Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Ren, L; Zuo, J; Li, G; Zheng, L; Zhang, Z; Ye, H; Xia, W; Feng, D
Effects of the Combination of Non-Phytate Phosphorus, Phytase and 25-Hydroxycholecalciferol on the Performance and Meat Quality of Broiler Chickens
Revista Brasileira de Ciência Avícola, vol. 17, núm. 3, julio-septiembre, 2015, pp. 371-380
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jul - Sept 2015 / v.17 / n.3 / 371-380

http://dx.doi.org/10.1590/1516-635x1703371-380

Effects of the Combination of Non-Phytate Phosphorus, Phytase and 25-Hydroxycholecalciferol on the Performance and Meat Quality of Broiler Chickens

Author(s)

Ren L[I,II]
Zuo J[I]
Li G[I]
Zheng L[I]
Zhang Z[I]
Ye H[I]
Xia W[I]
Feng D[I]

[I] College of Animal Science and Technology, South China Agriculture University, Guangzhou 510642,China
[II] College of Agriculture, Guangdong Ocean University, Zhanjiang 524000, China

Project funded by Nature Science Foundation of China

Mail Address

Corresponding author e-mail address
Prof. D. Y. Feng
College of Animal Science and Technology, South China Agricultural University, Room 1406, Building D, Xingfayuan, South Habin Road, Xiashan District, Zhanjiang city Guangzhou, 510642, China.
Phone: (+86) 13531102669
E-mail: fengdy@scau.edu.cn
renlils2004@163.com

Keywords

Broiler, non-phytate phosphorus, performance, phytase, 25-hydroxycholecalciferol.

Submitted: December/2014
Approved: May/2015

ABSTRACT

This experiment was conducted to evaluate the combination effect of low dietary non-phytate phosphorus (NPP) concentrations, phytase (PHY) levels, and 25-hydroxycholecalciferol (25-OH-D_3) levels on the growth performance and meat quality of broilers. Two levels of NPP, two levels of PHY, and two levels of 25-OH-D_3 resulted in a 2×2×2 factorial arrangements, with eight treatments (TRT). The birds on TRT 1-4 were fed diet 1 (NRC NPP was reduced by 0.1) and the birds on TRT 5-8 were fed with diet 2 (NRC NPP was reduced by 0.2). Each diet was mixed with different levels PHY and 25-OH-D_3. Performance and meat quality parameters were measured. Results showed that during entire experiment the most advantageous effects were obtained with TRT 3 (NRC NPP reduced by 0.1 + 600 U/kg phytase + 34.5µg/kg 25-OH-D_3) and TRT 4 (NRC NPP reduced by 0.1 + 600 U/kg phytase + 69µg/kg 25-OH-D_3). The lowest body weight gain (BWG) and feed intake(FI) were observed with TRT 5 (NRC NPP reduced by 0.2 + 300 U/kg phytase + 34.5µg/kg 25-OH-D_3). Lowering NRC NPP by 0.1 to 0.2 significantly reduced weight gain (WG) ($p< 0.05$) and FI ($p< 0.05$) during the starter phase (ST), while during grower phase (GF) lowering NRC NPP by 0.1 to 0.2 did not affect WG ($p>0.05$) and produced small decrease in FI. BWG, FI and feed conversion ratio were not influenced ($p>0.05$) by different PHY or 25-OH-D_3 levels. In addition, the meat color, pH, and shear force were not affected by the different NPP, PHY or 25-OH-D_3 levels.

INTRODUCTION

Phosphorus (P) pollution from animal waste has become a major environmental concern in recent years. Reducing dietary phosphorus is an effective method to minimize phosphorus pollution (Angel *et al.*, 2006; Powell *et al.*, 2008; Rama Rao *et al.*, 2009; Pillai *et al.*, 2009). Recent studies showed that the National Research Council (NRC,1994) recommendations of non-phytate phosphorus (NPP) exceed the requirements of modern broiler chickens (Angel *et al.*, 2000a,b; Yan *et al.*, 2001 and Angel *et al.*, 2005). It should be an important economic and environmental concern to formulate diets to precisely meet the phosphorus requirements of poultry (Summers,1997).

Another method to reduce phosphorus pollution is to use feed additives, such as microbial phytase (PHY), to increase the utilization of dietary phosphorus. Microbial phytases have been widely used in animal diets to increase phytate P (PP) availability and to reduce inorganic P supplementation (Lalpanmawia *et al.*, 2014) and P excretion (Pillai *et al.*, 2009;Naves *et al.*, 2014). Furthermore, several studies indicated that vitamin D_3 metabolites, such as 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$-D_3), 25-hydroxycholecalciferol (25-OH-D_3), and 1α-hydroxycholecalciferol (1α-OH-D_3), can enhance phytate phosphorus utilization (Biehl *et al*., 1995; Janocha 2*et al*., 2009 and Tatara *et al*., 2011). The 25-OH-D_3 has been successfully developed into a new feed additives to the industry today. Previous reports (Angel *et al*., 2005; Driver *et al*., 2005; Coto *et al*., 2008; Liem *et al*., 2009) suggested that cholecalciferol analog might work either alone or in combination with phytase to markedly improve utilization of dietary P. However, the combination effect of lower dietary NPP levels, PHY levels and 25-OH-D_3 levels has not been evaluated.

Supplementation of vitamin D_3 affects meat quality (Montgomery *et al*., 2004; Moron *et al*., 2008). Han *et al*. (2009) reported that 1α-OH-D_3 increase lightness and yellowness of the breast and thigh meat whereas it decrease the shear force and water-holding capacity of the thigh meat.

Our objective was to evaluate the combination effect of lower dietary NPP levels, PHY levels and 25-OH-D_3 levels on the growth performance and meat quality of the broilers.

MATERIALS AND METHODS

Birds, Diets, Feeding, and Management

The procedures of this experiment were approved by the Animal Care and Use Committee of the South China Agriculture University.

The experiment was conducted in a broiler house for 42 d during the fall. Two thousand and four hundred one-day-old yellow-feathered male broilers were randomly assigned to eight treatments according to the randomized complete block design, with five replicates per treatment. Birds were weighed and allocated to treatment groups in order to obtain similar initial average body weights among the treatment groups. The pens were located in a well-ventilated open sided house with pine-shavings. Birds were submitted to 24 h of light and same management practices. Each pen (260×320cm) was considered a replicate. The temperature inside the pen was maintained at 33 ºC on day 1 and reduced 3 ºC each week until 24 ºC was reached.

Birds were fed the experimental diets (in mash form) formulated to supply the NRC (1994) recommendations for all nutrients except for NPP. A two-phase feeding program was applied: starter diets from 1 to 21d and grower diets from 22 to 42d. The compositions of the starter and grower experimental diets are shown in Table 1. Basal diets, based on corn and soybean meal, were formulated and the treatment diets were obtained by supplementing the basal diets with monocalcium

Table 1 – Composition and nutritional levels of phosphorus-deficient basal diets

	Starter		Grower	
Ingredient (%)	Diet 1	Diet 2	Diet 1	Diet 2
Ground yellow corn	59.64	60.18	63.20	63.43
Soybean meal (dehulled)	29.20	29.10	25.27	25.68
Puffing soybean	3.00	3.00	3.00	3.00
Corn Gluten Meal	3.00	3.00	1.31	1.00
Soybean oil	0.95	0.76	3.30	3.22
Dicalcium phosphate	1.29	0.68	1.02	0.42
Limestone	1.30	1.66	1.36	1.71
L-Lysine	0.38	0.38	0.27	0.27
Iodized sodium chloride	0.27	0.27	0.27	0.27
DL-methionine	0.21	0.21	0.23	0.23
Sodium Bicarbonate	0.12	0.12	0.12	0.12
Choline chloride	0.10	0.10	0.10	0.10
Threonine	0.04	0.04	0.05	0.05
Mineral-vitamin premix[1,2]	0.50	0.50	0.50	0.50
Calculated composition[3]				
ME(MJ/kg)	12.10	12.10	12.70	12.70
CP,%	20.50	20.50	18.00	18.00
Ca,%	0.90	0.90	0.85	0.85
Total P,%	0.58	0.48	0.50	0.40
Non-phytate P,%	0.35	0.25	0.30	0.20
Determined composition				
CP,%,	20.36	20.41	18.18	17.89
Ca,%	0.93	0.89	0.84	0.87
Total P,%	0.57	0.48	0.52	0.43
Non-phytate P,%	0.34	0.26	0.31	0.22

[1] Mineral-vitamin premix provided the following (per kilogram of diet): thiamin-mononitrate, 2.4 mg; nicotinic acid, 44mg; riboflavin, 4.4 mg; D-Ca pantothenate,12 mg; vitamin B_{12} (cobalamin), 12.0μg; pyridoxine-HCl, 2.7 mg; D-biotin,0.11 mg; folic acid,0.55 mg; vitamin A,10000IU; vitamin E,30IU; menadione sodium bisulfate complex, 3.34 mg; manganese, 120mg; iron,100 mg; zinc, 100 mg; copper, 8 mg; iodine,0.7 mg; selenium,0.3mg.

[2]Vitamin D_3 was removed from the vitamin mixture.

[3]Based on the tables of feed composition of NRC(1994).

phosphate, limestone, PHY (Natuphos, BASF), or 25-OH-D_3 (DSM Nutritional Products). One unit (U) of phytase activity is defined as the amount of enzyme that releases 1 mol of inorganic P from 1.5 mM Na phytate at pH 5.5 and 37 °C. The birds in treatments 1-4 were fed with phosphorus deficient diet 1 (NPP level below NRC by 0.1 in each period), while the birds in treatments 5-8 were fed with phosphorus deficient diet 2 (NPP level below NRC by 0.2 in each period). The starter diets was formulated to contain 0.35% or 0.25% NPP and the grower diets were formulated to contain 0.3% or 0.25% NPP. Each phosphorus-deficient diet contained no Vitamin D_3 and was mixed with 300U/kg or 600U/kg phytase and 34.5μg /kg or 69μg /kg 25-OH-D_3. Two levels of NPP concentrations (NRC NPP reduced by 0.1 or 0.2), two levels of PHY (300U/kg or 600U/kg), and two levels of 25-OH-D_3 (34.5μg /kg diet or 69μg /kg diet) resulted in a 2×2×2 factorial design (NPP ×PHY × 25-OH-D_3), with a total of 8 experimental treatments (TRT), with 5 replicates (pens) per treatment (60 birds /pen). Normal level and lower level of phytase and 25-OH-D_3 was included in the diets in attempt to allow for any potential additive or synergistic effects. Samples of all feeds were assayed for crude protein, calcium, and total phosphorus. Prior to mixing the diets, corn-25-OH-D_3 premixes consisting of corn and an appropriate level of 25-OH-D_3-premix were prepared and used in the basal diets.

The broiler house had a curtain with an ultraviolet inhibition during the study to prevent birds from being exposure to ultraviolet radiation. Feed and water were provided *ad libitum* during the experiment.

Mortality and leg abnormality of the birds were checked daily. Leg abnormality, including the deformed legs, valgus/varus, and tibial dyschondroplasia, was assessed by observing the walking ability (walking gait and speed) of the birds when moving spontaneously in the rearing environment. On d 21 and 42, two birds per pen were killed by cervical dislocation and their tibiae were removed to determine the presence or absence of physical leg abnormalities. Feed and broilers were weighed on d 21 and 42 for the determination of body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR).

Meat Quality Measurement

At d 42, two birds per pen were killed by cervical dislocation and their right breast and thigh muscle were removed for pH, color, and shear force determination. According to the method of Lu *et al*. (2006), at 45min postmortem, the pH of the breast and thigh muscles were tested with a pH meter (PHS-3C, Shanghai Precision and Scientific Instrument Co. Ltd., Shanghai, China). At 24 h postmortem, lightness (L*), redness (a*), and yellowness (b*) values of the muscles were measured using a WSC-S Chroma Meter (Shanghai Precision and Scientific Instrument Co. Ltd.). According to the procedure described by Honikel (1998), shear force of the breast and thigh raw meat was measured by using a C-LM3 Digital Meat Tenderness Meter (Northeast Agricultural University, Harbin, China).

Statistical Analysis

Replicate means served as the experimental unit for statistical analysis. Analysis of variance was performed on all data for the experiment using the GLM procedure of SAS (SAS Institute, 2001) appropriate for a randomized block design. Treatment means were compared using Duncan's multiple range test (Duncan, 1955). P-values lower than 0.05 or 0.01 indicate statistical significance.

RESULTS

Body weight, survival rate, and leg abnormality rate

The survival rate of broilers in all treatments was high, of more than 99%, during the experimental periods, although NRC NPP was reduced by 0.2. No leg abnormality was observed during starter phase and grower phase of the experiment (Table 2). The 21 d BW of chicks on TRT 1-4 (fed with 0.35% NPP diet) was higher than that of chicks on TRT 5-8 (fed

Table 2 – Effect of the combination of NPP, phytase and 25-OH-D3 on BW, mortality rate and leg abnormality rate of broilers

				1-21d			22-42d		
TRT	NPP[1]	Phytase[2]	25-OH-D_3[3]	21d BW[4]	Survival rate	Leg abnormality rate	42d BW	Survival rate	Leg abnormality rate
	(%)	(U/kg)	(μg/kg)	(g/bird)	(%)	(%)	(g/bird)	(%)	(%)
1	0.35	300	34.5	360.2±2.87^{b}	99.33	0	979.6±12.6^{abc}	100	0
2	0.35	300	69	358.6±4.67^{b}	99.33	0	989.4±9.2^{abc}	100	0
3	0.35	600	34.5	368.4±3.04^{a}	99.00	0	1007.8±9.02^{a}	100	0
4	0.35	600	69	371.8±2.63^{a}	99.67	0	999.2±17.0^{ab}	100	0
5	0.25	300	34.5	345.8±3.73^{c}	99.67	0	952.2±14.2^{c}	100	0
6	0.25	300	69	343.4±4.79^{c}	99.33	0	959.2±15.3^{bc}	99.64	0
7	0.25	600	34.5	338.4±2.06^{c}	100	0	962.4±10.3^{bc}	100	0
8	0.25	600	69	333.2±5.77^{c}	99.33	0	968.0±11.5^{abc}	100	0

$^{a-c}$ Means with no common superscripts in the same column significantly differ ($p \le 0.05$).

1. NPP =non-phytate P. From 1 to 21 d, NPP levels for broilers on treatment 1-4 and treatment 5-8 were 0.35% and 0.25%, respectively. From 22 to 42 d, NPP levels for broilers on treatment 1-4 and treatment 5-8 were 0.3% and 0.2% respectively.

2. Natuphos, BASF, 1 U of PHY is defined as the amount of enzyme required to liberate 1 mol of inorganic P from 1.5 mM Na phytate at pH 5.5 and 37°C

3. 25-OH-D_3 =25-hydroxycholecalciferol, Hy-D, DSM Vitamins, Shanghai,Ltd, 69 μg/kg diet=2,760 IU/kg diet.

4. Means represent five pens of sixty birds each, n=5, Average initial weight of the chicks was 35.2g.

with 0.25% NPP diet). The broilers on TRT 4 (fed with 0.35% NPP diet + 600U/kg phytase + 69µg/kg 25-OH-D_3) grew better than those on any other TRT during starter phase. The BW of chicks on TRT 4 was 11.2% higher ($p< 0.05$) than that of chicks on TRT 8 at d 21. The broilers on TRT 3 (fed with 0.30% NPP diet + 600 U/kg phytase + 34.5µg/kg 25-OH-D_3) grew better ($p< 0.05$) than those on any other TRT at d 42. The BW of chicks on TRT 3 was 5.84% higher ($p< 0.05$) than that of chicks on TRT 5 at d 42.

Starter Phase (Hatch to 21 d of Age) Performance

The greatest BWG was obtained by broilers on TRT 4 (fed with 0.35% NPP diet + 600U/kg phytase + 69µg/kg 25-OH-D_3) in the starter phase (Table 3). FCR of chicks on TRT 4 (fed with 0.35% NPP diet + 600 U/kg phytase + 69µg/kg 25-OH-D_3) and TRT 3 (fed with 0.35% NPP diet + 600 U/kg phytase + 34.5µg/kg 25-OH-D_3) was significantly lower ($p< 0.05$) than that on any other TRT. The BWG and feed intake of chicks on TRT 1-4 (fed with 0.35% NPP diet) was significantly higher ($p< 0.05$) by 7.87% and 6.36% than that of chicks on TRT 5-8 (fed with 0.25% NPP diet). FCR of chicks on TRT 1-4 (fed with 0.35% NPP diet) was significantly lower ($p< 0.05$) than that of chicks on TRT 5-8 (fed with 0.25% NPP diet). The BWG, feed intake, and FCR were not influenced by different phytase or 25-OH-D_3 levels.

Grower Phase (22 to 42 d of age) Performance

In the grower phase, the BWG and FCR of chicks were not affected when NRC NPP was reduced by 0.1

Table 3 – Effect of the combination of NPP, phytase and 25-OH-D_3 on the performance of starter broilers (1 to 21 days).

TRT	NPP[1] (%)	Phytase[2] (U/kg)	25-OH-D_3 [3] (µg/kg)	BWG[4] (g)	Feed intake (g)	FCR (g/g)
1	0.35	300	34.5	325^{ab}	547^{a}	1.68^{ab}
2	0.35	300	69	323^{b}	545^{a}	1.69^{a}
3	0.35	600	34.5	333^{ab}	557^{a}	1.67^{b}
4	0.35	600	69	336^{a}	558^{a}	1.66^{b}
5	0.25	300	34.5	310^{c}	527^{b}	1.70^{a}
6	0.25	300	69	308^{c}	524^{b}	1.70^{a}
7	0.25	600	34.5	303^{c}	515^{bc}	1.70^{a}
8	0.25	600	69	298^{c}	509^{c}	1.71^{a}
SEM[5]				4.12	5.06	0.011
P				< 0.0001	< 0.0001	0.0146
NPP effect						
0.35				329^{a}	552^{a}	1.68^{b}
0.25				305^{b}	519^{b}	1.70^{a}
Phytase effect						
300				317	536	1.70
600				318	535	1.69
25-OH-D_3 effect						
34.5				318	537	1.69
69				316	534	1.69
Source of Variation						
NPP				0.0001	0.0001	0.0087
Phytase				0.662	0.790	0.234
25-OH-D_3				0.560	0.427	0.458
NPP × Phytase				0.0019	0.0012	0.233
Phytase × 25-OH-D_3				0.942	0.976	0.958
NPP × 25-OH-D_3				0.467	0.536	0.566
NPP × Phytase × 25-OH-D_3				0.513	0.658	0.638

$^{a-c}$ Means with no common superscripts in the same column significantly differ ($p \leq 0.05$).

1. NPP = non-phytate P
2. Natuphos, BASF
3. 25-OH-D_3 =25-hydroxycholecalciferol, Hy-D, DSM Vitamins, shanghai, Ltd, 69µg/kg diet=2,760 IU/kg diet.
4. Means represent five pens of sixty birds each, n=5, Average initial weight of the chicks was 35.2g ; BWG = body weight gain; FCR =feed conversion ratio.
5. Weighted average of the SEM

to 0.2, but feed intake decreased by 2.67%(from 1461 g/bird/d to 1422 g/bird/d) when NRC NPP was lowered by 0.1 to 0.2 (Table 4). The BWG, feed intake, and FCR of chicks were not influenced by different phytase or 25-OH-D_3 levels.

Cumulative (Hatch to 42 d of age) Performance

The broilers on TRT 3 (NRC NPP reduced by 0.1 + 600U/kg phytase + 34.5μg/kg 25-OH-D_3) and TRT 4 (NRC NPP reduced by 0.1 + 600 U/kg phytase + 69μg/kg 25-OH-D_3) grew better and ate more than those on any other TRT (Table 5). The BWG and feed intake of chicks on TRT 3 and TRT 4 were 973g and 2038g, and 964g and 2021g, respectively. The lowest BWG (917g) and feed intake (1930g) were observed in TRT 5 (NRC NPP reduced by 0.2 + 300U/kg phytase + 34.5μg/kg 25-OH-D_3). The BWG was 6.11% and 5.13% higher ($p< 0.05$) in birds on TRT 3 and TRT 4, respectively, compared with those on TRT 5.

The BWG and feed intake of chicks on TRT 1-4 (NRC NPP reduced by 0.1 plus phytase and 25-OH-D_3) was significantly greater ($p< 0.05$) by 3.68% and 3.71%, respectively, compared with TRT 5-8 (NRC NPP reduced by 0.2 plus phytase and 25-OH-D_3) from 1 to 42 d. No significant differences ($p>0.05$) were found in FCR of chicks from 1 to 42 d in any of TRT.

Meat Quality

There was no significant difference in the lightness (L* value; p=0.583), redness (a* value; p=0.273), yellowness (b* value; p=0.296), pH (p=0.878), or shear

Table 4 – Effect of the combination of NPP, phytase and 25-OH-D_3 on the performance of grower broilers (22 to 42 days).

TRT	NPP[1] (%)	Phytase[2] (U/kg)	25-OH-D_3[3] (μg/kg)	BWG[4] (g)	Feed intake (g)	FCR (g/g)
1	0.3	300	34.5	606	1435[ab]	2.37
2	0.3	300	69	615	1463[ab]	2.38
3	0.3	600	34.5	625	1481[a]	2.37
4	0.3	600	69	613	1463[ab]	2.38
5	0.2	300	34.5	591	1403[b]	2.37
6	0.2	300	69	601	1423[ab]	2.37
7	0.2	600	34.5	608	1419[ab]	2.34
8	0.2	600	69	619	1441[ab]	2.33
SEM[5]				11	19	0.02
P				0.524	0.029	0.710
NPP effect						
0.35				615	1461[a]	2.38
0.25				605	1422[b]	2.35
Phytase effect						
300				603	1431	2.37
600				616	1451	2.36
25-OH-D_3 effect						
34.5				608	1435	2.36
69				612	1448	2.37
Source of Variation						
NPP				0.227	0.0074	0.174
Phytase				0.113	0.152	0.398
25-OH-D_3				0.591	0.350	0.792
NPP × Phytase				0.609	0.520	0.234
Phytase × 25-OH-D_3				0.504	0.432	0.837
NPP × 25-OH-D_3				0.456	0.555	0.578
NPP × Phytase × 25-OH-D_3				0.488	0.389	0.976

[a-c] Means with no common superscripts in the same column significantly differ ($p \le 0.05$).

1. NPP =non-phytate P
2. Natuphos, BASF
3. 25-OH-D_3 =25-hydroxycholecalciferol, Hy-D, DSM Vitamins,Shanghai,Ltd, 69μg /kg diet=2,760 IU/kg diet.
4. Means represent five pens of sixty birds each, n=5 ; BWG = body weight gain; FCR =feed conversion ratio.
5. Weighted average of the SEM

Table 5 – Effect of the combination of NPP, phytase and 25-OH-D_3 on the performance of 1- to 42-d-old broilers

TRT	NPP[1] (%)	Phytase[2] (U/kg)	25-OH-D_3[3] (µg/kg)	BWG[4] (g)	Feed intake (g)	FCR (g/g)
1	0.35/0.3	300	34.5	945[abc]	1982[abc]	2.10
2	0.35/0.3	300	69	954[abc]	2008[ab]	2.10
3	0.35/0.3	600	34.5	973[a]	2038[a]	2.09
4	0.35/0.3	600	69	964[ab]	2021[a]	2.10
5	0.25/0.2	300	34.5	917[c]	1930[c]	2.10
6	0.25/0.2	300	69	924[bc]	1947[bc]	2.11
7	0.25/0.2	600	34.5	927[bc]	1934[c]	2.09
8	0.25/0.2	600	69	933[bc]	1950[bc]	2.09
SEM[5]				12	22	0.013
P				0.012	0.017	0.516
NPP effect						
0.35				959[a]	2012[a]	2.10
0.25				925[b]	1940[b]	2.10
Phytase effect						
300				935	1967	2.10
600				949	1986	2.09
25-OH-D_3 effect						
34.5				941	1971	2.10
69				944	1982	2.10
Source of Variation						
NPP				< 0.001	< 0.001	0.877
Phytase				0.110	0.217	0.212
25-OH-D_3				0.763	0.509	0.625
NPP × Phytase				0.523	0.313	0.657
Phytase × 25-OH-D_3				0.555	0.475	0.986
NPP × 25-OH-D_3				0.655	0.697	0.799
NPP × Phytase × 25-OH-D_3				0.673	0.501	0.762

[a-c] Means with no common superscripts in the same column significantly differ ($p \leq 0.05$).

1. NPP =non-phytate P. From 1 to 21 d, NPP levels for broilers on treatment 1-4 and treatment 5-8 were 0.35% and 0.25% respectively. From 22 to 42 d, NPP levels for broilers on treatment 1-4 and treatment 5-8 were 0.3% and 0.2% respectively.
2. Natuphos, BASF
3. 25-OH-D_3 =25-hydroxycholecalciferol, Hy-D, DSM Vitamins,shanghai,Ltd, 69 µg /kg diet=2,760 IU/kg diet.
4. Means represent five pens of sixty birds each, n=5, Average initial weight of the chicks was 35.2g ; BWG = body weight gain; FCR =feed conversion ratio.
5. Weighted average of the SEM

force (p=0.142) of breast meat among TRT (Table 6). The lightness (L* value), redness (a* value), yellowness (b* value), pH, and shear force of breast meat were not affected by different NPP, phytase, or 25-OH-D_3 levels.

Results (Table 7) showed that no significant difference was observed in the lightness (L* value; p=0.296), redness (a* value; p=0.421), yellowness (b* value; P =0.205), pH (p=0.527), or shear force (p=0.454) of thigh meat among treatments. The lightness (L* value), redness (a* value), yellowness (b* value), pH, and shear force of thigh meat were not affected by different NPP, phytase or 25-OH-D_3 levels.

DISCUSSION

Growth Performance

The current NPP recommendation for broilers is 0.45% from 1 to 21 d of age and 0.35% from 22 to 42 d of age. In this study, the level of dietary NPP was 0.1 and 0.2 below the NRC (1994) when phytase and 25-OH-D_3 were added in each period to spare NPP. Under this situation, no leg abnormality and deleterious effects on survival rate were observed. This is consistent with the findings of Coto *et al*. (2008), who reported that there were no negative effects on performance of feeding a phosphorus-deficient diet with the addition of phytase and 25-OH-D_3. Similarly, Biehl *et al*. (1995) reported that the correct combination of phytase and $1\alpha\text{-OH-D}_3$ could substantially reduce the required level of inorganic P supplementation, and consequently reduce the P level in excreta. Yan *et al*. (2003) suggested that the NPP requirement for optimal tibia ash content was 0.15 ± 0.049% at 49 d, when diets were supplemented with phytase.

During the entire experiment, lowering NRC NPP by 0.1 to 0.2 with the addition of phytase and 25-

Table 6 – Effect of the combination of NPP, phytase and 25-OH-D_3 on breast meat quality of 42-d-old broilers

TRT	NPP[1] (%)	Phytase[2] (U/kg)	25-OH-D_3[3] (µg/kg)	L* value[4] (lightness)	a* value (redness)	b* value (yellowness)	pH	Shear force (N)
1	0.3	300	34.5	52.60	15.40	9.52	6.04	13.18
2	0.3	300	69	51.72	16.60	9.44	6.05	13.17
3	0.3	600	34.5	51.88	16.95	9.72	6.04	13.20
4	0.3	600	69	53.06	15.65	8.74	6.02	13.23
5	0.2	300	34.5	52.13	16.53	9.59	6.02	13.21
6	0.2	300	69	51.39	16.76	8.61	6.03	13.19
7	0.2	600	34.5	51.69	16.36	9.54	6.04	13.15
8	0.2	600	69	52.04	16.11	9.23	6.04	13.18
SEM[5]				1.18	0.83	0.42	0.05	0.08
P				0.583	0.273	0.296	0.878	0.142
NPP effect								
0.35				52.32	16.15	9.35	6.04	13.20
0.25				51.82	16.44	9.24	6.03	13.18
Phytase effect								
300				51.96	16.33	9.29	6.04	13.19
600				52.17	16.27	9.31	6.04	13.19
25-OH-D_3 effect								
34.5				52.08	16.31	9.59	6.04	13.19
69				52.06	16.28	9.01	6.04	13.19
Source of Variation								
NPP				0.210	0.402	0.518	0.553	0.571
Phytase				0.939	0.871	0.669	0.572	0.219
25-OH-D_3				0.651	0.927	0.146	0.530	0.326
NPP × Phytase				0.806	0.299	0.359	0.774	0.716
Phytase × 25-OH-D_3				0.084	0.134	0.718	0.340	0.079
NPP × 25-OH-D_3				0.896	0.950	0.717	0.384	0.248
NPP × Phytase × 25-OH-D_3				0.408	0.147	0.833	0.676	0.187

[a-c] Means with no common superscripts in the same column significantly differ ($p \leq 0.05$).

1. NPP =non-phytate P
2. Natuphos, BASF
3. 25-OH-D_3 =25-hydroxycholecalciferol, Hy-D, DSM Vitamins,shanghai,Ltd, 69µg/kg diet=2,760 IU/kg diet.
4. Means represent five pens of sixty birds each,n =5.
5. Weighted average of the SEM

OH-D_3 reduced BWG and feed intake of chicks. This result is consistent with previous reports (Angel *et al*., 2000 a,b; Karimi *et al*., 2013) indicating that lowering dietary NPP decreased the BWG and feed intake.

In this experiment, BWG and feed intake of starter chicks on TRT 1-4 (fed with 0.35% NPP diet) was higher by 7.87% and 6.36% than that of chicks on TRT 5-8 (fed with 0.25% NPP diet), while the BWG and FCR of grower chicks on TRT 1-4 were similar to that on TRT 5-8. This indicated that reducing NRC NPP by 0.1 to 0.2 has a stronger effect on broiler performance more during the starter phase than during the grower phase. This result is consistent with the findings of Yan *et al*. (2005), who studied the ability of broiler chickens to adapt to early moderate P deficiencies. Yan *et al*. (2005) found that broiler chickens fed a low NPP diet weighed less ($p< 0.05$) than those fed a control diet at 18 d; however, by 23 d, these broilers had caught up with the control birds, and no BW differences ($p> 0.05$) were observed on d 28 and 32. Hence, the author inferred that modern broilers have capacity to adapt when exposed to dietary P restrictions.

The information obtained in this study indicated that BWG, feed intake, and FCR were not influenced by the different phytase or 25-OH-D_3 levels. This corroborates the findings of Ana *et al*. (2013), who reported that the supplementation of the basal diet with 25-OH-D_3 had no effect on BW. Additionally, Angel & Mitchell (2006) reported that feeding diets low in phosphorus together with phytase and 25-OH-D_3 did not affect broiler performance. However, our results are contrary to those reported by Keshavarz (2003), Snow *et al*. (2004), and Emami *et al*. (2013), indicating that phytase addition increased ADG and ADFI in diets deficient in NPP.

Table 7 – Effect of the combination of NPP, phytase and 25-OH-D3 on thigh meat quality of 42-d-old broilers

TRT	NPP[1] (%)	Phytase[2] (U/kg)	25-OH-D_3[3] (μg/kg)	L* value[4] (lightness)	a* value (redness)	b* value (yellowness)	pH	Shear force (N)
1	0.3	300	34.5	49.86	19.39	6.65	6.39	17.17
2	0.3	300	69	49.32	18.83	6.53	6.45	17.06
3	0.3	600	34.5	50.22	19.36	6.47	6.44	16.87
4	0.3	600	69	49.36	19.04	6.49	6.43	16.99
5	0.2	300	34.5	51.07	18.45	6.68	6.41	16.93
6	0.2	300	69	49.85	18.90	6.57	6.41	17.09
7	0.2	600	34.5	50.57	18.54	6.43	6.38	16.89
8	0.2	600	69	49.67	19.03	6.75	6.43	16.98
SEM[5]				0.94	0.53	0.30	0.06	0.25
P				0.296	0.421	0.205	0.527	0.454
NPP effect								
0.3				49.69	19.16	6.53	6.43	17.02
0.2				50.29	18.73	6.61	6.41	16.97
Phytase effect								
300				50.03	18. 90	6.61	6.42	17.06
600				49.95	18.99	6.53	6.42	16.93
25-OH-D_3 effect								
34.5				50.44	18.93	6.56	6.41	16.97
69				49.55	18.95	6.59	6.43	17.03
Source of Variation								
NPP				0.208	0.278	0.105	0.282	0.609
Phytase				0.541	0.672	0.083	0.647	0.359
25-OH-D_3				0.093	0.948	0.098	0.207	0.994
NPP × Phytase				0.972	0.966	0.122	0.609	0.506
Phytase × 25-OH-D_3				0.629	0.764	0.066	0.813	0.783
NPP × 25-OH-D_3				0.896	0.071	0.673	0.845	0.859
NPP × Phytase × 25-OH-D_3				0.434	0.339	0.297	0.101	0.177

[a-c] Means with no common superscripts in the same column significantly differ ($p \leq 0.05$).

1. NPP =non-phytate P
2. Natuphos, BASF
3. 25-OH-D_3 =25-hydroxycholecalciferol, Hy-D, DSM Vitamins, shanghai, Ltd, 69μg/kg diet=2,760 IU/kg diet.
4. Means represent five pens of sixty birds each,n =5.
5. Weighted average of the SEM

Meat Quality

There are no reports on the combined effects of NPP, PHY, and 25-OH-D_3 on the meat quality of broilers. More studies previously reported that the supplementation with vitamin D_3 before slaughter to cattle improved meat color (Wilborn *et al*., 2004;Lobo *et al*., 2012), decreased shear force (Tipton *et al*., 2007; Moron *et al*., 2008). Another study indicated that supplementation with vitamin D_3 improved steak tenderness by affecting muscle Ca concentrations, μ-calpain activities, and muscle proteolysis (Montgomery *et al*., 2004).

Some paper reported the effect of 25-OH-D_3 on meat quality. Lawrence *et al*. (2006) and Carnagey *et al*. (2008) reported that the supply of an oral bolus of 25-OH-D_3 to cows before slaughter influenced some muscle characteristics known to enhance beef tenderness, despite no effect on tenderness was not observed. Cho *et al*. (2006) studied the effects of 25-hydroxy-vitamin D_3 (25-OH-D_3) on carcass traits and beef tenderness and observed that, although Ca concentrations of the lean muscle numerically increased in response to 25-OH-D_3 supplementation, no statistical differences in Warner-Bratzler shear force or sensory traits were detected. In the present experiment, lightness (L* value), redness (a* value), yellowness (b* value), pH, and shear force of thigh meat were not affected by different NPP, phytase, or 25-OH-D_3 levels. This result is consistent with those mentioned above.

The different effects of 25-OH-D_3 and vitamin D_3 on meat quality are probably due differences in supplementation route, supplemental dose, animal type, etc. In most studies, vitamin D_3 was orally supplemented before slaughter and the experimental animals were cattle, whereas in the present study, 25-OH-D_3 was supplemented in the diet from the beginning to the end of the experiment and the experimental animals were broilers. Hence, further investigations on these factors are needed.

CONCLUSION

1. During the entire experimental period, lowering NRC NPP by 0.2 and adding phytase and 25-OH-D_3 did not affect the survival rate of broilers or caused leg abnormalities.

2. In the starter phase, reducing NRC NPP by 0.1 to 0.2 with the addition of phytase and 25-OH-D_3 reduced broiler BWG and feed intake. In the grower phase, reducing NRC NPP by 0.1 to 0.2 and adding phytase and 25-OH-D_3 to the diet did not affect BWG or FCR, produced a small decrease in feed intake of broilers.

3. Different levels of NPP, phytase, or 25-OH-D_3 did not have significant effects on meat color, pH, or shear force.

ACKNOWLEDGEMENTS

This research was supported by Nature Science Foundation of China (Beijing,China; No.30901033).

REFERENCES

Angel R, Applegate TJ, Christman M. Effect of dietary non-phytate phosphorus (NPP) on performance and bone measurements in broilers fed on a four-phase feeding system [abstract]. Poultry Science 2000a;79(Suppl1): 21-22.

Angel R, Applegate TJ, Christman M, Mitchell AD. Effect of dietary non-phytate phosphorus (NPP) level on broiler performance and bone measurements in the starter and grower phase [abstract]. Poultry Science 2000b;79(Suppl 1):22.

Angel R, Saylor WW, Dhandu AS, Powers W, Applegate TJ. Effects of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on performance of broiler chickens grown in floor pens. Poultry Science 2005; 84:1031-1044.

Angel R, Saylor WW, Mitchell AD, Powers W, Applegate TJ. Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. Poultry Science 2006;85:1200-1211.

AOAC - Association of Official Analytical Chemists. Official methods of analysis of the association of official analytical chemists. Arlington; 1990.

Biehl RR, Baker DH, Deluca HF. 1-Hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc, and manganese utilization in chicks fed soy-based diets. Journal of Nutrition 1995;125:2407-2416.

Carnagey KM, Huff-Lonergan EJ, Lonergan SM, Trenkle A, Horst RL, Beitz DC. Use of 25-hydroxyvitamin D3 and dietary calcium to improve tenderness of beef from the round of beef cows. Journal of Animal Science 2008;86:1637- 1648.

Cho YM, Choi H, Hwang IH, Kim YK, Myung KH. Effects of 25-hydroxyvitamin D3 and manipulated dietary cation-anion difference on the tenderness of beef from cull native Korean cows. Journal of Animal Science,2006;84:1481-1488.

Coto C, Yan F, Cerrate S, Wang Z, Sacakli P. Effects of dietary levels of calcium and non-phytate phosphorus in broiler starter diets on live performance, bone development and growth plate conditions in male chicks fed a wheat based diet. International Journal of Poultry Science 2008;2:101-109.

Driver JP, Pesti GM, Bakalli RI, Edwards HM Jr. Phytase and 1α-Hydroxycholecalciferol supplementation of broiler chickens during the starting and growing/finishing phases. Poultry Science 2005;84:1616-1628.

Duncan DB. Multiple range and multiple-F tests. Biometrics 1955;11:1-42.

Emami NK, Naeini SZ, Ruiz-Feria CA. Growth performance, digestibility, immune response and intestinal morphology of male broilers fed phosphorus-deficient diets supplemented with microbial phytase and organic acids. Livestock Science 2013;157:506-513.

Garcia FQM, Murakami AE, Duarte CAD, Rojas ICO, Picoli KP, Pugotti MM. Use of vitamin D3 and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. Asian- Australasian Journal of Animal Science 2013;26:408-415. (Corrigir no texto de Ana et al. 2013 na p. 11 para Garcia et al. 2013)

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

Honikel KO. Reference methods for the assessment of physical characteristics of meat. Meat Science 1998;49: 447-457.

Janocha A, Osek M, Klocek B. Effect of adding 25-hydroxycholecalciferol in plant diets with and without fish meal on rearing results and bones of broiler chickens. Annals of Animal Science 2009;9:415-423.

Karimi A, Coto C, Mussini F, Goodgame S, Lu C, Yuan J, Bedford MR, Waldroup PW. Interactions between phytase and xylanase enzymes in male broiler chicks fed phosphorus-deficient diets from 1 to 18 days of age. Poultry Science 2013;92:1818-1823.

Keshavarz K. A Comparison between Cholecalciferol and 25-OH-cholecalciferol on performance and eggshell quality of hens fed different levels of calcium and phosphorus. Poultry Science 2003;82:1415-1422.

Lalpanmawia H, Elangovan AV, Sridhar M, Shet D, Ajith S, Pal DT. Efficacy of phytase on growth performance, nutrient utilization and bone mineralization in broiler chicken. Animal Feed Science and Technology 2014;6(192):81-89.

Lawrence RW, Doyle J , Elliott R, Loxton I, McMeniman JP, Norton BW, Reid DJ, Tume RW. The efficacy of a vitamin D3 metabolite for improving the myofibrillar tenderness of meat from Bos indicus cattle. Meat Science 2006;72:69-78.

Liem A,Pesti GM, Atencio A, Edwards HM. Experimental approach to optimize phytate phosphorus utilization by broiler chickens by addition of supplements. Poultry Science 2009;88:1655-1665.

Lobo AR , Delgado EF, Mourao GB ,Pedreira, ACMS, Berndt A, Demarchi JJAA. Interaction of dietary vitamin D-3 and sunlight exposure on B. indicus cattle: Animal performance, carcass traits, and meat quality. Livestock Science 2012;145:196-204.

Lu L, Ji C, Luo XG, Liu B, Yu SX. The effect of supplemental manganese in broiler diets on abdominal fat deposition and meat quality. Animal Feed Science and Technology 2006;129: 49-59.

Montgomery JL, King MB, Gentry JG, Barham AR, Barham BL, Hilton GG, Blanton Jr JR, Horst RL, Galyean ML, Morrow Jr KJ, Wester DB, Miller MF. Supplemental vitamin D3 concentration and biological type of steers. II. Tenderness, quality, and residues of beef. Journal of Animal Science 2004;82:2092-2104.

Moron FO, Araujo FO, Pietrosemoli S , Gutierrez J ,Machado L, Martinez J. Effect of Vitamin D3 Supplementation on Meat Quality in Grazing Bovine Crossbred. Revista Cientifica-Facultad de Ciencias Veterinarias 2008;18:692-698.

National Research Council. Nutrient requirements of Poultry. 9th rev.ed. Washington: National Academy Press; 1994.

Naves LD, Rodrigues PB, Bertechini AG, Correa AD, Oliveira DH, Oliveira EC, Duarte WF, Cunha MRR. Comparison of methodologies to quantify phytate phosphorus in diets containing phytase and excreta from broilers. Asian-Australasian Journal of Animal Sciences 2014,27:1003-1012.

Pillai UP, Manoharan V, Lisle A, Li X, Bryden W. Phytase supplemented poultry diets affect soluble phosphorus and nitrogen in manure and manure-amended soil. Journal of Environmental Quality 2009;38:1700-1708.

Powell S, Johnston S, Gaston L, Southern LL. The effect of dietary phosphorus level and phytase supplementation on growth performance, bone-breaking strength, and litter phosphorus concentration in broilers. Poultry Science 2008;87:949-957.

Rama Rao SV, Raju MVLN, Panda AK, Shyam Sunder G, Sharma RP. 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. British Poultry Science 2009;50:528-535.

SAS Institute. SAS user`s guide. Version 8 ed. Cary; 2001.

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

Summers JD. Precision phosphorus nutrition. Journal of Applied Poultry Science 1997;6:495–500.

Tatara MR, Krupski W, Jankowski M, Zduńczykd Z, Jankowskic J, Studzińskie T. Effects of dietary calcium content and vitamin D source on skeletal properties in growing turkeys. British Poultry Science 2011;52:718-729.

Tipton NC, King DA, Paschal JC, Hale DS, Savell JW. Effects of oral vitamin D3 supplementation and supplement withdrawal on the accumulation of magnesium, calcium, and vitamin D in the serum, liver, and muscle tissue and subsequent carcass and meat quality of Bos indicus influenced cattle. Meat Science 2007;75:150-158.

Wilborn BS, Kerth CR, Owsley WF, Jones WR, Frobish LT. Improving pork quality by feeding supranutritional concentrations of vitamin D3. Journal of Animal Science, 2004;82: 218-224.

Yan F, Kersey JH, Waldroup PW. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poultry Science 2001;80: 455-459.

Yan F, Kersey JH, Fritts CA, Waldroup PW. Phosphorus requirements of broiler chicks six to nine weeks of age as influenced by phytase supplementation. Poultry Science 2003;82: 294-300.

Yan F, Angel R, Ashwell C, Mitchell A, Christman M. Evaluation of the broiler's ability to adapt to an early moderate deficiency of phosphorus and calcium. Poultry Science 2005;84: 1232 -1241.