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## Effects of Microbial Phytase Supplementation on Mineral Composition of Tibia and Mineral Utilization in Broiler Fed Maize - Based Diets

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**Abstract:** An experiment was conducted to determine effect of supplementing maize - based diet with different levels of microbial phytase on apparent nutrient bioavailability and tibia bone mineral of broilers. Eighty, day-old broiler were randomly allotted to five dietary treatments. The control diet contained 0 FTU/kg microbial phytase which was replaced by 200, 400, 600 and 800 FTU/kg microbial phytase of the basal feed combinations. Phytase supplementation of diet increased P, Ca, Zn, Cu and Phytate - P bioavailability significantly with 800 FTU/kg microbial phytase inclusion. No significant ( $p>0.05$ ) difference was observed in the calcium content ( $r = 0.94$ ) of the tibia bone of birds fed diets 400 (38.90%), 600 (38.91%) and 800 (39.08%) FTU/kg microbial phytase. Although, the phosphorus contents of birds fed diets containing 200 FTU/kg, 400 FTU/kg and 600 FTU/kg were not significantly ( $p>0.05$ ) different, the copper and iron levels were however vary significantly. The result also showed a significant correlation ( $r = 0.98$ ) between the phytase level and phytate phosphorus.

**Key words:** Phytase levels, phytate and maize - based diet, broiler

### INTRODUCTION

Phosphorus is one of the major minerals required by broilers and other categories of livestock to maintain proper bone development and egg shell qualities in layers. Essentially, the mineral phosphorus is of greater importance in the growth of poultry and other livestock and the consequences of inadequacy of this mineral both quality and quantity are physiological and economically disastrous. Thus nutritionists provide a margin of safety for phosphorus in their diets. Proper management of this valuable resource is a key to maintaining poultry industry viability while promoting environmental stewardship in areas where poultry production is concentrated. Because of the growing concerns regarding the potential contribution of phosphorus in poultry faeces on eutrophication of surface water, increasing pressure is being placed to limit the amount of excess phosphorus in diets and thus reduce the fecal output.

It had been reported that phosphorus excretion by monogastric could be reduced by about 30% by supplementing phytase in their diets (Sabastian *et al.*, 1998). The utilization of originally bound form of phosphorus (phytate) is of considerable practical importance since most poultry diets must be supplemented with inorganic phosphorus for optimum performance.

Phytate content of many ingredients vary considerably, the availability of phytate to exogenous phytase hydrolysis varies from ingredient to ingredient (Ravindran *et al.*, 1999). The consequence of such observation is that for practical purposes a significant safety margin needs to be employed in estimation of the

phosphorus contribution as a result of phytate hydrolysis.

The study was carried out to examine the response of broilers to microbial phytase added to maize - based diets on the mineral composition of the tibia bone and mineral bioavailability.

### MATERIALS AND METHODS

**Diets and plan of experiment:** The five experimental diets contained 0, 200, 400, 600 and 800 FTU/ Kg diet of microbial phytase. The microbial phytase used was Natuphos® 500, a commercial preparation of (BASF Corporation, Ludwigshafen, Germany) with phytase activity of 500 units/ g. The level of other ingredients remained the same in the five experimental diets (Table 1 and 2). The proximate compositions of the diets are in Table 3.

**Experimental animals and their management:** A total of 80 unsexed day old broiler chicks were obtained for this study. They were randomly allotted into five treatment groups of 16 birds/ treatment and were replicated four times. The birds were managed on deep litter system. The birds were fed *ad libitum* with regular water feed supply. Vaccination programme for broiler birds was carefully observed. The birds were placed on the experimental diets for a period of eight weeks during which data were collected.

**Chemical analysis:** Tibia bones from sacrificed birds were collected and oven dried for mineral analysis. In addition, the feeds and faecal samples were analyzed for mineral determination, using methods

Table 1: Gross composition of starter diets (%)

Ingredients (%)	Diets				
	A (0.00)	B (200.00)	C ( 400.00)	D ( 600.00)	E (800.00)
Maize	55.80	55.80	55.80	55.80	55.80
Groundnut meal	18.00	18.00	18.00	18.00	18.00
Soyabean meal	17.30	17.30	17.30	17.30	17.30
Fish meal	5.00	5.00	5.00	5.00	5.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.40	0.40	0.40	0.40	0.40
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Phytase (FTU/kg) diet	0.00	200.00	400.00	600.00	800.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated Composition of Starter diet					
Metabolizable Energy (kcal/kg DM)	3037.47	3037.47	3037.47	3037.47	3037.47
Crude Protein (%)	24.54	24.54	24.54	24.54	24.54
% Phosphorus	1.24	1.24	1.24	1.24	1.24
% Calcium	1.50	1.50	1.50	1.50	1.50

Table 2: Gross composition of finisher diets (%)

Ingredients (%)	Diets				
	A (0.00)	B (200.00)	C (400.00)	D (600.00)	E (800.00)
Maize	60.00	60.00	60.00	60.00	60.00
Groundnut meal	15.00	15.00	15.00	15.00	15.00
Soyabean meal	15.00	15.00	15.00	15.00	15.00
Wheat Bran	1.65	1.65	1.65	1.65	1.65
Fish meal	2.00	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Premix	2.50	2.50	2.50	2.50	2.50
Salt	0.40	0.40	0.40	0.40	0.40
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Phytase (FTU/kg) diet	0.00	200.00	400.00	600.00	800.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated Composition of Finisher diet					
Metabolizable Energy (kcal/kg DM)	3037.47	3037.47	3037.47	3037.47	3037.47
Crude Protein (%)	24.54	24.54	24.54	24.54	24.54
% Phosphorus	1.24	1.24	1.24	1.24	1.24
% Calcium	1.50	1.50	1.50	1.50	1.50

Table 3: Proximate composition of the diets (%)

Composition	Starter diet	Finisher diet
Dry matter	91.38	91.76
Moisture content	8.62	8.24
Crude protein	22.75	19.81
Crude fibre	4.16	4.26
Ash	11.21	5.86
Ether extracts	3.74	9.74
Nitrogen free extracts	58.14	60.33

recommended by VDLUFA (Naumann *et al.*, 1976). Phytate phosphorus was analyzed according to Harland and Oberleas (1986), using the anion exchange chromatographic method. Mineral bioavailability was determined using the formula:

$$\frac{\text{mineral intake} - \text{faecal mineral output}}{\text{mineral intake}}$$

**Statistical analysis:** The birds were subjected to completely randomized design and data obtained were subjected to statistical analysis of variance. The analysis was conducted using the general linear modeling procedure (SAS institute, 1999) while Duncan multiple range test of the same software was used to test significant differences among the treatment means.

## RESULTS

The mineral composition of Tibia bone and apparent mineral bioavailability of broiler bird fed phytase supplemented diets are shown in Tables 4 and 5. No significant ( $p > 0.05$ ) difference was observed in the calcium content ( $r = 0.94$ ) of the tibia bone of birds fed diets C (38.90%), D (38.91%) and E (39.08%) FTU/kg microbial phytase. Although, the phosphorus contents of birds on diet B, C and D were not significantly ( $p < 0.05$ ) different, the copper and iron levels were however vary

Table 4: Mineral composition of tibia bone of broilers fed graded levels of phytase supplementation in maize- based diets

Parameter	Diets					r	SEM
	A (0.00)	B (200.00)	C (400.00)	D (600.00)	E (800.00)		
Calcium (%)	38.35 <sup>b</sup>	38.38 <sup>b</sup>	38.90 <sup>a</sup>	38.91 <sup>a</sup>	39.08 <sup>a</sup>	0.94	0.56
Phosphorus (%)	15.92 <sup>b</sup>	16.50 <sup>a</sup>	16.23 <sup>b</sup>	16.38 <sup>b</sup>	16.70 <sup>a</sup>	0.77	1.02
Nitrogen (%)	0.14	0.52	0.75	0.63	0.56	0.65	0.04
Zinc (%)	0.31	0.32	0.35	0.35	0.38	0.97	0.03
Copper (%)	19.00 <sup>a</sup>	17.99 <sup>b</sup>	16.50 <sup>c</sup>	17.51 <sup>c</sup>	16.99 <sup>bc</sup>	-0.74	1.25
Iron (ppm)	860.50 <sup>a</sup>	864.50 <sup>a</sup>	844.00 <sup>c</sup>	840.50 <sup>c</sup>	851.00 <sup>b</sup>	-0.65	15.63

<sup>a,b,c</sup>Mean on the same row with different superscripts are significantly (p<0.05) different. r = Correlation factor. SEM = Standard Error of means

Table 5: Apparent bioavailability of minerals of broilers fed maize- based diets with phytase supplementation

Parameter(%)	Diets					r	SEM
	A (0.00)	B (200.00)	C (400.00)	D (600.00)	E (800.00)		
Calcium	86.82 <sup>c</sup>	88.31 <sup>b</sup>	88.14 <sup>b</sup>	88.43 <sup>b</sup>	91.61 <sup>a</sup>	0.69	1.35
Phosphorus	53.03 <sup>a</sup>	61.76 <sup>d</sup>	68.89 <sup>c</sup>	80.40 <sup>b</sup>	91.52 <sup>a</sup>	0.92	1.28
Nitrogen	49.60 <sup>a</sup>	37.19 <sup>d</sup>	41.64 <sup>c</sup>	46.23 <sup>b</sup>	52.35 <sup>a</sup>	0.41	3.02
Zinc	67.99 <sup>d</sup>	78.81 <sup>c</sup>	88.17 <sup>b</sup>	85.82 <sup>b</sup>	93.55 <sup>a</sup>	0.93	4.89
Copper	67.95 <sup>d</sup>	64.29 <sup>a</sup>	73.31 <sup>c</sup>	90.88 <sup>b</sup>	92.17 <sup>a</sup>	0.91	5.12
Iron	85.71 <sup>a</sup>	82.57 <sup>c</sup>	84.00 <sup>ab</sup>	85.39 <sup>a</sup>	86.95 <sup>a</sup>	0.49	1.02
Phytate Phosphorus	68.08 <sup>d</sup>	69.33 <sup>d</sup>	75.81 <sup>c</sup>	80.62 <sup>b</sup>	88.83 <sup>a</sup>	0.98	2.34

<sup>a,b,c</sup>Mean on the same row with different superscripts are significantly (p<0.05) different. r = Correlation factor. SEM = Standard Error of means.

Table 6: Regression equation of mineral composition of tibia bone of broilers

	Prediction Equation	Coefficient of variation
Calcium	38.32+0.0009x	0.42
Phosphorus	16.05+ 0.0007x	3.72
Nitrogen	0.33+0.0005x	34.47
Zinc	0.31+0.00009x	11.12
Copper	18.49-0.002x	8.13
Iron	18.49-0.002x	8.13

Table 7: Regression equation of apparent bioavailability of mineral in tibia bone of broilers

	Prediction Equation	Coefficient of variation
Calcium	86.74+0.004x	1.66
Phosphorus	53.59+0.04x	8.44
Nitrogen	45.49+0.007x	12.46
Zinc	71.24+0.03x	4.34
Copper	62.72+0.04x	6.97
Iron	84.01+0.002x	2.19
Phytate Phosphorus	65.98+0.03x	2.40

significantly. Among all the treatments, significant differences were not observed in the tibia nitrogen and zinc contents of the birds. Apparent mineral bioavailability of the birds revealed that birds on diet E (800FTU/kg) had the highest retention of minerals analyzed. However, no significant (p>0.05) difference was observed between the nitrogen contents of the birds on the control diet (A) and those on diet E. The same trend was observed for iron content. The result also showed a strong correlation (r = 0.98) between the phytase level and phytate phosphorus.

## DISCUSSION

During recent years there has been considerable interest in the use of microbial phytase to release phytate - bond phosphorus to improve overall phosphorus availability in poultry diets. The present results is in agreement with numerous previous reports (Simon *et al.*, 1990; Vogt, 1996) which demonstrated that supplemental phytase is effective in improving diets contain low qualities of non - phytate phosphorus for growth and bone mineralization.

Phytase supplementation increased the retention of phosphorus, calcium, zinc and iron in the tibia bone. This agrees with the previous studies of Young *et al.* (1993) where increased retention of phosphorus and calcium in bone and higher bone ash were observed in pigs receiving phytase supplemented diets. Removal or reduction of phytate in food generally improves zinc bioavailability (Reddy *et al.*, 1982). This study demonstrated that supplementation of phytase in maize - soyabean meal - groundnut cake based diet greatly increased tibia bone and zinc retention. The increase in bone composition of the minerals analyzed (Calcium, phosphorus, zinc, copper and iron) that arose from phytase supplementation of diet would be primarily due to a reduction in the inhibitory effect of phytic acid on mineral absorption through its hydrolysis by phytase. The works of Heaney *et al.* (1991) and Lesern (1993) indicated a better utilization of phosphorus and calcium when microbial phytase was added in the feed. The phytate addition had been found to balance the gastrointestinal phytate degradation. The apparent

bioavailability of phosphorus and calcium increased with inclusion of phytase levels in the dietary treatments. The minerals bioavailability values showed that 800FTU/Kg diet inclusion gave the highest percentage of calcium (91.61%) and phosphorus (91.52%) retention. The work of Pointillart (1991), which indicated a better utilization of phosphorus in rye - based diet supplemented with microbial phytase also tends support to the present findings.

Apparent zinc and copper bioavailability also followed the same trend as Ca and P with 800FTU/Kg level having the highest bioavailability mean values for the two minerals 93.55% and 92.77% respectively. This result also confirms the findings of Zacharias *et al.* (2003), who reported an increased availability of copper and zinc content in bone due to microbial phytase supplementation. Apparent iron bioavailability showed no significant ( $p>0.05$ ) difference among the mean values of the dietary treatments. Despite this observation, 800FTU/Kg diet supplementation still had the highest mean value of 88.95%. This still showed that 800FTU/Kg diet phytase supplementation is an optimum inclusion level in the diet which could enhance growth performance and mineral bioavailability.

The result of this present study showed that microbial phytase supplementation enhanced phytate bioavailability ranging 68.09% in the unsupplemented basal diets to 88.83% at 800FTU/Kg inclusion rate. This result confirms the findings of Xingen *et al.* (1993) that increased microbial phytase supplementation improve the phytate phosphorus availability. Similar findings were reported by Carlos and Edwards (1998) that microbial phytase supplementation enhanced phytate phosphorus retention.

In conclusion, the result of the present study showed that supplemented phytase counteract the negative influence of phytic acid in most plant sources used as ingredients in poultry feeds, hence the improved performance observed in this work.

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