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Influence of Dietary Non-phytate Phosphorous Levels and Phytase Supplementation on the Performance and Bone Characteristics of Broilers

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ABSTRACT

Precise phosphorus nutrition is important for significant reductions in both P pollution and ration costs. The influence of different levels (%) of dietary nPP fed from 0 to 20 d (0.45, 0.40, 0.35, 0.30, 0.25, compared with feeding 0.20 nPP with and without 500 F.T.U. of phytase per kg of diet) and from 21 to 36 d of age (0.414, 0.364, 0.314, 0.264, 0.214, compared with 0.164 nPP with and without 500 F.T.U. of phytase per kg of diet) were evaluate using a total of 588 day-old commercial broiler chicks. Each treatment was replicated four times in a completely randomized design. Body weight (BW), daily gain (DG), feed intake (DFI), feed conversion ratio, plasma P level and bone characteristics were determined, and from these data the P equivalency of the phytase was estimated. Feeding diets containing less than 0.40% of nPP to birds between 0 and 20d resulted in inferior BW, DFI, plasma P level and bone characteristics compared with the control. However, optimum FCR and mortality was supported at lower levels of nPP (0.25%). Between 21 and 36 d, 0.364% was enough to optimise BW, DFI, and femur ash (%); whilst only 0.314% or greater was needed to support optimum DG, toe and tibia ash and only 0.214 to 0.264% was required to optimise shank, femur and tibia lengths; FCR and survivability. A broken line analysis also showed that the nPP (%) requirement ranged from 0.267 to 0.410 and 0.272 to 0.380% during 0 to 20 and 21 to 36 d, respectively. Phytase supplementation improved performance and bone criterion and its P equivalency, depending upon the response of interest and birds age, ranged from 0.00 to 0.110%. In conclusion, the results showed that the combination of a lower level of nPP and phytase may be used to increase dietary P utilization, without severe changes in performance and bone quality.

INTRODUCTION

Phosphorus (P) is one of the most important and expensive macro minerals required by poultry and plays a vital role in the development and maintenance of the skeletal system and in numerous biochemical reactions in the body. The role of phosphorus and determination of its requirement in broiler nutrition has received a great deal of attention, and continues to do so, especially in the context of pollution from phosphorus run-off from agricultural operations (Waldroup, 1999; Bedford, 2000; Kies *et al.*, 2001; Adeola & Sands, 2003). An important aspect of phosphorus nutrition is the digestibility of phytate phosphorus, a form of phosphorus that naturally occurs in feedstuffs of plant origin. In legumes and grains, phytate phosphorus represents about 50 to 75 percent of the total phosphorus content. Swine and chickens lack sufficient endogenous phytase to meaningfully digest the phosphorylated cyclic sugar alcohols, or phytate, present in their feed (Bedford, 2000; Adeola & Sands, 2003; Selle *et al*., 2006).

The poor utilization of phytin-bound P by monogastric animals and its consequences on diet cost, the environment, and digestibility of minerals and protein have lead to extensive research efforts to understand the process of phytic acid digestion (Bedford, 2000; Kies *et al*., 2001; Adeola & Sands, 2003; Powell *et al*., 2008). During the last decade, several alternative methods for reducing the aforementioned negative impacts of phytate P on the environment and poultry performance have been recommended. Feeding birds with diets formulated closer to their nPP requirements, coupled with use of microbial phytase, are among the most successful strategies that have attracted scientific and practical attention (Simons *et al*., 1990; N.R.C, 1994; Summers, 1997; Sohail & Roland, 1999; Waldroup, 1999). It has been shown that the supplementation of poultry diets with phytase increases trace mineral absorption and amino acid digestibility, and reduces the amount of phosphorus in the manure. The extent of the observed effects, however, will depend on diet type (i.e., main cereals/oilseed meals), phytase inclusion rate, degree of inorganic phosphorus replacement, and dietary phosphorus content relative to the animal needs (Correl, 1999; Waldroup, 1999; Bedford, 2000; Kies *et al*., 2001; Adeola & Sands, 2003; Yan & Waldroup, 2006; Olukosi *et al*., 2007, 2008). With regards to the effect of dietary phosphorus, researchers have found that lower concentrations of dietary phosphorus tend to increase the utilization of phytate phosphorus (Kilburn & Edwards, 2001) even in the absence of phytase.

The first objective of this study was to evaluate the influence of dietary nPP level on the performance and bone length and mineralization of broilers. The second objective was to estimate inorganic P equivalency of phytase considering different performance and bone criteria as dependent variables.

MATERIALS AND METHODS

Birds, housing and management

Five hundred and eighty eight one-day-old Ross® 308 broiler chicks (mixed sex) were used in a 36-day trial. Chicks were housed in 28 floor pens (1.25x1.4m), with wood shavings used as litter, throughout the experiment. Lighting was continuous for the first day post hatching, after which a 23L: 1D schedule was maintained for the duration of the experiment. Temperature was maintained between 30 and 32ºC at the beginning of the rearing period and was gradually decreased by 2 to 3 °C each week to a final temperature of 22 °C at the end of rearing period. Chicks were given free access to the mash diets and to water during the experimental period. Care and management of the chicks were in accordance with commercial guidelines and were approved by University of Kurdistan Animal Ethics Committee.

Treatments and experimental design

Seven treatments were applied with different nPP concentrations (%), with the lowest nPP level being offered with or without microbial phytase at 500 F.T.U./kg feed. Each experimental treatment was replicated four times and each replicate pen contained 21 chicks in a completely randomized experimental design.

Nutritional composition of the diets

The corn-soybean meal-based starter diets were formulated to meet or exceed the requirements (NRC, 1994) for all nutrients, with the exception of nPP (Tables 1 and 2). Experimental diets formulated to contain different nPP levels (%) during 0 to 20 d (0.450, 0.400, 0.350, 0.300, 0.250, 0.200, and 0.200+500 F.T.U. kg^{-1} of phytase*) and 21 to 36 d (0.414, 0.364, 0.314, 0.264, 0.214, 0.164, and 0.164+500 F.T.U. kg^{-1} of phytase). Basal diets were formulated to be isonitrogenous and isocaloric (Tables 1 and 2). The nutritional contents of the dietary ingredients were estimated from the feed composition tables of the National Research Council (1994); however, P and Ca contents in $CaCO_3$ and DCP were measured by the supplier. The microbial phytase (EC 3.1.3.8; 10000 Ug^{-1}, one unit of phytase activity is defined as the quantity of enzyme required to produce 1 μmol of inorganic P/min from 5.1 mmol /L of sodium phytate at a pH of 5.5 and a temperature of 37 °C) used in this study was an *Aspergillus niger* product (Natuphos, BASF) obtained from Vetaque company (BASF product distributor), P.O. Box 14655/ 174, Tehran, IRAN.

Parameters

Birds were weighed as a group on arrival and at 20 and 36 days of age on a pen basis. Feed intake recorded at 20 and 36 days. Feed conversion ratio (FCR) was calculated as feed intake/weight gain. Mortality was daily recorded and feed intake data

* Natuphos, BASF Aktiengesellschaft Strategic Marketing Animal Nutrition, 67056 Ludwigshafen, Germany

Table 1 - Composition (g/kg) and calculated analysis of starter diets (1-20d).

	Dietary NPP Levels(g/kg)						
	2.0	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**
Phytase[1](FTU/kg)	500	0	0	0	0	0	0
Ingredients	($g\ kg^{-1}$)						
Corn grain	566.6	566.6	566.6	566.6	566.6	566.6	566.6
SBM (440 $g\ kg^{-1}$ CP)	368.0	368.0	368.0	368.0	368.0	368.0	368.0
Fish meal (630 $g\ kg^{-1}$ CP)	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Soy oil	14.9	14.9	14.9	14.9	14.9	14.9	14.9
$CaCO_3$	19.6	19.6	17.8	16.0	14.2	12.4	10.7
D.C.P[2]	0.00	0.00	3.0	5.9	8.9	11.8	14.8
Washed sand	5.8	5.8	4.6	3.5	2.3	1.2	0.00
Common Salt	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral premix[3]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin Premix[4]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL. Methionine	1.6	1.6	1.6	1.6	1.6	1.6	1.6
L-Lysine HCL	0. 6	0.6	0.6	0.6	0.6	0.6	0.6
Calculated nutrient composition (g/kg)							
ME (MJ\Kg)	12.14	12.14	12.14	12.14	12.14	12.14	12.14
CP	222.3	222.3	222.3	222.3	222.3	222.3	222.3
CA	9.4	9.4	9.4	9.4	9.4	9.4	9.4
Total Phosphorus	4.4	4.4	4.9	5.4	5.9	6.4	6.9
Non-Phytate Phosphorus	2.0	2.0	2.5	3.0	3.5	4.0	4.5
Methionine	5.4	5.4	5.4	5.4	5.4	5.4	5.4
TSAA	8.9	8.9	8.9	8.9	8.9	8.9	8.9
Lysine	13.3	13.3	13.3	13.3	13.3	13.3	13.3
Na	1.5	1.5	1.5	1.5	1.5	1.5	1.5

1 - Natuphos, 10000 F.T.U. /g , BASF Aktiengesellschaft Strategic Marketing Animal Nutrition, 67056 Ludwigshafen, Germany. 2 - Contain 250 $g\ kg^{-1}$ Ca and 180 $g\ kg^{-1}$ P. 3 - Provides per kg of diet: Vit. A, 9000 I.U.; Vit. D3, 2000 I.U.; Vit. E, 18 I.U.; Menadion, 2 mg; Thiamine, 1.8 mg; Riboflavin, 6.6 mg; Niacin, 30 mg; Pyridoxine, 3 mg; Vit B12, 15 mcg; D-Pantothenic acid , 100. mg; Folic acid, 1 mg; Biotin , 0.1 mg; Choline chloride, 500mg; Antioxidant, 100 mg. 4 - Provides per Kg of diet: Manganese, 100 mg; Zinc, 84.7 mg; Iron, 50 mg; Copper, 10 mg; Iodine, 1 mg; Se, 0.2 mg.

Table 2 - Composition (g/kg) and calculated analysis of grower diets (21-36d).

	Dietary NPP Levels (g/kg)						
Phytase[1](FTU/kg)	**1.64**	**1.64**	**2.14**	**2.64**	**3.14**	**3.64**	**4.14**
Ingredients	500	0	0	0	0	0	0
Corn grain	($g\ kg^{-1}$)						
SBM (440 $g\ kg^{-1}$ CP)	603.3	603.3	603.3	603.3	603.3	603.3	603.3
Fish meal (630 $g\ kg^{-1}$ CP)	330.8	330.8	330.8	330.8	330.8	330.8	330.8
Soy oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0
$CaCO_3$	24.7	24.7	24.7	24.7	24.7	24.7	24.8
D.C.P[2]	19.6	19.6	17.8	16.1	14.3	12.5	10.7
Washed sand	0.00	0.00	2.9	5.9	8.8	11.8	14.8
Common Salt	5.9	5.9	4.7	3.6	2.4	1.2	0.00
Mineral premix[3]	3.1	3.1	3.1	3.1	3.1	3.1	3.1
Vitamin Premix[4]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL. Methionine	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L-Lysine HCL	1.9	1.9	1.9	1.9	1.9	1.9	1.9
	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Calculated nutrient composition (g/kg)							
ME(KJ\kg)	12.56	12.56	12.56	12.56	12.56	12.56	12.56
CP	203.4	203.4	203.4	203.4	203.4	203.4	203.4
Ca	8.8	8.8	8.8	8.8	8.8	8.8	8.8
Total Phosphorus	3.98	3.98	4.48	4.98	5.48	5.98	6.48
Non-Phytate Phosphorus	1.64	1.64	2.14	2.64	3.14	3.64	4.14
Methionine	5.3	5.3	5.3	5.3	5.3	5.3	5.3
TSAA	8.0	8.0	8.5	8.5	8.5	8.5	8.5
Lysine	11.9	11.9	11.9	11.9	11.9	11.9	11.9
Na	1.5	1.5	1.5	1.5	1.5	1.5	1.5

1 - Natuphos, 10000 F.T.U. /g , BASF Aktiengesellschaft Strategic Marketing Animal Nutrition, 67056 Ludwigshafen, Germany. 2 - Contain 250 $g\ kg^{-1}$ Ca and 180 $g\ kg^{-1}$ P. 3 - Provides per kg of diet: Vit. A, 9000 I.U.; Vit. D3, 2000 I.U.; Vit. E, 18 I.U.; Menadion, 2 mg; Thiamine, 1.8 mg; Riboflavin, 6.6 mg; Niacin, 30 mg; Pyridoxine, 3 mg; Vit B12, 15 mcg; D-Pantothenic acid , 100. mg; Folic acid, 1 mg; Biotin , 0.1 mg; Choline chloride, 500mg; Antioxidant, 100 mg. 4 - Provides per Kg of diet: Manganese, 100 mg; Zinc, 84.7 mg; Iron, 50 mg; Copper, 10 mg; Iodine, 1 mg; Se, 0.2 mg.

were corrected for body weight of dead birds. Average body weight (BW), daily gain (DG), daily feed intake (DFI), and FCR were determined for each period and for the overall experiment. At the age of 20 and 36 days, four birds from each treatment, each as close to the pen average weight as possible, were selected for bone measurements. Birds were weighed, killed by cervical dislocation, and the whole left leg (including femur, tibia, shank) from each of the chicks was excised and defleshed without boiling. Legs were individually sealed in plastic bags to minimize moisture loss, and stored at -20 °C until analysis. Legs were thawed before bone characteristics were determined. The femur, tibia and shank of each leg were excised by making a cut at the adjacent anterior and posterior joints. Femur, tibia and shank length was measured with a calliper with an accuracy of 0.001 cm. After removing the fat by extraction (femurs and tibias) in refluxing petroleum ether in a *Soxhlet apparatus* and drying at 105 °C until a consistent weight was obtained, bone ash was determined by ashing in tarred ceramic crucibles for 12h at 605 °C (AOAC,1990). The ash content was expressed as grams of ash per 100 grams of the defatted dry weight. The middle toe ash content was also determined at 36 d following the procedure above; but without defatting.

On 20d, blood samples were taken from four birds per dietary treatment to determine the phosphorus level of plasma, using an enzymatic kit produced by Zist Chimie Company (Tehran, Iran).

Statistical analysis

Pen average was used as the experimental unit for statistical analysis. Data were analyzed according to the General Linear Models (GLM) procedure of SAS statistical package (SAS institute, 1991) as a completely randomized experimental design. Mortality data were transformed to $\sqrt{x+1}$ prior to analysis. Significant differences among treatments were determined at p<0.05 using Duncan's new multiple range tests. In addition, nonlinear regression analysis was conducted to estimate nPP levels (inflection points) required for maximum performance parameters and bone characteristics using the PROC NLIN procedure of SAS (SAS Institute Inc., 1991), incorporating the SAS macro of Robbins (1979, 1986). Linear ($Y=a+bx$), logarithmic ($Y=a+\ln(x)$) and quadratic ($Y=ax^2+bx+c$) equations were derived from nPP (%) concentration (x as independent variable) and the variables of interest (Y as dependent variable). These equations were solved to calculate optimal nPP concentrations and P equivalency values of the phytase. The relationship between nPP level and performance and bone characteristics was studied using the CORR procedure of SAS (SAS institute, 1991).

RESULTS AND DISCUSSION

Effect of nPP level

The results of the experiment are summarized in Tables 3 to 9.

Starter period (0 to 20 d)

BW, DG, DFI, and plasma P level were significantly (p<0.05) reduced when diets containing less than 0.40 % nPP were offered (Table 3). FCR, on the other hand, significantly (p<0.05) deteriorated only when dietary nPP level was lower than 0.30%, whilst mortality increased only when nPP levels were as low as 0.2%. Feeding the 0.20% nPP diet had severe negative impacts on all assessed performance parameters.

Bone ash content and length was also significantly affected by reducing dietary nPP levels during the 0 to 20d period (Table 3). Tibia and femur ash content (%) deteriorated when dietary nPP was reduced below 0.40%. The length of different parts of the leg, including shank, tibia and femur, was also reduced (p<0.05) when dietary nPP level was reduced. The femur (optimal nPP level not lower than 0.45%) was more sensitive than the shank (0.40% or more required), which in turn was more sensitive than the tibia (0.35%).

Non-linear regression analysis (Table 8) showed that while 0.38 to 0.40 % nPP was sufficient to support most performance parameters between 0 and 20 d, higher nPP levels (>0.41%) were needed to maintain bone ash content and length. Pearson correlation analysis (Table 6) also demonstrated a highly positive correlation between dietary nPP level and most performance and bone parameters, while significant negative correlations were observed between dietary nPP level and feed conversion ratio and mortality rate during this period.

Grower period (21-36 d)

BW and DFI were significantly (p<0.05) decreased when dietary nPP was reduced to less than 0.364% (Table 4), which was considerably more nPP than required to maintain DG (0.314%); FCR (0.214%), and bird survival (0.264%). It is important to note, however, that BW data are a composite of the growth rate during the 0-20 and 20-36 day periods, whereas all other parameters were exclusive to the 20-36 period.

Table 3 - Effect of nPP levels and phytase supplementation on performance and bone characteristics of broiler chicks from 0 to 20 d fed corn-soybean meal based diet.

NPP (%)	Phytase (F.T.U./kg)	BW, g (20 d)	DG, g	DFI, g	FCR, (g/g)	Plasma P level	Shank length (mm)	Tibia		Femur		
								Length (mm)	Ash (%)	Length (mm)	Ash (%)	Mort. (%)
0.45	0	605a	28.5a	48.6a	1.71c	6.90a	54.5a	68.8a	36.5a	53.5a	38.2a	6.0b
0.40		632a	29.6a	50.1a	1.70c	5.79ab	53.6ab	67.1ab	36.6a	50.8b	34.0ab	7.1b
0.35		557b	26.1b	44.1b	1.69c	5.27b	52.3bc	66.1abc	33.8b	50.6b	31.58bc	6.0b
0.30		498c	23.2c	40.6bc	1.75c	4.68b	51.6bc	64.3bcd	29.3dc	47.9c	26.9dc	4.8b
0.25		415d	18.9d	41.1bc	2.18b	4.53b	51.8bc	63.2cde	30.1c	46.6c	25.8d	7.1b
0.20		312e	13.0e	37.3c	2.54a	4.51b	48.7d	61.0e	26.7d	43.9d	22.7d	25.0a
0.20	500	428d	19.2d	42.3b	2.21b	4.92b	50.6dc	62.7ed	28.6dc	45.8dc	26.1d	9.5b
P- value		0.0001	0.0001	0.0001	0.0001	0.0141	0.0004	0.0002	0.0001	0.0001	0.0001	0.0001
SEM		13.016	0.570	1.431	0.057	0.461	0.729	0.982	1.026	0.828	1.828	2.220

BW, body weight; DG, daily gain; DFI, daily feed intake; FCR, feed conversion ratio; Mor., mortality.

Table 4 - Effect of nPP levels and phytase supplementation on performance and bone characteristics of broiler chicks from 21 to 36 d fed corn-soybean meal based diet.

NPP (%)	Phytase (F.T.U./kg)	BW, g (36 d)	DG, g	DFI, g	FCR (g/g)	Toe ash (%)	Shank length (mm)	Tibia		Femur		
								Length (%)	Ash (%)	Length (%)	Ash (%)	Mor. (mm)
0.414	0	1640ab	65.7a	133.2ab	2.03bc	13.3a	78.7a	95.4a	31.4a	71.9a	40.0a	5.1d
0.364		1712a	68.1a	136.2a	2.00bc	13.2a	77.2a	97.6a	30.5ab	72.3a	39.8a	6.5d
0.314		1557b	63.3a	124.5b	1.97c	12.6a	75.0ab	92.3a	30.0ab	69.4a	33.1b	5.1d
0.264		1329c	51.6b	111.3c	2.17b	11.1b	74.5ab	91.8a	28.6bc	68.8a	32.5b	10.0dc
0.214		1051de	36.1dc	76.3e	2.12bc	10.8b	69.2bc	85.2b	26.1d	60.3b	28.5b	19.2b
0.164		928e	31.2d	73.7e	2.36a	8.8c	65.1c	82.7b	24.7d	58.5b	27.6b	39.9a
0.164	500	1100d	41.3c	86.9d	2.11bc	9.7bc	63.3c	82.5b	27.8cd	62.6b	33.0b	13.2bc
P- value		0.0001	0.0001	0.0001	0.0017	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SEM		43.648	1.781	3.416	0.057	0.465	2.130	2.072	0.751	1.572	1.274	2.334

BW, body weight; DG, daily gain; DFI, daily feed intake; FCR, feed conversion ratio; Mor., mortality.

Table 5 - Effect of nPP levels and phytase supplementation on performance and bone characteristics of broiler chicks from 0 to 36 d fed corn-soybean meal based diet.

NPP (%)		Phytase (F.T.U./kg)	DG, g	DFI, g	FCR (g/g)	Mor. (%)
0-20 d	21-36d					
0.45	0.414	0	44.6ab	85.2a	1.91c	10.7d
0.40	0.364		46.1a	87.1a	1.89c	13.1d
0.35	0.314		42.2b	78.8b	1.87c	10.7d
0.30	0.264		35.5c	71.1c	2.01bc	14.3dc
0.25	0.214		26.1d	55.9d	2.14b	25.0b
0.20	0.164		19.5e	50.3e	2.56a	54.8a
0.20	0.164	500	28.3d	60.7d	2.15b	21.4bc
P- value			0.0001	0.0001	0.0001	0.0001
SEM			0.918	1.753	0.054	2.800

BW, body weight; DG, daily gain; DFI, daily feed intake; FCR, feed conversion ratio; Mor., mortality.

Whilst feeding a diet containing 0.314% of nPP was enough to sustain toe and tibia ash (%, Table 4), a higher nPP level was required to maintain femur ash content (0.364%) as compared to the control. Bone length data showed that less nPP was required to support the length of different leg segments (0.264% nPP) as compared to ash. Nonlinear regression estimates of the nPP requirement between 21 and 36d (Table 9) also showed that, as compared to 0 to 20d, less nPP was required to maintain BW, DG, DFI, and bone characteristics. However, the amount of nPP (%) required for optimal FCR and bird survival remained approximately at the same level during the 0 to 20 d growth period.

Pearson correlation analysis (Table 6, 21 to 36d) also showed highly significant positive correlations

Table 6 - Correlation (Pearson) coefficients matrix between nPP level and performance & bone parameters.

Criteria	0 to 20 d		21 to 36 d	p-value
	Coefficient	p-value	Coefficient	
Performance				
BW, g	0.9355	0.0001	0.9253	0.0001
DG, g	0.9355	0.0001	0.9262	0.0001
DFI, g	0.8183	0.0001	0.9202	0.0001
FCR, $g.g^{-1}$	-0.8096	0.0001	-0.6398	0.0008
Mor.	-0.5771	0.0003	-0.8066	0.0001
Bone ash, %				
Toe	-		0.8461	0.0001
Femur	0.8727	0.0001	0.9146	0.0001
Tibia	0.8789	0.0001	0.8794	0.0001
Bone length, mm				
Femur	0.8883	0.0001	0.8621	0.0001
Tibia	0.8242	0.0001	0.7972	0.0001
Shank	0.7817	0.0001	0.7663	0.0001
Blood serum P	0.6750	0.0003	-	

BW, body weight; DG, daily gain; DFI, daily feed intake; FCR, feed conversion ratio; Mor, mortality.

between dietary nPP level and most measured traits and a negative correlation between dietary nPP and FCR and mortality rate.

Dietary nPP levels had significant effects on DG, DFI, FCR and mortality rate (%) over the whole study period (Table 5). Chicks fed diets containing 0.40 % nPP between 1 to 21d, and 0.364% nPP between 21 to 36d had the best ($p<0.05$) DG and DFI. Feeding diets containing lower levels of nPP (0.25 and 0.214% during 0 to 20d and 21 to 36d, respectively) was adequate to minimize total mortality rate and FCR.

The fact that reducing nPP levels during the starter period to 0.40% did not adversely affect broiler performance, but further reduction to less than 0.35 % did, indicates that the broiler's requirement for phosphorus is lower than current recommendations. Data from literature on nPP requirements during the various growth periods is equivocal, with some papers suggesting lower nPP levels than those recommended by the NRC (1994) are required to constrain growth (Sohail & Roland, 1999; Waldroup, 1999) and others suggesting these levels are already deficient (NRC, 1994).

The results of the present experiment also indicated that the bird's response to dietary nPP levels depends on growth phase (starter vs. grower) and the criteria used as an indicator of phosphorus adequacy. These finding are also consistent with the results reported on nPP requirements of broiler chicks in the finisher and withdrawal periods (NRC, 1994; Waldroup, 1999), in which P requirements of broilers decrease with age. This is partly due to an age-related increase in feed intake, but also to the maturation of skeletal systems and thus reduced P requirements for bone mineralization.

Table 7 - Calculated phosphorus releasing ability (equivalency) of phytase using different performance and bone parameters as assessment criterion.

Criteria	0 to 20 d				21 to 36 d			
	Linear	Logarithmic	Quadratic	Average	Linear	Logarithmic	Quadratic	Average
Performance								
BW	0.06	0.06	0.06	0.060	0.04	0.04	0.04	0.040
DG	0.06	0.06	0.05	0.057	0.05	0.05	0.05	0.050
DFI	0.10	0.09	0.10	0.097	0.05	0.04	0.04	0.040
FCR	0.04	0.04	0.04	0.040	0.12	0.12	0.09	0.110
Mor.	0.12	0.12	0.08	0.107	0.13	0.12	0.09	0.110
Bone ash, %								
Toe	-	-	-	-	0.03	0.03	0.03	0.030
Femur	0.06	0.06	0.07	0.063	0.11	0.11	0.11	0.110
Tibia	0.04	0.04	0.04	0.040	0.05	0.05	0.04	0.047
Bone length, mm								
Femur	0.04	0.04	0.04	0.040	0.05	0.06	0.06	0.057
Tibia	0.04	0.04	0.04	0.040	0.00	0.00	0.01	0.003
Shank	0.05	0.05	0.05	0.050	0.00	0.00	0.00	0.000
Blood serum P	0.09	0.08	0.11	0.093	-	-	-	-

BW, body weight; DG, daily gain; DFI, daily feed intake; FCR, feed conversion ratio; Mor, mortality. Linear $y=a+bx$; logarithmic $y=a+\ln(x)$, quadratic $y=ax^2+bx+c$.

Table 8 - Nonlinear regression estimate of nPP requirements of broiler chicks from 0 to 20d.

	Inflection point[1]	Value at inflection	Asymptotic standard error	Asymptotic 95% confidence interval
Performance				
BW at 20d, g	0.3806	619.0	0.0105	0.3587-0.4025
DG, g	0.3745	29.03	0.0093	0.3551-0.3939
DFI, g	0.4040	48.59	0.0345	0.3323-0.4757
FCR, g:g	0.3076	1.70	0.0084	0.2901-0.3251
Mor., %	0.2667	5.95	0.0206	0.2239-0.3095
Bone ash, %				
Femur	0.4793	39.23	.	.
Tibia	0.4102	36.47	0.0274	0.3532-0.4672
Bone length, mm				
Femur	0.3879	52.14	0.0282	0.3297-0.4466
Tibia	0.4482	68.81	0.0450	0.3546-0.5418
Shank	0.4443	54.53	0.0511	0.3381-0.5506
Serum P level,	0.3208	4.70	0.0525	0.2112-0.4304

1 - Defined as the break point of dietary nPP concentrations as a function of the selected variables according to a nonlinear least squares analysis (Robbins, 1989; SAS Institute, 1991). BW, body weight; DG, daily gain; DFI, daily feed intake; FCR, feed conversion ratio; Mor, mortality.

Table 9 - Nonlinear regression estimate of nPP requirements of broiler chicks from 21 to 36d.

	Inflection point[1]	Value at inflection	Asymptotic standard error	Asymptotic 95% confidence interval
Performance				
BW at 36d, g	0.3452	1675.80	0.0129	0.3184-0.3720
DG, g	0.3344	66.91	0.0103	0.3131-0.3558
DFI, g	0.3413	134.70	0.0138	0.3126-0.3699
FCR, g:g	0.3140	2.00	0.0492	0.2116-0.4164
Mor., %	0.2725	5.53	0.0097	0.2524-0.2927
Bone ash, %				
Toe	0.3429	13.26	0.0256	0.2896-0.3963
Femur	0.3798	40.01	0.0230	0.3319-0.4277
Tibia	0.3759	31.44	0.0277	0.3182-0.4336
Bone Length, mm				
Femur	0.3344	72.07	0.0202	0.2924-0.3764
Tibia	0.3589	96.49	0.0358	0.2842-0.4334
Shank	0.3723	78.70	0.0428	0.2833-0.4613

1 - Defined as the break point of dietary nPP concentrations as a function of the selected variables according to a nonlinear least squares analysis (Robbins, 1989; SAS Institute, 1991). BW, body weight; DG, daily gain; DFI, daily feed intake; FCR, feed conversion ratio; Mor, mortality.

Similar results were reported by Sohail & Roland (1999), in which neither performance nor bone strength was significantly influenced as a result of nPP reduction from 0.425 to 0.325% nPP fed to 3-to-6 wk broiler chicks, further supporting lower nPP requirements for broilers than those currently suggested by the NRC (1994). The current results suggest that feeding 0.40% followed by 0.35 % nPP diets during starter and grower periods respectively results in sufficient retainable phosphorus to support optimal live weight and feed intake, with such levels actually being in excess of that required for maintaining feed conversion ratio or mortality rate comparable to controls.

The higher sensitivity of the femur as compared to the shank and tibia to reduced nPP levels mirrors the degree of maturation of these bones at the ages investigated. It also suggests that the selection of specific bones as indicators of bone development should take into account the age of the bird at the time of sampling. It is clearly of interest to ensure that the bone selected is still growing and mineralising to maximise the likelihood of detecting differences between treatments.

Phytase effect

The results of the present experiment suggest that between 0 and 20 d, the addition of 500 F.T.U. of phytase to the nPP deficient diet (0.20 % nPP) significantly ($p<0.05$) improved BW, DG, DFI and FCR, and decreased mortality rate (Table 3). However, the effects on plasma P level, bone length, and ash content were not significant ($p>0.05$). The addition of 500 F.T.U. of phytase to the diet between 21 and 36 d significantly ($p<0.05$) improved BW, DG, DFI, FCR, mortality rate, tibia and femur ash weights, but the effects on toe ash (%), shank length, tibia and femur lengths, and ash percentage were not significant ($p>0.05$) as compared to the control treatment (Table 4). Over the whole experimental period the addition of phytase significantly ($p<0.05$) improved DG, DFI, FCR, and decreased morality rate (Table 5).

The P equivalency of the phytase (500 F.T.U./kg) was estimated using linear, logarithmic, and quadratic equations linking dietary nPP level with each performance and bone parameters of interest. Estimates of phosphorus equivalency of the phytase treatment (Table 7) varied and were dependent on the performance or bone characteristic used as the criterion for assessment. For example, during the 0 to 20 d growth period, whilst the phosphorus equivalency of phytase ranged between 0.04 and 0.06 when BW, DG and FCR, respectively, were used as assessment criteria, it increased to approximately 0.10% when DFI or mortality rate were considered. Furthermore, P equivalency of phytase for some variables such as DFI, FCR, and femur ash changed with age (some reducing and some increasing with age); for the remaining variables these values remained relatively constant.

The beneficial effects of phytase on broiler chicks have been reported previously by many researchers. Sohail & Roland (1999) observed that when the nPP level of diets fed to broiler chicks between 4-6 wk of age were set at 3.30 g kg^{-1}, there was little, if any, positive effect of added phytase on performance. The response to phytase became significant at lower nPP levels (2.25 g kg^{-1}). Low nPP diets typically result in reduced gain and feed intake and sometimes there is a mildly adverse effect on FCR. Performance is often restored with the addition of phytase (Simons *et al*., 1990; Sohail & Roland, 1999; Yan *et al*., 2001; Punna & Roland, 2001; Ravindran *et al*., 2001; Lan *et al*., 2002; Viveros *et al*., 2002; Yan *et al*., 2003; Shirley & Edwards, 2003; Karimi, 2006), provided the initial deficiency was not too severe and the addition rates of phytase were adequate.

The multifaceted effects of phytase in practical diets are increasingly appreciated. It is possible that the observed performance response may reflect the release of P, available amino acids, and energy, alone or in combination. Generally, an improvement of 1.5 to 3.0 percentage points in performance was often observed in piglets and broilers when phytase was included in the diet, even when the diet met the digestible/available P requirement (Kies *et al*., 2001), suggesting significant extra-phosphoric effects of this enzyme. Nevertheless, the response of broilers to phytase is still not predictable, because there are many factors, including, concentration and source of phytin, protein quality, concentrations of divalent cations, vitamin D, mineral chelators in the diet, along with animal factors such as species, genetics and sex, and even diet processing, that may potentially affect the bird's response to dietary phytase supplementation (Kies *et al*., 2001; Adeola & Sands, 2003). The findings of this experiment indicate that the scale of the positive effect of phytase for broilers varies and is very much dependent upon the age and assessment criterion employed. Caution is therefore needed when utilising a phytase product, and it must be taken into account that P equivalency of a phytase is not a constant that can be used in practical diet formulation.

In conclusion, the results of this experiment showed that feeding low nPP diets reduced bird performance and bone mineralization; however, the response to nPP level is not constant and depends on the measured trait and/or bird age. In addition, though supplementation of phytase is effective in offsetting at least some of the negative effects of lowering dietary nPP level, caution must be taken when setting rigid P equivalency values for a phytase product in practical diet formulation. Despite the numerous studies that have been undertaken to assess the effectiveness of phytase in broiler nutrition, more research is needed to determine more accurately P equivalency of phytase under different nutritional and management practices.

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