Dietary Calcium and Non-phytin Phosphorus Interaction on Growth, Bone Mineralization and Mineral Retention in Broiler Starter Chicks

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ABSTRACT : An experiment was conducted to study the requirement of calcium (Ca) and non-phytin phosphorus (NPP) in commercial broilers during starter phase. Seven hundred and twenty day-old *Vencob* male broiler chicks were randomly distributed into 144 stainless steel battery brooders, 5 birds in each. Four levels each of Ca (6, 7, 8, and 9 g/kg) and NPP (3, 3.5, 4, and 4.5 g/kg diet) were fed in a factorial design in a corn-soya basal diet. Levels of dicalcium phosphate and oyster shell grit were adjusted to obtain the desired levels of Ca and NPP. Each diet was fed *ad libidum* to chicks in 9 battery brooders from one d to 21 d of age. Body weight gain and feed intake were depressed (p<0.01) by increasing the dietary Ca level (8 and 9 g/kg) at lower levels of NPP (3 and 3.5 g/kg diet). The growth depression observed at lower NPP level was alleviated by reducing the Ca content to 6 g/kg diet. The tibia ash content and tibia breaking strength increased with increase in both Ca (>6 g/kg) and NPP (>3 g/kg) levels. The leg abnormality score decreased (p<0.01) with increase in NPP content in the diet at all levels of Ca tested. The serum Ca and inorganic P levels were increased with increase in the level of the respective mineral in the diet, but the serum concentration of Ca and P were inversely related to the level of NPP and Ca, respectively /kg diet. In general, the excretion of macro minerals (Ca, and P), and micro minerals {zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu)} was significantly lower at lower levels of Ca and NPP tested (6 and 3 g/kg diet, respectively). The mineral excretion increased with increase in dietary Ca and NPP levels, more conspicuously at the disproportionate ratio of these minerals (>2:1, Ca and NPP). Similarly, the retention of Zn, Mn, and Fe in liver was significantly higher (p<0.01) at lower levels of Ca and NPP tested. Results from this study indicate that the commercial broilers do not require more than 3 g NPP and 6g Ca/kg diet during starter phase (up to 21 d of age) for optimum weight gain, feed efficiency and utilization of Ca, P, Zn, Mn, Fe and Cu. However, the requirements of these minerals for optimum bone mineralization were higher than the levels suggested above. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 5 : 719-725)***

Key Words : Calcium, Non-phytin Phosphorus, Broilers Growth, Tibia Ash, Tibia Breaking Strength, Trace Mineral Retention

INTRODUCTION

The requirement of calcium (Ca) and phosphorus (P) has been the subject of research for the last 3-4 decades. These two minerals are co-existing in many biological functions. However, several researchers determined the requirement Ca (Kiaei and Michie, 1994; Hossain et al., 1994) or P (Mohamad et al., 1998; Rama Rao et al., 1999) keeping the other mineral at a constant level. It is well established that the level of Ca or P influence the utilization of these macro minerals and also other trace mineral like manganese (Mn), zinc (Zn), copper (Cu), iron (Fe) etc. (Underwood, 1981; Georgievskii et al., 1982) in chicken. Therefore, determination of Ca and P requirements at different levels of these minerals would be more prudent compared to those studies aimed at determining the requirement of these minerals at a constant level of other mineral (Smith and Kabaiji, 1985; Rensburg et al., 1992).

Recently growing concern over the environmental pollution from large scale poultry farming is primarily associated with excretion of un-utilized mineral compounds like phytin phosphorus (PP) (Paik, 2000). The PP chelates other minerals like Ca, Cu, Zn, Fe etc. (Eardman and De Paepe, 1979; Kornegay et al., 1996) and made them not available to chicken. However, the PP can be made available by maintaining proper Ca and P ratio (Scheideler and Sell, 1987; Qian et al., 1997), thereby the pollution is minimized and the cost on supplemental P can be reduced. Therefore, in the present study an attempt was made to study the interaction between Ca and NPP in broiler starter chicken on the growth performance, leg abnormality score, bone mineralization, serum bio-chemical profile, excretion of minerals and retention of trace minerals in liver.

MATERIALS AND METHODS

Birds and management

Seven hundred and twenty commercial day old *Vencob* male broilers were wing banded on d one and randomly distributed in to 144 raised wire floor stainless steel battery brooder pens at the rate of five birds per pen (47.5"×29.5"× 17"). The brooder temperature was maintained at 34±1°C up to 7 d of age and was gradually decreased to 26±1°C by 21 d of age. Ground maize was provided *ad libitum* on d one followed by respective experimental diets. Uniform management and vaccination schedule was followed for all the birds.

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Table 1. Composition (g/kg) of basal diets fed to commercial broilers

Feed ingredient	g/kg
Yellow maize	600.8
Soyabean meal	390.0
DL methionine	1.5
Trace mineral premix[1]	0.1
Vitamin premix[2]	0.1
Choline chloride	2.5
Common salt	4.0
Antibiotic[3]	0.5
Coccidiostat[4]	0.5
Nutrient composition	
Analyzed	
Crude protein	230.8
Calcium	1.87
Non-phytin phosphorus*	1.51
Calculated	
Metabilizable energy (kcal/kg)	2,883
Methionine	5.20
Lysine	11.98
Arginine	15.82
Tryptophan	3.25

[1] Vitamin premix provided (mg/kg diet): thiamin 1; pyridodine, 2; cyanocobalamani, 0.01; niacin, 1.5; pantothenic acid, 10; tocopherol, 10; riboflavin, 5; menadione, 1; retinal acetate, 8,250 IU and cholecalciferol, 1,200 ICU.
[2] Trace mineral premix provided: (mg/kg): $ZnSO_4$ 60 g; $MnSO_4$, 60; $FeSO_4$, 30; $CuSO_4$, 4.3 g/100 kg.
[3] Furazolidone, 22.4%W/W.
[4] Coban TM (monensin sodium 10 w/w).
* Calculated based on the NPP content of individual feed ingredient.

Diets

Crude protein, Ca, TP and PP (Haugh and Lantzsch, 1983) were analyzed in maize and soybean meal. Dicalcium phosphate and oyster shell grit were analyzed for Ca and or P. Non-phytin phosphorus content in maize and soybean meal was calculated by subtracting the PP from TP. A broiler starter basal diets were prepared to contain about 2,883 kcal metabolizable energy and 230.8 g crude protein/kg (Table 1). Basal diet were supplemented with dicalcium phosphate and oyster shell grit to obtain the four levels of Ca (6, 7, 8 and 9 g/kg) and four NPP (3, 3.5, 4 and 4.5 g/kg). Each level of Ca was tested with four levels of NPP, resulting in sixteen experimental diets. Each experimental diet was assigned at random to 9 battery brooder pens and the diets were fed *ad libitum*. Completely randomized design was followed while allotting the diets to different replicates.

Traits measured

Body weight gain, feed intake and leg abnormality score (Watson et al., 1970) were recorded at weekly intervals. Two to three ml of blood was drawn from brachial vein of each bird and one bird from each replicate at 21 d of age. The sera was pooled and analyzed for Ca (Atomic Absorption Spectrophotometer) and inorganic P (Fiske and Subba Row, 1925). Bone mineralization was studied in terms of leg abnormality score, tibia ash content and bone breaking strength. At 22 d of age, five birds from each treatment were randomly selected and sacrificed by cervical dislocation. Both the tibiae were freed of soft tissue. The dried (100°C/3 h) bone samples were defattened in petroleum ether for 48 h. The right tibia of each bird was used to determine the breaking strength (EZ Test, Shimidzu, Japan). Both tibiae of a bird were ashed together at 600±20°C/2 h. Samples from liver were collected and pooled treatment wise to analyze the concentration of Zn, Fe and Mn (Atomic Absorption Spectrophotometer, Perkin Elmer, Analyst 100, Operation Manual). The total excreta voided on 20th d from five replicates per treatment was collected and pooled to estimate the concentration of Ca, P, Mn, Zn, Fe and Cu.

Table 2. Body weight gain (g) and feed intake (g/b) in commercial male broilers at 21 d of age fed different levels (g/kg diet) of calcium and non-phytin phosphorus

Calcium	Non-phytin phosphorus			
	3.0	3.5	4.0	4.5
Body weight gain (g)			n=9, SEM = 5.12	
6	554.3^{ax}	577.0^{ax}	575.4^{ax}	572.5^{ax}
7	519.3^{aby}	559.4^{abxy}	581.9^{ax}	552.2^{axy}
8	489.6^{by}	526.7^{abxy}	574.4^{ax}	564.4^{ax}
9	398.7^{cz}	512.1^{by}	567.5^{ax}	568.4^{ax}
Feed intake (g/b)			n=9, SEM = 7.34	
6	883^{ax}	888^{ax}	875^{ax}	897^{ax}
7	800^{aby}	837^{abxy}	879^{ax}	851^{axy}
8	743^{bz}	795^{bcyz}	875^{ax}	853^{axy}
9	617^{cz}	756^{cy}	815^{axy}	860^{ax}

^{a,b,c} Means bearing common superscript(s) in a column for each parameter do not vary significantly ($p<0.01$).
^{x,y,z} Means bearing common superscript(s) in a row do not vary significantly ($p<0.01$).

Statistical analysis

Two factorial analysis was carried out following the Completely Randomized Design (Snedecor and Cochran, 1980) with levels of Ca and NPP as factors. When the interaction was not found significant the effect of individual factors were considered. When interactions were significant, separate analyses were conducted within each main effect. Comparisons among means were made by Duncan's multiple range test (Duncan, 1955).

RESULTS

The NPP content in maize and soybean meal is about 1.20 and 2.03 g/kg, respectively. The interaction between the levels of Ca and NPP was significantly influenced the body weight gain, feed intake, leg abnormality score, tibia breaking strength, tibia ash content, serum Ca and inorganic

Table 4. Serum calcium and inorganic phosphorus contents (mg/dl) in broilers (at 22 d of age) fed different levels (g/kg) of calcium and non-phytin phosphorus

Calcium	Non-phytin phosphorus			
	3	3.5	4	4.5
Calcium			n=9, SEM =0.497	
6	28.0^{bx}	22.2^{by}	17.9^{cz}	22.2^{by}
7	20.3^{cy}	30.7^{ax}	19.9^{bcy}	17.4^{cy}
8	34.4^{ax}	28.0^{ay}	22.0^{bz}	26.9^{ay}
9	36.1^{ax}	20.9^{by}	33.3^{ax}	22.5^{by}
Inorganic phosphorus			n=9, SEM =0.129	
6	6.94^{az}	7.24^{ay}	7.90^{ax}	8.07^{aw}
7	4.16^{bz}	6.99^{by}	7.56^{bx}	7.03^{by}
8	3.75^{cz}	4.97^{cy}	6.08^{cw}	5.71^{cx}
9	3.51^{dz}	3.60^{dy}	5.33^{dw}	5.11^{dx}

^{a,b,c} Means with a common superscript(s) in a column for each parameter don't vary significantly ($p<0.01$).
^{x,y,z} Means with a common superscript(s) in a row don't vary significantly ($p<0.01$).

P content, the concentration of Ca, P and trace minerals (Fe, Zn, Mn and Cu) in excreta and concentration of trace minerals in liver. However, either interaction or the individual effect of dietary Ca and NPP levels did not influence ($p>0.05$) the feed/body weight gain.

Performance

Interaction between the levels of NPP and Ca on 21 d body weight gain and feed intake was found significant (Tables 2 and 3, respectively). At 3 g NPP, weight gain and feed intake were significantly ($p<0.01$) depressed by increasing the dietary Ca level to 8 g/kg diet, whereas at 3.5g NPP growth depression was observed at 9 g Ca/kg diet. At 4 and 4.5 g NPP, the level of Ca in diet did not affect the body weight gain and feed intake. Similarly, Ca at 6 g/kg diet, body weight gain and feed intake were not affected by the level of dietary NPP. The growth depression and reduced feed intake observed at 8 and 9 g Ca/kg diet, was alleviated by increasing the NPP content to 3.5 and 4 g/kg diet, respectively.

Leg abnormality score and bone mineralization

The leg abnormality score was significantly ($p\leq0.01$) higher at lower levels of NPP (3 g/kg) in diet and decreased by increasing the NPP from 3 to 4.5 g/kg diet (Table 3). The leg abnormality score was not affected due to dietary Ca level at different P levels tested except at 3.5 gNPP/kg diet where the score increased with increase in the dietary Ca level from 6 to 9 g/kg.

The tibia breaking strength was significantly ($p\leq0.01$) increased by increasing the dietary Ca level from 6 to 7 g Ca/kg diet at all levels of NPP tested (Table 3). Similarly, at 7 and 9 gCa/kg diet, the bone strength was significantly increased with increase in NPP up to 4 g/kg diet beyond which the bone strength showed declining trend. The bone strength was not affected due to increase in NPP content at

Table 3. Leg abnormality score, tibia breaking strength (N) and tibia ash content (g/kg) in commercial male broilers at 21 d of age fed different levels (g/kg diet) of calcium and non-phytin phosphorus

Calcium	Non-phytin phosphorus			
	3.0	3.5	4.0	4.5
Leg abnormality score			n=9, SEM = 0.063	
6	3.53^{aw}	2.53^{bx}	2.49^{ax}	2.77^{awx}
7	3.29^{aw}	3.24^{abw}	2.97^{aw}	2.67^{aw}
8	3.79^{aw}	3.32^{abwx}	2.93^{ax}	2.71^{ax}
9	3.92^{aw}	3.71^{aw}	3.31^{aw}	2.50^{ax}
Tibia breaking strength (N)			n=9, SEM = 1.087	
6	31.61^{bx}	36.20^{bx}	33.38^{cx}	34.92^{cx}
7	39.87^{abz}	50.24^{ay}	62.03^{ax}	56.90^{axy}
8	43.39^{ax}	46.40^{ax}	45.91^{bx}	38.5^{bcx}
9	38.50^{aby}	44.62^{axy}	54.03^{abx}	45.9^{bxy}
Tibia ash content (g/kg)			n=9, SEM = 2.02	
6	420^{aby}	438^{abxy}	454^{ax}	446^{bx}
7	427^{ay}	451^{ax}	464^{ax}	460^{abx}
8	414^{aby}	432^{aby}	465^{ax}	471^{ax}
9	407^{bz}	429^{by}	447^{ay}	468^{abx}

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^{x,y,z} Means with a common superscript(s) in a row don't vary significantly ($p<0.01$).

other levels of Ca tested (6 and 8 g/kg diet). The maximum bone strength was observed at 7 g Ca/kg diet when the NPP content was 3.5 and above. In general, the bone strength showed declining trend with increase in dietary Ca levels beyond 7 g/kg.

At lower levels of NPP (3 and 3.5 g/kg diet) the bone ash contents showed a declining trend with increase in the level of Ca in diet (Table 3). The bone ash content was significantly ($p<0.01$) increased by increasing the dietary NPP to 4 g/kg diet at all levels of Ca tested except 7 g/kg diet compared to those fed 3 gNPP/kg diet. But, at 7 g Ca/kg diet increased the bone ash content was observed with increase in dietary NPP up to 3.5 g/kg diet and further increase in NPP didn't affect the bone ash content. However, at 9 gCa/kg diet, the tibia ash content showed a significant increase with the level of NPP up to 4.5 g/kg diet. This trend indicates the higher NPP requirement at higher dietary Ca level to maintain optimum bone calcification.

Serum Ca and inorganic P

The serum Ca content increased significantly ($p<0.01$) with increase in the dietary Ca level at 3 and 4 gNPP/kg diet (Table 4). But the increase in serum Ca level at other levels of NPP (3.5 and 4.5 g/kg) was observed only up to 8 g/kg diet. In general, the concentrations of serum Ca and inorganic P decreased with increase levels of NPP and Ca, respectively in the diet. The serum inorganic P level increased significantly ($p<0.01$) with the level of NPP in the diet up to 4 g/kg diet (Table 4). Increase in P level beyond 4 g/kg diet, showed a declining trend in serum inorganic P level, except at 6 g Ca, where a significant increase in

Table 5. Calcium, phosphorus (g/kg), zinc, manganese, iron and copper contents (mg/kg) in excreta of broilers (at 22 d of age) fed different levels (g/kg) of calcium and non-phytin phosphorus

Calcium	Non-phytin phosphorus			
	3	3.5	4	4.5
Calcium			n=4, SEM =0.36	
6	9.73^{cy}	17.4^{cw}	17.43^{aw}	16.93^{cx}
7	9.75^{cz}	17.48^{ax}	18.1^{bw}	17.0^{cy}
8	14.55^{bz}	15.93^{by}	17.05^{dx}	17.72^{bw}
9	11.65^{ay}	16.52^{bx}	18.8^{aw}	18.95^{aw}
Phosphorus			n=4, SEM = 0.182	
6	7.76^{ay}	7.56^{by}	8.85^{ax}	8.85^{abx}
7	4.98^{cy}	8.91^{ax}	8.91^{ax}	8.67^{abx}
8	5.14^{cy}	8.99^{ax}	9.08^{ax}	8.44^{bx}
9	6.02^{bz}	8.27^{aby}	9.38^{ax}	9.46^{ax}
Zinc			n=4, SEM = 1.36	
6	207^{cy}	224^{aw}	219^{abx}	207^{ay}
7	197^{by}	199^{dy}	221^{aw}	213^{ax}
8	186^{dy}	216^{bw}	218^{bw}	203^{dx}
9	190^{cz}	202^{cy}	214^{cw}	210^{bx}
Iron			n=4, SEM = 18.5	
6	722^{cy}	780^{bxy}	841^{bx}	914^{bw}
7	684^{cy}	995^{abw}	$1{,}013^{aw}$	890^{bx}
8	776^{by}	958^{bw}	904^{bwx}	849^{bx}
9	899^{ay}	$1{,}050^{ax}$	$1{,}072^{ax}$	$1{,}225^{aw}$
Manganese			n=4, SEM = 4.5	
6	335^{ay}	418^{aw}	323^{dz}	364^{ax}
7	296^{by}	323^{dx}	367^{bw}	363^{aw}
8	282^{cz}	377^{cx}	389^{aw}	364^{ay}
9	279^{cz}	411^{bw}	337^{cx}	324^{by}
Copper			n=4, SEM = 0.32	
6	29.0^{ax}	34.5^{aw}	33.8^{aw}	34.5^{aw}
7	28.7^{ay}	35.9^{aw}	33.3^{ax}	33.8^{ax}
8	29.0^{ax}	32.7^{cw}	33.4^{aw}	33.8^{aw}
9	28.6^{ax}	34.0^{bw}	33.7^{aw}	33.6^{aw}

[a,b,c] Means with a common superscript(s) in a column for each parameter don't vary significantly ($p<0.01$).

[x,y,z] Means with a common superscript(s) in a row don't vary significantly ($p<0.01$).

serum inorganic P was observed with increase in NPP from 4 to 4.5 g/kg diet.

Mineral content in excreta

Calcium : In general, the Ca content in excreta was significantly ($p<0.01$) increased by increasing the dietary Ca level from 6 to 9 g/kg diet except at 3.5 gNPP/kg diet (Table 5). At later concentration of NPP, the Ca content in excreta decreased significantly with increase in dietary Ca from 7 to 8 g/kg. The Ca excretion was also increased ($p<0.01$) by increasing the dietary NPP from 3 to 3.5 g/kg at all levels of Ca tested. However, at 7 gCa/kg diet the excreta Ca contents was progressively increased up to 4 g NPP/kg diet. A further, increase in dietary NPP to 4.5 g/kg resulted in significant decrease in excreta Ca content. At 8 g Ca, the excreta Ca showed a significant increase with NPP level up to 4.5 g/kg. However, at 9 gCa/kg diet, the excreta Ca content was not altered due to increase in NPP content from 4.0 to 4.5 g/kg diet.

Phosphorus : At 3 gNPP, the amount of P in excreta was significantly ($p\leq0.01$) decreased with increase in dietary Ca content from 6 to 7 g/kg (Table 5). At 3.5 gNPP, the excreta P content was significantly increased by increase in dietary Ca from 6 to 7 g/kg. Significant increase in the amount of P in excreta was observed with increase in level of P up to 3.5 and 4 g NPP/kg diet, respectively at 7 and 8 g Ca and 6 and 9 g Ca/kg diet. A further increase in NPP didn't influence the amount of P in the excreta.

Zinc : Excretion of Zn increased with the level of P in diet up to 4 gCa/kg. At 6 gCa/kg diet, the excretion of Zn increased with the levels of NPP up to 3.5 g/kg diet. However, at higher levels of Ca (7, 8, and 9 g) the Zn excretion increased with the level of NPP up to 4 g/kg diet (Table 5), a further increase in NPP level to 4.5 g reduced the concentration of Zn in excreta. In general, the excreta Zn concentration was progressively and significantly decreased with the levels of Ca in the diet.

Iron : Increasing the levels of Ca and NPP in diet significantly increased the excretion of Fe. The amount of Fe in the excreta was significantly ($p<0.01$) lower at 3 gNPP compared to those fed higher levels of NPP (3.5 & above). Similarly, the excretion of Fe was significantly increased with the level of Ca in the diet and it was lowest at 6 g and highest at 9 gCa and intermediate in groups in fed 7 and 8 g Ca/kg diet (Table 5).

Manganese : Increasing the dietary NPP level increased the excretion of Mn. The excreta Mn content was significantly ($p<0.01$) increased with increase in level of NPP from 3 to 3.5 g or higher levels/kg diet at all levels of Ca tested (Table 5). In general, at all levels of NPP except 3.5 g NPP/kg diet, the concentration of Mn in excreta was significantly increased with increase in level of Ca in diet up to 8 g/kg and a further increase in Ca level to 9 g/kg depressed the Mn content in excreta.

Copper : In general, the Cu content in excreta was significantly ($p<0.01$) increased with increase in NPP content from 3 to 3.5 gNPP/kg diet and further increase in NPP level resulted no further change in concentration of Cu excreta at 6, 8 and 9 g Ca/kg diet (Table 5). However, at. 7 g Ca, increase in NPP level beyond 3.5 g/kg diet, significantly reduced the Cu content in excreta. The excreta Cu content was not affected by the level of Ca in the diet at 3. 4 and 4.5 g NPP/kg diet, but at 3.5 g Ca the rank order of Cu content in excreta among different Ca levels is 7>(6=8)>9.

Mineral retention in liver

Zinc : The deposition of Zn in liver was maximum at the lowest levels of NPP and Ca tested (3 and 6 g, respectively/kg diet) and the Zn concentration was decreased significantly ($p<0.01$) by increase in the levels of either Ca or NPP in diet (Table 6). The Zn content was

Table 6. Retention of zinc, iron, manganese and copper (mg/kg) in liver of broilers (at 22 d of age) fed different levels (g/kg) of calcium and non-phytin phosphorus

Calcium	Non-phytin phosphorus			
	3	3.5	4	4.5
Zinc			n=6, SEM = 4.1	
6	204^{aw}	128^{bz}	170^{ax}	135^{by}
7	193^{bx}	153^{ay}	118^{cz}	245^{aw}
8	149^{dw}	119^{cyz}	121^{bxy}	113^{dz}
9	188^{cw}	102^{dy}	104^{dy}	124^{cx}
Iron			n=6, SEM = 4.7	
6	322^{aw}	280^{ay}	319^{aw}	305^{ax}
7	284^{bx}	234^{cy}	224^{bz}	307^{aw}
8	214^{dx}	251^{bw}	205^{cy}	255^{bw}
9	225^{cx}	286^{aw}	161^{dz}	210^{cy}
Manganese			n=6, SEM = 0.428	
6	15.7^{aw}	14.17^{ax}	11.56^{ay}	9.67^{az}
7	9.58^{bw}	6.53^{bx}	5.78^{by}	6.09^{by}
8	3.54^{dxy}	3.94^{cx}	3.45^{cy}	4.70^{cw}
9	4.04^{cw}	2.13^{dx}	2.27^{dx}	2.34^{dx}

[a,b,c] Means with a common superscript(s) in a column for each parameter don't vary significantly ($p<0.01$).
[x,y,z] Means with a common superscript(s) in a row don't vary significantly ($p<0.01$).

significantly highest in broiler fed 3 g NPP/kg diet compared to other P levels at all levels of Ca tested except at 7 g Ca and 4.5 g NPP/kg diet. In general, the retention of Zn in liver was significantly reduced with increase in dietary Ca level at all NPP levels tested.

Iron : The concentration of Fe in liver was progressively and significantly decreased with the level of Ca in broiler diet at all levels of NPP tested except at 3.5 g. The retention of Fe was significantly reduced by increasing the Ca level from 6 to 7 g /kg, and further increase in Ca level to 9 g resulted in significantly increase in Fe content similar to those fed 6 g Ca/kg diet (Table 6). Though the level of NPP influenced the concentration of Fe in liver, no specific trends could be observed.

Manganese : The Mn concentration in liver was significantly ($p<0.01$) decreased with increase in level of Ca at all levels of NPP tested (Table 6). Similarly, the retention of Mn in liver was significant decreased as the level of NPP increased at different levels of Ca tested except at 8 g Ca/kg diet. At the later concentration of Ca, the liver Mn content was significantly increased by increase in NPP level from 4.0 to 4.5 g/kg diet. However, at 9 g Ca/kg diet, the retention of Mn in liver was significantly reduced by increasing the P from 3 to $\geq$3.5 gNPP/kg diet.

DISCUSSION

The body weight gain and feed intake were optimum at the lowest levels of Ca and NPP tested (6 and 3 g/kg diet, respectively). The growth depression and reduced feed intake observed by increasing the dietary levels of Ca from 6 to 8/9 g/kg at 3 and 3.5 g NPP/kg may be due to improper utilization of these minerals at wider Ca and NPP ratio (2.33, 2.67 & 3:1). At higher Ca and NPP ratio these two minerals tend to form calcium phosphate, an insoluble complex in the chicken gut and resulting in poor absorption of these mineral (Underwood, 1981; Georgievskii et al., 1982). The depressed performance (weight gain, feed intake and leg abnormalities) observed in broilers fed higher levels of Ca (8 and 9 g/kg) at low dietary P (3 and 3.5 g NPP/kg) was alleviated by increasing the NPP content to 4 and 4.5 g/kg diet. Results indicate that the dietary levels of P should be altered in proportion to the Ca level to maintain optimum ratio between Ca and NPP (2:1). Based on these results, broilers do not require more than 3 gNPP and 6 g Ca/kg diet for optimum growth. Contrary to these findings, several workers suggested higher Ca (Hulan et al., 1985; Muramoto et al., 1998) and NPP (Orban and Roland, 1990; Yu et al., 1990; Rama Rao et al., 1999) levels (9-14.7 g and 3.9-5.1 g/kg diet, respectively) for broiler during starter phase. The higher levels of Ca (9-14.7 g/kg diet) and P (3.9-5.1 g NPP/kg diet) reported by these authors might be due to higher levels of P (3.9 to 6.7 g NPP/kg diet) and Ca (10 g Ca/kg diet), respectively used in their studies. Few authors used higher levels of P (Orban and Roland, 1990; Tortuero et al., 1994) and Ca (Ahmad et al., 1982; Kr-steva et al., 1986; Hossain et al., 1994) and reported the lowest levels used as the requirement. Similar to the results of the present study, several authors (Waldroup et al., 1962 and 1963; Burnell et al., 1990; Scheideler et al., 1995) recommended lower levels of Ca (6 to 7.6) and NPP (2.6 to 3.8 g/kg diet) for optimum performance of broilers. Contrary to the results of the present study, the growth was significantly depressed in broilers fed 3.5 and 3 g NPP/kg diet during starter and finisher phases, respectively compared to those fed 4.5 and 3.5 g NPP/kg diet during the same periods (Lim et al., 2001). The growth depression observed in broilers fed 3.5 and 3 gNPP/kg diet may be due to the higher Ca level (10 g/kg) used in their study.

The data of the present study and also the results of the above authors, suggest that the levels of Ca or NPP should be adjusted to maintain the desired Ca and P ratio (2:1) for obtaining optimum broiler performance. Though Hulan et al. (1985 and 1986) reported higher Ca (11.1-14.7 g/kg) and NPP (6.3 to 6.7 g/kg diet) requirements compared to the levels reported in the present study, the ratio between Ca and NPP used in their study was nearer 2:1. It is well established that the utilization of Ca and P (Rensburg et. al., 1992) is better at 2:1 Ca and P ratio in broilers. The wide variation observed in Ca and NPP requirements for broilers may be due to difference in concentration of vitamin D_3 (Nelson, 1967), PP (Ballam et. al., 1984), energy and protein (Florescu, 1972; Waldroup et. al., 1975) in the diet, environmental temperature (Orban and Roland, 1990) and housing method (Ademosun and Kalango, 1973), which are known to influence the availability or utilization of Ca and P in chicken.

The higher ($p<0.01$) incidence of leg abnormalities at disproportionate ratio of Ca (8 & 9 g/kg) and NPP (3 & 3.5 g/kg) in diet also suggests poor availability of these minerals at wider Ca and P ratio. Similar to these findings, Edwards and Veltmann (1983) also reported high incidence of tibia dyschondroplacia at disproportionate ratio of Ca and P in broiler diet. The data on tibia ash and tibia breaking strength suggest higher P requirement for bone mineralization compared to body weight gain. Similar to these findings few authors reported higher P (Torturo et al., 1994; Rama Rao et al., 1999) and Ca (Waldroup et al., 1963) requirements for bone ash mineralization compared to optimum body weight in broilers.

The decreased concentrations of both Ca and inorganic P in serum with increase in levels of NPP and Ca, respectively could be due to formation of calcium phosphate, an insoluble complex at disproportionate ratio of Ca and NPP in the diet. The calcium phosphate is known to be excreted without being absorbed in to the system from the gut (Underwood. 1981), therefore, the concentration of Ca and P (Table 5) in excreta were higher with increased concentration of NPP and Ca, respectively in the diet. Increased excretion of Zn and Fe (Table 5) with increase in level of NPP from 3 to 4 and 4.5 g NPP/kg diet, respectively and Fe with increase in dietary Ca level (6 to 9 g/kg) also indicate reduced mineral utilization at wider Ca and P ratio in the diet. Similarly, excretion of Mn and Cu (Table 5) increased by increasing the dietary NPP from 3 to 3.5 g/kg. Increased elimination of trace minerals (Mn, Zn, Cu etc.) at higher dietary Ca levels was also reported in the literature (Georgievskii et al., 1982; Russell and McDowell, 1992) owing to the inhibition of their absorption. Decreased concentrations of Zn, Fe and Mn (Table 6) in liver with increase in level of Ca and or NPP in diet also suggest the reduced availability of these minerals at disproportionate or higher levels of Ca and P in diet.

Based on the results it can be concluded that the requirement of NPP and Ca in broiler starter diet is not more than 3 and 6 g/kg, respectively at 1,200 icuD_3/kg for optimum growth and feed intake. Bone mineralization (tibia ash and tibia breaking strength) increased with increase in Ca and NPP in diet. Higher levels of Ca (>6 g/kg) and NPP (>3 g/kg) in diet increased the excretion of Ca, P. Mn, Zn. Fe and reduced the retention of Mn, Fe and Zn in liver.

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