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Effects of calcium to non-phytate phosphorus ratio and different sources of vitamin D on growth performance and bone mineralization in broiler chickens

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ABSTRACT - A 7 × 2 factorial experiment was designed to test the effects of calcium (Ca) to non-phytate phosphorus (NPP) ratio (1.14, 1.43, 1.71, 2.00, 2.29, 2.57, and 2.86) and different sources of vitamin D (1α-hydroxycholecalciferol (1α-OH-D_3) and 25-hydroxycholecalciferol (25-OH-D_3)) on growth performance and bone mineralization in 1- to 42-d-old broiler chickens. On the day of hatch, 700 female Ross 308 broilers were weighed and randomly assigned to 14 treatments with five stainless steel cages of 10 birds each. Dietary Ca levels were 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 g kg^{-1} and the NPP content was 3.5 g kg^{-1}. The dose of 1α-OH-D_3 or 25-OH-D_3 was 5 µg kg^{-1}. Diets were not supplemented with cholecalciferol (vitamin D_3). Results showed that the Ca to NPP ratio, vitamin D source, and their interaction affected body weight gain (BWG), feed intake (FI), feed efficiency (FE), and carcass and breast yields, as well as tibia weight and length and ash weight in broiler chickens from 1 to 42 d of age. Broilers fed 1α-OH-D_3 had higher BWG and FI as well as tibia breaking strength, weight, length, diameter, and ash weight than birds fed 25-OH-D_3 at 42 d of age. The Ca to NPP ratio had a quadratic effect on BWG, FI, mortality, as well as tibia breaking strength, weight, length, ash weight, and ash and P contents in 42-d-old broilers. Broiler chickens at 42 d of age obtain optimal growth performance and bone mineralization at the Ca to NPP ratio of 2.32 when 1α-OH-D_3 or 25-OH-D_3 are used as the vitamin D source.

Key Words: broiler chicken, 1α-hydroxycholecalciferol, 25-hydroxycholecalciferol

Introduction

The imbalance between dietary calcium (Ca) and phosphorus (P) damages growth performance and bone development in poultry (Li et al., 2012). Usually, the Ca to P ratio is used to evaluate the balance between dietary Ca and P. The Ca to non-phytate phosphorus (NPP) system has been used for evaluating the requirement of Ca and P of poultry (NRC, 1994). The NRC (1994) recommended the Ca and NPP requirements of 10.0 and 4.5 g kg^{-1} (Ca/NPP = 2.22) in 1- to 21-d-old birds, and 9.0 and 3.5 g kg^{-1} (Ca/NPP = 2.57) in 22- to 42-d-old broilers. Further research showed that the highest performance and tibia mineralization of broilers were observed at a Ca to NPP ratio of 2.0 (Bar et al., 2003; Rao et al., 2007). These data suggest that the Ca to NPP ratio ranges from 2.0 to 2.6 in broiler diets. However, another study reported that optimal Ca to NPP ratios were 1.1, 1.4, and 1.6 for body weight gain (BWG), feed efficiency (FE), and tibia ash percentage in broilers from 1 to 16 d of age (Driver et al., 2005). These data revealed the uncertainty in the Ca to NPP ratio of broiler diets.

Cholecalciferol (vitamin D_3) was used as a vitamin D source in the abovementioned research (Bar et al., 2003; Driver et al., 2005; Rao et al., 2007). 1α-hydroxycholecalciferol (1α-OH-D_3) and 25-hydroxycholecalciferol (25-OH-D_3) are derivatives of vitamin D. Their bioavailability is higher than that of vitamin D_3. 25-OH-D_3 is nearly twice as active as vitamin D_3 (Soares et al., 1995) and 1α-OH-D_3 is 5 to 8 times as effective as vitamin D_3 in broilers (Edwards et al., 2002; Han et al., 2013). 25-OH-D_3 has been approved for use in feed for broiler chickens, laying hens, and turkeys in Europe, USA, and China. In contrast, 1α-OH-D_3 is not widely used as a feed additive. The effect of Ca to NPP ratio on growth of broiler chickens has not been examined when 1α-OH-D_3 or 25-OH-D_3 was used as a vitamin D source.

Therefore, the objective of the present study was to assess the influence of dietary Ca to NPP ratio and different sources of vitamin D (1α-OH-D_3 or 25-OH-D_3) on growth performance and bone mineralization in 1- to 42-d-old broiler chickens.

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Material and Methods

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

On the day of hatch, 700 female Ross 308 broilers were weighed and randomly assigned to 14 treatments with five stainless steel starter cages (70 × 70 × 30 cm) of 10 birds each. On d 13, the broilers were transferred to stainless steel finisher cages (190 × 50 × 35 cm). The experiment lasted until the birds reached 42 d of age. A 7 × 2 factorial experiment was designed to test the Ca to NPP ratios of 1.14, 1.43, 1.71, 2.00, 2.29, 2.57, and 2.86 in combination with two sources of vitamin D (1α-OH-D_3 and 25-OH-D_3). Dietary Ca levels were 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 g kg^{-1}, and the NPP content was 3.5 g kg^{-1} (Table 1). The dose of 1α-OH-D_3 or 25-OH-D_3 was 5 μg kg^{-1}. Diets were not supplemented with vitamin D_3. Birds were given access to mash feed and water *ad libitum*. The lighting program consisted of 23 h of light from d 1 to 3, 20 h of light from d 4 to 21, and 18 h of light from d 22 to 42. Room temperature was controlled at 33 °C from d 0 to 3, and then gradually decreased by 3 °C per week to a final temperature of 21 °C on d 42.

The crystalline 1α-OH-D_3 and 25-OH-D_3 were supplied by Taizhou Healtech Chemical Co., Ltd. (Taizhou, China) and Changzhou Book Chemical Co., Ltd. (Changzhou, China), respectively. The crystalline 1α-OH-D_3 and 25-OH-D_3 were weighed and dissolved in ethanol. Then, they were diluted to a final concentration of 10 mg L^{-1} of 1α-OH-D_3 or 25-OH-D_3 in a solution of 5% ethanol and 95% propylene glycol (Han et al., 2013). After preparation, the 1α-OH-D_3 or 25-OH-D_3 solution was supplemented to the diets.

Birds were weighed on d 21 and d 42. Ten chickens per treatment were selected randomly for the collection of blood and tibiae. Plasma samples (5 mL) were collected through cardiac puncture on d 21 and through the wing vein on d 42. These samples were centrifuged for 10 min at 3,000 *g* at 20 °C. Birds were killed after collecting the blood samples. Carcass and breast (with bones) were weighed. Carcass and breast yield was calculated as percentage of the live body weight of the birds. The left and right tibiae of individual birds were excised and frozen at –20 °C for further analysis (breaking strength, weight, length, diameter, ash weight, and percentage contents of ash, Ca, and P).

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

Following the method of Hall et al. (2003), the left tibiae were boiled for 5 min to loosen muscle tissues. Meat, connective tissue, and fibula bone were completely removed using scissors and forceps. Tibiae were placed in a container of ethanol for 48 h (removing water and polar

Table 1 - Composition of the experimental diets

	Calcium (Ca) level in starter diet (1-21 days)							Ca level in finisher diet (22-42 days)						
	4.0	5.0	6.0	7.0	8.0	9.0	10.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Ingredient (g kg^{-1})														
Corn	608.7	602.8	597.1	591.4	585.7	580.3	574.2	650.0	643.5	638.1	632.2	626.7	621.0	615.4
Soybean meal (43%)	320.0	320.0	320.0	320.0	320.0	320.0	320.0	280.0	280.0	280.0	280.0	280.0	280.0	280.0
Soybean oil	15.0	15.0	15.0	15.0	15.0	15.0	15.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Swine lard	0.0	2.1	4.1	6.1	8.1	10.0	12.2	0.0	2.3	4.2	6.3	8.2	10.2	12.2
Soy protein isolate	34.1	34.9	35.7	36.5	37.3	38.0	38.8	23.2	24.1	24.8	25.6	26.4	27.2	27.9
Limestone	0.0	3.0	5.9	8.7	11.6	14.4	17.4	0.0	3.3	6.0	9.0	11.8	14.7	17.6
Dicalcium phosphate	13.1	13.1	13.1	13.2	13.2	13.2	13.3	13.3	13.3	13.4	13.4	13.4	13.4	13.4
L-lysine•HCl	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
DL-methionine	1.4	1.4	1.4	1.4	1.4	1.4	1.4	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Trace mineral premix[1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[2]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride (50%)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sodium chloride	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Nutrient composition														
Metabolizable energy (kcal kg^{-1})	2972	2972	2972	2972	2972	2972	2972	3069	3069	3069	3069	3069	3069	3069
Analyzed crude protein (g kg^{-1})	205.8	216.2	215.5	217.2	208.5	212.8	209.6	195.8	189.5	192.1	191.1	196.5	192.7	191.6
Analyzed calcium (g kg^{-1})	3.8	5.4	6.3	7.2	8.3	8.9	10.5	4.0	4.9	5.8	6.5	7.7	8.8	10.2
Analyzed total phosphorus (g kg^{-1})	5.4	6.1	5.7	5.4	5.3	5.5	5.6	5.2	5.1	5.1	5.2	5.3	5.4	5.1
Non-phytate phosphorus (g kg^{-1})	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Lysine (g kg^{-1})	11.0	11.0	11.0	11.0	11.0	11.0	11.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Methionine (g kg^{-1})	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.1	4.1	4.1	4.1	4.1	4.1	4.1

[1] The trace mineral premix provided the following (per kg of diet): iron - 100 mg; zinc - 100 mg; copper - 8 mg; manganese - 120 mg; iodine - 0.7 mg; selenium - 0.3 mg.
[2] The vitamin premix provided the following (per kg of diet): vitamin A - 8,000 IU; vitamin E - 20 IU; menadione - 0.5 mg; thiamine - 2.0 mg; riboflavin - 8.0 mg; niacin - 35 mg; pyridoxine - 3.5 mg; vitamin B_{12} - 0.01 mg; pantothenic acid - 10.0 mg; folic acid - 0.55 mg; biotin - 0.18 mg.

lipids) after cleaning. Afterward, the bones were extracted in anhydrous ether for 48 h (removing non-polar lipids). Tibiae were dried at 105 °C for 24 h before weighing. Tibia diameter was determined at the medial point. Tibia ash content was determined by ashing the bone in a muffle furnace for 48 h at 600 °C.

The right tibia was used to analyze the breaking strength, which was determined using an all-digital electronic universal testing machine (Shenzhen Hengen Instrument Co. Ltd., Shenzhen, China). Tibiae were cradled on two support points measuring 4 cm apart. A 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^{-1} (Jendral et al., 2008).

The Ca and total P content in diets and tibiae were determined by the method of Han et al. (2013). The crude protein content in diets was determined using a PN-1430 Kjeldahl apparatus (Barcelona, Spain).

Replicate means are the experimental units in statistical analysis. All data were analyzed with the two-way ANOVA procedure of SAS software (Statistical Analysis System, version 9.0). Means were compared by Tukey's test when probability values were significant (P<0.05). Polynomial contrasts were used to test the linear and quadratic effects of dietary Ca to NPP ratio on growth performance and bone mineralization. Nonlinear regression analysis was conducted using the PROC NLIN procedure of the SAS software to estimate the optimal Ca to NPP ratio based on growth performance and tibia mineralization. The model was $y = ax^2 + bx + c$, in which y is the response and x is the Ca to NPP ratio.

Results

The Ca to NPP ratio, vitamin D source, and their interaction affected body weight gain (BWG), feed intake (FI), and feed efficiency (FE) of broiler chickens from 1 to 42 d of age (P<0.05, Table 2). Broilers fed 1α-OH-D_3 had higher BWG and FI and lower FE than birds fed 25-OH-D_3 at 42 d of age (P<0.05). No differences in mortality were observed between broilers fed 1α-OH-D_3 and 25-OH-D_3 (P>0.05). The Ca to NPP ratio influenced BWG, FI, FE, and mortality quadratically in 42-d-old broilers (P<0.05). Birds obtained the greatest BWG and FI at 42 d of age when the dietary Ca to NPP ratio ranged from 2.00 to 2.29.

The Ca to NPP ratio, vitamin D source, and their interaction affected the carcass and breast yield (P<0.05, Table 3). Broilers fed 1α-OH-D_3 had lower carcass and breast yield than birds fed 25-OH-D_3 (P<0.05). The Ca to NPP ratio affected the meat yield quadratically (P<0.05).

Table 2 - Effects of Ca to NPP ratio and different sources of vitamin D on growth performance of broiler chickens

Vitamin D	Ca/NPP	Weight gain (g)		Feed intake (g)		Feed efficiency		Mortality (%)	
		Days 1-21	Days 1-42	Days 1-21	Days 1-42	Days 1-21	Days 1-42	Days 1-21	Days 1-42
1α-OH-D_3	1.14	410	1210	733	2581	1.79	2.14	0	13
	1.43	505	1667	906	3522	1.80	2.12	0	7
	1.71	617	1912	996	4074	1.61	2.13	2	11
	2.00	625	2045	1035	4150	1.66	2.03	0	0
	2.29	656	2031	998	4036	1.52	1.99	0	2
	2.57	660	2006	1012	4008	1.53	2.00	2	7
	2.86	613	1985	939	3992	1.53	2.01	7	7
25-OH-D_3	1.14	306	605	619	1761	2.02	2.91	4	31
	1.43	629	1741	952	3512	1.52	2.03	0	4
	1.71	640	1809	979	3595	1.53	1.99	0	2
	2.00	697	1996	1030	4109	1.48	2.06	0	0
	2.29	697	1973	1034	3952	1.48	2.00	2	2
	2.57	684	1905	1018	3888	1.49	2.04	0	2
	2.86	689	1897	1026	3849	1.49	2.03	0	2
SEM		14	47	15	81	0.02	0.03	0.5	1
Main effect									
1α-OH-D_3		584b	1836a	946	3766a	1.63a	2.06b	2	7
25-OH-D_3		620a	1704b	951	3524b	1.57b	2.15a	1	6
Source of variation									
Vitamin D		<0.001	<0.001	0.557	<0.001	<0.001	<0.001	0.496	0.872
Ca/NPP		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.473	<0.001
D × Ca/NPP		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.065	0.021
Contrast analysis of Ca/NPP effect									
Linear		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.399	<0.001
Quadratic		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.070	<0.001

a-b - means in the same column without a common letter differ significantly (P<0.05).
NPP - non-phytate phosphorus; SEM - standard error of the mean.

The lowest Ca to NPP ratio resulted in the lowest yield of carcass and breast.

The Ca to NPP ratio and vitamin D source affected plasma Ca concentration in 21-d-old birds (P<0.05). Plasma Ca in birds fed 1α-OH-D_3 was lower than that of birds fed 25-OH-D_3 (P<0.05). The Ca to NPP ratio influenced plasma Ca quadratically (P<0.05). The greatest plasma Ca was at the Ca to NPP ratio of 2.57. No interactions in plasma Ca or inorganic phosphorus concentration in 21- or 42-d-old broilers were observed between the Ca to NPP ratio and vitamin D source (P>0.05).

Vitamin D source affected tibia breaking strength, weight, length, diameter, and ash weight in broilers at 21 and 42 d of age (P<0.05, Tables 4 and 5). Birds fed 1α-OH-D_3 had greater tibia breaking strength, weight, length, diameter, and ash weight than those fed 25-OH-D_3 at 42 d of age (P<0.05). The percentages of tibia ash, Ca, and P in 42-d-old broilers were not affected by the vitamin D source (P>0.05).

The Ca to NPP ratio influenced tibia breaking strength, weight, length, ash weight, and ash and P contents quadratically in 42-d-old broilers (P<0.05). Interaction effects between vitamin D source and the Ca to NPP ratio were observed on tibia weight, length, ash weight, and ash percentage (P<0.05). The lowest Ca to NPP ratio resulted in the lowest values of the above parameters.

Quadratic relationships between the Ca to NPP ratio and growth performance or tibia mineralization were observed. Non-linear regression analysis showed that the highest values of BWG, FI, tibia weight, length, ash weight, and ash percentage were observed at the Ca to NPP ratios of 2.33, 2.28, 2.45, 2.21, 2.46, and 2.44 in broiler chickens fed 1α-OH-D_3 as the vitamin D source, respectively (Table 6). The above values of Ca to NPP ratio were 2.27, 2.29, 2.25, 2.29, 2.26, and 2.31 in birds fed 25-OH-D_3, respectively. In general, the Ca to NPP ratio ranged from 2.21 to 2.46 and the average Ca to NPP ratio was 2.32 in 1- to 42-d-old broilers fed 1α-OH-D_3 or 25-OH-D_3 as the vitamin D source.

Discussion

Our unpublished data indicated that the relative bioavailability of 1α-OH-D_3 was higher than that of 25-OH-D_3 in broiler chicken diets. Thus, 42-d-old broilers fed 1α-OH-D_3 had higher BWG and FI than birds fed 25-OH-D_3 in the present study.

Table 3 - Effects of Ca to NPP ratio and different sources of vitamin D on carcass yield and plasma mineral concentration of broiler chickens

Vitamin D	Ca/NPP	Carcass yield				Plasma mineral			
		Carcass (g kg^{-1})		Breast (g kg^{-1})		Ca (mg dL^{-1})		IP (mg dL^{-1})	
		Day 21	Day 42	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
1α-OH-D_3	1.14	635.3	718.9	110.6	148.3	6.78	10.41	6.45	5.46
	1.43	661.2	734.0	112.3	155.0	8.31	10.42	4.87	5.33
	1.71	669.8	750.6	125.4	155.4	9.15	9.94	5.48	5.83
	2.00	648.9	754.4	121.2	162.5	8.86	10.31	5.52	5.91
	2.29	658.7	755.1	136.5	168.9	9.29	10.70	5.55	5.89
	2.57	667.0	742.4	134.7	157.7	9.84	10.61	5.29	6.15
	2.86	649.9	756.7	128.8	170.3	9.23	9.92	5.19	6.31
25-OH-D_3	1.14	628.2	688.3	125.3	126.4	6.96	9.30	6.42	5.12
	1.43	689.8	756.9	169.9	186.3	9.22	10.82	5.08	6.36
	1.71	684.4	754.4	146.2	182.7	8.56	10.19	5.74	5.78
	2.00	684.0	754.9	161.7	175.6	9.78	10.14	6.04	5.33
	2.29	685.8	789.5	168.6	186.2	9.65	10.42	6.07	6.20
	2.57	673.7	778.5	157.2	197.1	10.29	10.23	5.97	5.82
	2.86	692.4	771.9	168.1	179.7	9.64	9.67	5.59	5.46
SEM		3.0	3.6	2.7	2.5	0.14	0.10	0.14	0.09
Main effect									
1α-OH-D_3		655.8b	744.6b	124.2b	159.7b	8.78b	10.33	5.48	5.84
25-OH-D_3		676.9a	756.3a	156.7a	176.3a	9.16a	10.11	5.84	5.72
Source of variation									
Vitamin D		<0.001	0.013	<0.001	<0.001	0.021	0.288	0.203	0.501
Ca/NPP		<0.001	<0.001	<0.001	<0.001	<0.001	0.192	0.228	0.301
D × Ca/NPP		0.041	0.004	<0.001	<0.001	0.201	0.546	0.996	0.101
Contrast analysis of Ca/NPP effect									
Linear		<0.001	<0.001	<0.001	<0.001	<0.001	0.955	0.409	0.088
Quadratic		<0.001	<0.001	<0.001	<0.001	<0.001	0.059	0.600	0.344

a-b - means in the same column without a common letter differ significantly (P<0.05).
NPP - non-phytate phosphorus; IP - inorganic phosphorus; SEM - standard error of the mean.

Table 4 - Effects of Ca to NPP ratio and different sources of vitamin D on tibia growth of broiler chickens

Vitamin D	Ca/NPP	Breaking strength (N)		Weight (g)		Length (cm)		Diameter (cm)	
		Day 21	Day 42	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
	1.14	25.9	191.9	0.83	3.04	5.40	7.46	0.48	0.67
	1.43	43.6	279.3	1.01	3.77	5.77	8.62	0.49	0.69
	1.71	66.3	329.5	1.22	4.90	6.30	9.40	0.50	0.72
1α-OH-D_3	2.00	79.8	394.1	1.33	5.61	6.50	9.63	0.53	0.77
	2.29	77.8	438.4	1.35	5.43	6.30	9.46	0.55	0.79
	2.57	76.6	462.2	1.39	5.67	6.19	8.99	0.54	0.83
	2.86	70.1	426.4	1.37	5.45	6.12	9.16	0.54	0.78
	1.14	26.8	104.6	0.69	1.69	4.99	5.94	0.43	0.63
	1.43	73.0	272.2	1.23	4.32	6.14	9.00	0.49	0.72
	1.71	75.9	278.1	1.24	4.34	6.16	9.13	0.51	0.73
25-OH-D_3	2.00	101.8	291.6	1.54	4.73	6.45	9.17	0.51	0.72
	2.29	116.3	314.6	1.56	5.14	6.42	9.47	0.53	0.69
	2.57	111.5	313.0	1.60	4.56	6.53	9.53	0.51	0.68
	2.86	99.6	304.7	1.52	4.46	6.33	9.24	0.50	0.70
SEM		3.6	14.1	0.03	0.14	0.06	0.12	0.01	0.01
Main effect									
1α-OH-D_3		62.88b	360.3a	1.22b	4.84a	6.08	8.96a	0.52a	0.75a
25-OH-D_3		86.42a	268.4b	1.34a	4.18b	6.15	8.78b	0.50b	0.70b
Source of variation									
Vitamin D		<0.001	<0.001	<0.001	<0.001	0.224	0.048	0.030	0.007
Ca/NPP		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.055
D × Ca/NPP		0.075	0.495	0.033	0.014	0.002	<0.001	0.787	0.166
Contrast analysis of Ca/NPP effect									
Linear		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.009
Quadratic		<0.001	0.002	<0.001	<0.001	<0.001	<0.001	0.005	0.086

a-b - means in the same column without a common letter differ significantly (P<0.05).
NPP - non-phytate phosphorus; SEM - standard error of the mean.

Table 5 - Effects of Ca to NPP ratio and different sources of vitamin D on tibia mineralization of broiler chickens

Vitamin D	Ca/NPP	Ash (g)		Ash (g 100 g^{-1})		Ca (g 100 g^{-1})		P (g 100 g^{-1})	
		Day 21	Day 42	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
	1.14	0.28	1.30	34.47	42.81	10.04	15.76	6.59	8.05
	1.43	0.40	1.67	39.36	44.24	13.80	16.70	7.54	8.45
	1.71	0.56	2.18	45.57	44.72	16.45	16.78	8.44	8.45
1α-OH-D_3	2.00	0.61	2.56	45.62	45.68	16.80	17.17	8.68	8.65
	2.29	0.62	2.62	45.78	48.30	16.35	17.33	8.69	8.93
	2.57	0.63	2.75	45.45	48.65	17.81	17.90	8.73	8.27
	2.86	0.57	2.48	41.47	45.52	16.53	17.11	8.09	8.15
	1.14	0.24	0.67	34.96	40.02	12.83	15.16	7.09	7.53
	1.43	0.56	1.93	45.68	44.94	17.07	16.10	9.05	8.68
	1.71	0.57	2.03	45.92	46.49	17.00	16.96	8.65	8.84
25-OH-D_3	2.00	0.78	2.19	50.43	46.16	18.77	17.06	9.56	8.89
	2.29	0.83	2.35	52.73	45.71	19.92	16.81	9.93	8.36
	2.57	0.83	2.12	51.52	46.48	19.13	17.42	9.63	8.67
	2.86	0.78	2.07	51.03	46.35	19.45	17.29	9.70	8.53
SEM		0.02	0.07	0.69	0.32	0.33	0.11	0.13	0.07
Main effect									
1α-OH-D_3		0.52b	2.22a	42.53b	45.70	15.40b	16.96	8.11b	8.42
25-OH-D_3		0.65a	1.91b	47.47a	45.17	17.74a	16.69	9.09a	8.50
Source of variation									
Vitamin D		<0.001	<0.001	<0.001	0.243	<0.001	0.120	<0.001	0.517
Ca/NPP		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
D × Ca/NPP		<0.001	0.008	<0.001	0.031	<0.001	0.741	0.009	0.101
Contrast analysis of Ca/NPP effect									
Linear		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.127
Quadratic		<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001

a-b - means in the same column without a common letter differ significantly (P<0.05).
NPP - non-phytate phosphorus; SEM - standard error of the mean.

Table 6 - Regression analysis of the relationship between Ca to NPP ratio (x) and performance or tibia mineralization (y) in 1- to 42-d-old broiler chickens

Vitamin D	Criterion	Formula	P-value	R^2	Ca/NPP
	Growth performance				
	Weight gain	$y = -585.3x^2 + 2730.5x - 1091.1$	<0.001	0.91	2.33
	Feed intake	$y = -1170.1x^2 + 5324.3x - 1818.1$	<0.001	0.84	2.28
1α-OH-D_3	Tibia mineralization				
	Weight	$y = -1.5927x^2 + 7.8089x - 3.8857$	<0.001	0.77	2.45
	Length	$y = -1.7380x^2 + 7.6864x + 1.1094$	<0.001	0.72	2.21
	Ash weight	$y = -0.8296x^2 + 4.0830x - 2.3512$	<0.001	0.84	2.46
	Ash content	$y = -2.9344x^2 + 14.2999x + 29.8018$	<0.001	0.43	2.44
	Growth performance				
	Weight gain	$y = -991.6x^2 + 4511.6x - 3027.3$	<0.001	0.82	2.27
	Feed intake	$y = -1603.6x^2 + 7334.3x - 4204.2$	<0.001	0.83	2.29
25-OH-D_3	Tibia mineralization				
	Weight	$y = -2.4203x^2 + 10.8815x - 7.1101$	<0.001	0.67	2.25
	Length	$y = -2.4182x^2 + 11.0860x - 2.9227$	<0.001	0.76	2.29
	Ash weight	$y = -1.1872x^2 + 5.3578x - 3.6679$	<0.001	0.69	2.26
	Ash content	$y = -4.2775x^2 + 19.7638x + 24.1524$	<0.001	0.50	2.31
Mean					2.32

NPP - non-phytate phosphorus.

The Ca to NPP ratio influenced BWG, FI, FE, and mortality quadratically in 42-d-old broilers. The lowest Ca to NPP ratio of 1.14 damaged growth performance of broilers. Birds obtained the greatest BWG and FI at 42 d of age when the Ca to NPP ratio ranged from 2.27 to 2.33 in diets with 1α-OH-D_3 or 25-OH-D_3 as vitamin D. Previous research showed that the high Ca to NPP ratio of 4.0 depressed the BWG and FI of broilers (Li et al., 2012) and the optimal Ca to NPP ratio was 2.0 in broiler diets with vitamin D_3 as the vitamin D source (Bar et al., 2003; Rao et al., 2007).

A high dietary Ca to NPP ratio negatively affected P utilization because of the formation of Ca-P complexes in the gastrointestinal tract, which is not available for the birds. Growth improvement by lowering the Ca to NPP ratio results from the increase in phytase activity, P digestibility, and P retention in broiler chickens (Qian et al., 1997), turkeys (Qian et al., 1996), and pigs (Liu et al., 1998; Stein et al., 2011). Lowering the dietary Ca to NPP ratio also increases the absorbed and retained P (Al-Masri, 1995).

Decreasing the available phosphorus of 21- to 42-d-old broilers from 4.0 to 2.5 g kg^{-1} decreased the BWG and carcass yield (Chen and Moran, 1995). Birds fed 4.0 g kg^{-1} Ca (Ca/NPP = 1.14) had the lowest carcass and breast yields in the present study. These data indicated that deficiency of Ca or P decreased the muscle growth and meat production of broiler chickens.

We have found that 1α-OH-D_3 is about two times as effective as 25-OH-D_3 in promoting growth performance and bone mineralization in broiler chicken diets (unpublished). Therefore, birds fed 1α-OH-D_3 had greater tibia breaking strength, weight, length, diameter, and ash weight than those fed 25-OH-D_3 at 42 d of age.

Interaction effects between vitamin D source and the Ca to NPP ratio were observed on tibia weight, length, ash weight, and ash percentage. The Ca to NPP ratio influenced tibia mineralization quadratically. The highest values of tibia weight, length, ash weight, and ash percentage were observed when the dietary Ca to NPP ratio ranged from 2.21 to 2.46 in 42-d-old broilers.

The dietary Ca to P ratio regulates bone mineralization and turnover by affecting the intestinal Ca and P transports in mice (Masuyama et al., 2003). The imbalance between Ca and P also impaired bone mineralization of pigs (Létourneau-Montminy et al., 2010). Similar results were observed in poultry studies. Dietary Ca or P restriction increased duodenal calbindin and decreased bone ash weight in both 22- and 43-d-old broiler chickens, but the effect on bone ash was less noticeable in the 43-d-old birds than in the younger birds (Bar et al., 2003). A Ca- and P-deficient diet resulted in low tibia minerals and breaking strength of broilers; all tibia parameters were further decreased when the Ca to P ratio was relatively higher (Li et al., 2012). In the present study, deficiency of Ca resulted in poor tibia mineralization, in particular, low levels of breaking strength, length, weight, ash weight, and ash and Ca contents.

The optimal Ca to NPP ratio in broiler chickens fed vitamin D_3 has been studied by several researchers. Growth performance and bone ash percentage decreased with the increase of Ca to NPP ratio from 2.1 to 3.8; the highest values were observed at the Ca to NPP ratio of 2.1 in broilers

(Qian et al., 1997). Phosphorus requirements for growth and bone ash are similar and are as high in older chickens as in younger ones, and the Ca requirement for growth and bone ash is 10.0 g kg^{-1} in broilers (Bar et al., 2003). Further research showed that the highest performance and tibia quality were observed when the dietary Ca to NPP ratio was 2.0 (Rao et al., 2007). These data indicated that the Ca to NPP ratio in 22- to 42-d-old broilers was lower than that (Ca/NPP = 2.57) recommended by the NRC (1994).

Previous research has shown that addition of 25-OH-D_3 helps to relieve leg problems when broiler chickens were fed diets with suboptimal Ca to NPP ratios (Coto et al., 2008). 1α-OH-D_3 exerts the highest activities at lower concentrations of dietary Ca (Han et al., 2012). In the present study, 42-d-old broilers fed 1α-OH-D_3 or 25-OH-D_3 as the vitamin D source obtained the highest BWG and FI when the Ca to NPP ratio was from 2.27 to 2.33. In contrast, the Ca to NPP ratio of 2.21 to 2.46 was associated with optimal bone mineralization. The average Ca to NPP ratio is about 2.32 for growth and bone quality in broiler chickens from 1 to 42 d of age.

Conclusions

Broiler chickens at 42 days of age obtain optimal growth performance and bone mineralization at the dietary calcium to non-phytate phosphorus ratio of 2.32 when 1α-OH-D_3 or 25-OH-D_3 are used as the vitamin D source.

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