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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-D_3) to cholecalciferol (vitamin D_3) 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 D_3 was fed at 0, 2.5, 5.0, 10.0, and 20.0 μg/kg, and 25-OH-D_3 was fed at 1.25, 2.5, 5.0, and 10.0 μg/kg. The RBV of 25-OH-D_3 was determined using vitamin D_3 as the standard source by the slope ratio method. Vitamin D_3 and 25-OH-D_3 intake was used as the independent variable for regression analysis. The linear relationships between the level of vitamin D_3 or 25-OH-D_3 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 value of 25-OH-D_3 to vitamin D_3 was 1.85. Using the mineralization of the femur, tibia, and metatarsus as criteria, the RBV of 25-OH-D_3 to vitamin D_3 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-D_3 is approximately 2.03 times as active as vitamin D_3 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 D_3) has been used as a feed additive to regulate calcium (Ca) and phosphorus (P) metabolism and bone development in animals for many years. Vitamin D_3 undergoes 25-hydroxylation in animal livers to transform 25-hydroxycholecalciferol (25-OH-D_3). The commercial 25-OH-D_3 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-D_3 were greater than those of the birds fed with vitamin D_3 (Yarger et al., 1995; Fritts and Waldroup, 2003). Replacing vitamin D_3 by 25-OH-D_3 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-D_3 is higher than that of vitamin D_3.

However, no consistent results have been obtained in the RBV of 25-OH-D_3 to vitamin D_3. Soares et al. (1995) reviewed that the RBV of 25-OH-D_3 to vitamin D_3 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-D_3 to vitamin D_3 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-D_3 to vitamin D_3 among the studies. The RBV of 25-OH-D_3 to vitamin D_3 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-D_3 to vitamin D_3.

Therefore, the present study was conducted to investigate the effects of 25-OH-D_3 and vitamin D_3 on the growth performance and development of the femur, tibia, and metatarsus and to re-evaluate the RBV of 25-OH-D_3 to vitamin D_3 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 D_3 was fed at 0, 2.5, 5.0, 10.0, and 20.0 μg/kg and 25-OH-D_3 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-D_3 and vitamin D_3

Crystalline 25-OH-D_3 (98%) and vitamin D_3 (99%) were supplied by Changzhou Book Chemical Co., Ltd. (Changzhou, China) and Jiaxing Tianhecheng Biological Technology Co., Ltd. (Jiaxing, China), respectively. The 25-OH-D_3 and vitamin D_3 solutions were prepared by the method of Biehl and Baker (1997). Crystalline 25-OH-D_3 and vitamin D_3 were weighed and dissolved in ethanol. Then, they were diluted to a final concentration of 10 mg/L of 25-OH-D_3 or vitamin D_3 in a solution of 5% ethanol and 95% propylene glycol. After the preparation, the solution of 25-OH-D_3 or vitamin D_3 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 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.

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

[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; and 0.15 mg selenium.

[2] The 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 B_{12}; 10.0 mg pantothenic acid; 0.55 mg folic acid; and 0.18 mg biotin.

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-D_3 was determined using vitamin D_3 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 D_3 intake, x_2 is 25-OH-D_3 intake, a is the intercept, and b_1 and b_2 are the slope of vitamin D_3 and 25-OH-D_3, respectively. Orthogonal comparisons were performed to determine the linear and quadratic effects of the 25-OH-D_3 or vitamin D_3 levels on growth performance and bone mineralization. The basal diet treatment was included for both vitamin D_3 and 25-OH-D_3 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-D_3 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 D_3 levels also influenced the above parameters ($p<0.05$). 25-OH-D_3 or vitamin D_3 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-D_3 or vitamin D_3 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-D_3 or vitamin D_3 linearly improved the tibia breaking-strength ($p<0.05$, Table 4). Similar results were observed in the relationship between the level of 25-OH-D_3 or vitamin D_3 and the weight, length, ash weight, and the percentage of ash, Ca, and P of the tibia.

Increasing the 25-OH-D_3 or vitamin D_3 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-D_3 to vitamin D_3

The slope ratio method was used to evaluate the RBV of 25-OH-D_3 to vitamin D_3 in broiler chickens (Table 6). Vitamin D_3 and 25-OH-D_3 intake was used as the independent variable for regression analysis. The slopes of

Table 2. Effects of vitamin D_3 and 25-OH-D_3 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-D_3, 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 D_3 and 25-OH-D_3 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-D_3, 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 D_3 and 25-OH-D_3 were 16.706 and 30.862, respectively, when BWG was used as the criterion. Thus, the RBV value of 25-OH-D_3 to vitamin D_3 was 1.85 (namely 185%, 30.862 divided by 16.706).

Using BWG as the criterion, the RBV value of 25-OH-D_3 to vitamin D_3 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-D_3 to vitamin D_3 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-D_3 to vitamin D_3 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-D_3 is higher than that of vitamin D_3 in broilers. The average RBV of 25-OH-D_3 to vitamin D_3 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 D_3 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 D_3 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 D_3 and 25-OH-D_3 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-D_3, 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 D_3 and 25-OH-D_3 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-D_3, 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-D_3 to vitamin D_3 at the 3.125 μg/kg level, the RBV of 25-OH-D_3 to vitamin D_3 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-D_3 and vitamin D_3 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-D_3 to vitamin D_3 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-D_3 or vitamin D_3 level should not exceed their requirement when evaluating the RBV of 25-OH-D_3 to vitamin D_3.

The optimal level of 25-OH-D_3 was 10 μg/kg for promoting bone ash in broiler chicken diets (Goodgame et al., 2011). Thus, the level of 25-OH-D_3 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 D_3 ranging from 25 to 1,000 μg/kg (Han et al., 2013). Therefore, vitamin D_3 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-D_3 or vitamin D_3 level and the performance or bone mineralization in broiler chickens.

The positive effects of 25-OH-D_3 or vitamin D_3 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 D_3 (Qian et al., 1997), 25-OH-D_3 (Ledwaba and Roberson, 2003), 1α-OH-D_3 (Shirley, 2003; Han et al., 2012), or 1,25-$(OH)_2$-D_3 (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-D_3 to vitamin D_3 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-D_3 to vitamin D_3 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-D_3 to vitamin D_3 (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-D_3 to vitamin D_3 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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