Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Oct - Dec 2015 / v.17 / n.4 / 503-510
http://dx.doi.org/10.1590/1516-635x1704503-510

Effects of Non-phytate Phosphorus and 1α-Hydroxycholecalciferol on Growth Performance, Bone Mineralization, and Carcass Traits of Broiler Chickens

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■Keywords

Non-phytate phosphorus, 1α-hydroxycholecalciferol, growth, bone, broiler chicken.

Submitted: August/2014
Approved: March/2015

ABSTRACT

This study evaluated the effects of dietary non-phytate phosphorus (NPP) and 1α-hydroxycholecalciferol (1α-OH-D_3) on the growth performance, bone mineralization, and carcass traits of 1- to 21-day-old broiler chickens. On the day of hatch, 600 male Ross 308 chicks were weighed and randomly assigned to 12 treatments, with five cages of 10 birds each. A 6 × 2 factorial arrangement was applied, consisting of 0.20%, 0.25%, 0.30%, 0.35%, 0.40%, or 0.45% NPP and 0 or 5 μg/kg of 1α-OH-D_3. The basal diet contained 0.52% calcium (Ca) and was not supplemented with vitamin D_3. Dietary NPP levels significantly affected growth performance and tibia mineralization (except width) of broilers; by contrast, meat yield and organ relative weight were not influenced by NPP. The inclusion of 1α-OH-D_3 improved growth performance, tibia mineralization, and carcass and breast yield, whereas it decreased the relative weights of the liver, heart, and kidney. A significant interaction between NPP and 1α-OH-D_3 was observed for body weight gain (BWG), feed efficiency (FE), mortality, serum Ca and P levels, tibia breaking-strength, ash weight, and Ca content, as well as breast yield and heart relative weight. These results suggest that broilers fed with 5 μg of 1α-OH-D_3 per kg of diet obtain optimal growth performance and tibia mineralization when dietary NPP level was 0.30% and the analyzed Ca to NPP ratio was 1.97.

INTRODUCTION

Intestinal mucosa phytase activity increases and more phytate phosphorus (PP) is hydrolyzed at low dietary calcium (Ca, 0.40%) compared with high Ca (0.90%) in broiler chickens from 14 to 24 days of age (Applegate *et al*.,2003). Broiler growth rate and tibia ash responses to supplemental phytase are the greatest at low non-phytate phosphorus (NPP) levels and high Ca levels, and these responses decrease when the Ca level decreases or when the NPP level increases (Driver *et al*., 2005). These data indicate that dietary Ca and phosphorus (P) affect the efficacy of endogenous and exogenous phytase in broiler chickens.

Vitamin D efficacy maybe also influenced by dietary Ca and P in poultry. Chickens obtained the highest growth rate, bone ash, and Ca and P retention when they were fed with diets of Ca to total phosphorus (tP) ratios ranging from 1.1 to 1.4:1 (Qian *et al*., 1997). The metabolite of vitamin D, 1α-hydroxycholecalciferol (1α-OH-D_3), is 5 to 8 times as active as vitamin D_3 in promoting growth and tibia ash content (Edwards *et al*., 2002; Han *et al*., 2013). The compound 1α-OH-D_3 had positive effects on growth and bone mineralization in broiler chickens (Biehl and Baker, 1997). However, the efficacy of 1α-OH-D_3 negatively responded to dietary Ca levels (Han *et al*., 2012). These data indicate that dietary Ca affects vitamin D bioavailability.

Han *et al*. (2009c) reported that 1α-OH-D_3 improved growth performance and bone mineralization of broilers fed diets with 0.21% NPP. However, 1α-OH-D_3 did not significantly improve broiler growth when dietary NPP was increased to 0.29% (Han *et al*., 2009b). Edwards (2002) also found that 1α-OH-D_3 did not improve body weight gain (BWG) or feed efficiency (FE) in 1- to 16-day-old broilers when the dietary NPP level reached 0.30%. These results suggest that NPP may influence the bioavailability of 1α-OH-D_3.

However, the relationship between NPP and 1α-OH-D_3 has not been examined. Thus, the objective of the present study was to evaluate the effects of dietary NPP and 1α-OH-D_3 on the growth performance, bone mineralization, and carcass traits of broiler chickens.

MATERIAL AND METHODS

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

Birds, diets, and management

On the day of hatch, 600 male Ross 308 broiler chicks were weighed and randomly assigned to 12 treatments, and were housed in five stainless steel cages (70 × 70 × 30 cm) of 10 birds each. The chicks were transferred into stainless steel growing-finishing cages (190 × 50 × 35 cm) on day 14. A 6×2 factorial arrangement was applied to test 0.20%, 0.25%, 0.30%, 0.35%, 0.40% and 0.45% NPP combined with 0 and 5 μg/kg of 1α-OH-D_3 in a basal diet (Table 1). The basal diet contained 0.52% Ca and was not supplemented with vitamin D_3. The birds were given access to mash feed and water *ad libitum*. The lighting system consisted of 23 h of light from day 0 to 21. Room temperature was controlled at 33°C from day 0 to 3 and then gradually reduced by 3°C per week until the final temperature of 24°C was reached.

Crystalline 1α-OH-D_3

The crystalline 1α-OH-D_3 product was supplied by Taizhou Healtech Chemical Co., Ltd. (Taizhou, China). The1α-OH-D_3 solution was prepared using the method of Han *et al*. (2012). Briefly, 1α-OH-D_3 was dissolved in ethanol and then diluted to a final concentration of 10 mg/L of 1α-OH-D_3 in a solution of 5% ethanol and 95% propylene glycol.

Sample collection

The chicks were individually weighed on day 21. One chick which body weight was close to the average weight of the replicate was selected for the collection of blood and tibias. The live body weight of the chicks was determined after fasting for 12 hours. Blood samples (5 mL) were collected by cardiac puncture on day 21 and centrifuged for 10 min at 3000*g* at 20°C. The chicks were sacrificed after blood samples

Table 1 – Ingredients and nutrient composition of the experimental diets.

Ingredient (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Corn	60.78	60.55	60.30	60.06	59.83	59.60
Soybean meal (43% CP)	32.00	32.00	32.00	32.00	32.00	32.00
Soybean oil	1.50	1.50	1.50	1.50	1.50	1.50
Swine lard	0.08	0.16	0.25	0.34	0.42	0.50
Soy protein concentrate	3.41	3.44	3.47	3.50	3.53	3.56
Limestone	0.90	0.72	0.54	0.36	0.18	0.00
Dicalcium phosphate	0.42	0.72	1.03	1.33	1.63	1.93
L-Lysine·HCl	0.14	0.14	0.14	0.14	0.14	0.14
DL-Methionine	0.14	0.14	0.14	0.14	0.14	0.14
Trace mineral premix[1]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[2]	0.03	0.03	0.03	0.03	0.03	0.03
Choline chloride (50%)	0.20	0.20	0.20	0.20	0.20	0.20
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Nutrient composition						
AME (kcal/kg)	2975	2975	2975	2975	2975	2975
Analyzed crude protein (%)	21.24	21.65	21.09	21.44	21.81	21.43
Analyzed calcium (%)	0.56	0.56	0.59	0.58	0.59	0.56
Analyzed total phosphorus (%)	0.40	0.46	0.51	0.56	0.60	0.65
Non-phytate phosphorus (%)	0.20	0.25	0.30	0.35	0.40	0.45

[1] The trace mineral premix provided the following (per kilogram of diet): 100 mg iron; 100 mg zinc; 8 mg copper; 120 mg manganese; 0.7 mg iodine; 0.3 mg selenium.

[2] The vitamin premix provided the following (per kilogram 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.

were collected. The carcass, breast (with bones), leg quarter, liver, heart, and kidney were weighed. The meat yield and organ relative weight were calculated as the percentage of the live body weight of chicks. The left and right tibias of the individual chicks were excised and frozen at -20°C for further analysis (breaking-strength, weight, length, width, ash weight, and percentage of ash, Ca and P).

Sample analysis

Serum Ca and inorganic phosphate (Pi) were determined using a Shimadzu CL-8000 analyzer (Shimadzu Corp., Kyoto, Japan) following the manufacturer's instructions.

Following the method by Hall *et al*. (2003), the left tibias were boiled for 5 min to loosen muscle tissues. The meat, connective tissue, and the fibula bone were completely removed using scissors and forceps. The tibias were placed in a container with ethanol for 48 h (to remove water and polar lipids) after cleaning. The bones were then further extracted in anhydrous ether for 48 h (removing non-polar lipids). Tibias were dried at 105°C for 24 h before weighing. Tibia width was determined at the medial point. Tibia ash content was determined by burning the bone in a muffle furnace for 30 hour at 600°C.

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

Calcium and total P in diet and tibia were determined by the method of Han *et al.* (2013). Crude protein was determined using the Kjeldahl method (PN-1430, Barcelona, Spain).

Statistical analyses

The data were analyzed by one-way and two-way ANOVA procedures of SAS (SAS Institute, 2002). Means were compared by Tukey's test when probability values were significant ($p< 0.05$).

RESULTS

Growth performance

Dietary NPP levels significantly affected BWG, feed intake (FI), FE, and mortality (Table 2). Vitamin D deficiency decreased BWG, FI, and FE, and caused

Table 2 – Effects of non-phytate phosphorus (NPP) and 1α-hydroxycholecalciferol (1α-OH-D_3) on the growth performance of 1- to 21-day-old broiler chicks.

NPP (%)	1α-OH-D_3 (μg/kg)	Growth[1]				Serum[2]	
		BWG[3] (g)	FI[3] (g)	FE[3] (BWG/FI)	Mortality (%)	Ca[3] (mg/100mL)	Pi[3] (mg/100mL)
0.20	0	176 ^{e}	470 ^{c}	0.372 ^{e}	56 ^{a}	9.74 ^{a}	3.21 ^{b}
0.25	0	251 ^{d}	515 ^{c}	0.498 ^{d}	36 ^{ab}	8.94 ^{ab}	3.05 ^{b}
0.30	0	290 ^{cd}	540 ^{c}	0.534 ^{cd}	32 ^{ab}	7.67 ^{ab}	6.75 ^{a}
0.35	0	287 ^{cd}	548 ^{c}	0.525 ^{cd}	28 ^{b}	7.91 ^{ab}	6.16 ^{a}
0.40	0	329 ^{c}	548 ^{c}	0.580 ^{bc}	16 ^{bc}	7.58 ^{ab}	5.28 ^{ab}
0.45	0	299 ^{c}	551 ^{c}	0.552 ^{cd}	22 ^{bc}	7.68 ^{ab}	6.34 ^{a}
0.20	5	597 ^{b}	923 ^{b}	0.647 ^{ab}	0 ^{c}	7.45 ^{b}	4.72 ^{ab}
0.25	5	649 ^{a}	1024 ^{a}	0.635 ^{ab}	0 ^{c}	7.76 ^{ab}	4.76 ^{ab}
0.30	5	667 ^{a}	1037 ^{a}	0.643 ^{ab}	0 ^{c}	7.72 ^{ab}	5.36 ^{ab}
0.35	5	668 ^{a}	1051 ^{a}	0.636 ^{ab}	0 ^{c}	7.90 ^{ab}	5.31 ^{ab}
0.40	5	664 ^{a}	1031 ^{a}	0.645 ^{ab}	2 ^{c}	8.81 ^{ab}	4.84 ^{ab}
0.45	5	660 ^{a}	1001 ^{ab}	0.660 ^{a}	0 ^{c}	9.01 ^{ab}	5.74 ^{a}
SEM[3]		25	33	0.012	3	0.15	0.20
P-value		<0.001	<0.001	<0.001	<0.001	0.011	<0.001
				Source of variance			
NPP		<0.001	0.011	<0.001	0.011	0.414	<0.001
1α-OH-D_3		<0.001	<0.001	<0.001	<0.001	0.584	0.976
NPP × 1α-OH-D_3		0.019	0.858	<0.001	0.006	0.002	0.016

[a-e] Means in the same column without a common superscript significantly differ ($p< 0.05$).

[1]Data are the means of five replicate cages consisting of 10 chicks per cage.

[2] Data are the means of five replicate cages consisting of one chick per replicate cage.

[3]BWG = body weight gain, FI = feed intake, FE = feed efficiency, Ca = calcium, Pi= inorganic phosphate, SEM= pooled standard error of the mean.

severe mortality of broilers in groups 1 to 6. Addition of 1α-OH-D_3 improved BWG, FI, and FE, and decreased mortality of birds in groups 7 to 12. No significant differences were observed in BWG, FI, FE, and mortality among groups fed 0.25% to 0.45% NPP plus 1α-OH-D_3. Significant interaction between dietary NPP and 1α-OH-D_3 was observed for BWG, FE, and mortality.

Serum minerals

Dietary NPP increased serum P when 1α-OH-D_3 was not added; by contrast, it did not affect serum Ca (Table 2). The addition of 1α-OH-D_3 did not affect serum Ca or P. An interaction between NPP and 1α-OH-D_3 was observed for serum Ca and P levels.

Tibia mineralization

Dietary NPP levels influenced tibia breaking-strength, weight, length, ash weight and the percentage of ash, Ca, and P (Table 3). Vitamin D deficiency caused low levels of tibia mineralization of broilers in groups 1 to 6. Tibia mineralization was improved by 1α-OH-D_3. No significant differences were observed in tibia parameters (except breaking-strength) among groups fed 0.30% to 0.45% NPP plus 1α-OH-D_3. Significant interaction between NPP and 1α-OH-D_3 was observed in tibia breaking-strength, ash weight, and Ca content.

Carcass traits

Dietary NPP levels did not affect meat yield or organ relative weights (Table 4). Vitamin D deficiency reduced muscle growth and meat production of broilers. Carcass and breast yields of groups 1 to 6 was significantly lower than those of groups 7 to 12 supplemented with 1α-OH-D_3. The addition of 1α-OH-D_3 increased carcass and breast meat yields and decreased the relative weights of the liver, heart, and kidney. However, it did not affect leg yield. A significant interaction between NPP and 1α-OH-D_3 was observed for breast meat yield and heart relative weight.

DISCUSSION

Growth performance

Dietary NPP levels significantly affected BWG, FI, FE, and mortality of broilers fed 0.52% Ca in this study. Augspurger & Baker (2004) reported that 0.10% to 0.30% NPP levels linearly improved the growth performance of 8- to 22-day-old broilers. Literature studies mentioned below showed that the response of broilers to P is affected by Ca levels. The BWG of 1- to 42-day-old broilers was not affected by 0.30% to 0.45% NPP when Ca level was 0.6 to 0.8%; however, the same levels of NPP increased BWG when Ca reached 0.90% (Rao *et al*., 2006). Driver *et al*. (2005)

Table 3 – Effects of non-phytate phosphorus (NPP) and 1α-hydroxycholecalciferol (1α-OH-D_3) on tibia mineralization parameters of 1- to 21-day-old broiler chicks[1].

NPP (%)	1α-OH-D_3 (μg/kg)	BS[2] (N)	Weight (g)	Length (cm)	Width (cm)	Ash (g)	Ash (%)	Ca[2] (%)	P[2] (%)
0.20	0	12.94 ^{e}	0.60 ^{d}	4.12 ^{d}	0.43 ^{cd}	0.16 ^{d}	25.67 ^{e}	7.95 ^{d}	4.65 ^{e}
0.25	0	18.32 ^{e}	0.66 ^{d}	4.20 ^{cd}	0.48 ^{abcd}	0.19 ^{d}	28.21 ^{de}	8.86 ^{cd}	5.18 ^{de}
0.30	0	22.58 ^{e}	0.77 ^{d}	4.70 ^{b}	0.43 ^{d}	0.22 ^{d}	28.55 ^{cde}	9.45 ^{cd}	5.27 ^{de}
0.35	0	23.02 ^{e}	0.67 ^{d}	4.52 ^{bcd}	0.45 ^{bcd}	0.22 ^{d}	32.71 ^{c}	11.46 ^{b}	5.88 ^{d}
0.40	0	23.15 ^{e}	0.77 ^{d}	4.58 ^{bc}	0.45 ^{bcd}	0.24 ^{d}	30.61 ^{cd}	10.42 ^{bc}	5.75 ^{d}
0.45	0	20.98 ^{e}	0.70 ^{d}	4.55 ^{bc}	0.43 ^{cd}	0.23 ^{d}	32.15 ^{cd}	11.49 ^{b}	5.99 ^{d}
0.20	5	71.10 ^{d}	1.21 ^{c}	6.04 ^{a}	0.55 ^{a}	0.58 ^{c}	46.95 ^{b}	17.46 ^{a}	8.62 ^{c}
0.25	5	84.40 ^{c}	1.32 ^{bc}	6.26 ^{a}	0.53 ^{abc}	0.63 ^{bc}	47.87 ^{ab}	17.43 ^{a}	8.87 ^{bc}
0.30	5	103.97 ^{a}	1.56 ^{a}	6.40 ^{a}	0.56 ^{a}	0.77 ^{a}	49.18 ^{ab}	17.91 ^{a}	9.20 ^{abc}
0.35	5	97.27 ^{ab}	1.40 ^{abc}	6.26 ^{a}	0.54 ^{ab}	0.73 ^{ab}	51.71 ^{a}	18.81 ^{a}	9.78 ^{a}
0.40	5	90.45 ^{bc}	1.40 ^{abc}	6.31 ^{a}	0.53 ^{abc}	0.70 ^{ab}	49.89 ^{ab}	18.22 ^{a}	9.38 ^{abc}
0.45	5	100.08 ^{ab}	1.44 ^{ab}	6.31 ^{a}	0.55 ^{a}	0.74 ^{a}	51.27 ^{ab}	18.44 ^{a}	9.56 ^{ab}
SEM[2]		4.86	0.05	0.12	0.01	0.03	1.34	0.55	0.26
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
				Source of variance					
NPP		<0.001	<0.001	<0.001	0.971	<0.001	<0.001	<0.001	<0.001
1α-OH-D_3		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NPP × 1α-OH-D_3		0.047	0.228	0.276	0.431	0.035	0.776	0.005	0.771

[a-e] Means in the same column without a common superscript significantly differ ($p < 0.05$).

[1]Data are the means of five replicate cages consisting of one chick per replicate cage.

[2]BS = Breaking-strength, Ca = calcium, P= phosphorus, SEM= pooled standard error of the mean.

Table 4 – Effects of non-phytate phosphorus (NPP) and 1α-hydroxycholecalciferol (1α-OH-D_3) on the carcass traits of 1- to 21-day-old broiler chicks[1,2]

NPP (%)	1α-OH-D_3 (μg/kg)	Carcass (%)	Breast (%)	Leg quarter (%)	Liver (%)	Heart (%)	Kidney (%)
0.20	0	64.92 ^{c}	8.82 ^{e}	18.23	4.00 ^{a}	1.26 ^{a}	0.82
0.25	0	67.54 ^{c}	10.86 ^{de}	17.87	3.46 ^{ab}	1.07 ^{ab}	0.65
0.30	0	67.08 ^{c}	12.63 ^{cde}	19.33	3.54 ^{ab}	0.82 ^{b}	0.75
0.35	0	67.43 ^{c}	12.88 ^{bcde}	19.50	3.31 ^{ab}	0.92 ^{ab}	0.66
0.40	0	67.77 ^{bc}	12.66 ^{cde}	19.41	3.38 ^{ab}	0.97 ^{ab}	0.59
0.45	0	66.36 ^{c}	11.54 ^{de}	19.89	3.59 ^{ab}	1.06 ^{ab}	0.67
0.20	5	71.35 ^{a}	17.59 ^{a}	18.52	2.90 ^{b}	0.73 ^{b}	0.65
0.25	5	71.16 ^{ab}	17.02 ^{ab}	17.99	2.98 ^{ab}	0.73 ^{b}	0.66
0.30	5	71.28 ^{a}	15.11 ^{abcd}	19.72	2.83 ^{b}	0.85 ^{b}	0.52
0.35	5	72.17 ^{a}	16.91 ^{abc}	20.67	2.75 ^{b}	0.79 ^{b}	0.55
0.40	5	71.20 ^{a}	14.86 ^{abcd}	21.21	3.23 ^{ab}	0.90 ^{b}	0.56
0.45	5	72.33 ^{a}	14.55 ^{abcd}	19.26	2.73 ^{b}	0.84 ^{b}	0.54
SEM[3]		0.37	0.41	0.32	0.08	0.03	0.02
P-value		<0.001	<0.001	0.629	0.002	<0.001	0.118
Source of variance							
NPP		0.276	0.387	0.249	0.524	0.277	0.270
1α-OH-D_3		<0.001	<0.001	0.433	<0.001	<0.001	0.009
NPP × 1α-OH-D_3		0.194	0.004	0.922	0.353	0.007	0.549

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

[1] Data are the means of five replicate cages consisting of one chick per cage.

[2] The meat yield (%) and organ relative weight (%) were calculated as the percentage of the live body weight of chicks.

[3] SEM = pooled standard error of the mean.

also found that the BWG of 1- to 16-day-old broilers was not affected by the NPP levels when dietary Ca level was 0.44%; however, bird BWG linearly increased as a function the NPP levels when dietary Ca level ranged from 0.85% to 1.04%. These data indicate that dietary NPP and Ca to NPP ratios affected the growth of broilers.

Vitamin D deficiency reduced BWG, FI, and FE, and caused severe mortality of broilers in groups 1 to 6. The addition of1α-OH-D_3 improved the growth performance of broilers in the present study, which is in agreement with the findings of Biehl & Baker (1997), Edwards (2002), Snow *et al*. (2004), and Han *et al*. (2009c, 2012).

Serum minerals

Dietary NPP increased serum P when 1α-OH-D_3 was not added to the diets in this study. This result agrees with the findings of Mohammed *et al*. (1991) and Han *et al*. (2009a), who found that the increase in dietary NPP level increased plasma P level, but reduced plasma Ca levels in 42-day-old broilers.

The addition of 1α-OH-D_3 did not significantly affect serum P levels in our study. Previous studies have shown that high levels of vitamin D_3 increased blood P levels (Mohammed *et al*., 1991; Rao *et al*., 2007). As the metabolite of vitamin D, 1α-OH-D_3 also has positive effects on serum P levels (Edwards, 2002; Han *et al*., 2009c; 2012). The compound 1α-OH-D_3 is rapidly metabolized to 1,25-$(OH)_2$-D_3 in chicks (Edelstein *et al*., 1978). Active 1,25-$(OH)_2$-D_3 increases ^{32}P uptake in isolated chick intestinal cells (Zhao & Nemere, 2002). Further research has shown that PKCα and PKCβ in protein kinase C (PKC) are involved in steroid-stimulated phosphate uptake in isolated intestinal epithelial cells from vitamin D-replete chicks (Tunsophon & Nemere, 2010).

Studies have shown that blood Ca levels of broiler chickens may be increased (Rao *et al*., 2007), not affected (Mohammed *et al*., 1991), or even reduced (Edwards, 2002) by dietary vitamin D_3. Han *et al*. (2009c) found that 1α-OH-D_3 decreases plasma Ca levels. The present study showed that 1α-OH-D_3 did not significantly affect serum Ca level. The different response of blood Ca to vitamin D among studies may be related to the dietary Ca to P levels and their ratios. Vitamin D_3 and its metabolites regulate the balance between Ca and P in animal blood.

Tibia mineralization

Low dietary P levels decreased tibia weight, length, ash, and Ca and P content in broilers (Mohammed *et al*., 1991). Similar results were found in the present

study. Another study showed that the dietary Ca to P ratio regulated bone mineralization and turnover by affecting the intestinal Ca and P transport in vitamin D receptor knockout mice (Masuyama *et al*., 2003). Onyango *et al*. (2003) reported that BWG, FI, FE, bone ash content, bone mineral content (BMC), and bone mineral density (BMD) of broilers increased linearly as dietary Ca and P levels increased. Rao *et al*. (2006) found that 42-day-old broilers presented the highest tibia breaking-strength and ash content when the ratio of dietary Ca to NPP was 2.0. The present study showed that chicks fed 1α-OH-D_3 presented the greatest tibia breaking-strength, weight, length, width, and ash weight values when dietary NPP level was 0.30% and the analyzed Ca to NPP ratio was 1.97.

Vitamin D deficiency impaired bone mineralization, and resulted in low tibia breaking-strength, weight, length, width, ash weight values and low ash, Ca and P percentages in broilers in groups 1 to 6 in the present study. High levels of vitamin D_3 increased bone growth and mineral deposition in broiler chickens (Whitehead *et al*., 2004; Kim *et al*., 2011). As a metabolite of vitamin D, 1α-OH-D_3 has higher bioavailability than vitamin D_3 (Edwards *et al*., 2002). The present study showed that 1α-OH-D_3 significantly improved tibia growth and mineralization in chicks. The positive effect of 1α-OH-D_3 on bone calcification was demonstrated by Biehl & Baker (1997), Edwards (2002), Driver (2004) and Han *et al*. (2009c; 2012). Addition of 1α-OH-D_3 stimulates the absorption and retention of Ca and P after it is converted into 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$-D_3). Ichikawa *et al*. (1995) found that the expression of vitamin D_3 25-hydroxylase mRNA was the highest in the liver, followed by the duodenum, calvaria, lung, kidney, skin, and long bone, and lowest in the spleen. Those authors found that 1α-OH-D_3 was converted into 1,25-$(OH)_2$-D_3 in the skeletal tissues of mouse by hydroxylation at the 25-position. Active 1,25-$(OH)_2$-D_3 increased the bone ash content of chicks (Edwards, 1989; Mitchell *et al*., 1997).

Carcass traits

Inadequate P levels reduce broiler carcass yield (Chen & Moran, 1995). However, in the present study, dietary NPP levels did not affect carcass yield or relative weights of the liver, heart, or kidney.

The carcass and breast meat yields of broilers increased when phytase and 1α-OH-D_3 were added to low P diets (Driver, 2004). Similar results were found in the present study. Vitamin D deficiency reduced muscle growth and meat production of broilers in groups 1 to 6. The addition of 1α-OH-D_3 increased carcass and breast yield by decreasing the relative weights of the organs in 21-day-old chicks.

CONCLUSION

Dietary NPP levels affected BWG, FI, FE, mortality, serum P level, and tibia mineralization parameters (except width). Vitamin D deficiency impaired broiler growth, bone quality, and meat yield, and increased mortality. The addition of 1α-OH-D_3 improved growth performance and tibia mineralization as well as carcass and breast yields, but decreased the relative weights of the liver, heart, and kidney. A significant interaction between NPP and 1α-OH-D_3 was observed for BWG, FE, mortality, serum Ca and Pi levels, tibia breaking-strength, ash weight, Ca content, and breast meat yield and heart relative weight. These data suggest that broilers fed with 5 μg of1α-OH-D_3 per kg of diet achieve optimal growth performance and tibia mineralization when dietary NPP was 0.30% and the analyzed Ca to NPP ratio was 1.97 in the present study.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (31101732), Innovation Scientists and Technicians Troop Construction Projects of Henan Province, and Shangqiu Normal University Foundation (2013GGJS10).

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