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Growth, Bone Mineralization and Mineral Excretion in Broiler Starter Chicks Fed Varied Concentrations of Cholecalciferol

S. V. Rama Rao*, M. V. L. N. Raju, G. Shyam Sunder, A. K. Panda and P. Pavani
Project Directorate on Poultry, Indian Council of Agricultural Research, Rajendranagar, Hyderabad 500 030, India

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ABSTRACT : An experiment was conducted to study the growth performance, bone mineralization and mineral excretion in broiler starter chicks fed high levels of cholecalciferol (CC) at sub-optimal levels of calcium (Ca) and non-phytate phosphorus (NPP). Five hundred and sixty day-old *Vencobb* female broiler chicks were housed in raised wire floor stainless steel battery brooder pens (24”×30”×18”) at the rate of five chicks per pen. A maize-soyabean meal basal diet was supplemented with dicalcium phosphate, oyster shell powder and synthetic CC to arrive at two levels each of Ca (0.50 and 0.60%), and NPP (0.25 and 0.30%) and four levels of CC (200, 1,200, 2,400 and 3,600 ICU/kg) in a 2×2×4 factorial design. Each diet was fed *ad libitum* to chicks in 7 pens from 2 to 21days of age. Body weight gain, feed intake and bone weight increased ($p<0.05$) with increase in level of CC at both the Ca and NPP levels tested. The CC levels required to obtain significant improvement in body weight gain and feed intake reduced (2,400 ICU/kg vs. 1,200 ICU/kg) with increase in levels of P in diet (0.25% vs. 0.3%, respectively). The feed conversion ratio was significantly improved ($p<0.05$) with increase in level of CC from 200 to 1,200 ICU/kg diet at 0.5% Ca, while at 0.6% Ca, the level of CC in diet did not influence the feed efficiency. Tibia mineralization (density, breaking strength and ash content) and Ca and P contents in serum increased significantly ($p<0.05$) with increase in levels of CC in diet. The CC effect on these parameters was more pronounced at lower levels of Ca and NPP (0.5 and 0.25%, respectively). The data on body weight gain and feed intake indicated that NPP level in diet can be reduced from 0.30 to 0.25% by increasing CC from 200 to 2,400 ICU/kg. Similarly, the bone mineralization (tibia weight, density and ash content) increased non-linearly ($p<0.01$) with increase in CC levels in diet. Concentrations of P and Mn in excreta decreased ($p<0.01$), by increasing CC level from 200 to 2,400 ICU/kg diet. It can be concluded that dietary levels of Ca and NPP could be reduced to 0.50 and 0.25%, respectively by enhancing the levels of cholecalciferol from 200 to 2,400 ICU/kg with out affecting body weight gain, feed efficiency and bone mineralization. Additionally, phosphorus and manganese excretion decreased with increase in levels of CC in broiler diet. (**Key Words :** Broilers, Cholecalciferol, Calcium, Non-phytate Phosphorus, Bone Mineralization, Mineral Retention)

INTRODUCTION

Environmental pollution from intensive poultry farming is primarily associated with excretion of un-utilized mineral compounds like phytate phosphorus (PP) (Paik, 2000). The phosphoric acid chelates several essential minerals like Ca, Cu, Zn, Fe, etc. in cereals and oilseed byproducts (Kornegay et al., 1996), thereby inhibiting their availability to chickens resulting in their excretion into the environment. Cholecalciferol (CC) enhances utilization of PP by increasing synthesis or activity of intestinal phytase (Shafey et al., 1991), increasing phytate hydrolysis (Mohamed et al., 1991; Biehl and Baker, 1997; Qian et al., 1997) and increasing absorption of Ca and P (Wasserman and Taylor, 1973; Biehl and Baker, 1997).

The requirement of CC for broiler chicken fed diets containing adequate levels of Ca and non-phytate P (NPP) is about 200-500 ICU/kg (NRC, 1994; Baker et al., 1998; Edwards et al., 2002). However, the suggested requirement of CC was higher in diets containing sub-optimal levels of Ca and P (Waldroup et al., 1965; Edwards, 1976; Mc Naughton et al., 1977; Baker et al., 1998). Supplementation of higher levels of CC in layer diet containing adequate levels of Ca and NPP was reported to enhance the concentration of the vitamin in eggs (Park et al., 2005). The recent literature (Rama Rao et al., 2003a) indicated that the requirements of Ca and NPP for broiler starter chicks were 0.60 and 0.30%, respectively for optimum growth, feed efficiency and mineral utilization at 1,200 ICU CC/kg diet. CC is known to enhance utilization of Ca and P in chicken and it is hypothesized that the concentrations of dietary Ca and NPP can be reduced further by increasing the level of

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* Corresponding Author: S.V. Rama Rao. Tel: +91-40-24017000, Fax: +91-40-24017002, E-mail: svramarao1@rediffmail.com
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Table 1. Composition of basal diet fed to commercial broilers

Ingredient	g/kg
Yellow maize	574.0
Soyabean meal	390.0
DL-methionine	1.5
Lysine-HCl	0.8
Common salt	4.0
Choline chloride (50%)	1.3
Additives[1]	28.4
Nutrient composition (g/kg)	
ME (kcal/kg[2])	2,875
Crude protein	231.0
Calcium	1.59
NPP[2]	0.95
Lysine	11.10
Methionine	4.77

[1] Additives contained (/kg^{-1}): Zn = 60 mg; Mn = 55 mg; Fe = 38 mg; Cu = 4 mg; thiamin = 1 mg; pyridoxine = 2 mg; cyanocobalamine = 0.01 mcg; niacin = 1.5 mg; pantothenic acid = 10 mg; tocopherol = 10 mg; riboflavin = 10 mg; menadione = 2 mg; retinal acetate = 1,650 IU; monensin sodium = 0.5 mg.

[2] Calculated based on the NPP content of individual feed ingredient.

CC in diet. Since, the cost (per unit of diet) of synthetic CC ($0.03/ton) is less than that of supplemental P as dicalcium phosphate ($6.67/ton), reducing P and Ca levels in diet by increasing levels of CC would reduce the cost of feed considerably besides reducing the P pollution from the intensive poultry operations. Therefore, in the present study, an attempt was made to study the possibility of reducing dietary levels of P and Ca in broiler starter diet by increasing the levels of supplemental CC in the diet.

MATERIALS AND METHODS

Birds and management

Five hundred and sixty commercial day-old *Vencobb* female broiler chicks were wing banded on day one and randomly distributed into 112-raised wire floor battery brooder pens. The battery brooders were made up of stainless steel in vertical three tire system, in each tire (pen) (24”×30”×18”) five birds were housed. Each pen was provided with individual linear feeder (1.5 feet) and a plastic waterer (1.5 liter). The bottom of the floor was also made up of stainless steel wire mesh. An excreta tray was fitted under the wire floor to remove the excreta. Battery brooders were placed in an open sided house having optional curtains on both the sides to regulate the in house temperature. The brooder house temperature was maintained at 34±1°C up to 7 d of age and was gradually reduced to environmental temperature (27 to 33°C) by 21 d of age. Incandescent electrical bulbs were used in the brooders to provide the required temperature and light. Ground maize was provided *ad libitum* on day one and the respective experimental diets from 2 to 21 d of age. All the birds were vaccinated with HVT virus (Marek’s disease) on day one, Lasota virus (Newcastle disease) on 8th day and infectious bursal disease virus on 15th day to protect them from these diseases.

Diets

Maize and soyabean meal were analyzed for Ca, total P (TP) and PP (Haugh and Lantzsch, 1983). Dicalcium phosphate and oyster shell powder were analyzed for Ca and or P. NPP content in maize and soyabean meal was calculated by subtracting PP from TP. A basal diet was prepared to contain about 2,875 kcal ME (calculated), 23.1% crude protein, 0.159% Ca and 0.095% NPP (Table 1). The basal diet was supplemented with dicalcium phosphate and oyster shell powder to obtain two levels each of Ca (0.5 and 0.60%) and NPP (0.25 and 0.30%) in a factorial manner. Each combination of Ca and NPP was supplemented with four levels of CC (200, 1,200, 2,400 and 3,600 ICU/kg diet) in 2×2×4 factorial design with feed grade crystalline CC (Duphar Interfran, Mumbai, India). Each experimental diet was assigned at random to 7 battery brooder pens in a completely randomized design and the diets were fed *ad libitum* from 2 to 21 d of age.

Traits measured

Body weight gain and feed intake were recorded at weekly intervals by weighing all the birds in a pen and feed residue on an electronic weighing balance having one g accuracy. Feed conversion ratio was calculated, as feed required producing a unit body weight gain. The degree of leg abnormality was scored as 1 = normal hock joint; 2 = slight swelling of hock joint; 3 = marked swelling of the joint; 4 = marked swelling of the joint and slight slipping of the Achilles tendon and 5 = marked swelling and complete slipping of the tendon (Watson et al., 1970). Three ml of blood was drawn from the brachial vein of a bird selected at random from each replicate on 21st day of age. The sera were analyzed for Ca with an Atomic Absorption Spectrophotometer using 0.1% lanthanum chloride and inorganic P (Fiske and Subba Row, 1925). Bone mineralization was assessed by measuring tibia weight, density (AG 285 Densitometer, Mettler-Toledo GmbH, Switzerland), breaking strength and ash content. At 22 days of age, seven birds from each treatment were randomly selected and sacrificed by cervical dislocation. Both the tibiae were freed from soft tissue. The dried (100°C/3 h) bone samples were defattened by extracting with petroleum ether for 48 h. The right tibia of each bird was used to determine the breaking strength (EZ Test, Shimadzu, Japan) with 3-point method, 40 mm as guage length and 5 mm velocity of the load cell per minute. Both tibiae of each bird were ashed together at 600±20°C/6 h in a microwave muffle furnace (BR 600521, Phoenix, CEM Corporation, USA). Clean excreta trays were placed under the wire floor

Table 2. Interaction between dietary levels of calcium (Ca)/non-phytate phosphorus (NPP) and cholecalciferol (CC) on weight gain, feed intake, feed efficiency and tibia weight in broiler chicks (up to 21 d of age)

Ca/NPP (%)	CC (ICU/kg)	Weight gain^{1} (g)	Feed intake^{1} (g)	Feed conversion ratio^{2} (g feed/g weight gain)	Bone weight^{1} (g)
0.50/0.25	200	328.7^{d}	587.8^{c}	1.727^{ab}	2.109^{b}
0.50/0.25	1,200	408.6^{b}	687.6^{b}	1.630^{d}	2.254^{ab}
0.50/0.25	2,400	428.9^{ab}	735.3^{ab}	1.661^{cd}	2.474^{ab}
0.50/0.25	3,600	449.9^{a}	770.7^{a}	1.745^{a}	2.424^{ab}
0.60/0.30	200	371.5^{c}	622.5^{c}	1.731^{ab}	2.181^{ab}
0.60/0.30	1,200	425.0^{ab}	714.1^{b}	1.710^{abc}	2.459^{ab}
0.60/0.30	2,400	445.0^{ab}	723.1^{b}	1.699^{abc}	2.542^{a}
0.60/0.30	3,600	431.6^{a}	733.6^{ab}	1.675^{bcd}	2.532^{a}
SEM ±		4.8	6.9	0.0093	0.047
n		7	7	7	7
Mean sum of squares					
Ca		1,283	9,108*	0.005	0.053
NPP		5,700*	246	0.115**	0.166
Ca×NPP		966	49	0.039*	0.008
CC					
Linear		114,353**	298,304**	0.002	0.055
Non-linear		17,030.9**	26,401**	0.0305**	0.243
Ca×CC		1,754	1,527	0.028**	1.088**
NPP×CC		4,378*	7,912*	0.018	0.674*
Ca×NPP×CC		420	4,681	0.015	0.462
Error		1,180	1,944	0.007	0.213

$^{a, b, c, d}$ Means having a common superscript in a column do not vary significantly (** p<0.01, * p<0.05).

^{1} NPP×CC; ^{2} Ca×CC.

of each pen on 20th day of age and the total excreta voided in 24 hours by all five birds was collected from four replicates per treatment to estimate concentrations of Ca, P (AOAC, 1990), Fe, Mn. Zn and Cu (Atomic Absorption Spectrophotometer, Perkin Elmer, Analyst 100, Operation Manual).

Statistical analysis

Three-way factorial analysis was carried out following the Completely Randomized Design (Snedecor and Cochran, 1980) with levels of Ca, NPP and CC as factors. When the three-way interaction was not found significant, a two-way interaction was undertaken. The effects of individual factors were considered only when the interactions were not significant. Comparisons among means were made by the Duncan Multiple Range test (Duncan, 1955). Contrast analysis was carried out to find out the effect of graded levels of CC on various parameters.

RESULTS

Body weight gain, feed intake, feed conversion ratio, tibia weight and leg abnormality score were not significantly (p>0.05) influenced by the three-way interaction among Ca, NPP and CC levels in diet (Table 2). However, these parameters were influenced (p<0.05) by interaction between CC and NPP (weight gain, feed intake and tibia weight) and CC and Ca (feed conversion ratio). The level of CC in diet significantly (p<0.05) influenced the leg abnormality score. The three-way interaction among these nutrients significantly influenced tibia density, breaking strength, ash content (Table 3), serum Ca and inorganic P levels and excretion of minerals (Ca, P, Fe, Mn & Cu) (Table 4) except Zn, which was affected by the two-way interaction between Ca and NPP.

Production performance

Body weight gain and feed intake increased significantly (p<0.05) by increasing level of CC up to 2,400 ICU/kg diet at 0.25% NPP (Table 2). However, at 0.30% NPP, improvement in these parameters was observed up to 1,200 ICU CC/kg diet. Further increase in the vitamin level did not show any additional benefits on these parameters at both levels of NPP. At 200 ICU CC/kg, weight gain increased significantly (p<0.05) due to increase in NPP from 0.25 to 0.30%, however, such an improvement was not observed at higher levels of CC in the diet (≥1,200 ICU/kg). The levels of Ca (0.5/0.6%) tested did not influence body weight gain and feed efficiency. In general, body weight gain, feed intake and feed efficiency improved non-linearly (p<0.01) due to increase in levels of CC in diet. At 0.50% Ca in diet, feed efficiency improved significantly with increases in CC to 1,200 ICU/kg, but further increase in CC level to 3,600 ICU significantly (p<0.05) reduced the feed efficiency, which was similar to those fed 200 ICU CC/kg diet (Table 2). However, the feed efficiency was not

Table 3. Tibia mineralization parameters in broiler starter chicks (21 d of age) fed different levels of calcium (Ca), non-phytate phosphorus (NPP) and cholecalciferol (CC)

Ca (%)	NPP (%)	CC (ICU/kg)	Density (g cm^{-3})	Breaking strength (N)	Ash (g/kg)
0.50	0.25	200	0.288^{gh}	33.9^{c}	252^{h}
0.50	0.25	1,200	0.340^{fgh}	36.0^{c}	287^{g}
0.50	0.25	2,400	0.567^{cde}	45.9^{abc}	359^{a}
0.50	0.25	3,600	0.606^{cd}	47.7^{abc}	290^{fg}
0.50	0.30	200	0.386^{fgh}	32.0^{c}	236^{h}
0.50	0.30	1,200	0.419^{cfgh}	41.5^{abc}	313^{de}
0.50	0.30	2,400	0.439^{defg}	45.5^{abc}	335^{bc}
0.50	0.30	3,600	0.492^{def}	55.2^{ab}	340^{b}
0.60	0.25	200	0.255^{h}	39.9^{bc}	251^{h}
0.60	0.25	1,200	0.490^{def}	42.0^{abc}	296^{efg}
0.60	0.25	2,400	0.449^{defg}	44.7^{abc}	314^{de}
0.60	0.25	3,600	0.481^{def}	53.5^{ab}	325^{bcd}
0.60	0.30	200	0.441^{def}	40.7^{abc}	253^{h}
0.60	0.30	1,200	0.504^{cdef}	56.6^{a}	317^{cd}
0.60	0.30	2,400	0.607^{cd}	55.3^{ab}	318^{cd}
0.60	0.30	3,600	0.594^{cd}	54.1^{ab}	336^{bc}
n			7	7	6
SEM±			0.0182	1.10	0.38
Mean sum of squares					
Ca			0.018	729.1*	1.990
NPP			1.112**	493.8	8.797*
Ca×NPP			0.005	103.3	2.680
CC					
Linear			0.180**	40.91	793.8**
Non-linear			0.163**	286.4	65.6**
Ca×CC			0.081**	653.6**	12.84**
NPP×CC			0.145**	275.5	38.88**
Ca×NPP×CC			0.526	657.3**	23.00**
Error			0.015	131.6	1.477

$^{a, b, c, d, e, f, g, h, I, j, k}$ Means having a common superscript in a column do not vary significantly (** p<0.01, * p<0.05).

affected due to variation in the CC level in the diet at 0.60% Ca.

Leg abnormality score and bone mineralization

The tibia density, weight and ash content were not increased (p>0.05) with the level of Ca in diet from 0.50 to 0.60%, suggesting that the lower level of Ca tested (0.50%) was adequate for optimum bone mineralization.

The leg abnormality score progressively and significantly (p<0.01) reduced (4.14, 2.64 and 2.28) with each incremental level of CC up to 2,400 ICU/kg diet. Increase in CC level beyond 2,400 ICU/kg diet did not further reduce the leg abnormality (2.094). Similarly, leg abnormality score reduced (p<0.01) significantly (2.906 vs. 2.669) by increasing the dietary Ca from 0.50 to 0.60%. The NPP levels tested did not influence the magnitude of leg abnormality in broilers.

The two-way interaction between NPP and CC significantly (p<0.05) affected tibia weight (Table 2). The bone weight was significantly lower in groups fed the lowest level of Ca, NPP and CC (0.5%, 0.25% and 200 ICU/kg, respectively) compared to those fed 0.60% Ca, 0.30% NPP and CC≥2,400 ICU/kg. The bone weight in broilers fed other combinations of these nutrients was intermediate. In general, tibia density decreased and the bone ash content increased non-linearly (p<0.01), while tibia-breaking strength was not affected by increasing the levels of CC in diet from 200 to 3,600 ICU/kg (Table 3). The density and ash content of tibia increased with dietary CC levels up to 2400 ICU/kg and further increase in CC level to 3,600 ICU/kg diet did not show any improvement. Though, the tibia breaking strength increased with CC level in diet, the difference was not significant at different Ca and NPP levels tested, except at 0.50% Ca and 0.30% NPP, where the tibia breaking strength was significantly (p<0.01) higher at 3,600 ICU/kg compared to 200 ICU CC/kg diet.

Serum Ca and inorganic P

At all combinations of CC and Ca tested, the serum Ca concentration increased significantly (p<0.01) by increasing dietary NPP content from 0.25 to 0.30% (Table 4). The concentration of serum Ca was maximum (16.68 to 17.33 mg/kg) in broilers fed 0.60% Ca and 0.30% NPP when compared to those fed other combinations of these two

Table 4. Serum Ca and inorganic P and mineral concentration in excreta of broiler starter chicks (21 d of age) fed different levels of calcium (Ca), non-phytate phosphorus (NPP) and cholecalciferol (CC)

Ca (%)	NPP (%)	CC (ICU/kg)	Serum (mg/dl)		Excreta					
			Ca	P	Ca	P	Zn	Fe	Mn	Cu
					% DM		mg/kg dry matter			
0.50	0.25	200	14.43^{ef}	4.88^{hi}	1.205^{g}	0.853^{e}	279	$1{,}735^{j}$	376^{I}	37.3^{cd}
0.50	0.25	1,200	11.17^{g}	4.98^{gh}	1.505^{e}	0.785^{hi}	316	$1{,}656^{n}$	382^{k}	38.0^{bc}
0.50	0.25	2,400	14.48^{ef}	7.19^{b}	2.065^{b}	0.740^{l}	320	$1{,}788^{f}$	341^{o}	36.1^{d}
0.50	0.25	3,600	11.44^{g}	7.38^{a}	2.175^{a}	0.752^{k}	282	$1{,}660^{m}$	392^{l}	39.2^{ab}
0.50	0.30	200	15.87^{d}	3.72i	1.730^{c}	0.932^{c}	237	$1{,}846^{e}$	466^{c}	36.0^{d}
0.50	0.30	1,200	16.50_{c}	4.84^{i}	1.395^{f}	0.810^{g}	216	$1{,}651^{o}$	422^{g}	31.0^{g}
0.50	0.30	2,400	16.12^{cd}	5.01^{g}	1.407^{f}	0.781^{i}	282	$1{,}786^{g}$	427^{f}	37.0^{cd}
0.50	0.30	3,600	17.33^{a}	5.85^{e}	1.493^{e}	0.760^{j}	203	$1{,}692^{l}$	372^{m}	31.3^{fg}
0.60	0.25	200	14.73^{e}	4.92^{ghi}	1.255^{g}	0.990^{a}	232	$1{,}770\ ^{1}$	445^{d}	34.0^{e}
0.60	0.25	1,200	10.34^{h}	3.91^{k}	1.740^{c}	0.945^{b}	264	$1{,}722^{k}$	387^{j}	32.5^{f}
0.60	0.25	2,400	11.43^{g}	4.38^{j}	1.520^{e}	0.840^{f}	291	$1{,}926^{b}$	443^{e}	36.6^{cd}
0.60	0.25	3,600	13.98^{f}	3.83^{k}	1.610^{d}	0.885^{d}	286	$1{,}767^{I}$	368^{n}	34.5^{e}
0.60	0.30	200	17.09^{ab}	4.84^{i}	1.710^{c}	0.985^{a}	277	$1{,}734^{j}$	485^{b}	36.3^{d}
0.60	0.30	1,200	16.68^{bc}	5.58^{f}	1.073^{h}	0.942^{b}	261	$1{,}865^{d}$	490^{a}	34.0^{e}
0.60	0.30	2,400	17.14^{ab}	6.04^{d}	1.410^{f}	0.849^{e}	290	$1{,}997^{a}$	442^{e}	39.5^{a}
0.60	0.30	3,600	17.33^{a}	6.37^{c}	1.425^{f}	0.790^{h}	255	$1{,}879^{c}$	396^{0}	31.1^{g}
N			6	4	4	4	4	4	4	4
SEM±			0.245	0.135	0.055	0.012	6.85	12.27	5.411	0.35
Mean sum of squares										
Ca			0.714*	3.99**	0.380	0.092**	127	179,474**	19,283**	13.66**
NPP			385.3**	0.152**	0.371	0.036**	1,5266*	10,070**	9,691**	40.94**
Ca×NPP			4.463**	29.19**	0.048	0.051**	1,8364*	1,225**	5,156**	95.14**
CC							NS			
Linear			0.029	0.266**	0.501*	0.085**		6049**	8,917**	4.353**
Non-linear			22.17**	9.043**	0.059	0.051**		101,586**	8,037**	63.77**
Ca×CC			6.242**	3.287**	0.108	0.005*	1,211	37,931**	385**	11.65**
NPP×CC			16.50**	1.669**	0.651**	0.026**	2,856	8418**	14,131**	44.21**
Ca×NPP×CC			10.94**	2.204**	0.466*	0.027**	846	22,721**	5,110**	7.17**
Error			0.133	0.003	0.144	0.001	2,580	0.576	0.818	0.492

$^{a, b, c, d, e, f, g, h, I, j, k, l, m, n}$ Means having a common superscript in a column do not vary significantly (** p<0.01, * P<0.05).

minerals at all levels of CC.

Mineral content in excreta

In general, the concentration of Ca in excreta increased linearly (p<0.01), while the P, Fe, Mn and Cu decreased non-linearly (p<0.01) with increase in CC levels in diet from 200 to 3,600 ICU/kg. Increasing the NPP content from 0.25 to 0.30% in diet, significantly reduced the Ca content in excreta at all levels of CC except at 200 ICU/kg (Table 4). At the latter concentration of CC, Ca content in excreta increased significantly (p<0.01) with increase in P content in diet. At 2,400 and 3,600 ICU CC/kg diet, the Ca content in excreta was significantly (p<0.05) higher at lower levels of Ca and NPP tested, compared to those fed other levels of these minerals. At 0.25% NPP and 0.50% Ca, the Ca content in excreta increased significantly with increase in level of CC up to 3,600 ICU/kg. But, at 0.30% NPP and at both the levels of Ca, increasing the CC level from 200 to 1200 ICU/kg diet significantly (p<0.05) decreased the Ca content in excreta.

The P content in excreta was significantly (p<0.01) increased at higher Ca level compared to those fed low Ca diet at all levels of CC except at 200 ICU/kg. At 200 ICU CC and 0.50% Ca/kg diet, the excreta P content increased significantly with increase in NPP from 0.25% to 0.30% in diet. At 0.60% Ca, the variation in concentration of P in diet did not influence the P content in excreta. In general, the excretion of P decreased (p<0.01) non-linearly with increase in CC level in diet.

The level of Ca, NPP and CC tested did not influence the concentration of Zn in excreta (Table 4). The concentrations of Fe, Mn and Cu were significantly influenced (p<0.01) by the three-way interaction among Ca, NPP and CC in diet. The concentrations of Fe and Mn were higher at higher levels of Ca and NPP in diet. Excretion of Mn, Cu and Fe were maximum at 3,600 ICU CC/kg at 2:1, Ca and NPP ratio.

Economics

Reducing the levels of NPP from 0.30 to 0.25% equals to 2.95 kg dicalcium phosphate per ton of feed, which amounts to \$1.14 saving for one ton feed. Amount required for additional supplementation of CC need \$0.33 per ton of feed. Therefore, by adopting this technology, the cost of boiler starter feed can be reduced to \$0.81 per ton based on the present cost of CC and dicalcium phosphate.

DISCUSSION

The data on body weight gain and feed intake (Table 3) suggest that the levels of NPP can be reduced from 0.30 to 0.25% in diet, by increasing the CC level to 2,400 ICU/kg. The literature also suggests higher requirement of CC at sub-optimal levels of Ca and P in diet for optimum body weight gain and bone mineralization (Edwards, 1976; Mc Naughton et al., 1977; Baker et al., 1998). A higher level of CC in the diet is known to stimulate hydrolysis of phytate (Shafey et al., 1991; Mohamed et al., 1991) by increasing the activity of intestinal phytase (Davies et al., 1970; Pointillart et al., 1985), thereby improving the utilization of PP and Ca.

The levels of Ca and NPP used in the present study were considerably lower than the levels recommended for commercial broiler chicken (NRC, 1994). The recent literature (Vogt, 1992; Sebastian et al., 1996; Sohail and Roland, 1999; Rama Rao et al., 2003a, b) also recommended lower dietary levels of NPP (0.26 to 0.325%) and Ca (0.60 to 0.76%) for broilers (up to 21-35 d of age) for optimum performance. Better utilization of minerals at their sub optimal levels in the diet might be responsible for obtaining optimum performance at the above levels tested (Rennie et al., 1995; Elliot and Edwards, 1997). Sub optimal levels of Ca and NPP are also known to stimulate the CC metabolism, which in turn enhances utilization of Ca and P (Bar et al., 1978).

The bone mineralization parameters in broilers fed 0.50% Ca and 0.25% NPP and 2,400 ICU CC/kg were either similar (bone density and bone breaking strength, $p<0.01$) or higher (bone ash content, $p<0.01$) compared to those fed the highest levels of these nutrients in the diet (0.60%, 0.30% and 3,600 ICU/kg, respectively). These results thus indicated that for optimum bone mineralization, 0.50% Ca and 0.25% NPP were adequate in broiler starter chicks fed diet containing 2,400 ICU CC/kg.

The severity of leg abnormality decreased ($p<0.05$) with increase in CC from 200 to 2,400 ICU/kg diet. Increasing dietary CC content significantly improved weight and ash content of tibia at majority of Ca ($p<0.01$) and NPP ($p<0.05$) levels tested. Increased tibia density with increase in levels of CC also support the reduced incidence ($p<0.01$) of leg abnormalities with increasing CC concentration in diet.

The lack of response to variation in Ca and NPP levels on tibia mineralization parameters at the higher level of CC (2,400 ICU/kg diet) tested (Table 3) suggest adequacy of lower concentrations of Ca and NPP tested for bone mineralization at 2,400 ICU CC/kg diet. Increasing the levels of CC up to 2,400 ICU/kg diet reduced ($p<0.05$) the incidence of leg abnormality and increased body weight gain similar to those fed the highest levels of Ca, NPP and CC (0.6%, 0.3% and 3.600 ICU CC/kg diet, respectively). On the contrary, Cruickshank and Sim (1987) and Lofton and Soares (1986) reported higher incidence of leg abnormality at higher levels of CC (800 to 4,000 ICU/kg) in diets containing recommended levels of Ca and P. Lack of such ill effects at higher levels of CC used in the present study on leg abnormality may be due to the sub-optimal levels of Ca and NPP used in the present study. At optimal levels of Ca and NPP (1.0 and 0.45%, respectively), the surfeit levels of CC in diet might have exerted toxic effects through hypercalcemia and hyper phosphatemia. Similarly, other authors (Waldroup et al., 1965; Meixner et al., 1979; Biehl and Baker, 1997; Baker et al., 1998) have also reported beneficial effects of supplementing higher levels of CC on broiler performance when fed sub optimal levels of Ca and P.

In general, the serum P level increased significantly ($p<0.01$) by increasing the level of CC at all combinations of Ca and NPP tested, except at 0.60% Ca and 0.25% NPP. The concentration of P in serum was significantly increased irrespective of CC level when the ratio between Ca and NPP was 2:1 compared to those fed disproportionate ratios (> or <2) of these minerals. Better utilization and absorption of Ca and P at 2:1 ratio (Underwood, 1981; Georgievskii et al., 1982; Rama Rao et al., 2003a; 2006) might have resulted in higher concentrations of these minerals in serum.

The higher levels of NPP and CC in the diet might have a beneficial effect on the utilization of Ca and P, respectively, as reflected in higher concentrations of the respective mineral in serum (Table 4). Reduced excretion of Ca with increase in dietary NPP level suggested better utilization of Ca at lower Ca and NPP ratio and at CC $\geq$ 1,200 ICU/kg diet. Supplementing additional levels of CC might have increased the utilization of dietary P, which was evident in reduced the mineral content in excreta. Reduced excretion of P in birds fed metabolites of CC was also reported (Biehl and Baker, 1997). At sub optimal levels of CC, the utilization of Ca might be lower and further increase in NPP resulted in wider Ca and NPP ratio with a consequent rise in excretion of Ca (Table 4), perhaps due to formation of calcium phosphate in the gut.

Excretion of Fe and Mn was higher at higher levels of Ca and or NPP in diet, probably due to inhibitory effect of these minerals on absorption of trace minerals (Underwood, 1981; Georgievskii, 1982). The concentration of Fe, Cu and Mn, particularly at the highest level of CC (3,600 ICU/kg) showed maximum excretion at 2:1 Ca and NPP ratio, contradicting the popular assumption of 2:1 as the ideal Ca and NPP ratio for mineral utilization. At 2:1 Ca and NPP ratio, the utilization of these two minerals in chicken is known to be maximum and therefore, the possibility of their interference with utilization of trace minerals was less at 2:1 Ca and P ratio (NRC, 1980). The contradictory findings of the present study may be due to the sub-optimal levels of Ca and NPP used in the present study. The literature indicates increased utilization of trace minerals (Baker and Halpin, 1988; Biehl et al., 1995) with CC metabolites in diet. However, further studies are required to determine the specific effect of varied levels of Ca and NPP on trace mineral utilization.

Based on the results, it could be concluded that the dietary NPP levels can be reduced from 0.30 to 0.25% without affecting the weight gain, leg abnormality score, bone mineralization and mineral retention at 0.50% Ca by increasing dietary CC to 2,400 ICU/kg diet with a net saving of $0.81 per ton of feed. Excretion of P and Mn also decreased at 2,400 ICU/kg diet than at 200 ICU/kg diet.

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