition of 1α-OH-D_3 in feeds improved growth performance, bone mineralization, and P utilization in 1- to 21-day-old broiler chickens fed with P-deficient diets (Biehl and Baker, 1997; Snow *et al*., 2004). Broiler chickens fed Ca- and P-inadequate diet with 5 μg/kg of 1α-OH-D_3 and adequate vitamin D_3 showed insufficient for bone formation (Landy and Toghyani, 2018; Ghasemi *et al*., 2019). Another research has shown that supplementation with 15 to 25 μg/kg of 1α-OH-D_3 inhibited body weight (BW) and feed intake (FI) and resulted in kidney mineralization in 1- to 42-day-old broiler chickens fed with Ca- and P-adequate diets (Pesti and Shivaprasad, 2010). These data suggest that the responses of broilers to 1α-OH-D_3 are affected by dietary P levels. However, the optimal 1α-OH-D_3 levels in P-adequate diets of broiler chickens have not been examined.

The promotion of P absorption in the small intestine by dietary 1α-OH-D_3 contributes to the improvement of growth performance and bone mineralization of broiler chickens. When 1α-OH-D_3 is transformed into 1,25-dihydroxycholecalciferol [1,25-$(OH)_2$-D_3], the latter binds to vitamin D

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Correspondence: Dr. Jincheng Han, Department of Animal Science, College of Life Science, Shangqiu Normal University, Shangqiu, China.
(E-mail: j.c.han@hotmail.com)
Xue Yang and Ning Zhang contributed equally to this work.

receptor (VDR) to regulate P absorption in the small intestine. Two types of VDRs are found in poultry, which are nuclear vitamin D receptor (nVDR) and membrane vitamin D receptor (mVDR). nVDR is located in the nucleus of the epithelial cells of the small intestine. mVDR, which is also called the membrane-associated rapid response steroid binding (MARRS) receptor or ERp57/GRp58, is located in the basal lateral membrane of the small intestinal cells (Khanal and Nemere, 2007; Nemere *et al*., 2012). Type IIb sodium-phosphate cotransporter (NaPi-IIb) is a P transporter protein in the apical membranes of the epithelial cells in the small intestine of broiler chickens (Yan *et al*., 2007; Forster *et al*., 2013). 1,25-$(OH)_2$-D_3 regulates NaPi-IIb mRNA expression levels in the small intestine of rats (Xu *et al*., 2002). However, the relationships between VDR and NaPi-IIb mRNA expressions in the small intestine and 1α-OH-D_3 levels in broiler diets with adequate P have not been examined.

Therefore, the objective of this study was to evaluate the effects of 1α-OH-D_3 levels on growth performance, bone mineralization, and mRNA expression levels of VDR and NaPi-IIb in the small intestine and to estimate the optimal dietary levels of 1α-OH-D_3 in broiler chickens from 1 to 42 days of age.

Materials and Methods

Birds, Diets and Management

All animal care procedures adopted in this study were approved by Henan Agricultural University and Shangqiu Normal University.

The Ca and non-phytate phosphorus (NPP) levels in the basal diets were respectively 1.00% and 0.45% for broilers of 1 to 21 days old and 0.90% and 0.35% in birds of 22 to 42 days old in experiments 1, 2, and 3 (Table 1) (NRC, 1994). The basal diets did not contain supplemental vitamin D_3 in the three experiments.

In experiment 1, 150 males of 1-day-old Ross 308 broilers were weighed and randomly allotted to five treatment groups with three replicate cages containing 10 birds each. Five levels of 1α-OH-D_3 (0, 1.25, 2.5, 5 and 10 μg/kg) were added to the basal diet. At 12 days old, three chickens per replicate cage (9 birds per treatment) were randomly selected and euthanized by cervical dislocation to collect the tibia and the mucosa samples from the duodenum. The tibia was excised and then frozen at −20℃. The duodenal mucosa was scraped off 3 cm at half of the individual duodenal segments using a glass microscope slide on ice. This was immediately frozen in liquid nitrogen, and then stored at −80℃.

Table 1. **Ingredients and nutrient composition of the experimental diets (as-fed basis)**

Item	Exp. 1	Exp. 2		Exp. 3	
	1 to 12 days	1 to 21 days	22 to 42 days	1 to 21 days	22 to 42 days
Ingredient (%)					
Corn	58.11	58.12	63.28	58.10	63.27
Soybean meal (45% CP)	32.07	32.07	27.52	32.07	27.52
Soybean oil	2.22	2.20	3.00	2.22	3.00
Soy protein powder (65% CP)	3.50	3.50	2.74	3.50	2.74
Limestone	1.36	1.36	1.45	1.36	1.47
Dicalcium phosphate	1.94	1.94	1.36	1.94	1.35
L-Lysine·HCl (98%)	0.14	0.14	0.14	0.14	0.14
DL-Methionine (98%)	0.14	0.14	0.08	0.14	0.08
Trace mineral premix[1]	0.01	0.01	0.01	0.01	0.01
Vitamin premix[2]	0.01	0.02	0.02	0.02	0.02
Choline chloride (50%)	0.20	0.20	0.10	0.20	0.10
Sodium chloride	0.30	0.30	0.30	0.30	0.30
Nutrient composition (%)					
Metabolizable energy (kcal/kg)	2951	2950	3054	2951	3053
Crude protein	21.07	21.07	19.08	21.07	19.08
Calcium (Ca)	1.00	1.00	0.90	1.00	0.90
Analyzed Ca	1.01	0.96	0.92	1.03	1.00
Total phosphorus (tP)	0.69	0.69	0.58	0.69	0.57
Analyzed tP	0.68	0.69	0.58	0.70	0.61
Non-phytate phosphorus (NPP)	0.45	0.45	0.35	0.45	0.35
Lysine	1.10	1.10	0.99	1.10	0.99
Methionine	0.50	0.50	0.41	0.50	0.41

[1] The trace mineral premix provided the following (per kg of diet): 80 mg iron; 40 mg zinc; 8 mg copper; 60 mg manganese; 0.35 mg iodine; 0.15 mg selenium.

[2] The vitamin premix (without vitamin D_3) provided the following (per kg of diet): 8,000 IU vitamin A; 20 IU vitamin E; 0.5 mg menadione; 2.0 mg thiamine; 8.0 mg riboflavin; 35 mg niacin; 3.5 mg pyridoxine; 0.01 mg vitamin B_{12}; 10.0 mg pantothenic acid; 0.55 mg folic acid; 0.18 mg biotin.

Table 2. **Primer sequences for quantitative real-time PCR**

Gene	Accession	Orientation	Primer sequence (5′–3′)	Size (bp)
nVDR	AF011356.1	Forward	AAGTCATCGACACCCTCCTG	173
		Reverse	GCCAAAGACATCGTTGGAGT	
mVDR	NM_204110.3	Forward	CTACTGGCGCAACCGAGTTA	136
		Reverse	CTCACCCACGCTGTTGTCTA	
NaPi-IIb	NM_204474.1	Forward	TCGGTCCGTTCACTCTGTTG	164
		Reverse	GCCACGTTGCCTTTGTGATT	
GAPDH	NM_204305.1	Forward	GAACATCATCCCAGCGTCCA	133
		Reverse	ACGGCAGGTCAGGTCAACAA	

In experiment 2, 300 males of 1-day-old Ross 308 broilers were weighed and randomly allotted to six treatment groups with five replicate cages containing 10 birds each. Six different amounts of 1α-OH-D_3 (0.625, 1.25, 2.5, 5, 7.5, and 10 μg/kg) were added to the basal diet. At 42 days old, three chickens per replicate cage (15 birds per treatment) were randomly selected and euthanized by cervical dislocation for collection of the femur and the tibia. The leg bones were excised and then frozen at −20℃.

In experiment 3, 350 males of 1-day-old Ross 308 broilers were weighed and randomly allotted to seven treatment groups with five replicate cages of 10 birds each. Seven levels of 1α-OH-D_3 (2, 2.5, 3, 3.5, 4, 4.5, and 5 μg/kg) were added to the basal diet. At 42 days old, three broilers per replicate cage (15 birds per treatment) were randomly selected and euthanized. Femur and tibia samples were collected and frozen at −20℃.

The broilers were reared in stainless steel cages (190 cm × 50 cm × 35 cm). The birds were provided *ad libitum* access to mash feed and water during the 42 days of the experiment and were exposed to 20 h of light from incandescent bulbs and 4 h of darkness. The room temperature was controlled at 33℃ from 0 to 3 days, 30℃ from 4 to 7 days, 27℃ from 8 to 21 days, and 24℃ from 22 to 42 days.

1α-OH-D_3

The crystalline 1α-OH-D_3 was supplied by Taizhou Healtech Chemical Co., Ltd. (Taizhou, China). The solution was prepared using the following method described by Biehl and Baker (1997). In brief, crystalline 1α-OH-D_3 was weighed, dissolved in ethanol, and diluted with propylene glycol (5% ethanol and 95% propylene glycol). The concentration of 1α-OH-D_3 solution was analyzed using high-performance liquid chromatography (HPLC) method by Shanghai Fuxin Chemical Technology Service Co., Ltd. (Shanghai, China). The concentration of 1α-OH-D_3 solution was determined as 9.25 μg/mL. An appropriate quantity of 1α-OH-D_3 solution was pipetted and added to the diets.

Sample Collection and Analysis

All broilers were weighed at 12 days (experiment 1) and 42 days of age (experiments 2 and 3). The FI, body weight gain (BWG), and feed conversion ratio (FCR) of the broilers were calculated. All the birds that died spontaneously during the experiment were weighed, and the weight was used to correct the FI.

The mineralization of the femur and tibia was analyzed as described by Hall *et al*. (2003). The bones were dried at 105℃ for 24 h, and then weighed. The bone ash weight was determined by ashing the bone in a muffle furnace at 600℃ for 48 h. The Ca and total phosphorus (tP) contents in the diets and the bones were determined as described by Han *et al*. (2017).

Total RNA Extraction, Reverse Transcription, and Quantitative Real-Time Polymerase Chain Reaction (PCR)

The total RNA was isolated from the mucosa of the duodenum of the chickens using TRIzol reagent (Tiangen Biotech Co. Ltd., Beijing, China) in accordance with the manufacturer's instructions. RNA concentration was determined spectrophotometrically. The OD260/280 values ranged from 1.8 to 2.0 to assure the purity of the total RNA. All samples were stored at −80℃.

Reverse transcription was performed with 1 μg of the total RNA using primescript reverse transcription reagent kit (Takara Biotechnology Co. Ltd., Dalian, China) in accordance with manufacturer's instructions. The primers of nuclear vitamin D receptor (nVDR), membrane vitamin D receptor (mVDR), type IIb sodium-phosphate cotransporter (NaPi-IIb) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China, Table 2).

Quantitative real-time PCR was performed using SYBR Premix PCR Kit (Takara Biotechnology Co. Ltd., Dalian, China) on a Thermo Scientific PikoReal Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). The reactions were conducted in a 10 μL reaction system containing 5 μL of SYBR Green Premix I PCR mix (Tli RNaseH Plus) (2×), 0.4 μL of forward primer (10 μM), 0.4 μL of reverse primer (10 μM), 1.0 μL of cDNA, and 3.2 μL of RNase-free water. The program was set at 95℃ for 60 s, followed by 40 cycles of 95℃ for 10 s, 60℃ for 30 s, and 72℃ for 30 s. Each gene was amplified in triplicates. The standard curve was determined using pooled samples. The gene expression levels relative to the endogenous control of GAPDH for each sample were calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical Analysis

Replicate means are the experimental units in statistical analysis. The data were analyzed using the general linear model (GLM) procedure of the SAS software (SAS Institute,

2002). Polynomial comparisons were performed to determine the linear and quadratic effects of 1α-OH-D_3 levels on the growth performance, bone mineralization, and mRNA expression levels of nVDR, mVDR, and NaPi-IIb.

Results

Experiment 1

The addition of 0 to 10 μg/kg of 1α-OH-D_3 quadratically improved the FI, BWG, and FCR of broiler chickens from 1 to 12 days of age (Table 3). Compared to the basal diet, the addition of 1.25 μg/kg of 1α-OH-D_3 increased the BWG. No significant differences in BWG were observed among broilers fed with 1.25, 2.5 and 5 μg/kg of 1α-OH-D_3. The BWG of broilers fed with 10 μg/kg of 1α-OH-D_3 was lower than that of birds fed with 1.25 and 2.5 μg/kg of 1α-OH-D_3.

Dietary 1α-OH-D_3 quadratically affected tibia mineralization of broiler chickens at 12 days of age (Table 3). Increasing 1α-OH-D_3 levels from 0 to 1.25 μg/kg enhanced the weight, length, ash weight, and P percentage of the tibia. No significant differences in tibia quality were found in birds fed with 1.25, 2.5, and 5 μg/kg of 1α-OH-D_3. In contrast, the weight and ash weight of the tibia of broilers fed with 10 μg/kg of 1α-OH-D_3 were lower than those of birds fed with 1.25, 2.5, and 5 μg/kg of 1α-OH-D_3.

Dietary 1α-OH-D_3 quadratically affected the mRNA expression levels of nVDR, mVDR, and NaPi-IIb in the duodenum of broiler chickens at 12 days old (Table 3). Compared to the basal diet, the addition of 1.25 μg/kg of 1α-OH-D_3 enhanced the mRNA expression levels of the three genes. No significant differences in the mRNA expression levels of the three genes observed in birds fed with 1.25 and 2.5 μg/kg of 1α-OH-D_3. Increasing 1α-OH-D_3 levels from 2.5 to 10 μg/kg decreased the mRNA expression levels. The mRNA expression levels of nVDR and NaPi-IIb in broilers fed with 10 μg/kg of 1α-OH-D_3 were lower than those of birds fed with 1.25, 2.5, and 5 μg/kg of 1α-OH-D_3. The lowest mRNA expression levels of nVDR and mVDR were observed in birds fed with basal diet. In contrast, the lowest NaPi-IIb mRNA expression level was detected in birds fed with 10 μg/kg of 1α-OH-D_3.

Experiment 2

Supplementation with 0.625 to 10 μg/kg of 1α-OH-D_3 quadratically improved FI and BWG but linearly increased the mortality of broiler chickens from 1 to 42 days of age (Table 4). Growth performance of the chickens was enhanced as dietary 1α-OH-D_3 levels increased from 0.625 to 2.5 μg/kg. The broilers showed the highest FI and BWG when they were fed with 2.5 and 5 μg/kg of 1α-OH-D_3. However, the performance of the birds declined when dietary 1α-OH-D_3 levels increased from 5 to 10 μg/kg. The FI and BWG of broilers fed with 7.5 and 10 μg/kg of 1α-OH-D_3 were lower than those of birds fed with 2.5 and 5 μg/kg of 1α-OH-D_3. The lowest FI and BWG were observed in broilers fed with 0.625 and 10 μg/kg of 1α-OH-D_3. The highest mortality was recorded in birds fed with 7.5 and 10 μg/kg of 1α-OH-D_3.

Dietary levels of 0.625 to 10 μg/kg 1α-OH-D_3 were quadratically related to the weight, length, and ash weight of the femur and the tibia of the broiler chickens at 42 days old (Table 4). The lowest bone weight and ash weight was observed in broilers fed with 0.625 μg/kg of 1α-OH-D_3. The bone quality improved when the 1α-OH-D_3 levels increased from 0.625 to 2.5 μg/kg. The highest bone mineralization values were recorded in birds fed with 2.5 to 5 μg/kg of 1α-OH-D_3. However, the leg bone quality declined when dietary 1α-OH-D_3 levels increased from 5 to 10 μg/kg. The bone weight and ash weight of broilers fed with 10 μg/kg of 1α-OH-D_3 were lower than those of birds fed with 2.5 and 5 μg/kg of 1α-OH-D_3.

Table 3. **Effects of dietary levels of 1α-OH-D_3 on growth performance, tibia mineralization, and mRNA expression levels of nVDR, mVDR, and NaPi-IIb in the small intestine of broiler chickens from 1 to 12 days of age (experiment 1)**

Item	1α-OH-D_3 (μg/kg)					SEM	*P*-value		
	0	1.25	2.5	5	10		ANOVA	Linear	Quadratic
Growth performance from 1 to 12 days of age[1]									
BWG (g/bird)	206^{b}	282^{a}	274^{a}	249^{ab}	208^{b}	9	<0.001	0.376	<0.001
FI (g/bird)	294	358	346	328	296	10	0.104	0.677	0.015
FCR	1.43	1.27	1.26	1.33	1.43	0.03	0.243	0.761	0.034
Tibia mineralization at 12 days of age[2]									
Weight (g)	0.44^{c}	0.76^{a}	0.73^{a}	0.67^{a}	0.56^{b}	0.03	<0.001	0.050	<0.001
Length (cm)	4.26^{c}	4.83^{a}	4.78^{ab}	4.52^{abc}	4.49^{bc}	0.06	0.001	0.482	<0.001
Ash (g)	0.15^{c}	0.37^{a}	0.36^{a}	0.33^{a}	0.26^{b}	0.02	<0.001	0.001	<0.001
P (%)	6.66^{c}	8.81^{a}	8.67^{ab}	8.56^{ab}	8.01^{b}	0.22	<0.001	<0.001	<0.001
mRNA expression levels at 12 days of age[2]									
nVDR	1.00^{b}	2.40^{a}	2.00^{a}	1.98^{a}	1.05^{b}	0.15	<0.001	0.306	<0.001
mVDR	1.00^{b}	2.33^{a}	2.36^{a}	1.53^{b}	1.29^{b}	0.15	<0.001	0.542	<0.001
NaPi-IIb	1.00^{c}	2.63^{a}	2.50^{a}	1.83^{b}	0.18^{d}	0.25	<0.001	<0.001	<0.001

[1] Values are means of 3 replicates of 10 chickens per replicate (n=3).
[2] Values are means of 3 replicates of 3 chickens per replicate (n=3).

Table 4. **Effects of dietary levels of 1α-OH-D_3 on growth performance and bone mineralization of broiler chickens from 1 to 42 days of age (experiment 2)**

Item	1α-OH-D_3 (μg/kg)						SEM	*P*-value		
	0.625	1.25	2.5	5	7.5	10		ANOVA	Linear	Quadratic
Growth performance from 1 to 42 days of age[1]										
BWG (g/bird)	1028^{d}	1921^{bc}	2299^{a}	2180^{ab}	1868^{c}	1235^{d}	90	<0.001	0.159	<0.001
FI (g/bird)	2574^{c}	3934^{b}	4523^{a}	4494^{a}	3657^{b}	2665^{c}	150	<0.001	0.641	<0.001
FCR	2.53^{a}	2.05^{b}	1.97^{b}	2.07^{b}	1.96^{b}	2.16^{b}	0.04	<0.001	<0.001	<0.001
Mortality (%)	2	2	2	4	14	16	2	0.054	0.004	0.194
Femur mineralization at 42 days of age[2]										
Weight (g)	2.34^{c}	3.21^{bc}	4.33^{a}	4.41^{a}	3.85^{ab}	2.53^{c}	0.17	<0.001	0.119	<0.001
Length (cm)	5.43^{b}	6.63^{a}	6.93^{a}	6.81^{a}	6.62^{a}	5.92^{b}	0.11	<0.001	0.032	<0.001
Ash (g)	0.80^{d}	1.28^{bc}	1.75^{a}	1.85^{a}	1.58^{ab}	0.99^{cd}	0.08	<0.001	0.015	<0.001
P (%)	5.44	6.72	7.19	6.86	6.33	6.98	0.19	0.070	0.085	0.069
Tibia mineralization at 42 days of age[2]										
Weight (g)	2.63^{c}	3.89^{b}	5.60^{a}	5.87^{a}	5.13^{a}	3.77^{bc}	0.23	<0.001	<0.001	<0.001
Length (cm)	7.24^{c}	8.84^{ab}	9.47^{a}	9.30^{a}	9.09^{a}	8.21^{b}	0.16	<0.001	0.001	<0.001
Ash (g)	1.13^{c}	1.69^{b}	2.48^{a}	2.67^{a}	2.28^{a}	1.67^{b}	0.11	<0.001	<0.001	<0.001
P (%)	7.45	7.59	7.85	7.92	7.84	7.10	0.10	0.154	0.627	0.013

[1] Values are means for 5 replicate cages of 10 chickens per cage (n=5).
[2] Values are means for 5 replicate cages of 3 chickens per cage (n=5).

Table 5. **Effects of dietary levels of 1α-OH-D_3 on growth performance and bone mineralization of broiler chickens from 1 to 42 days of age (experiment 3)**

Item	1α-OH-D_3 (μg/kg)							SEM	*P*-value		
	2	2.5	3	3.5	4	4.5	5		ANOVA	Linear	Quadratic
Growth performance from 1 to 42 days of age[1]											
BWG (g/bird)	2359	2418	2305	2380	2226	2272	2320	21	0.208	0.094	0.567
FI (g/bird)	4654	4592	4337	4409	4293	4252	4458	57	0.448	0.113	0.154
FCR	1.97	1.90	1.88	1.86	1.93	1.88	1.92	0.02	0.935	0.705	0.355
Mortality (%)	2	2	2	4	2	4	0	1	0.820	0.852	0.335
Femur mineralization at 42 days of age[2]											
Weight (g)	4.58	4.76	4.20	4.35	4.61	4.57	4.36	0.08	0.512	0.570	0.649
Length (cm)	6.91^{a}	6.84^{a}	6.43^{b}	6.72^{ab}	6.48^{b}	6.62^{ab}	6.69^{ab}	0.05	0.039	0.073	0.023
Ash (g)	2.06	2.12	1.98	1.86	2.10	2.09	2.03	0.04	0.640	0.978	0.427
P (%)	7.65^{b}	8.00^{ab}	8.56^{a}	7.80^{ab}	8.23^{ab}	7.95^{ab}	7.70^{b}	0.08	0.025	0.778	0.010
Tibia mineralization at 42 days of age[2]											
Weight (g)	6.02	6.13	5.76	5.95	5.96	6.36	5.83	0.11	0.878	0.966	0.917
Length (cm)	9.33	9.34	8.89	9.13	8.80	8.93	9.17	0.06	0.089	0.088	0.042
Ash (g)	2.81	2.73	2.83	2.69	2.82	3.07	2.71	0.06	0.721	0.685	0.950
P (%)	7.47^{b}	7.97^{ab}	8.56^{a}	7.91^{ab}	8.66^{a}	8.42^{a}	7.35^{b}	0.11	<0.001	0.578	<0.001

[1] Values are means for 5 replicate cages of 10 chickens per cage (n=5).
[2] Values are means for 5 replicate cages of 3 chickens per cage (n=5).

Experiment 3

Dietary levels of 2 to 5 μg/kg 1α-OH-D_3 did not affect the FI, BWG, FCR, or mortality of broiler chickens from 1 to 42 days of age (Table 5). No relationship was observed between 1α-OH-D_3 levels and the growth of broilers. Similarly, dietary levels of 2 to 5 μg/kg 1α-OH-D_3 did not influence the weight or ash weight of the femur and the tibia of the broiler chickens at 42 days old (Table 5). In contrast, dietary 1α-OH-D_3 quadratically influenced P percentages in the femur and the tibia. The lowest values of P percentages were observed in birds fed with 2 and 5 μg/kg of 1α-OH-D_3.

Discussion

Growth Performance and Bone Mineralization

1α-OH-D_3 was hydroxylated by 25-hydroxylase to 1,25-$(OH)_2$-D_3 in the liver of chickens. 1,25-$(OH)_2$-D_3 is the final active form of 1α-OH-D_3. The bioactivity of 1α-OH-D_3 is similar to that of 1,25-$(OH)_2$-D_3 in broiler chicken diets (Edwards, 2002). Compared to basal diet without vitamin D, the addition of 2 μg/kg of 1,25-$(OH)_2$-D_3 improved the BW

and tibia ash percentage of 16-day-old broilers (Aburto *et al*., 1998). Similar results were obtained in the present study. The BWG and tibia ash weight of 1- to 12-day-old broilers was enhanced when dietary 1α-OH-D_3 levels increased from 0 to 2.5 μg/kg (experiment 1). The BWG, FI, and the weight and ash weight of the femur and the tibia of the 42-day-old broilers fed with 0.625 and 1.25 μg/kg of 1α-OH-D_3 were lower than those of the birds fed with 2.5 μg/kg of 1α-OH-D_3 (experiment 2). These data suggest that 1.25 μg/kg of 1α-OH-D_3 failed to satisfy the required level for growth performance and bone mineralization of the broilers.

The BW of 16-day-old broilers fed with 16 μg/kg of 1,25-$(OH)_2$-D_3 was lower than that of birds fed with 2 to 4 μg/kg of 1,25-$(OH)_2$-D_3 (Aburto *et al*., 1998). Moreover, compared to the control diet with adequate vitamin D, the addition of 10 and 15 μg/kg of 1,25-$(OH)_2$-D_3 decreased the BW in 1- to 21-day-old chickens (Rennie *et al*., 1993; Mitchell *et al*., 1997). Similar results were observed in 1α-OH-D_3 studies. High 1α-OH-D_3 levels (25 μg/kg) resulted in a severe kidney mineralization and growth inhibition in broiler chickens (Pesti and Shivaprasad, 2010). In the present study, the BWG and FI of the 42-day-old broilers fed with 7.5 and 10 μg/kg of 1α-OH-D_3 were lower than those of birds fed with 2.5 and 5 μg/kg of 1α-OH-D_3 (experiment 2). The highest mortality and the poor leg bone mineralization were recorded for the 42-day-old birds fed with 10 μg/kg of 1α-OH-D_3 (experiment 2). These data suggest that 7.5 and 10 μg/kg of 1α-OH-D_3 exceeded the optimal amount for growth and bone quality of broiler chickens from 1 to 42 days of age.

Research has shown that the addition of 5 μg/kg of 1,25-$(OH)_2$-D_3 increased the tibia ash percentage of 21-day-old broilers (Elliot *et al*., 1995; Mitchell *et al*., 1997; Han *et al*., 2009). In contrast, the addition of 5 μg/kg of 1,25-$(OH)_2$-D_3 (Elliot *et al*., 1995) or 1α-OH-D_3 (Pesti and Shivaprasad, 2010) did not affect the growth of broilers. These data suggest that 5 μg/kg of 1α-OH-D_3 or 1,25-$(OH)_2$-D_3 may be the maximum required amount for chickens. Aburto *et al*. (1998) did not find significant differences in growth between birds fed with 2 and 4 μg/kg of 1,25-$(OH)_2$-D_3. In the present study, the highest growth performance and bone mineralization values was detected in the 42-day-old chickens fed with 2.5 to 5 μg/kg of 1α-OH-D_3 (experiment 2). No difference was observed in the growth and leg bone quality of the 42-day-old birds fed with 2 to 5 μg/kg of 1α-OH-D_3 (experiment 3). Thus, the optimal 1α-OH-D_3 level for growth performance and bone mineralization in chickens should range from 2 to 5 μg/kg.

mRNA Expression Levels of nVDR, mVDR, and NaPi-IIb

The improvement of growth performance and bone development by 1α-OH-D_3 is related to P absorption and the P transporter gene expressions. 25-OH-D_3 is a derivative of vitamin D_3. Research has shown that the required 25-OH-D_3 amount is 12.5 μg/kg for broiler chickens from 1 to 42 days of age (Chen *et al*., 2017). Compared to the basal diet without vitamin D_3, the addition of 12.5 μg/kg of 25-OH-D_3 increased the mRNA expression levels of nVDR, mVDR, and NaPi-IIb in the duodenum of broilers (Han *et al*., 2018). In the present study, the mRNA expression levels of nVDR, mVDR, and NaPi-IIb in the duodenum were quadratically affected by 1α-OH-D_3 levels. The addition of 1.25 and 2.5 μg/kg of 1α-OH-D_3 enhanced the mRNA expression levels compared to the basal diet. Increasing the 1α-OH-D_3 levels from 2.5 to 10 μg/kg decreased the mRNA expression levels of nVDR, mVDR, and NaPi-IIb. These data suggest that the addition of 1.25 and 2.5 μg/kg of 1α-OH-D_3 promoted the mRNA expression levels of nVDR, mVDR, and NaPi-IIb in the duodenum.

Optimal 1α-OH-D_3 Levels

Vitamin D_3 and 25-OH-D_3 are used to regulate Ca and P metabolism in poultry. The recommended levels of vitamin D_3 in China are 25 and 19 μg/kg for broilers from 1 to 21 days and 22 to 42 days of age, respectively. The estimated 25-OH-D_3 levels are from 10 to 20 μg/kg in 1- to 42-day-old broiler chickens (Goodgame *et al*., 2011; Chen *et al*., 2017). The presence of 1α-OH-D_3 in broiler chicken diets is more active than vitamin D_3 and 25-OH-D_3 (Edwards *et al*., 2002; Han *et al*., 2017). Thus, the optimal dietary 1α-OH-D_3 level (2−5 μg/kg) is lower than those of the two vitamin D.

In conclusion, the 1α-OH-D_3 levels were quadratically related to growth, leg bone mineralization, and the mRNA expression levels of nVDR, mVDR, and NaPi-IIb in the small intestine of broilers. The highest values of BWG, bone weight, and mRNA expression levels of the three genes were detected in chickens fed with 2.5 to 5 μg/kg of 1α-OH-D_3. No significant difference was observed in growth or bone quality of the 42-day-old birds fed with 2 to 5 μg/kg of 1α-OH-D_3. These data indicated that 2 to 5 μg/kg of 1α-OH-D_3 was sufficient for growth performance and bone mineralization of broiler chickens from 1 to 42 days of age.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Aburto A, Edwards HM and Britton WM. The influence of vitamin A on the utilization and amelioration of toxicity of cholecalciferol, 25-hydroxycholecalciferol, and 1,25 dihydroxycholecalciferol in young broiler chickens. Poultry Science, 77: 585−593. 1998

Biehl RR and Baker DH. Utilization of phytate and nonphytate phosphorus in chicks as affected by source and amount of vitamin D_3. Journal of Animal Science, 75: 2986−2993. 1997.

Chen GH, Zhang JL, Wang JG, Zhang N, Qu HX, Wang ZX, Yan YF and Han JC. Requirement of 25-hydroxycholecalciferol for broilers. Chinese Journal of Animal Nutrition, 29: 2335−2347. 2017.

Edwards HM. Studies on the efficacy of cholecalciferol and derivatives for stimulating phytate utilization in broilers. Poultry Science, 81: 1026−1031. 2002.

Edwards HM, Shirley RB, Escoe WB and Pesti GM. Quantitative evaluation of 1α-hydroxycholecalciferol as a cholecalciferol substitute for broilers. Poultry Science, 81: 664−669. 2002.

Elliot MA, Roberson KD, Rowland GN and Edwards HM. Effects of dietary calcium and 1,25-dihydroxycholecalciferol on the development of tibial dyschondroplasia in broilers during the starter and grower periods. Poultry Science, 74: 1495−1505. 1995.

Forster IC, Hernando N, Biber J and Murer H. Phosphate transporters of the SLC20 and SLC34 families. Molecular Aspects of Medicine, 34: 386−395. 2013.

Ghasemi P, Toghyani M and Landy N. Effects of dietary 1alpha-hydroxycholecal- ciferol in calcium and phosphorous-deficient diets on growth performance, tibia related indices and immune responses in broiler chickens. Animal Nutrition, 5: 134−139. 2019.

Goodgame SD, Mussini FJ, Lu C, Bradley CD, Watkins SE and Waldroup PW. Evaluation of a fermentation source of 25-hydroxycholecalciferol in broiler diets. International Journal of Poultry Science, 10: 295−299. 2011.

Hall LE, Shirley RB, Bakalli RI, Aggrey SE, Pesti GM and Edwards HM. Power of two methods for the estimation of bone ash of broilers. Poultry Science, 82: 414−418. 2003.

Han JC, Chen GH, Zhang JL, Wang JG, Qu HX, Yan YF, Yang XJ and Cheng YH. Relative biological value of 1α-hydroxycholecalciferol to 25-hydroxycholecalciferol in broiler chicken diets. Poultry Science, 96: 2330−2335. 2017.

Han JC, Yang XD, Zhang T, Li H, Li WL, Zhang ZY and Yao JH. Effects of 1α-hydroxycholecalciferol on growth performance, parameters of tibia and plasma, meat quality, and type IIb sodium phosphate cotransporter gene expression of one- to twenty-one-day-old broilers. Poultry Science, 88: 323−329. 2009.

Han JC, Zhang JL, Zhang N, Yang X, Qu HX, Guo Y, Shi CX and Yan YF. Age, phosphorus, and 25-hydroxycholecalciferol regulate mRNA expression of vitamin D receptor and sodium-phosphate cotransporter in the small intestine of broiler chickens. Poultry Science, 97: 1199−1208. 2018.

Khanal R and Nemere I. Membrane receptors for vitamin D metabolites. Critical Reviews in Eukaryotic Gene Expression, 17: 31−47. 2007.

Landy N and Toghyani M. Evaluation of one-alpha-hydroxycholecalciferol alone or in combination with cholecalciferol in Ca-P deficiency diets on development of tibial dyschondroplasia in broiler chickens. Animal Nutrition, 4: 109−112. 2018.

Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods, 25: 402−408. 2001.

Mitchell RD, Edwards HM, Mcdaniel GR and Rowland GN. Dietary 1,25-dihydroxy- cholecalciferol has variable effects on the incidences of leg abnormalities, plasma vitamin D metabolites, and vitamin D receptors in chickens divergently selected for tibial dyschondroplasia. Poultry Science, 76: 338−345. 1997.

Nemere I, Garbi N, Hammerling G and Hintze KJ. Role of the $1,25D_3$ -MARRS receptor in the $1,25\ (OH)_2\ D_3$ -stimulated uptake of calcium and phosphate in intestinal cells. Steroids, 77: 897−902. 2012.

National Research Council (NRC). Nutrient requirements of poultry, 9th revised edition. The National Academies Press, Washington, DC, USA. 1994.

Pesti GM and Shivaprasad HL. The influence of excessive levels of 1α-hydroxycholecalciferol on the growth and tissue appearance of market weight chickens. Journal of Applied Poultry Research, 19: 349−353. 2010.

Rennie JS, Whitehead CC and Thorp BH. The effect of dietary 1,25-dihydroxycholecalciferol in preventing tibial dyschondroplasia in broilers fed on diets imbalanced in calcium and phosphorus. British Journal of Nutrition, 69: 809−816. 1993.

Statistical Analysis System (SAS) Institute. SAS User's Guide. Version 9.0. SAS Institute, Cary, NC, USA. 2002.

Snow JL, Baker DH and Parsons CM. Phytase, citric acid, and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poultry Science, 83: 1187−1192. 2004.

Xu H, Bai L, Collins JF and Ghishan FK. Age-dependent regulation of rat intestinal type IIb sodium-phosphate cotransporter by $1,25\text{-}(OH)_2$ vitamin D_3. American Journal of Physiology-Cell Physiology, 282: 487−493. 2002.

Yan F, Angel R and Ashwell CM. Characterization of the chicken small intestine type IIb sodium phosphate cotransporter. Poultry Science, 86: 67−76. 2007.