



Evaluation of Relative Bioavailability of 25-Hydroxycholecalciferol to Cholecalciferol for Broiler Chickens

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ABSTRACT: This study was conducted to evaluate the relative bioavailability (RBV) of 25-hydroxycholecalciferol (25-OH-D₃) to cholecalciferol (vitamin D₃) in 1- to 21-d-old broiler chickens fed with calcium (Ca)- and phosphorus (P)-deficient diets. On the day of hatch, 450 female Ross 308 broiler chickens were assigned to nine treatments, with five replicates of ten birds each. The basal diet contained 0.50% Ca and 0.25% non-phytate phosphorus (NPP) and was not supplemented with vitamin D. Vitamin D₃ was fed at 0, 2.5, 5.0, 10.0, and 20.0 µg/kg, and 25-OH-D₃ was fed at 1.25, 2.5, 5.0, and 10.0 µg/kg. The RBV of 25-OH-D₃ was determined using vitamin D₃ as the standard source by the slope ratio method. Vitamin D₃ and 25-OH-D₃ intake was used as the independent variable for regression analysis. The linear relationships between the level of vitamin D₃ or 25-OH-D₃ and body weight gain (BWG) and the weight, length, ash weight, and the percentage of ash, Ca, and P in femur, tibia, and metatarsus of broiler chickens were observed. Using BWG as the criterion, the RBV of 25-OH-D₃ to vitamin D₃ was 1.85. Using the mineralization of the femur, tibia, and metatarsus as criteria, the RBV of 25-OH-D₃ to vitamin D₃ ranged from 1.82 to 2.45, 1.86 to 2.52, and 1.65 to 2.05, respectively. These data indicate that 25-OH-D₃ is approximately 2.03 times as active as vitamin D₃ in promoting growth performance and bone mineralization in broiler chicken diets. (**Key Words:** 25-Hydroxycholecalciferol, Cholecalciferol, Relative Bioavailability, Growth, Bone, Broiler Chicken)

INTRODUCTION

Cholecalciferol (vitamin D₃) has been used as a feed additive to regulate calcium (Ca) and phosphorus (P) metabolism and bone development in animals for many years. Vitamin D₃ undergoes 25-hydroxylation in animal livers to transform 25-hydroxycholecalciferol (25-OH-D₃). The commercial 25-OH-D₃ has been produced and approved for use in poultry and swine feed in China in 2014.

Previous research has shown that body weight and feed efficiency of broiler chickens fed with 25-OH-D₃ were greater than those of the birds fed with vitamin D₃ (Yarger

et al., 1995; Fritts and Waldroup, 2003). Replacing vitamin D₃ by 25-OH-D₃ at 50% has a beneficial effect on the growth performance of broilers (Koreleski and Swiatkiewicz, 2005). These data indicate that the relative bioavailability (RBV) of 25-OH-D₃ is higher than that of vitamin D₃.

However, no consistent results have been obtained in the RBV of 25-OH-D₃ to vitamin D₃. Soares et al. (1995) reviewed that the RBV of 25-OH-D₃ to vitamin D₃ ranged from 1.0 to 4.0 when Ca absorption, plasma Ca, bone ash, bone strength, and tibial dyschondroplasia were used as the criteria. Atencio et al. (2005) found that the RBV values of 25-OH-D₃ to vitamin D₃ were 1.38, 1.33, 1.28, and 1.11 for egg production, hatchability, late embryo mortality, and body ash of the progeny in broiler breeder hen diets, respectively. These data reveal the differences in the RBV of 25-OH-D₃ to vitamin D₃ among the studies. The RBV of 25-OH-D₃ to vitamin D₃ in poultry diets should be further clarified.

Researchers usually use the tibia to evaluate the RBV of

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vitamin D derivatives. In fact, the differences in growth and mineralization among the femur, tibia, and metatarsus in poultry have been observed (Applegate and Lilburn, 2002; Goetting-Fuchs et al., 2012; Han et al., 2015). The femur and metatarsus should also be used as criteria to evaluate the RBV of 25-OH-D₃ to vitamin D₃.

Therefore, the present study was conducted to investigate the effects of 25-OH-D₃ and vitamin D₃ on the growth performance and development of the femur, tibia, and metatarsus and to re-evaluate the RBV of 25-OH-D₃ to vitamin D₃ in broilers fed with Ca- and P-deficient diets.

MATERIALS AND METHODS

Birds, diets, and management

All of the procedures adopted in this study were approved by the Animal Care Committee of Shangqiu Normal University.

On the day of hatch, 450 female Ross 308 broiler chickens were assigned to nine treatments, with five replicates of ten birds each. The initial body weight of broiler chickens was 46.5±1.9 g. Birds from 1 to 13 d of age were reared in stainless steel starter cages (70 cm×70 cm×30 cm). At 14 d, the broilers were transferred to stainless steel grower cages (190 cm×50 cm×35 cm). The basal diet contained 0.50% Ca and 0.25% non-phytate phosphorus (NPP) and was not supplemented with vitamin D. Vitamin D₃ was fed at 0, 2.5, 5.0, 10.0, and 20.0 µg/kg and 25-OH-D₃ was fed at 1.25, 2.5, 5.0, and 10.0 µg/kg. The birds were provided mash diet (Table 1) and water *ad libitum*. The lighting system consisted of 23 h of light from 1 d to 3 d and 20 h of light from 4 d to 21 d. Room temperature was controlled at 33°C from 0 d to 3 d, 30°C from 4 d to 7 d, 27°C from 8 d to 14 d, and 24°C from 15 d to 21 d.

25-OH-D₃ and vitamin D₃

Crystalline 25-OH-D₃ (98%) and vitamin D₃ (99%) were supplied by Changzhou Book Chemical Co., Ltd. (Changzhou, China) and Jiaxing Tianhecheng Biological Technology Co., Ltd. (Jiaxing, China), respectively. The 25-OH-D₃ and vitamin D₃ solutions were prepared by the method of Biehl and Baker (1997). Crystalline 25-OH-D₃ and vitamin D₃ were weighed and dissolved in ethanol. Then, they were diluted to a final concentration of 10 mg/L of 25-OH-D₃ or vitamin D₃ in a solution of 5% ethanol and 95% propylene glycol. After the preparation, the solution of 25-OH-D₃ or vitamin D₃ was added to the diets.

Sample collection

The birds were weighed on d 21 after 12 h of fasting. Ten chicks per treatment were randomly selected for the collection of blood, femur, tibia, and metatarsus. Plasma

Table 1. Ingredients and nutrient composition of the basal diet

Items	Basal diet
Ingredient (%)	
Corn	60.73
Soya bean meal (43% CP)	32.00
Soya bean oil	1.60
Soya bean protein isolate (65% CP)	3.47
Limestone	0.67
Dicalcium phosphate	0.71
L-lysine·HCl (98%)	0.14
DL-methionine (98%)	0.14
Trace mineral premix ¹	0.01
Vitamin premix ²	0.03
Choline chloride (50%)	0.20
Sodium chloride	0.30
Nutrient composition	
Metabolizable energy (kcal/kg)	2,975.20
Crude protein (CP, %)	21.24
Analyzed calcium (Ca, %)	0.52
Analyzed total phosphorus (TP, %)	0.49
Non-phytate phosphorus (NPP, %)	0.25

¹The 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; and 0.15 mg selenium.

²The vitamin premix 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 B₁₂; 10.0 mg pantothenic acid; 0.55 mg folic acid; and 0.18 mg biotin.

samples (5 mL) were collected through cardiac puncture and centrifuged for 10 min at 3,000×g at 20°C. The birds were killed after collecting the blood samples. The femur, tibia, and metatarsus of the individual birds were excised and frozen at -20°C for analysis.

Sample analysis

Plasma Ca and inorganic phosphorus (Pi) were determined using a Shimadzu CL-8000 analyzer (Shimadzu Corp., Kyoto, Japan) following the instructions of the manufacturer.

The left femur, tibia, and metatarsus were boiled for 5 min to loosen the muscle tissues using the method of Hall et al. (2003). The meat, connective tissue, and fibula bone were completely removed using scissors and forceps. The bones were placed in a container of ethanol for 24 h (removing water and polar lipids) after cleaning. Afterward, the bones were further extracted in anhydrous ether for 24 h (removing non-polar lipids). The bones were dried at 105°C for 24 h before weighing. The bone ash content was determined by ashing the bone in a muffle furnace for 48 h at 600°C.

The right tibia was utilized to analyze the breaking-strength, which was determined using an all-digital electronic universal testing machine (Shenzhen Hengen Instrument Co. Ltd., Shenzhen, China). The tibias were

cradled on two support points measuring 4 cm apart. Force was applied to the midpoint of the same face of each tibia using a 50 kg load cell with a crosshead speed of 10 mm/min (Jendral et al., 2008).

Dietary and bone Ca were determined by the ethylene diamine tetraacetic acid titration method, and P was determined by photometric methods after reaction with ammonium molybdate and ammonium metavanadate (Han et al., 2013).

Statistical analysis

Replicate means are the experimental units in the statistical analysis. Data were analyzed with a general linear model of the SAS software (SAS Institute, 2002). The RBV of 25-OH-D₃ was determined using vitamin D₃ as the standard source by the slope ratio method (Littell et al., 1997). Feed intake differed for the dietary treatments so that vitamin D intake rather than vitamin D content was used as the independent variable for regression analysis. The model is as follows: $y = a + b_1x_1 + b_2x_2$, where y is the response, x_1 is vitamin D₃ intake, x_2 is 25-OH-D₃ intake, a is the intercept, and b_1 and b_2 are the slope of vitamin D₃ and 25-OH-D₃, respectively. Orthogonal comparisons were performed to determine the linear and quadratic effects of the 25-OH-D₃ or vitamin D₃ levels on growth performance and bone mineralization. The basal diet treatment was included for both vitamin D₃ and 25-OH-D₃ when conducting orthogonal polynomial contrast test. Means were compared by conducting Tukey test when probability values were significant ($p < 0.05$).

RESULTS

Growth performance

Dietary 25-OH-D₃ linearly affected body weight gain (BWG), feed intake (FI), feed efficiency, and mortality in 1- to 21-d-old broiler chickens ($p < 0.05$, Table 2). Vitamin D₃ levels also influenced the above parameters ($p < 0.05$). 25-OH-D₃ or vitamin D₃ did not affect the plasma Ca or Pi concentration ($p > 0.05$).

Bone mineralization

The femur, tibia, and metatarsus are three leg bones in poultry. They reflect the body bone quality of birds. The linear relationships between the level of 25-OH-D₃ or vitamin D₃ and the weight, length, ash weight, and the percentage of ash, Ca, and P of the femur in broiler chickens were observed ($p < 0.05$, Table 3).

Dietary 25-OH-D₃ or vitamin D₃ linearly improved the tibia breaking-strength ($p < 0.05$, Table 4). Similar results were observed in the relationship between the level of 25-OH-D₃ or vitamin D₃ and the weight, length, ash weight, and the percentage of ash, Ca, and P of the tibia.

Increasing the 25-OH-D₃ or vitamin D₃ level linearly increased the weight, length, ash weight, and the percentage of ash, Ca, and P of the metatarsus ($p < 0.05$, Table 5).

Relative bioavailability of 25-OH-D₃ to vitamin D₃

The slope ratio method was used to evaluate the RBV of 25-OH-D₃ to vitamin D₃ in broiler chickens (Table 6). Vitamin D₃ and 25-OH-D₃ intake was used as the independent variable for regression analysis. The slopes of

Table 2. Effects of vitamin D₃ and 25-OH-D₃ on growth performance and plasma mineral concentration in 1- to 21-d-old broiler chickens

Vitamin D ₃ (µg/kg)	25-OH-D ₃ (µg/kg)	Growth				Plasma	
		BWG (g/bird)	FI (g/bird)	FE (BWG/FI)	Mortality (%)	Ca (mg/dL)	Pi (mg/dL)
0	0	233 ^d	431 ^d	0.542 ^c	26 ^a	5.45	3.07
2.5		338 ^c	621 ^{bc}	0.548 ^{bc}	12 ^b	6.69	3.27
5.0		433 ^b	709 ^b	0.612 ^a	0 ^b	6.90	3.83
10.0		584 ^a	932 ^a	0.627 ^a	0 ^b	6.93	4.82
20.0		602 ^a	955 ^a	0.631 ^a	0 ^b	6.98	4.64
	1.25	321 ^c	585 ^c	0.549 ^{bc}	10 ^b	5.72	3.24
	2.5	426 ^b	702 ^b	0.606 ^{ab}	0 ^b	5.70	3.60
	5.0	556 ^a	866 ^a	0.642 ^a	0 ^b	6.70	3.66
	10.0	574 ^a	927 ^a	0.619 ^a	0 ^b	6.73	4.17
SEM		19	27	0.007	2	0.16	0.18
p value							
Vitamin D ₃	Linear	<0.001	<0.001	<0.001	<0.001	0.043	0.022
	Quadratic	0.002	0.006	0.363	0.010	0.171	0.890
25-OH-D ₃	Linear	<0.001	<0.001	<0.001	<0.001	0.013	0.038
	Quadratic	0.036	0.089	0.067	<0.001	0.732	0.797

25-OH-D₃, 25-hydroxycholecalciferol; BWG, body weight gain; FI, feed intake; FE, feed efficiency; Ca, calcium; Pi, inorganic phosphorus; SEM, standard error of the mean.

^{a-d} Means in the same column without a common superscript differ significantly ($p < 0.05$).

Table 3. Effects of vitamin D₃ and 25-OH-D₃ on femur mineralization in 1- to 21-d-old broiler chickens

Vitamin D ₃ (µg/kg)	25-OH-D ₃ (µg/kg)	Weight (g)	Length (cm)	Ash (g)	Ash (%)	Ca (%)	P (%)
0	0	0.56 ^d	3.43 ^f	0.17 ^d	26.64 ^e	10.30 ^e	4.92 ^f
2.5		0.63 ^{cd}	3.75 ^{ef}	0.20 ^d	33.89 ^{cd}	12.70 ^{cd}	6.04 ^{de}
5.0		0.78 ^b	4.34 ^{bcd}	0.29 ^c	37.55 ^{bc}	13.01 ^{cd}	6.78 ^{cde}
10.0		1.01 ^a	4.69 ^{ab}	0.40 ^{ab}	39.55 ^b	14.02 ^{abc}	7.13 ^{bc}
20.0		1.07 ^a	4.87 ^a	0.45 ^a	43.71 ^a	15.20 ^{ab}	7.88 ^{ab}
	1.25	0.69 ^{bc}	3.85 ^{def}	0.23 ^{cd}	33.07 ^d	11.31 ^{de}	5.91 ^e
	2.5	0.80 ^b	4.10 ^{cde}	0.29 ^c	36.37 ^{bcd}	13.48 ^{bc}	6.68 ^{cde}
	5.0	0.98 ^a	4.58 ^{abc}	0.38 ^b	38.64 ^b	14.52 ^{abc}	6.95 ^{cd}
	10.0	1.02 ^a	4.77 ^{ab}	0.45 ^a	44.14 ^a	15.86 ^a	8.22 ^a
SEM		0.03	0.08	0.02	0.81	0.28	0.16
p value							
Vitamin D ₃	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.352	0.268	0.288	0.033	0.318	0.207
25-OH-D ₃	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.424	0.590	0.457	0.368	0.761	0.936

25-OH-D₃, 25-hydroxycholecalciferol; SEM, standard error of the mean.

^{a-f} Means in the same column without a common superscript differ significantly (p<0.05).

vitamin D₃ and 25-OH-D₃ were 16.706 and 30.862, respectively, when BWG was used as the criterion. Thus, the RBV value of 25-OH-D₃ to vitamin D₃ was 1.85 (namely 185%, 30.862 divided by 16.706).

Using BWG as the criterion, the RBV value of 25-OH-D₃ to vitamin D₃ was 1.85. When the weight, length, ash weight, and the percentage of ash, Ca, and P of the femur were used as the criteria, the RBV of 25-OH-D₃ to vitamin D₃ were 1.88, 1.82, 2.00, 2.03, 2.45, and 2.22, respectively. Using the same parameters of the tibia as the criteria, the RBV of 25-OH-D₃ to vitamin D₃ were 2.12, 1.86, 2.17, 2.13, 2.52, and 2.52, respectively. Metatarsus mineralization was also used as a criterion. The above RBV values were 2.05, 1.89, 2.00, 1.76, 1.73, and 1.65, respectively.

Generally, the bioavailability of 25-OH-D₃ is higher than that of vitamin D₃ in broilers. The average RBV of 25-OH-D₃ to vitamin D₃ is approximately 2.03 (namely 203%) in promoting growth performance and bone mineralization in 1- to 21-d-old broiler chicken diets.

DISCUSSION

Previous research has shown that vitamin D₃ levels linearly improve growth and bone quality when broilers are fed with Ca- and NPP-deficient diets; by contrast, growth is quadratically or not significantly affected by vitamin D₃ levels when the Ca and NPP contents are sufficient (Aburto et al., 1998; Baker et al., 1998; Rao et al., 2009). Thus, the

Table 4. Effects of vitamin D₃ and 25-OH-D₃ on tibia mineralization in 1- to 21-d-old broiler chickens

Vitamin D ₃ (µg/kg)	25-OH-D ₃ (µg/kg)	BS (N)	Weight (g)	Length (cm)	Ash (g)	Ash (%)	Ca (%)	P (%)
0	0	20.28 ^d	0.66 ^d	4.71 ^d	0.18 ^e	26.82 ^f	9.76 ^e	5.08 ^d
2.5		24.94 ^d	0.80 ^d	5.26 ^c	0.26 ^d	32.70 ^{de}	11.51 ^d	6.00 ^{cd}
5.0		39.87 ^c	1.01 ^{bc}	5.71 ^{bc}	0.37 ^c	36.70 ^{cd}	13.18 ^{bc}	6.83 ^{bc}
10.0		54.10 ^b	1.32 ^a	6.23 ^a	0.51 ^b	39.61 ^{abc}	13.81 ^{bc}	7.14 ^{ab}
20.0		68.91 ^a	1.38 ^a	6.38 ^a	0.57 ^{ab}	41.65 ^{ab}	14.13 ^{ab}	7.15 ^{ab}
	1.25	21.51 ^d	0.83 ^{cd}	5.38 ^c	0.26 ^d	31.81 ^e	11.23 ^{de}	5.53 ^d
	2.5	39.83 ^c	1.06 ^b	5.52 ^c	0.39 ^c	36.88 ^{cd}	12.44 ^{cd}	6.77 ^{bc}
	5.0	49.32 ^{bc}	1.32 ^a	6.12 ^{ab}	0.50 ^b	37.76 ^{bc}	13.14 ^{bc}	6.91 ^{abc}
	10.0	71.18 ^a	1.39 ^a	6.30 ^a	0.59 ^a	42.93 ^a	15.59 ^a	7.77 ^a
SEM		2.88	0.04	0.09	0.02	0.78	0.27	0.14
p value								
Vitamin D ₃	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.088	0.633	0.125	0.765	0.059	0.003	0.015
25-OH-D ₃	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.009	0.282	0.195	0.581	0.269	0.288	0.715

25-OH-D₃, 25-hydroxycholecalciferol; BS, breaking-strength; SEM, standard error of the mean.

^{a-f} Means in the same column without a common superscript differ significantly (p<0.05).

Table 5. Effects of vitamin D₃ and 25-OH-D₃ on metatarsus mineralization in 1- to 21-d-old broiler chickens

Vitamin D ₃ (µg/kg)	25-OH-D ₃ (µg/kg)	Weight (g)	Length (cm)	Ash (g)	Ash (%)	Ca (%)	P (%)
0	0	0.50 ^c	3.56 ^f	0.12 ^c	22.20 ^e	7.68 ^e	3.67 ^f
2.5		0.58 ^c	3.84 ^{ef}	0.17 ^c	29.02 ^{cd}	10.23 ^d	4.94 ^{de}
5.0		0.80 ^b	4.29 ^{bc}	0.25 ^b	31.69 ^{bcd}	11.77 ^{bc}	5.81 ^{abcd}
10.0		0.91 ^{ab}	4.61 ^{ab}	0.30 ^{ab}	33.06 ^{abc}	12.01 ^{abc}	5.93 ^{abc}
20.0		0.97 ^a	4.65 ^a	0.35 ^a	36.71 ^a	13.20 ^a	6.68 ^a
	1.25	0.59 ^c	3.92 ^{de}	0.16 ^c	27.41 ^d	10.39 ^d	4.92 ^e
	2.5	0.80 ^b	4.23 ^{cd}	0.25 ^b	29.50 ^{cd}	10.84 ^{cd}	5.40 ^{cde}
	5.0	0.94 ^{ab}	4.55 ^{abc}	0.30 ^{ab}	31.65 ^{bcd}	11.32 ^{cd}	5.67 ^{bcde}
	10.0	0.94 ^{ab}	4.58 ^{abc}	0.34 ^a	35.86 ^{ab}	12.88 ^{ab}	6.35 ^{ab}
SEM		0.03	0.06	0.01	0.69	0.25	0.14
p value							
Vitamin D ₃	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.133	0.052	0.582	0.029	<0.001	0.007
25-OH-D ₃	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.071	0.024	0.519	0.593	0.076	0.104

25-OH-D₃, 25-hydroxycholecalciferol; SEM, standard error of the mean.

^{a-f} Means in the same column without a common superscript differ significantly (p<0.05).

Ca- and NPP-deficient diet was designed in the present study. The optimal dietary Ca to NPP ratio is 2.0 to promote growth performance and bone mineralization in broiler chickens (Bar et al., 2003; Rao et al., 2007). Therefore, the Ca and NPP levels were 0.50% and 0.25%, respectively.

In the comparison of 25-OH-D₃ to vitamin D₃ at the

3.125 µg/kg level, the RBV of 25-OH-D₃ to vitamin D₃ ranged from 1.08 to 4.00 in broiler breeder hens (Atencio et al., 2005). However, no statistical differences in the performance between 25-OH-D₃ and vitamin D₃ were observed when their level reached 12.5 µg/kg in hens (Atencio et al., 2005) or 50 µg/kg in broiler chickens (Fritts

Table 6. Relative bioavailability (RBV) of 25-OH-D₃ to vitamin D₃ based on vitamin D intake (µg/bird) in 1- to 21-d-old broiler chickens with the slope ratio method

Criteria	Intercept	Slope±SE		p value	R ²	RBV±SE
		Vitamin D ₃	25-OH-D ₃			
Growth performance						
Weight gain	334.545	16.706±1.799	30.862±3.726	<0.001	0.73	1.85±0.23
Femur mineralization						
Weight	0.666	0.024±0.003	0.045±0.005	<0.001	0.73	1.88±0.24
Length	3.813	0.065±0.008	0.118±0.017	<0.001	0.66	1.82±0.29
Ash weight	0.216	0.014±0.001	0.028±0.003	<0.001	0.81	2.00±0.20
Ash percentage	32.147	0.669±0.075	1.356±0.156	<0.001	0.73	2.03±0.21
Ca percentage	11.776	0.198±0.030	0.485±0.061	<0.001	0.66	2.45±0.41
P percentage	5.797	0.121±0.015	0.269±0.031	<0.001	0.71	2.22±0.29
Tibia mineralization						
Weight	0.838	0.033±0.004	0.070±0.008	<0.001	0.70	2.12±0.31
Length	5.242	0.070±0.009	0.130±0.019	<0.001	0.64	1.86±0.29
Ash weight	0.267	0.018±0.002	0.039±0.004	<0.001	0.79	2.17±0.23
Ash percentage	31.847	0.594±0.083	1.263±0.171	<0.001	0.65	2.13±0.32
Ca percentage	11.216	0.187±0.027	0.472±0.056	<0.001	0.68	2.52±0.40
P percentage	5.876	0.085±0.017	0.214±0.034	<0.001	0.53	2.52±0.59
Metatarsus mineralization						
Weight	0.632	0.020±0.003	0.041±0.006	<0.001	0.59	2.05±0.37
Length	3.919	0.046±0.007	0.087±0.014	<0.001	0.59	1.89±0.34
Ash weight	0.175	0.010±0.001	0.020±0.002	<0.001	0.71	2.00±0.27
Ash percentage	26.868	0.570±0.073	1.004±0.152	<0.001	0.65	1.76±0.26
Ca percentage	9.767	0.202±0.027	0.350±0.057	<0.001	0.62	1.73±0.24
P percentage	4.733	0.113±0.016	0.187±0.033	<0.001	0.60	1.65±0.27

and Waldroup, 2003). These data indicate that the 25-OH-D₃ or vitamin D₃ level should not exceed their requirement when evaluating the RBV of 25-OH-D₃ to vitamin D₃.

The optimal level of 25-OH-D₃ was 10 µg/kg for promoting bone ash in broiler chicken diets (Goodgame et al., 2011). Thus, the level of 25-OH-D₃ ranged from 1.25 to 10 µg/kg in the present study. No significant differences were observed in the tibia ash, breaking-strength, and the contents of Ca and P among the broilers fed with vitamin D₃ ranging from 25 to 1,000 µg/kg (Han et al., 2013). Therefore, vitamin D₃ levels were lower than those of our previous research (Han et al., 2013) and were at 2.5, 5, 10, and 20 µg/kg in the present study. Our results revealed the linear relationship between the 25-OH-D₃ or vitamin D₃ level and the performance or bone mineralization in broiler chickens.

The positive effects of 25-OH-D₃ or vitamin D₃ on growth performance and tibia weight, breaking-strength, and the percentage of ash, Ca, and P in broiler chickens have been observed (Fritts and Waldroup, 2003; Rao et al., 2006; Han et al., 2012). The improvement of vitamin D derivatives on performance and bone of birds was caused by the increase of Ca and P utilization. Research has shown that addition of vitamin D₃ (Qian et al., 1997), 25-OH-D₃ (Ledwaba and Roberson, 2003), 1 α -OH-D₃ (Shirley, 2003; Han et al., 2012), or 1,25-(OH)₂-D₃ (Edwards, 2002) improves the retention of Ca and P in broiler chicken diets.

Previous research has shown that when bone ash was used as criteria, the RBV of 25-OH-D₃ to vitamin D₃ ranged from 1.0 to 2.5 (Soares et al., 1995). The RBV was from 1.11 to 1.38 in broiler breeder hens (Atencio et al., 2005). The present study showed that the RBV of 25-OH-D₃ to vitamin D₃ is approximately 2.03 for promoting growth performance and bone mineralization in 1- to 21-d-old broiler chickens. The differences in RBV values among the criteria were observed. Tibia gave the highest RBV of 25-OH-D₃ to vitamin D₃ (2.22) and followed by femur (2.07). The BWG and metatarsus criteria yielded the lowest values (1.85).

CONCLUSIONS

The present study indicates that the average RBV of 25-OH-D₃ to vitamin D₃ is approximately 2.03 (namely 203%) for promoting growth performance and bone mineralization in 1- to 21-d-old broiler chickens fed with Ca- and P-deficient diets.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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REFERENCES

- Aburto, A., H. M. Edwards Jr., and W. M. Britton. 1998. The influence of vitamin A on the utilization and amelioration of toxicity of cholecalciferol, 25-hydroxycholecalciferol, and 1,25 dihydroxycholecalciferol in young broiler chickens. *Poult. Sci.* 77:585-593.
- Applegate, T. J. and M. S. Lilburn. 2002. Growth of the femur and tibia of a commercial broiler line. *Poult. Sci.* 81:1289-1294.
- Atencio, A., G. M. Pesti, and H. M. Edwards. Jr. 2005. Twenty-five hydroxycholecalciferol as a cholecalciferol substitute in broiler breeder hen diets and its effect on the performance and general health of the progeny. *Poult. Sci.* 84:1277-1285.
- Baker, D. H., R. R. Biehl, and J. L. Emmert. 1998. Vitamin D₃ requirement of young chicks receiving diets varying in calcium and available phosphorus. *Br. Poult. Sci.* 39:413-417.
- Bar, A., D. Shinder, S. Yosefi, E. Vax, and I. Plavnik. 2003. Metabolism and requirements for calcium and phosphorus in the fast-growing chicken as affected by age. *Br. J. Nutr.* 89:51-60.
- Biehl, R. R. and D. H. Baker. 1997. Utilization of phytate and nonphytate phosphorus in chicks as affected by source and amount of vitamin D₃. *J. Anim. Sci.* 75:2986-2993.
- Edwards Jr., H. M. 2002. Studies on the efficacy of cholecalciferol and derivatives for stimulating phytate utilization in broilers. *Poult. Sci.* 81:1026-1031.
- Fritts, C. A. and P. W. Waldroup. 2003. Effect of source and level of vitamin D on live performance and bone development in growing broilers. *J. Appl. Poult. Res.* 12:45-52.
- Goetting-Fuchs, C., R. Günther, V. G. Liesner, B. G. Liesner, M. Beyerbach, and J. Kamphues. 2012. Investigations on skeletal development, bone mineralisation as well as calcium and phosphorus levels in blood of male fattening turkeys. *Europ. Poult. Sci.* 76:121-130.
- Goodgame, S. D., F. J. Mussini, C. Lu, C. D. Bradley, S. E. Watkins, and P. W. Waldroup. 2011. Evaluation of a fermentation source of 25-hydroxycholecalciferol in broiler diets. *Int. J. Poult. Sci.* 10:295-299.
- Hall, L. E., R. B. Shirley, R. I. Bakalli, S. E. Aggrey, G. M. Pesti, and H. M. Edwards. Jr. 2003. Power of two methods for the estimation of bone ash of broilers. *Poult. Sci.* 82:414-418.
- Han, J. C., H. X. Qu, J. G. Wang, G. H. Chen, Y. F. Yan, J. L. Zhang, F. M. Hu, L. Y. You, and Y. H. Cheng. 2015. Comparison of the growth and mineralization of the femur, tibia, and metatarsus of broiler chicks. *Braz. J. Poult. Sci.* 17:333-339.
- Han, J. C., H. X. Qu, J. Q. Wang, J. H. Yao, C. M. Zhang, G. L. Yang, Y. H. Cheng, and X. S. Dong. 2013. The effects of

- dietary cholecalciferol and 1 α -hydroxycholecalciferol levels in a calcium- and phosphorus-deficient diet on growth performance and tibia quality of growing broilers. *J. Anim. Feed Sci.* 22:158-164.
- Han, J. C., Y. Liu, J. H. Yao, J. Q. Wang, H. X. Qu, Y. F. Yan, J. Yue, J. L. Ding, Z. T. Shi, and X. S. Dong. 2012. Dietary calcium levels reduce the efficacy of one alpha-hydroxycholecalciferol in phosphorus-deficient diets of broilers. *J. Poult. Sci.* 49:34-38.
- Jendral, M. J., D. R. Korver, J. S. Church, and J. J. R. Feddes. 2008. Bone mineral density and breaking strength of white leghorns housed in conventional, modified, and commercially available colony battery cages. *Poult. Sci.* 87:828-837.
- Koreleski, J. and S. Swiatkiewicz. 2005. Efficacy of different limestone particle size and 25-hydroxycholecalciferol in broiler diets. *J. Anim. Feed Sci.* 14:705-714.
- Ledwaba, M. F. and K. D. Roberson. 2003. Effectiveness of twenty five hydroxycholecalciferol in the prevention of tibial dyschondroplasia in Ross cockerels depends on dietary calcium level. *Poult. Sci.* 82:1769-1777.
- Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. *J. Anim. Sci.* 75:2672-2683.
- Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium: Total phosphorus ratio in broiler diets. *Poult. Sci.* 76:37-46.
- Rao, S. V. R., M. V. L. N. Raju, A. K. Panda, G. S. Sunder, and R. P. Sharma. 2006. Effect of high concentrations of cholecalciferol on growth, bone mineralization, and mineral retention in broiler chicks fed suboptimal concentrations of calcium and nonphytate phosphorus. *J. Appl. Poult. Res.* 15:493-501.
- Rao, S. V. R., M. V. L. N. Raju, A. K. Panda, G. S. Sunder, and R. P. Sharma. 2009. Performance and bone mineralisation in broiler chicks fed on diets with different concentrations of cholecalciferol at a constant ratio of calcium to non-phytate phosphorus. *Br. Poult. Sci.* 50:528-535.
- Rao, S. V. R., M. V. L. N. Raju, and M. R. Reddy. 2007. Performance of broiler chicks fed high levels of cholecalciferol in diets containing sub-optimal levels of calcium and non-phytate phosphorus. *Anim. Feed Sci. Tech.* 134:77-88.
- SAS Institute. 2002. SAS User's Guide. 9th edn. SAS Inst. Inc., Cary, NC, USA.
- Shirley, R. B. 2003. Evaluation of Phytase, Vitamin D₃ Derivatives, and Broiler Breed Differences on Nutrient Utilization, Broiler Performance, Leg Disorders, and the Expression of Intestinal Calbindin-28 kd mRNA and Protein. Ph.D. Dissertation. University of Georgia, Athens, GA, USA.
- Soares Jr., J. H., J. M. Kerr, and R. W. Gray. 1995. 25-hydroxycholecalciferol in poultry nutrition. *Poult. Sci.* 74:1919-1934.
- Yarger, J. G., C. A. Saunders, J. L. McNaughton, C. L. Quarles, B. W. Hollis, and R. W. Gray. 1995. Comparison of dietary 25-hydroxycholecalciferol and cholecalciferol in broiler chickens. *Poult. Sci.* 74:1159-1167.