

Relative Bio-Availability of Different Phosphorus Supplements in Broiler and Layer Chicken Diets

S. V. Rama Rao* and V. Ramasubba Reddy

College of Veterinary Science, Rajendranagar, Hyderabad 500 030, India

ABSTRACT : Two experiments on commercial broiler chickens (3-37 d) and WL layers (252-364 d) were conducted to study the relative bioavailability of phosphorus (P) from different P supplements in comparison to tricalcium phosphate (TCP), at constant dietary calcium (Ca):P ratio. The P sources tested were dicalcium phosphate (DCP), steam sterilized bone meal (SSBM), commercial mineral mixture (CMM), phosphoric acid (PA) and a combination of CMM + PA. Fluorine (F) content in CMM and SSBM was 13.12 and 0.14 g/kg, respectively. In commercial broiler diets, DCP, SSBM or PA could be used as supplemental P sources without affecting ($p < 0.05$) weight gain, feed intake, tibia ash and, P and Ca contents in tibia ash when compared to TCP. Severity of leg abnormality and deposition of F in bone were higher ($p < 0.05$) in group fed CMM. P retention and serum inorganic P content was significantly reduced ($p < 0.05$) in CMM fed birds compared to those fed TCP, SSBM, PA or CMM+PA. Among other P sources (TCP, SSBM, PA and CMM+PA) the serum inorganic P levels did not vary significantly ($p < 0.05$). The P retention also significantly reduced in CMM fed groups compared to those fed DCP or PA. The P retention significantly increased from 0.183 to 0.216 units by supplementation of PA to CMM diet. In layers, egg production was not affected by replacing TCP with DCP, SSBM, PA or CMM+PA, but significantly ($p < 0.05$) reduced with CMM. Feed (kg)/kg egg mass, egg weight, shell quality (shell weight and shell thickness) and serum Ca levels were not influenced by dietary variation in P source. The poor performance of both broilers and layers fed on CMM based diets could be attributed to the presence of higher levels of F (647.8 and 630.1 mg/kg, respectively) and low P utilization. Based on growth, bone mineralization and P retention it is concluded that DCP, SSBM or PA can be used as alternatives to TCP in broiler diets. In WL layer diets, in addition to above P sources, CMM can also be used as supplemental P source by replacing one half of P from CMM with PA without affecting egg production and shell quality. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 7 : 979-985)

Key Words : Phosphorus Sources, Fluorine, Weight Gain, Egg Production

INTRODUCTION

Phosphorus (P) is an expensive and critical nutrient in poultry diet. Due to high cost and at times non-availability of dicalcium phosphate (DCP), the traditional source of P in poultry feeds forces to utilize alternative P supplements like bone meals, commercial mineral mixture in poultry diets. However, the bio-availability of P from different inorganic sources varies greatly (Pensack, 1974; Senkoylu, 1983; Osorio and Jensen, 1986; Sullivan et al., 1994). The level of inclusion is one of the major factors determining the P bio-availability to the chicken. The P retention increases as the level of dietary P decreases (Rama Rao et al., 1999). But, majority of the P bioavailability studies conducted in the past (Nelson et al., 1990; Potter et al., 1995) utilized suboptimal levels of dietary P in order to quantify the P availability from different sources. Incorporation of P source at required levels of P in bioavailability studies would reflect the practical conditions and the results obtained from such studies could directly be applicable to field conditions. Therefore, two

experiments were conducted utilizing various P supplements (tricalcium phosphate (TCP), DCP, steam sterilized bone meal (SSBM), commercial mineral mixture (CMM), and phosphoric acid (PA) as a source of supplemental P in commercial broiler and WL layer diets.

MATERIALS AND METHODS

The P sources and feed ingredients were analysed for calcium (Ca), total phosphorus (TP), phytin phosphorus (Haugh and Lantzsich, 1983) and proximate principles (AOAC, 1990). Fluorine (F) content in P supplements, diets and tibia ash was analysed with an ion selective electrode (AOAC, 1990). The non-phytin phosphorus (NPP) content of feed ingredients was calculated by subtracting the phytin phosphorus (PP) content from the TP.

Two experiments were conducted on commercial broiler chickens and WL layers to study the bio-availability of P from DCP, SSBM (feed grade), TCP (analytical grade), PA (laboratory grade), CMM (M/S Venvet Chemicals, Hyderabad, India) and a combination of CMM and PA. The commercial mineral mixture was prepared using raw rock phosphate as P source. The mineral composition of these P sources is presented in table 3.

* Corresponding Author: S. V. Rama Rao. Project Directorate on Poultry, Rajendranagar, Hyderabad 500 030, India. Fax: +91-40-4017002, E-mail: pdpoultry@ap.nic.in.
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Broiler experiment

A reference maize-soya diet was prepared containing about 0.45% NPP and 1.0% Ca utilizing analytical grade TCP as P supplement. In the test diets, TCP was totally replaced with various P supplements (DCP, PA, SSBM and CMM) on P basis. Since, the CMM contained relatively high Ca (32.15%) and low P (6.57%), its inclusion level was restricted to meet the Ca level of 1.0%, resulting in 0.229% NPP in diet. In another diet, PA was supplemented to the CMM diet to provide the required levels of NPP (0.45%). Levels of yellow maize, P sources and oyster shell grit were adjusted to obtain the desired levels of NPP and Ca. Metabolizable energy, crude protein and essential amino acid contents were kept constant in all the experimental diets (table 1).

Two hundred and forty Hubbard day old broiler chicks were distributed equally at random into 24 electrically heated wire floored galvanised iron battery brooder cells. During the initial two days, chicks were

fed on ground yellow maize. Each diet was fed *ad libitum* to four replicate groups of ten chicks each, from 3 to 37 d of age. Individual body weight, feed intake of each replicate and leg abnormality score (Watson et al., 1970) of individual birds were recorded at weekly interval throughout the experimental period. Feed conversion ratio was calculated at weekly intervals as feed intake/weight gain. Blood samples were collected from three birds at random per replicate (twelve birds per treatment) at 38 d of age. Sera was pooled and analysed for Ca (AOAC, 1990) and inorganic P (Pi) contents (Fiske and Subba Row, 1925).

A metabolism trial of 3 d duration was conducted during 38-40 d of age. Three birds per replicate (twelve birds per treatment) were selected at random and were housed in raised wire floored battery brooders. Feed intake and excreta voided were estimated. The samples of feed and excreta were analysed for dry matter, Ca and TP contents. The

Table 1. Composition of diets (%) varying in phosphorus supplements fed to commercial broilers (3-37 d)

Ingredient	Phosphorus source					
	TCP	DCP	SSBM	CMM	PA	CMM+PA
Maize, yellow	66.45	65.47	64.19	65.53	65.04	65.40
Soybean meal	30.00	30.00	30.00	30.00	30.00	30.00
Common salt	0.40	0.400	0.400	0.400	0.400	0.400
Phosphorus source	1.927	1.922	4.165	2.298	0.00	2.298
Phosphoric acid					1.022	0.705
Oyster shell grit	0.020	1.01	0.050	0.570	2.340	0.00
DL-methionine	0.380	0.380	0.380	0.380	0.380	0.380
L-lysine HCl	0.320	0.320	0.320	0.320	0.320	0.320
Vitamin premix ¹	0.330	0.330	0.330	0.330	0.330	0.330
Trace minerals ²	0.120	0.120	0.120	0.120	0.120	0.120
Coccidiostat ³	0.050	0.050	0.050	0.050	0.050	0.050
Nutrient composition						
Analysed						
Total P	0.674	0.659	0.670	0.660	0.652	0.665
NPP ⁴	0.451	0.451	0.450	0.229	0.452	0.452
Calcium	1.029	1.030	1.029	1.029	1.028	1.029
Fluorine (mg/kg)			9.83	647.8		630.1
Crude protein	21.71	21.67	21.61	21.70	21.61	21.63
Calculated						
ME (Kcal/kg)	2,860	2,860	2,839	2,882	2,851	2,866
Lysine	1.431	1.431	1.429	1.432	1.430	1.431
Methionine	0.727	0.727	0.726	0.726	0.726	0.727
Methionine+cystine	1.044	1.044	1.042	1.046	1.043	1.044
Threonine	0.751	0.751	0.749	0.753	0.750	0.751

¹ Vitamin premix provided (mg/kg diet) : thiamin, 1; pyridoxine, 2; cyanocobalamine, 0.01; niacin, 1.5; pantothenic acid, 10; α -tocopherol, 10; riboflavin, 5; menadione, 1; retinol acetate, 8250 IU; cholecalciferol, 1200 ICU, choline 650.

² Trace mineral premix provided (mg/kg diet) zinc, 80; manganese, 90; iron, 60; copper, 5.

³ Coban TM (Monensin sodium 10% w/w).

⁴ Based on analysed values of phytin phosphorus in feed ingredients.

TCP=tricalcium phosphate; DCP=dicalcium phosphate; SSBM=steam sterilized bone meal; CMM=commercial mineral mixture; PA=phosphoric acid.

retention of Ca and P was calculated as proportion of the respective mineral retained per unit intake.

Three birds per replicate were selected at random and were killed by cervical dislocation on 41 d of age to collect both the tibiae. Tibiae were freed of soft tissue including the diaphysis and defatted by soaking in petroleum ether for 48 h. Dried bone samples were ashed at $600 \pm 30^\circ\text{C}$ for 12 h. Ca, P and F contents of the tibia ash were analysed (AOAC, 1990).

Layer experiment

Relative bio-availability of P from DCP, SSBM, CMM and PA in WL layers was studied in this experiment. TCP was used as P source in the reference diet. The P supplements were included in the test diets at levels sufficient to replace the amount of NPP provided by the TCP (2.62 g/kg) in the reference diet. (table 2). In the sixth diet, 50% CMM was replaced with PA. Ca and NPP levels were maintained at 3.5 and 0.35%, respectively, in all the experimental

diets. Water soaked and sun dried saw dust was used as inert material in the diets. Levels of P sources, oyster shell grit and saw dust were adjusted to arrive at the desired levels of Ca and NPP (table 2). Each diet was fed *ad libitum* to four replicates of 18 WL layers each. Birds were housed in individual California type cages, from 252 to 364 d of age. Light was provided for 17 h daily using incandescent bulbs.

Hen day egg production, body weight gain and feed (kg)/egg mass (kg) were recorded and compiled at every 28 d period. All the eggs produced during the last 3 consecutive days of every 28 d period were collected to measure egg weight, shell weight and shell thickness with Dial Thickness Gage (Mitutoyo, No. 7301). At the end of experiment, three birds were selected at random from each replicate and about 5 ml of blood was drawn from the left jugular vein of each hen and sera was pooled replicate wise. The Ca (AOAC, 1990) and inorganic P (Pi) contents (Fiske and Subba Row, 1925) in sera were analysed.

The experimental results were subjected to

Table 2. Composition of diets (%) varying in phosphorus supplements fed to WL layers (252-364 d)

Ingredient	Phosphorus source					
	TCP	DCP	SSBM	CMM	PA	CMM+PA
Maize, yellow	58.17	58.17	58.17	58.17	58.17	58.17
Soybean meal	19.00	19.00	19.00	19.00	19.0	19.00
Sunflower meal	9.00	9.000	9.000	9.000	9.00	9.00
Common salt	0.40	0.400	0.400	0.400	0.400	0.400
Phosphorus source	1.361	1.506	2.938	3.989	0.690	1.895+0.345
Oyster shell grit	8.280	8.858	8.261	5.946	9.834	8.969
Saw dust ³	3.549	2.826	1.991	3.255	2.666	1.981
DL-methionine	0.100	0.100	0.100	0.100	0.100	0.100
Vitamin premix ¹	0.035	0.035	0.035	0.035	0.035	0.035
Trace minerals ²	0.105	0.105	0.105	0.105	0.105	0.105
Nutrient composition						
Analysed						
Total P	0.608	0.608	0.601	0.621	0.611	0.612
NPP ⁴	0.350	0.350	0.350	0.350	0.350	0.350
Calcium	3.51	3.51	3.53	3.50	3.52	3.56
Fluorine (mg/kg)			4.11	523.4		309.1
Crude protein	17.79	17.79	17.79	17.79	17.79	17.79
Calculated						
ME (kcal/kg)	2,543	2,543	2,543	2,543	2,543	2,543
Lysine	0.834	0.834	0.834	0.834	0.834	0.834
Methionine	0.398	0.398	0.398	0.398	0.398	0.398
Methionine+cystine	0.669	0.669	0.669	0.669	0.669	0.669
Threonine	0.572	0.572	0.572	0.572	0.572	0.572

¹ Vitamin premix provided (mg/kg diet): thiamin, 1; pyridoxine, 2; cyanocobalamine, 0.01; niacin, 1.5; pantothenic acid, 10; tocopherol, 10; riboflavin, 5; menadione, 1; retinol acetate, 8250 IU; cholecalciferol, 1200 ICU.

² Trace mineral premix provided (mg/kg diet): manganese, 108; zinc, 104; copper, 4; iodine, 4.

³ Water soaked and sun dried.

⁴ Based on analysed values of phytin phosphorus in feed ingredients.

TCP=tricalcium phosphate; DCP=dicalcium phosphate; SSBM=steam sterilized bone meal; CMM=commercial mineral mixture; PA=phosphoric acid.

statistical analysis (Snedecor and Cochran, 1980) by using randomized block design. The differences among the treatment means were compared with Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The PP content in feed ingredients (table 3) was relatively high in comparison to those reported by previous workers (Nelson et al., 1968; Han, 1989; Eeckhout and De Paepe, 1994). The probable reasons for this difference might be due to variation in cultivar, processing conditions and fertilisation dose in soil (Maga, 1982). The Ca:P ratio in bone meal was maintained at about 2:1. SSBM and CMM contained 0.14 and 13.12 g F/kg, respectively. The presence of high amounts of F in CMM is due to inclusion of rock phosphates as P source.

Broiler experiment

Variation in the source of supplemental P in broiler diet had significant ($p < 0.01$) effect on body

weight gain, feed intake, tibia ash F content, Ca and Pi content in serum and retention of Ca and P. But, feed/gain, tibia ash content and its Ca and P contents were not affected due to variation in P source (table 4).

The weight gain was not affected in broilers fed DCP, SSBM or PA compared to those fed TCP. These sources can be used as the sole source of P in a practical broiler diet. CMM+PA also supported body weight compared to TCP, PA or SSBM, which indicates a possibility of this source to substitute for TCP in broiler ration. The growth depression in CMM group might be due to inadequate levels of NPP (2.29 g/kg) in the diet (table 1). Though the weight gain was improved by supplementation of PA to CMM diet, the growth depression effect was not totally alleviated, which indicates the possible toxic effects of F from CMM. The growth depression ($p < 0.05$) observed in CMM+PA fed chicks, in spite of similar P retention also, confirms the toxic effect of F (630.1 mg/kg) on growth. Reduction in weight gain and feed intake observed in CMM and CMM+PA could be due to the F present in these diets (647.8 and 630.1

Table 3. Analysed mineral composition (%) of feed ingredients and different calcium and phosphorus supplements

Feed ingredient	Calcium	Total phosphorus	Phytin phosphorus	Fluorine
Yellow maize	0.202	0.267	0.196	
Soybean meal	0.441	0.510	0.402	
Sunflower meal	0.595	0.785	0.492	
Tricalcium phosphate (TCP)	37.89	19.26		0.00
Dicalcium phosphate (DCP)	21.01	19.31		0.00
Steam sterilized bone meal (SSBM)	17.67	8.923		0.014
Commercial mineral mixture (CMM)	32.15	6.571		1.312
Phosphoric acid (PA)		31.65		
Oyster shell grit	33.00			

Table 4. Performance of commercial broilers (3-37 d) fed diets containing various sources of phosphorus

P source	Body weight gain (g)	Feed intake (g/b)	Feed/weight gain	Leg abnormality score	Tibia attribute (%)				Serum		Retention coefficient	
					Ash	Ca	P	F	Ca (mg/dl)	Pi (mg/dl)	Ca	P
Tricalcium phosphate	1516 ^{ab}	3256 ^a	2.147	0.00	47.47	17.50	9.547	0.046 ^c	12.6 ^{cd}	3.99 ^a	0.441 ^b	0.219 ^{bc}
Dicalcium phosphate	1575 ^a	3339 ^a	2.124	0.00	47.87	18.43	9.520	0.052 ^c	12.4 ^d	3.86 ^{ab}	0.329 ^c	0.225 ^b
Steam sterilized bone meal	1529 ^{ab}	3325 ^a	2.178	0.00	47.85	17.82	9.385	0.045 ^c	13.5 ^a	4.58 ^a	0.194 ^c	0.218 ^{bc}
Commercial mineral mixture	1200 ^c	2269 ^c	1.905	2.70	48.18	18.12	9.492	0.879 ^a	11.4 ^c	3.12 ^b	0.562 ^a	0.185 ^c
Phosphoric acid	1434 ^{ab}	2845 ^b	1.985	0.00	45.33	17.01	8.902	0.013 ^d	12.7 ^{bc}	4.19 ^a	0.356 ^c	0.342 ^a
CMM+PA	1390 ^b	2954 ^c	2.122	0.00	47.91	18.25	9.682	0.216 ^b	12.9 ^b	4.02 ^a	0.505 ^a	0.216 ^{bc}
SEM(±)	28.98	86.72	0.0378		0.583	0.143	0.222	0.0519	0.109	0.123	0.0264	0.0163
n	4	4	4		6	6	6	6	6	6	4	4

^{a,b,c} Means with different superscripts in a column differ significantly ($p \leq 0.05$).

CMM=commercial mineral mixture, PA=Phosphoric acid.

mg/kg). Growth depression and reduced feed intake was also reported by previous researchers (Suttie et al., 1984; Huyghebaert et al., 1988) when F was incorporated at 200 or 400 mg/kg diet. Some reports indicated that broilers could tolerate even up to 500 mg F/kg diet (Gardiner et al., 1959; Camps, 1985). The large variation observed regarding the level of F tolerance might be due to the differences in the concentration of Ca and P in diet, age and strain of the bird and period of ingestion of F diet (Huyghebaert and De Groote, 1986).

Broilers fed CMM could not stand properly and showed the symptoms of severe leg disorders as observed by leg weakness score (table 4). But, supplementation of PA to CMM diet alleviated the leg problems. The lameness observed in broilers fed CMM probably could be due to presence of toxic levels of F in these diets. Similarly, higher incidences of tibial dyschondroplastic plugs and epiphyseal bone with looser structure was observed in broilers fed 200 or 400 mg F/kg diet (Huyghebaert et al., 1988).

Supplementation of PA to CMM diet increased ($p<0.05$) the P availability which resulted in improved weight gain and feed intake and reduced incidence of lameness in comparison to those fed CMM alone as P source (table 4). Similar beneficial effects of PA addition to soft phosphate was also reported by Gardiner et al. (1959) and Summers et al. (1959) in broilers.

Dietary variation in source of P, though influenced the growth rate, did not exhibit any influence on bone mineralisation (table 4). Similarly, Senkoylu (1983) and Hahn and Guenter (1986) also reported lack of influence of P source and F content on bone mineralization in broilers. These results indicate that weight gain is a more sensitive criterion compared to bone mineralisation parameters. Summers et al. (1959) also reported weight gain as a valuable criterion in P utilization studies. Whereas, Nelson and Walker (1964) reported per cent bone ash as a sensitive criterion

compared to growth for assaying the P availability in poultry. The lack of response in bone calcification observed in the present study might be due to age of the birds at which the bone calcification parameters were studied. The zone of proliferation in developing bone of the young chicks is especially sensitive to nutritional changes and is quickly influenced by P deficiency. The F content of bone was significantly ($p<0.05$) higher in CMM followed by CMM+PA. Deposition of F in tibia did not vary significantly among groups fed TCP, DCP and SSBM. The amount of F deposition was significantly ($p<0.05$) lower in PA compared to those fed other P sources.

The serum Ca levels was significantly ($p<0.05$) lower in CMM fed broilers compared to other P sources. Similarly, serum Pi levels were also significantly lower in chicks fed diet containing CMM as compared to those fed other P sources except DCP. The Ca retention was significantly ($p<0.05$) higher in groups fed CMM or CMM+PA compared to those fed other P sources. The chicks fed PA retained higher P than those fed other P sources. The data on P retention shows that the availability of P from CMM was lowest. The higher dietary F levels in CMM based diets probably reduced the feed intake, P absorption and thereby the serum concentration of Pi and P retention ($p<0.05$) in these groups.

Layer experiment

Variation in dietary P source had significant ($p<0.05$) effect on hen day egg production, feed intake, weight gain and serum Pi content (table 5). Feed per egg mass, egg weight, shell quality (shell weight and shell thickness) and serum Ca levels, however, were not influenced by the source of P in layer diet.

DCP, SSBM, PA and CMM+PA are good sources of P for layers as indicated by their egg production (table 5). The egg production in CMM was significantly less compared to DCP, SSBM or PA.

Table 5. Performance of WL layers (252-364 d) fed diets containing different phosphorus supplements

P source	Egg Production, no/h/d	Feed intake, g/b/d	Feed (kg)/egg mass (kg)	Egg weight, g	Shell weight, g/kg	Shell thickness, mm	Body weight gain, g	Serum, mg/dl	
								Ca	Pi
Tricalcium phosphate	75.5 ^{ab}	114.5 ^b	2.847	53.0	8.485	0.325	120 ^{bc}	29.8	4.16 ^{ab}
Dicalcium phosphate	79.6 ^a	109.3 ^c	2.546	52.8	8.684	0.329	111 ^{cd}	30.1	5.21 ^a
Steam sterilized bone meal	80.8 ^a	118.2 ^a	2.873	53.6	8.608	0.325	124 ^{bc}	30.6	3.25 ^b
Commercial mineral mixture	72.2 ^b	105.3 ^d	2.781	55.3	8.327	0.328	326 ^a	30.3	2.74 ^b
Phosphoric acid	80.7 ^a	110.0 ^c	2.593	52.6	8.598	0.325	98 ^d	30.3	3.91 ^{ab}
CMM+PA	77.9 ^{ab}	108.4 ^c	2.627	54.0	8.640	0.327	135 ^b	30.7	3.95 ^{ab}
SEM (\pm)	0.84	0.89	0.0637	0.308	0.0503	0.0008	16.5	0.220	0.243
n	4	4	4	4	4	4	4	4	4

^{abc,d} Means with different superscripts in a column differ significantly ($p\leq 0.05$).

CMM=commercial mineral mixture, PA=phosphoric acid.

The depressed egg production observed in CMM group could be attributed to the poor availability and retention of P (0.185 vs 0.219) from these sources as observed in the present broiler experiment (table 4) and also to the higher F content in the diets (523.4 mg/kg). Depression in layer performance was reported by Said et al. (1979) due to F toxicity.

Feed intake was significantly decreased on CMM supplemented diet compared to other P sources (table 5). A progressive reduction in feed intake was also observed in other studies (Guenter, 1979; Tues, 1979; Guenter and Hahn, 1986) in chicks fed 400 mg or higher levels of F/kg diet, resulting in reduction in egg production and feed efficiency.

Though the egg production was significantly decreased in CMM fed birds, the dietary variations in source of P did not influence shell quality (table 5). Said et al. (1979) also found depression in egg production and feed efficiency without affecting the shell quality at 648 mg F/kg diet. The dietary F concentration in the present study appears to be too low to influence the egg weight and shell quality.

The serum Pi levels were influenced by the source of P, but no specific trend could be observed. The symptoms of F toxicity like reduced feed intake, and egg production (Van Toledo and Combs, 1984; Hahn and Guenter, 1986) were clearly observed in the present study in layers fed CMM incorporated diet. Significantly higher ($p < 0.05$) weight gain in layers fed CMM during the experimental period perhaps was due to relatively poor egg production in these groups with a resultant diversion of nutrients for body growth.

Based on the growth, bone mineralisation and P retention, it may be concluded that TCP, DCP, SSBM or PA can be used as sole source of supplemental P in broiler diets. In WL layer diet, along with the above P sources, a combination of CMM+PA can also be used as supplemental P source without affecting egg production and shell quality.

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