The Journal of Poultry Science, 45: 25–30, 2008
http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.45.25

Effect of Surfeit Concentrations of Vitamin D_3 on Performance, Bone Mineralization and Mineral Retention in Commercial Broiler Chicks

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An experiment was conducted to see the effect of higher concentrations of vitamin D_3 in diets containing sub optimal levels of calcium (Ca) and non-phytate phosphorus (NPP) in broiler chickens. Maize-soybean meal based starter and finisher reference diets (RD) prepared to contain 1.0% and 0.9% Ca, 0.45 and 0.35% NPP, respectively. The RD contained 1200 ICU D_3/kg. Another basal diet (BD) was prepared to contain 0.4% Ca and 0.2% NPP. The BD was supplemented with D_3 at 4 different concentrations such as 1200, 2400, 4800 and 9600 ICU/kg. Each diet was fed to 11 replicates of 5 chicks each during experimental period of 0 to 6 weeks of age. The body weight gain decreased significantly with reduction in levels of Ca and NPP in diet compared to those fed RD. Supplementation of vitamin D_3 to the low Ca and NPP diet, though improved the weight gain significantly but not at per with RD. The feed efficiency was not affected by concentrations of Ca, NPP and D_3 in diet at 36 d of age. The relative weight of tibia, tibia breaking strength and tibia ash content decreased significantly with reduction in Ca and NPP levels in diet. Supplemental D_3 replenished the tibia ash content and enhanced tibia weight and strength. Supplemental D_3 non-linearly increased the serum Ca, P, acid and alkaline phosphatase contents in serum. Concentrations of Ca, P, Fe, Zn, Mn and Cu in excreta decrease non-linearly with increase vitamin D_3 concentration in BD. It is concluded that higher levels of D_3 supplementation to low Ca and NPP diet improved the performance partially but not at per with diet containing the recommended concentrations of Ca and NPP in broiler diet.

Key words: bone mineralization, broiler chicks, calcium, growth, phosphorus, vitamin D_3

J. Poult. Sci., 45: 25–30, 2008

Introduction

Phosphorus (P) from plant feed ingredients is not completely available for chicken due to its complex bond with inositol and divalent cations like Ca, Mg, Cu, Zn, Fe, Mn, etc. The complex is called as phytate. Therefore, inverse relation exists between dietary phytate and the solubility/availability of these minerals to poultry (Nwokola and Bragg, 1977; Eardman, 1979; Kornegay *et al.,* 1996). Use of plant feed ingredients in chicken diet, result in excretion of P and other minerals bound with phytate in considerable quantities and cause environmental pollution. Dietary calcium (Ca) and P at their recommended concentrations is known to reduce the utilization of PP (Ballam *et al.,* 1985; Schoner *et al.,* 1993; Qian *et al.,* 1994).

The evidence of increased PP availability from plant feed ingredients at their sub-optimal concentrations of P (Davies *et al.,* 1970; Onyango *et al.,* 2006), existence of gut mucosal phytase activity (Bitar and Reinhold, 1972; Onyango *et al.,* 2001) and its enhancement with D_3 supplementation (Onyango et al., 2006) reduced cost on supplemental P (DCP) and to minimized mineral excretion (Rama Rao *et al.,* 2006b; 2007). Results of experiments conducted at our laboratory suggested the possibility of reducing dietary Ca and non-phytate P (NPP) to 0.6 and 0.3%, respectively at 1200 icu D_3/kg (Rama Rao *et al.,* 2006a) and further reduction in the mineral concentration to 0.5 and 0.25%, respectively with increase in D_3 concentration to 3600 icu/kg diet (Rama Rao *et al.,* 2006 b). Unpublished data from our laboratory indicated linear improvement in performance and bone mineralization with increase in concentration of D_3 from 300 to 1200 ICU/kg in diet containing 0.4% Ca and 0.2% NPP. This indicate the possibility of further improvement in these parameters at higher levels ($>$1200 ICU/kg) of D_3 in diet containing 0.4% Ca and 0.2% NPP. Therefore in the present study, an experiment was carried out to investigate whether the dietary Ca and NPP could be reduced further than 0.5 and 0.25%, respectively, by supplementing higher concentrations of D_3.

Received: April 5, 2007, Accepted: September 10, 2007
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Materials and Methods

Birds and management

Day old Cobb female broiler chicks ($n=275$) were randomly distributed at the rate of five birds per pen into 55 - raised wire floor stainless steel battery brooders (610 mm×762 mm×457 mm), which were kept in open sided poultry house. Ground maize was fed on d 1 and each experimental diet was allotted at random to 11 pens and fed *ad libitum* from 2 to 36 d of age. The brooder temperature was regulated depending on the age of the bird and environmental temperature (22 to 38℃). Uniform management and vaccination schedules were followed for all the birds.

Diets

Feed ingredients were analyzed for Ca and total P (TP) (AOAC, 1990) and PP (Haugh and Lantzsch, 1983). The NPP content in maize and soybean meal was calculated as the difference between TP and PP. Maize-soybean meal based broiler starter and finisher reference diets (RD) were prepared to contain 1.0 and 0.9% Ca, 0.45 and 0.35% NPP, respectively (Table 1). The RD contained 1200 ICU D_3/kg. Low Ca and NPP basal diets (BD) (0.4 and 0.2%, respectively) were prepared with similar energy and protein concentrations for starter and finisher phases. The levels of oyster shell powder, DCP, maize and vegetable oil were adjusted to obtain the desired concentrations of Ca, NPP, and other nutrients in the BD. The BD was supplemented with crystalline D_3 (Duphar Interfran, Mumbai, India) to arrive four gradient concentrations of D_3 i.e. 1200, 2400, 4800 and 9600 ICU/kg. Crystalline D_3 was mixed with the BD after dissolving in 200 m*l* propylene glycol (5:95 ethanol and propylene glycol, respectively V/V) as described in our earlier report (Rama Rao *et al.,* 2006b).

Table 1. Composition (%) of the reference and the basal diets fed during starter and finisher phases

Ingredient	Starter		Finisher	
	Reference	Basal	Reference	Basal
Yellow maize	52.53	57.42	57.29	61.09
Soybean meal	38.94	37.94	32.58	31.80
Common salt	0.40	0.40	0.40	0.40
Dicalcium phosphate	2.071	0.543	1.51	0.588
Oyster shell grit	1.457	0.687	1.53	0.66
DL methionine	0.198	0.195	0.131	0.129
L-lysine HCl	0.0	0.018	0.0	0.013
AB2 D_3K[2]	0.010	0.010	0.010	0.010
B complex Vitamins[2]	0.020	0.020	0.020	0.020
Choline chloride, 50%	0.13	0.13	0.10	0.10
Toxin binder	0.20	0.20	0.20	0.20
Trace mineral premix[1]	0.10	0.10	0.10	0.10
Anti biotic[3]	0.05	0.05	0.05	0.05
Coccidiostat[4]	0.05	0.05	0.05	0.05
Anti oxidant	0.02	0.02	0.02	0.02
Vegetable oil	3.81	2.196	5.97	4.72
Nutrient composition				
ME, kcal/kg*	3000	3000	3200	3200
Crude protein**	22.5	22.5	20.0	20.0
Lysine*	1.29	1.29	1.113	1.113
Methionine*	0.55	0.55	0.45	0.45
Calcium**	1.00	0.40	0.90	0.40
NPP**	0.45	0.20	0.35	0.20

[1] Trace minerals contained (mg/kg) manganese, 60 mg; zinc, 35 mg; iron, 30 mg and copper, 5 mg.
[2] Vitamin premix contained (units/kg): Vitamin A, 12375 IU; Ribiflavin, 7.5 mg; Cholecalciferol, 1800 ICU; Vitamin K, 1.5 mg; Thiamin, 1.2 mg; Pyridoxine, 2.4 mg; Cyanocobalamine, 12 mcg; Vitamin E, 12 mg; Pantothenic acid, 12 mg; Niacin, 18 mg.
[3] Antibiotic (Furazolidone 20% w/w).
[4] Coccidiostat (monensin sodium 10%).
* calculated; ** analysed.

Traits measured

Body weight gain and feed intake were recorded at 12, 24 and 36 d of age and leg abnormality score (LAS) were recorded at 36 d of age. The degree of leg abnormality was measured (Watson *et al.* 1970) by physical examination of hoc joint for swelling and bending of the joint and position of *Achilles* tendon (Rama Rao *et al.,* 2006). About 3 m*l* blood was collected from brachial vein from 6 birds per treatment at 36 d of age and analyzed for Ca (AOAC, 1990) and inorganic P (Fiske and Subba Row, 1925) contents and activities of acid phosphatase and alkaline phosphatase (73001 and 72011, respectively, Qualigens Fine Chemicals, Mumbai, India).

One bird representing the mean body weight of replicate (eleven birds per treatment) were selected and killed by cervical dislocation at 37 d of age. The right tibia was dissected from the carcass of the each bird and the soft tissue was freed from the bone. Fat from dried bone samples (100℃/3 h) were extracted by soaking them in petroleum ether for 48 h. The weight of the dried (70 ℃/24 h) bone was recorded. The breaking strength of the bone was measured using universal testing machine (EZ Test, Shimadzu, Japan). The bones were ashed independently at 600±20℃/6 h in microwave muffle furnace (BR 600521, Phoenix CEM Corp., NC). Liver and pancreas were collected from four birds per treatment to measure concentrations of Fe, Mn, Zn and Cu (AS 100, Perkin Elmer, an atomic absorption spectrophotometer operation manual).

About 100 g fresh excreta sample from six pens per treatment was collected at 10.0 A.M on 36 d of age from the total excreta voided by 5 birds in a replicate in 24 h period and dried separately. Excreta samples were digested to colorless solution, initially with concentrated nitric acid at 120℃ followed by perchloric acid at 200℃. The digested solution was analysed for Mn, Fe, Zn and Cu with an atomic absorption spectrophotometer (Perkin Elmer, Analyst 100, Operation Manual). The excreta samples were ashed at 600℃/2h to estimate the concentrations of Ca and TP (AOAC, 1990).

Statistical analysis

The data were subjected to one-way analysis of variance (Snedecor and Cochran, 1980) to compare the affects of supplementing D_3 to basal diet in relation to the RD. The relation between concentration of supplemental D_3 and various performance parameters were tested through regression equation.

Results and Discussion

Performance and bone mineralisation

The body weight gain was significantly affected by change in concentrations of Ca, NPP and vitamin D_3 at 12, 24 and 36 d of age (Table 2). At all the ages tested, the weight gain decreased significantly with reduction in levels of Ca and NPP in diet (BD) compared to those fed the reference diet. At 12 d of age, the body weight gain increased non-linearly with D_3 supplementation and the weight gain in groups fed $D_3 \geq 4800$ ICU/kg was similar to the in groups fed the RD. Similarly at 24 and 36 d of age, D_3 supplementation non-linearly ($P < 0.01$) increased the growth compared to the BD. But the weight gain in the latter group was not similar ($P > 0.05$) to those fed the RD. The feed efficiency at 12 and 36 d of age was not affected by the concentrations of Ca, NPP and D_3 in diet. At 24 d of age, the feed efficiency decreased significantly ($P < 0.05$) in birds fed BD. The feed efficiency improved non-linearly with increase in D_3. At 4800 ICU/kg the feed efficiency was similar to the RD fed broilers. Though, significant improvement in weight gain was observed with supplementation of higher concentrations of D_3 to diet containing 0.4% Ca and 0.2% NPP, the improvement is not similar to those fed the RD. Significant improvement in weight gain in birds fed BD supplemented with D_3 might be due to increased availability/utilization of PP and Ca at higher concentration of D_3 supplementation. Increased activity of intestinal phytase (Pointillart *et al.,* 1985; Schoner *et al.,* 1993; Onyango *et al.,* 2006) and production of Ca binding protein (Underwood, 1981) with D_3 supplementation might have respectively increased utilization of PP and Ca. Increased utilization of PP and or Ca with increase in concentrations of D_3 in chicken diet also reported in literature (Shafey *et al.,* 1990; Mohammed *et al.,* 1991; Edwards, 1993; Biehl *et al.,* 1995; Biehl and Baker, 1997; Qian *et al.,* 1997).

The relative weight of tibia, tibia breaking strength and tibia ash content decreased significantly with reduction in Ca and NPP levels in diet (Table 2). Supplementation of D_3 to BD, non-linearly improved tibia weight and tibia ash contents. The tibia breaking strength increased linearly due to supplementation of D_3 to BD. Similarly, supplementing the BD with 9600 ICU D_3/kg significantly increased the bone breaking strength compared to those fed the BD. However, the bone breaking strength in the later group was lower than the broilers fed the RD. The bone ash content increased with D_3 supplementation at >2400 ICU/kg BD similar to those fed the RD.

Progressive increase in response of majority of the parameters studied with increase in D_3 concentration might be due to higher requirement of the vitamin at lower Ca and NPP (BD) compared to those fed the recommended concentrations of Ca and NPP. It is well established that the requirement of D_3 is higher at sub-optimal concentrations of Ca and P in diet compared to optimum concentrations of these minerals. A level of 400 ICU D_3/kg diet was reported adequate at recommended concentrations of P or Ca in broiler diet (Mc Naughton *et al.,* 1977; Lofton and Soares, 1986; Baker *et al.,* 1998). But at sub-optimal concentrations of Ca and P in diet, birds required higher D_3 concentration in diet compared to those fed adequate concentrations of these minerals (Waldroup *et al.,* 1965).

Serum biochemical profile

Dietary variation in levels of Ca, NPP and D_3 in broiler diet significantly influenced the concentrations of Ca and iP, and the activities of acid and alkaline phosphatases in serum (Table 3). The Ca and iP concentrations in serum decreased significantly ($P < 0.01$) by reducing the levels of these minerals in the BD. However, supplementation of D_3 non-linearly increased the concentration of these minerals in serum. The serum Ca levels increased significantly with each incremental level, while the P level reached to

Table 2. Body weight gain, feed/gain and bone mineralisation parameters in broilers fed diets containing sub-optimal levels of Ca and NPP and higher concentrations of cholecalciferol

Ca: NPP, %	CC ICU/kg	Body weight gain, g			Feed/gain			Tibia		
		12 d	24 d	36 d	12 d	24 d	36 d	Weight, g/kg	Strength, N	Ash, %
1.0:0.45	1200	267.5[a]	941.6[a]	1656[a]	1.352	1.477[b]	1.819	3.213[a]	72.2[a]	49.9[a]
0.4:0.2	1200	253.7[ab]	737.0[d]	1380[c]	1.369	1.577[a]	1.768	2.786[b]	36.9[c]	36.5[b]
0.4:0.2	2400	249.7[ab]	808.4[c]	1493[b]	1.310	1.560[ab]	1.784	2.760[b]	47.6[bc]	45.3[a]
0.4:0.2	4800	234.9[b]	857.7[bc]	1505[b]	1.374	1.474[b]	1.788	2.458[b]	41.8[bc]	45.0[a]
0.4:0.2	9600	233.7[b]	896.3[b]	1523[b]	1.441	1.494[b]	1.797	2.646[b]	54.9[b]	47.9[a]
P		0.017	0.001	0.001	0.374	0.026	NS	0.01	0.01	0.01
N		11	11	11	11	11	11	11	11	11
SEM		3.74	12.54	16.39	0.0204	0.0134	0.0139	0.0501	2.373	0.954
Regression analysis (effect of D_3 supplementation to basal diet)										
Linear		0.218	0.186	0.002	0.126	0.280	0.310	0.155	0.004	0.001
Non-linear		0.011	0.001	0.017	0.574	0.026	0.399	0.026	0.117	0.018

Table 3. Serum biochemical profile in commercial broilers (36 d of age) fed diets containing sub-optimal levels of Ca and NPP and higher concentrations of cholecalciferol

Ca: NPP, %	CC, ICU/kg	Ca, mg%	P, mg%	Acid P, KA units	Alk. P, KA units
1.0:0.45	1200	15.88[a]	7.532[a]	1.555[a]	43.0[b]
0.4:0.2	1200	10.27[e]	3.822[c]	1.240[d]	23.7[d]
0.4:0.2	2400	12.30[d]	3.827[c]	1.357[c]	32.5[c]
0.4:0.2	4800	13.78[c]	4.677[b]	1.432[b]	42.2[b]
0.4:0.2	9600	14.75[b]	4.998[b]	1.575[a]	53.7[a]
P		0.01	0.01	0.01	0.01
N		6	6	6	6
SEM		0.38	0.257	0.024	1.915
Regression analysis (effect of D_3 supplementation to basal diet)					
Linear		0.001	0.001	0.001	0.001
Non-linear		0.001	0.001	0.004	0.001

Means with different alphabets/superscripts in a column differ significantly.
Acid P.=acid phosphatase; Alk. P.=alkaline phosphatase.

Table 4. Excretion of Ca (%), P (%) and trace minerals (mg/kg) in commercial broilers (36 d of age) fed diets containing sub-optimal levels of Ca and NPP and higher concentrations of cholecalciferol

Ca: NPP, g	CC, ICU	Ca	P	Fe	Zn	Mn	Cu
1.0:0.45	1200	2.090[a]	1.067[a]	867[d]	233[a]	426[a]	41.9[b]
0.4:0.2	1200	1.852[b]	0.950[b]	1018[a]	225[a]	401[b]	46.4[a]
0.4:0.2	2400	1.697[c]	0.857[c]	973[b]	203[b]	394[b]	31.7[c]
0.4:0.2	4800	1.545[d]	0.770[d]	936[c]	210[b]	369[c]	47.5[a]
0.4:0.2	9600	1.455[e]	0.760[d]	918[c]	175[c]	357[c]	39.2[b]
P		0.01	0.01	0.01	0.01	0.01	0.01
N		6	6	6	6	6	6
SEM		0.042	0.022	9.981	3.90	4.87	1.13
Regression analysis (effect of D_3 supplementation to basal diet)							
Linear		0.003	0.001	0.001	0.001	0.001	0.436
Non-linear		0.001	0.001	0.001	0.001	0.001	0.001

Means with different alphabets/superscripts in a column differ significantly.

significant level at 4800 ICU/kg diet. The levels of P in serum did not increase further with increase in CC to 9600 ICU/kg. The activities of acid phosphatase and alkaline phosphatase in serum decreased with reduction in Ca and P levels in the BD. But, the enzyme activities increased non-linearly with the level of D_3 in the diet. The activity of acid phosphatase at the highest concentration of D_3 was similar to those fed the RD, while the activity of alkaline phosphatase at this concentration was significantly higher than those fed the RD. Increased utilization of Ca and P with D_3 supplementation was also reported by Lofton and Soares (1986).

Mineral excretion

The concentration of Cu in broilers fed BD was significantly higher than those fed the RD (Table 4). The concentration of minerals in excreta decreased non-linearly with increase in D_3 concentration in basal diet. The trend of Cu concentration in excreta was inconsonant.

Homeostatic mechanism of increased utilization of Ca and P at their sub-optiamal concentrations in diet (Rennie *et al.,* 1995; Elliot and Edwards, 1997) also might have contributed to increased availability of these minerals in groups fed BD and consequently resulted in reduced concentration of these minerals in diet. Reduced mineral excretion with D_3 fortification to BD also confirms the possibility of activation of mucosal phytase and consequent hydrolysis of the complex phytate molecule. Similarly, Biehl and Baker (1997) reported decreased excretion of P in birds fed metabolites of D_3 in diet. Supplementation of D_3 to chicken diet was reported to increase availability of Mn (Wedekind *et al.,* 1991; Biehl *et al.,* 1995) and Zn (Baker and Halpin, 1988).

Mineral retention — liver and pancreas

The concentration of Cu, Mn and Fe in liver decreased significantly ($P<0.01$), while the concentration of Zn was not affected by reducing the Ca and NPP levels in diet. Increasing the levels of D_3 to 2400 ICU/kg non-linearly

Table 5. Mineral retention (mg/kg) in liver and pancreas of commercial broilers (36 d of age) fed diets containing sub-optimal levels of Ca and NPP and higher concentrations of cholecalciferol

Ca: NPP	CC	Liver				Pancreas			
		Cu	Mn	Zn	Fe	Cu	Mn	Zn	Fe
1.0:0.45	1200	8.633[d]	8.685[a]	81.3[c]	303.1[c]	2.680[b]	4.495[c]	107.7[ab]	76.63[c]
0.4:0.2	1200	7.692[e]	5.790[d]	82.1[c]	255.9[d]	2.752[b]	2.865[d]	92.32[c]	66.80[d]
0.4:0.2	2400	10.42[a]	7.085[c]	90.6[b]	303.6[c]	3.445[a]	3.132[d]	105.9[b]	87.01[b]
0.4:0.2	4800	9.370[b]	8.000[b]	105[a]	468.2[a]	3.702[a]	13.32[a]	94.66[c]	107.3[a]
0.4:0.2	9600	8.992[c]	7.128[c]	80.0[c]	345.6[b]	2.335[c]	8.853[b]	113.8[a]	102.4[a]
P		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
N		4	4	4	4	4	4	4	4
SEM		0.205	0.232	2.172	16.591	0.120	0.931	1.978	3.579
Regression analysis (effect of D_3 supplementation to basal diet)									
Linear		0.001	0.001	0.025	0.001	0.001	0.001	0.001	0.001
Non-linear		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Means with different alphabets/superscripts in a column differ significantly.

increased the concentration of all the trace minerals tested, compared to those fed the RD (Table 5). Further increase in the levels of D_3 to 4800 ICU/kg significantly increased the concentration of Zn, Fe and Mn compared to those fed 2400 ICU/kg diet. The mineral retention in liver showed a declining trend with further increase in CC to 9600 ICU/kg diet.

In general, the retention of trace minerals in pancreas decreased with reduction in Ca and P levels in diet except Cu, which was not effected due to the levels of Ca and P in the diet (Table 5). The retention of trace minerals increased non-linearly with the levels of D_3 in the diet. Maximum levels of Cu, Mn, Fe and Zn were retained in pancreas at 2400, 4800 and 9600 ICU D_3/kg diet, respectively. The retention at these levels was either higher or equal to that of the RD fed birds. Increased concentrations of Ca and P in serum, retention of trace minerals in visceral organs (liver and pancreas) and reduction in excretion of Ca, P and trace minerals with increase in level of D_3 in groups fed BD suggest increased utilization of PP due to fortification of low Ca and NPP diet with higher concentrations of D_3.

Based on the data, it could be concluded that the performance and mineral utilization (serum Ca and P levels; mineral retention in liver and pancreas and mineral excretion) in commercial broiler chicks fed sub-optimal levels of Ca and NPP increased with supplementation of synthetic D_3. Though mineral utilization increased at higher concentrations of D_3 in diets containing 0.4% Ca and 0.2% NPP, body weight gain, feed conversion efficiency, tibia weight and tibia strength remained inferior to those fed recommended concentrations of Ca and NPP (1.0 and 0.45%, respectively) in broiler diet.

Acknowledgments

The authors are thankful to the Indian Council of Agricultural Research, New Delhi, India for funding the research work under Young Scientist Award scheme.

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