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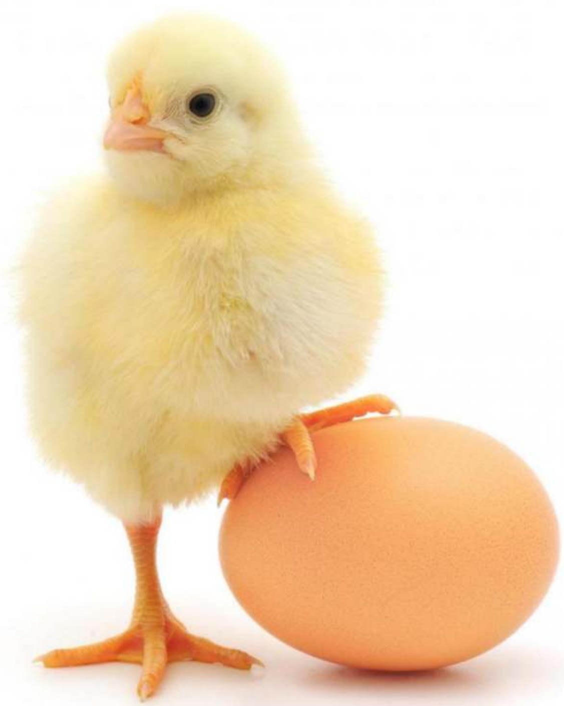

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Effect of Phytase Supplementation on the Calcium Requirement of Broiler Chicks[1]

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Abstract: It has been frequently demonstrated that addition of phytase to corn-soybean meal diets will improve the utilization of the phytate-bound P for the broiler chicken. The effect of phytase on release of other nutrients such as Ca is less clear-cut. A study was conducted to examine the effects of phytase supplementation on diets with various dietary levels of Ca and nonphytate P (nPP). A diet was formulated that provided nutrients in excess of NRC (1994) recommendations with 1.0% Ca and 0.50% nPP. By varying the amounts of dicalcium phosphate, limestone, and sand in aliquots of a common basal diet, diets were prepared with 1) Low-P and Low-Ca, 2) Low-P and High-Ca, 3) High-P and Low-Ca, and 4) High-P and High-Ca. The diets were analyzed for Ca and P content and blended as needed to provide test diets in a factorial arrangement of three Ca levels (0.5, 0.7, and 0.9%) with eight levels of nPP (0.15 to 0.50% in increments of 0.05%). These 24 diets were divided and half supplemented with 1000 units/kg of phytase. Each treatment was fed to six pens of six male broilers from one to 21 d of age. Response of both body weight and tibia ash to phytase supplementation in broilers was significantly affected by dietary Ca levels as well as dietary nPP levels. Without phytase supplementation, both body weight and tibia ash were depressed at the lowest level of nPP as dietary Ca level increased. Adding phytase to these diets improved both body weight and tibia ash as a result of increased availability of P. At both 0.5% and 0.7% Ca, the dietary Ca level was a limiting factor in maximizing tibia ash regardless of P level or phytase supplementation. These data indicate that minimal Ca appeared to be released by phytase and that no reduction in Ca level of broiler should be implemented with phytase supplementation.

Key words: Phytase, calcium, phosphorus, broilers

Introduction

Supplementation of broiler diets with microbial phytase has been shown by many to increase P availability, reduce the needs for supplemental P and therefore reduce P excretion in broilers (Simons *et al.*, 1990; Saylor *et al.*, 1991; Broz *et al.*, 1994; Denbow *et al.*, 1995; Qian *et al.*, 1996; Sebastian *et al.*, 1996a; Waldroup *et al.*, 2000; Yan *et al.*, 2000). The effects of phytase on releasing nutrients other than P are less clear-cut. In weak acid or neutral pH conditions of the gastrointestinal tract of chickens, each of the six phosphate groups of phytic acid has either one or two negative charges, which can bind various cations including Ca, potentially rendering them unavailable for intestinal absorption (Wise, 1983; Pallauf and Rimbach, 1997). Several reports have shown that phytase supplementation increased the availability of Ca for broiler chickens (Schoner *et al.*, 1991; Broz *et al.*, 1994; Kornegay *et al.*, 1996; Sebastian *et al.*, 1996a), which indicates that the Ca need of the broiler may also be reduced by phytase supplementation.

The phytate-Ca complex formed in the intestine is extremely insoluble (Nelson and Kirby, 1987), which is not only unavailable for intestinal absorption, but also resistant to phytase hydrolysis. It was also reported that the dietary Ca could directly repress phytase activity by competing for the active sites of the enzyme (Wise, 1983; Pointillart *et al.*, 1985). Being the most abundant mineral in the diet, dietary Ca has the potential to affect the efficiency of phytase supplementation in broiler diets.

Limited research has been conducted to study the response of broiler chickens to phytase supplementation as affected by dietary Ca levels. Adding phytase to broiler diets containing 0.6, 1.0, and 1.25% Ca, Sebastian *et al.* (1996b) reported that the response to supplemental phytase was significantly affected by dietary Ca levels and the optimum growth performance, retention of P, Ca, and N, ash content of the tibia shaft and plasma P and Cu were achieved at the lowest level of dietary Ca. Qian *et al.* (1996; 1997) reported that widening the Ca:total P ratio from 1.4 to 2.0% decreased phytase efficacy by 7.4 and 4.9% respectively for diets with 0.27 and 0.36% nPP in young turkeys, and by 11.1 and 12.2% respectively for diets with 66 and 660 μg/kg of vitamin D_3 in young broilers. A Ca:total P ratio range of 1.1 to 1.4 has been reported to be critical to the efficient use of supplemental phytase in corn-soybean meal broiler diets (Qian *et al.,* 1997).

The objective of the present study was to determine if dietary Ca needs of the young (0 to 21 d) broiler may be reduced by phytase supplementation and to examine the effects of dietary Ca and nPP levels on responses of broiler chicks to phytase supplementation.

Materials and Methods

Feed formulation: A diet was formulated that provided nutrients in excess of minimum NRC (1994) requirements with 1.0% Ca and 0.50% nPP. Dicalcium phosphate and ground limestone of known Ca and P content were used as major sources of these minerals. By varying the amounts of these two ingredients in the diet, along with the use of washed builder's sand as an inert ingredient, four basal diets were prepared with 1) Low-P and Low-Ca, 2) Low-P and High-Ca, 3) High-P and Low-Ca, and 4) High-P and High-Ca. Composition of the four basal diets was shown in Table 1. Samples of these four diets were analyzed[3] for Ca and total P, with the results of the assays used in formulating the final test diets.

Test diets: Based upon the assay results, the four basal diets were blended to provide a series of test diets that provided a factorial arrangement of three Ca levels (0.5, 0.7, and 0.9%) and eight nPP levels(0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50%) for a total of 24 test diets. These diets were then divided into two sublots; one portion was fed as prepared while the other portion was supplemented with 1000 units/kg of microbial phytase enzyme[4]. Samples of all test diets were assayed in quadruplicate for Ca and total P by the same laboratory conducting the assays for the basal diets, and the phytase supplemented diets were assayed for phytase activity by the manufacturer of the phytase.

Birds and housing: Day-old male chicks of a commercial strain[5] were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. They were randomly assigned to compartments in electrically heated brooders with raised wire floors. Six pens with six birds per pen were assigned to each of the 48 dietary treatments, randomized across tiers in batteries. Constant fluorescent lighting was used. The test diets and tap water were provided for *ad libitum* consumption. Care and management of the birds followed recommended guidelines (FASS, 1999).

Measurements: Birds were group weighed by pen at one and 21 d of age. Feed consumption was determined by weighing back uneaten feed in a pre-weighed feed container at the conclusion of the study. Birds were checked twice daily; any bird that died or removed for obvious leg disorders was weighed and the weight was used for adjustment of feed conversion (FCR, grams of feed consumed ÷ (weight of live birds + weight of dead birds). At 21 d, two representative birds per pen were killed by CO_2 inhalation; the right tibia was removed, cleaned of adhering tissue, and ash content of dried defatted bones determined as described by AOAC (1990). At 20 d, excreta samples were collected for a 24 hr period from each pen, freeze-dried, and total P content determined.

Statistical analysis: Pen means served as the experimental unit for statistical analysis. Data were subjected to analysis of variance as a factorial arrangement of treatments with nPP level, Ca level, and phytase supplementation as main factors; all possible interactions between and among the main effects were evaluated. Mortality data were transformed to $\sqrt{n+1}$ prior to analysis; data are presented as natural numbers. Analysis used the General Linear Model procedure of SAS (SAS Institute Inc., 1991). Significant differences between or among means were separated by repeated t-tests using the lsmeans option of SAS. Statements of probability were based on $P \leq 0.05$. Following this analysis, nonlinear regression analysis was conducted to estimate the requirements of the broiler for nPP under different Ca and phytase situations using the PROC NLIN procedure of SAS (SAS Institute Inc., 1991), incorporating the SAS macro of Robbins (1986).

Results and Discussion

Total P and Ca content of the basal diets prior to preparation of test diets were in agreement with calculated values (Table 1). There was good agreement between calculated and analyzed total P and Ca for the final test diets. Total P levels were generally within ±0.02% of the calculated levels (data not shown). The phytase supplemented diets were analyzed to have an average of 1298±257 PU/kg, which was higher than the calculated level of 1000 PU/kg.

Factorial analysis: The results of statistical analysis on body weight, feed conversion, mortality, and tibia ash are shown in Table 2. Body weight was significantly affected by main effects of nPP level, Ca level, phytase supplementation, and by interactions between levels of nPP and Ca, nPP level and phytase, Ca level and phytase, and among nPP level, Ca level, and phytase supplementation. These effects are best demonstrated in Fig. 1 and 2. In the absence of phytase (Fig. 1) increasing the Ca level at low levels of dietary nPP (0.25% or lower) significantly decreased body weight, most likely due to an exacerbated imbalance between Ca and P. The importance of the ratio between Ca and P in poultry diets has long been recognized (Bethke *et al.*, 1929; Hart *et al.*, 1930; Mussehl and Ackerson, 1932). At dietary nPP levels of 0.30 and 0.35%, birds fed 0.7% Ca were heavier than those fed 0.5 or 0.9% Ca, and there was no difference between birds fed 0.5 and 0.9% Ca. It appeared that when diets contained 0.30 or 0.35% nPP, a proper balance between Ca and P was

Table 1: Composition (g/kg) and analysis of basal diets

Ingredient	Low P Low Ca	Low P High Ca	High P Low Ca	High P High Ca
Yellow corn	510.91	510.91	510.91	510.91
Soybean meal	370.97	370.97	370.97	370.97
Poultry oil	74.99	74.99	74.99	74.99
Salt	4.56	4.56	4.56	4.56
DL-methionine	2.84	2.84	2.84	2.84
Trace minerals[1]	1.00	1.00	1.00	1.00
Broiler vitamins[2]	2.00	2.00	2.00	2.00
L-Threonine	0.37	0.37	0.37	0.37
L-Lysine Hcl	0.04	0.04	0.04	0.04
Dicalcium phosphate	0.00	0.00	20.48	20.48
Limestone	0.00	22.75	0.00	11.84
Washed sand	32.32	9.57	11.84	0.00
TOTAL	1000	1000	1000	1000
Nutrient analysis[3]				
ME kcal/kg (C)	3200.00	3200.00	3200.00	3200.00
CP, % (C)	22.34	22.34	22.34	22.34
Met, % (C)	0.62	0.62	0.62	0.62
TSAA, % (C)	1.00	1.00	1.00	1.00
Lys, % (C)	1.26	1.26	1.26	1.26
Thr, % (C)	0.90	0.90	0.90	0.90
Ca (C)	0.11	1.00	0.54	1.00
Ca (A)	0.15	0.99	0.55	0.93
Total P (C)	0.37	0.37	0.75	0.75
Total P (A)	0.36	0.36	0.73	0.75
Nonphytate P (C)	0.12	0.12	0.50	0.50

[1]Provides per kg of diet: Mn (from $MnSO_4.H_2O$) 100 mg; Zn (from $ZnSO_4.7H_2O$) 100 mg; Fe (from $FeSO_4.7H_2O$) 50 mg; Cu (from $CuSO_4.5H_2O$) 10 mg; I (from $Ca(IO_3)_2.H_2O$ 1 mg. [2] Provides per kg of diet: vitamin A (from vitamin A acetate) 7714 IU; cholecalciferol 2204 IU; vitamin E 16.53 IU; vitamin B_{12} 0.013; choline 465 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; thiamin (from thiamine mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg. [3]C = calculated; A = analyzed.

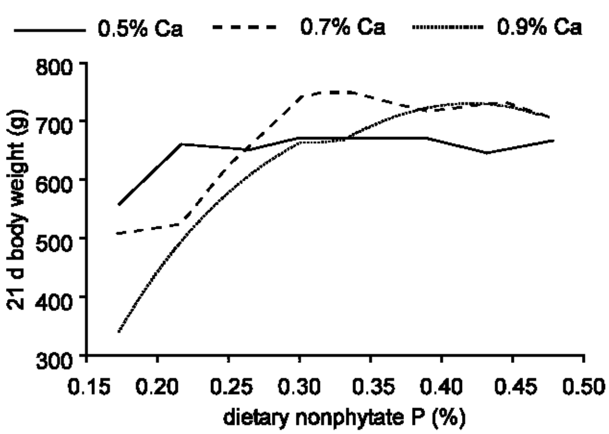

Fig. 1: Effects of dietary nonphytate P level on body weight of 21-d-old broilers fed diets with three levels of Ca in the absence of phytase

kept with 0.7% Ca, at 0.5 or 0.9% Ca, the level of Ca was either too low or too high, which again demonstrated the importance of a proper ratio between Ca and P.

The decrease in body weight related to dietary Ca was not observed when phytase was added to the diet (Fig. 2). Phytase supplementation of low nPP diets significantly improved body weight, and the negative impact of increasing dietary Ca disappeared, apparently because of the increased availability of P and the resulting improved balance between Ca and P. Adding phytase to these diets (nPP=0.30 or 0.35%) improved body weight at Ca of 0.9%, whereas had no effect at Ca of 0.5 or 0.7%. For these diets (nPP=0.30 or 0.35%), Ca was the limiting factor for growth at Ca of 0.5%, the proper balance of Ca and P was achieved at Ca of 0.7%, whereas P was the limiting factor for growth at Ca of 0.9%. The fact that phytase supplementation improved performance only at Ca of 0.9%, but not 0.5 or 0.7% suggested that the primary function of phytase in these diets was to release P, and it had minimum effect on Ca availability. When the diets contained 0.40% nPP or higher, there was no significant difference in body weights of birds on 0.7 and 0.9% Ca, which were generally higher than those of birds on 0.5% Ca. Phytase supplementation to these diets (nPP=0.40 or higher) had minimum effect on body weight, because the P in these diets was not a limiting factor for growth.

Table 2: Results of statistical analysis (Probability > F) of body weight, feed conversion, mortality, and tibia ash of 21-d-old broilers

Source	Body weight	Feed conversion	Mortality	Tibia ash
P level	0.0001	0.0001	0.0001	0.0001
Ca level	0.0001	0.0039	0.0205	0.0001
P level × Ca level	0.0001	0.0001	0.0001	0.0001
Phytase	0.0001	0.0037	0.0001	0.0001
P level × Phytase	0.0001	0.0002	0.0001	0.0001
Ca level × Phytase	0.0001	0.0143	0.0014	0.0001
P level × Ca level × Phytase	0.0001	0.0001	0.1201	0.0001

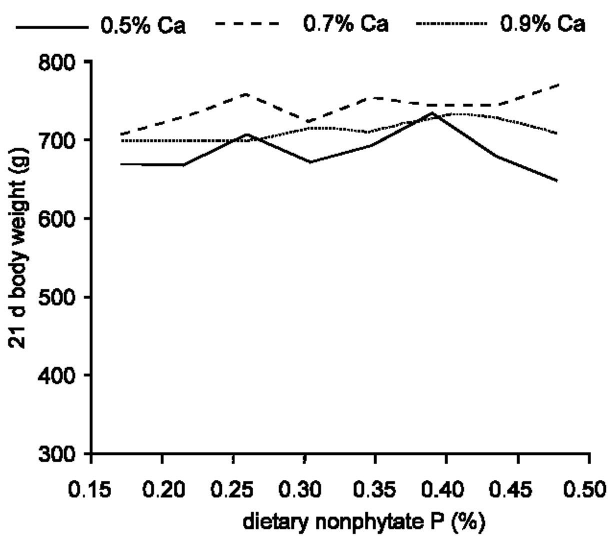

Fig. 2: Effects of dietary nonphytate P level on body weight of 21-d-old broilers fed diets with three levels of Ca in the presence of phytase

Regardless of phytase supplementation, a Ca level of 0.7% appeared to be sufficient to support optimum body weight. Schoner *et al.* (1993) reported that with phytase supplementation, a diet with 0.6% Ca resulted in higher body weight and feed intake than a diet with 0.9% Ca.

Feed conversion was significantly affected by main effects of nPP level, Ca level, phytase supplementation, and by interactions between levels of nPP and Ca, nPP level and phytase, Ca level and phytase, and among nPP level, Ca level, and phytase supplementation (Table 2, and 3). Among the 48 treatments, only the feed conversion by the birds fed the diet with the lowest level of nPP (0.15%) and the highest Ca level (0.90%) in the absence of phytase was significantly higher than those of the birds on the other 47 test diets. There was no significant difference in feed conversion among the rest of the treatments except the treatment with 0.25% nPP and 0.7% Ca had an unexplainable better feed conversion. The significantly higher feed conversion ratio of 2.339 for the treatment with the low nPP of 0.15% and high Ca of 0.9% mainly accounted for all the main effects and interactions.

Mortality was significantly affected by main effects of nPP level, Ca level, phytase supplementation, and interactions between levels of nPP and Ca, nPP level and phytase, and Ca level and phytase (Table 2, Fig. 3 and Fig. 4). Increasing dietary Ca level, especially in the absence of phytase, significantly increased mortality at the lower levels of dietary nPP and had no effect at the higher levels of nPP, accounting for the interaction between Ca and nPP levels. Regardless of dietary Ca level, phytase significantly reduced mortality when added to diets with 0.15 or 0.20% nPP and had no effect when nPP was 0.25% or higher, accounting for the P level × phytase interaction. Phytase supplementation significantly decreased mortality at each level of Ca; the response was much greater at Ca level of 0.9%, accounting for the Ca level × phytase interaction. In the absence of phytase, 0.20, 0.25, and 0.35% nPP appeared to be required to minimize mortality for birds fed 0.5, 0.7, and 0.9% Ca, respectively (Fig. 3). With phytase present in the feed, the lowest nPP level tested, which was 0.15%, was adequate to support optimum livability regardless of dietary Ca level (Fig. 4).

Tibia ash percentage was significantly affected by main effects of nPP level, Ca level, and phytase supplementation. There were significant interactions between nPP level and Ca level, nPP level and phytase, Ca level and phytase, and among nPP level, Ca level, and phytase (Table 2, Fig. 5 and Fig. 6). In the absence of phytase, when dietary nPP levels were 0.25% or lower, increasing Ca level resulted in significantly lower tibia ash, suggesting that the high levels of Ca created an imbalance between Ca and P in the P deficient diets. With phytase supplementation to these diets, the negative effect of Ca on tibia ash no longer existed, which indicated that phytase increased P utilization, and the balance between Ca and P was improved by phytase supplementation. When diets contained medium levels of nPP (0.30 or 0.35%) increasing Ca from 0.5 to 0.7% significantly increased tibia ash percentage; further increasing Ca level from 0.7% to 0.9% numerically decreased tibia ash percentage, but the difference was not significant, which indicated that Ca level of 0.9% created a slight imbalance between Ca and P. Adding phytase to these diets with 0.30 or 0.35% nPP increased tibia ash at 0.7 and 0.9% Ca with the response being

Table 3: Effect of dietary nonphytate P and Ca levels on mortality of broilers from d 1 to 21 with or without phytase supplementation

nPP (%)	Without Phytase				With Phytase				Mean	
	0.5%Ca	0.7% Ca	0.9% Ca	Mean	0.5% Ca	0.7% Ca	0.9% Ca	Mean	0.5%Ca	0.7% Ca
0.15	1.408b^{c}	1.450^{b}	2.339^{a}	1.732^{a}	1.367^{bc}	1.365^{bc}	1.361^{bc}	1.364^{b}	1.387^{b}	1.407^{b}
0.20	1.336b^{c}	1.383^{bc}	1.386^{bc}	1.368^{b}	1.375^{bc}	1.355^{bc}	1.362^{bc}	1.364^{b}	1.355^{b}	1.369^{b}
0.25	1.416^{bc}	1.239^{c}	1.383^{bc}	1.346^{b}	1.354^{bc}	1.319^{bc}	1.361^{bc}	1.345^{b}	1.385^{b}	1.279^{b}
0.30	1.406^{bc}	1.300^{bc}	1.369^{bc}	1.358^{b}	1.386^{bc}	1.342^{bc}	1.358^{bc}	1.362^{b}	1.396^{b}	1.321^{b}
0.35	1.386^{bc}	1.310^{bc}	1.374^{bc}	1.357^{b}	1.373^{bc}	1.290^{bc}	1.291^{bc}	1.318^{b}	1.380^{b}	1.300^{b}
0.40	1.454^{b}	1.337^{bc}	1.312^{bc}	1.368^{b}	1.302^{bc}	1.326^{bc}	1.327^{bc}	1.318^{b}	1.378^{b}	1.331^{b}
0.45	1.457^{b}	1.315^{bc}	1.365^{bc}	1.379^{b}	1.342^{bc}	1.398^{bc}	1.321^{bc}	1.354^{b}	1.399^{b}	1.356^{b}
0.50	1.369^{bc}	1.297^{bc}	1.391^{bc}	1.352^{b}	1.376^{bc}	1.273^{bc}	1.385^{bc}	1.345^{b}	1.373^{b}	1.285^{b}
Mean	1.404^{b}	1.329^{c}	1.490^{a}	1.407^{a}	1.359^{bc}	1.333^{bc}	1.346^{bc}	1.346^{b}	1.382^{a}	1.331^{b}

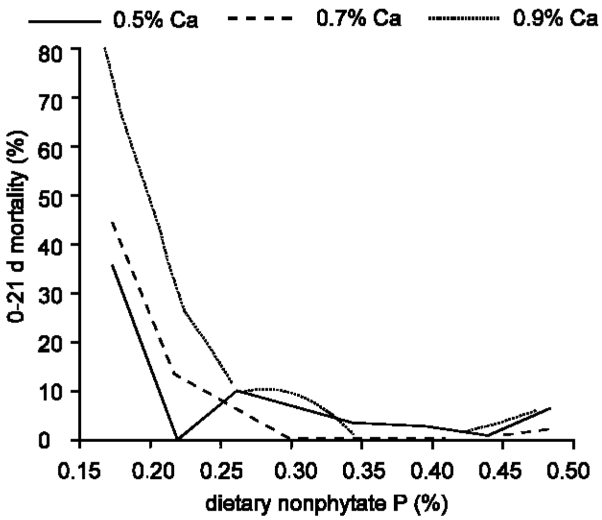

Fig. 3: Effects of dietary nonphytate P level on mortality of 21-d-old broilers fed diets with three levels of Ca in the absence of phytase

greater at 0.9%; whereas no effect was seen with 0.5% Ca, which indicated that phytase had minimum effects in releasing Ca. With phytase present in these diets (nPP=0.30 or 0.35%), the birds on 0.9% Ca had the highest tibia ash, followed by those fed 0.7% Ca, and 0.5% Ca. When dietary nPP was close to adequate or was adequate (0.40% or higher), tibia ash increased significantly with the increase in dietary Ca. Adding phytase to these diets had no effect on tibia ash, regardless of dietary Ca levels, which again suggested that minimum Ca was released by phytase. Regardless of phytase supplementation, tibia ash continued to increase with the increase of dietary Ca level from 0.5% to 0.9%, which indicated that 0.9% Ca was required to maximum tibia ash in the presence of adequate P, even with supplementation of phytase.

At dietary Ca level of 0.5%, increasing nPP level had minimum effect on tibia ash, regardless of phytase supplementation, which indicated that Ca is the limiting factor for bone development under this condition. At dietary Ca of 0.7 and 0.9%, increasing the dietary nPP level significantly increased tibia ash percentage regardless of phytase supplementation; however, a nPP level required for tibia ash to reach a plateau and the value of tibia ash content at this plateau depended on the Ca level and phytase supplementation. Regardless of phytase supplementation, the values of tibia ash percentage at the plateau increased with the increase in Ca levels, which again indicated that 0.7% dietary Ca was not adequate to support optimum tibia ash, even with phytase supplementation. Sebastian *et al.* (1996b) reported that when diets contained 0.30% nPP, phytase supplementation at the Ca level of 0.6% resulted in comparable tibia shaft ash content with 1.0% or 1.25% Ca plus phytase in 21-d-old chicks. However, the tibia head ash content at the Ca level of 0.6% with phytase supplementation was lower than that at the Ca level of 1.0 or 1.25% in the presence of phytase.

Responses (body weight and tibia ash) to phytase supplementation were affected by both dietary nPP and Ca levels. Responses were generally obvious under the condition of P deficiency. When the dietary nPP was approaching the adequate level, responses diminished which clearly indicated that the function of phytase in these diets was P related. Besides the dietary nPP level, dietary Ca levels had a significant impact on the responses to phytase. Even in P deficiency, no responses to phytase supplementation can be observed if dietary Ca was also a limiting factor at the same time. Dietary Ca level has been reported to be a key factor influencing the efficiency of phytase use in poultry. Zyla et al. (2000) reported that increasing dietary Ca from 0.59 to 0.79% in a diet with 0.17% nPP, thus widening the Ca:P ratio from 1.44 to 1.93, negatively influenced feed intake, weight gain, and toe ash of broilers fed wheat-based diets enriched with phytase. Qian *et al.* (1997) reported that a Ca to total P ratio range of 1.1 to 1.4 appeared to be critical to the efficient use of supplemental phytase in corn-soybean meal broiler diets. In the present experiment, responses to phytase supplementation were mainly determined by the balance between Ca and P and dietary nPP level. As

Table 4: Results of nonlinear regression analysis to estimate the nonphytate phosphorus requirement of male broilers fed three different calcium levels with and without phytase supplementation

Calcium level	Added Phytase[1]	Value at inflection	Inflection point[2]	Asymptotic standard error	Asymptotic 95% confidence interval
Body weight, g					
0.5	No	708	0.21	0.008	0.191-0.231
0.5	Yes	727	0.25	0.178	-0.209-0.708
0.7	No	766	0.30	0.026	0.234-0.367
0.7	Yes	790	0.25	NE[3]	NE
0.9	No	749	0.31	0.011	0.283-0.336
0.9	Yes	760	0.40	0.111	0.116-0.684
Tibia ash, %					
0.5	No	39.73	0.30	0.029	0.232-0.373
0.5	Yes	39.53	0.20	0.017	0.158-0.242
0.7	No	43.15	0.33	0.010	0.308-0.357
0.7	Yes	43.67	0.24	0.029	0.170-0.310
0.9	No	45.82	0.44	0.021	0.383-0.491
0.9	Yes	45.49	0.27	0.014	0.241-0.309

[1] With or without addition of 1000 units/kg phytase (Natuphos, BASF).
[2]Defined as the break point of dietary nonphytate phosphorus concentration as a function of the selected variables according to a nonlinear least squares analysis (Robbins, 1989; SAS Institute, 1991; Yu and Morris, 1999). [3]Non- estimable

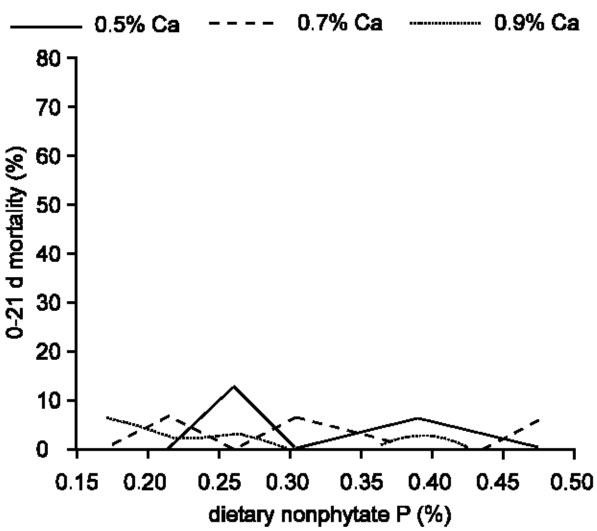

Fig. 4: Effects of dietary nonphytate P level on mortality of 21-d-old broilers fed diets with three levels of Ca in the presence of phytase

long as P was deficient and Ca was not a more limiting factor than P, birds responded to phytase by improving weight gain and tibia ash percentage. The ratio of Ca to total P did not seem to be a critical factor influencing the magnitude of the responses in this trial. At the low level of nPP, 0.15 or 0.20%, Ca to total P ratios ranging from 1.50 to 2.16 (at dietary Ca of 0.7 and 0.9%) did not seem to negatively affect the magnitude of the responses. Whether dietary Ca affects phytase efficiency by binding phytate or by repressing phytase activity, theoretically, the absolute dietary Ca level, not the ratio between Ca to P, should have more profound impact on the efficiency of phytase use. In this experiment, the highest Ca level used was 0.9%, which was probably not high enough to affect the responses to phytase to a significant degree. Moreover, in this experiment, the phytase supplemented diets contained a average of 1298 units phytase/kg diet, which was more than the phytase levels used in most other research (600 to 800 units/kg). It is possible that whatever magnitude of negative effects of increasing dietary Ca from 0.5 to 0.9% on phytase efficiency was masked by the abundance of phytase in the diets.

Regression analysis: Results of the nonlinear regression analysis to estimate the nPP levels required for optimum body weight and tibia ash at each Ca level with and without phytase supplementation are shown in Table 4. The requirement was established as the inflection point of the one-slope regression model (Robbins, 1986; Yu and Morris, 1999).

In the absence of phytase, the inflection point for maximum body weight was 0.21, 0.30, and 0.31% nPP at the Ca level of 0.5, 0.7, and 0.9% respectively. More P was needed for optimum body weight at higher Ca levels. The values of body weight at inflection points were 766 and 749 grams at the Ca levels of 0.7 and 0.9%, which were higher than 708 grams at the Ca level of 0.5%. When diets were supplemented with phytase, inflection points of 0.25, 0.25, and 0.40% nPP were obtained at 0.5, 0.7, and 0.9% Ca, respectively. However, the standard errors were large and the asymptotic 95% confidence intervals were either non-estimable or beyond the range of dietary nPP levels, which indicated that the estimated requirements for optimum body

Table 5: Effects of dietary nonphytate P levels, Ca levels, and phytase supplementation on excreta P percentage (mean ± SEM) of 21-d male broilers

Diet nonphytate P %	Without phytase[1]			With phytase[1]		
	0.5% Ca	0.7% Ca	0.9% Ca	0.5% Ca	0.7% Ca	0.9% Ca
0.15	0.49±0.04	0.43±0.06	0.53±0.17	0.59±0.08	0.47±0.04	0.52±0.09
0.20	0.59±0.04	0.55±0.07	0.59±0.08	0.69±0.04	0.56±0.03	0.49±0.08
0.25	0.70±0.06	0.63±0.09	0.71±0.07	0.75±0.04	0.64±0.04	0.64±0.03
0.30	0.85±0.10	0.79±0.05	0.80±0.13	0.95±0.15	0.79±0.04	0.78±0.05
0.35	0.96±0.07	1.02±0.20	0.97±0.09	0.93±0.09	0.88±0.05	0.91±0.08
0.40	1.16±0.08	1.03±0.10	1.01±0.09	1.11±0.05	1.11±0.08	1.04±0.08
0.45	1.21±0.09	1.18±0.21	1.11±0.10	1.31±0.08	1.17±0.09	1.19±0.11
0.50	1.33±0.09	1.30±0.18	1.26±0.08	1.38±0.11	1.37±0.09	1.33±0.10[1]

With or without the addition of 1000 units/kg of phytase (Natuphos, BASF).

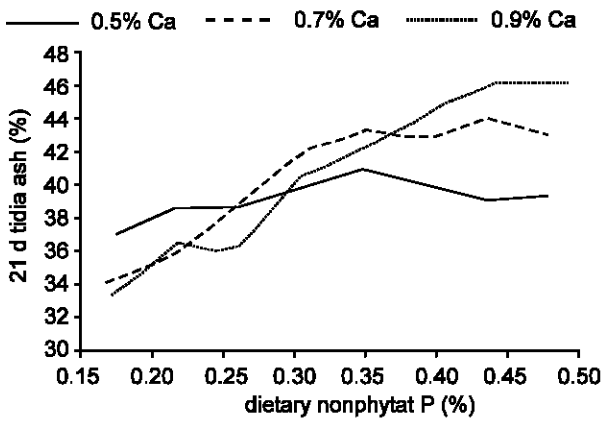

Fig. 5: Effects of dietary nonphytate P level on tibia ash content of 21-d-old broilers fed diets with three levels of Ca in the absence of phytase

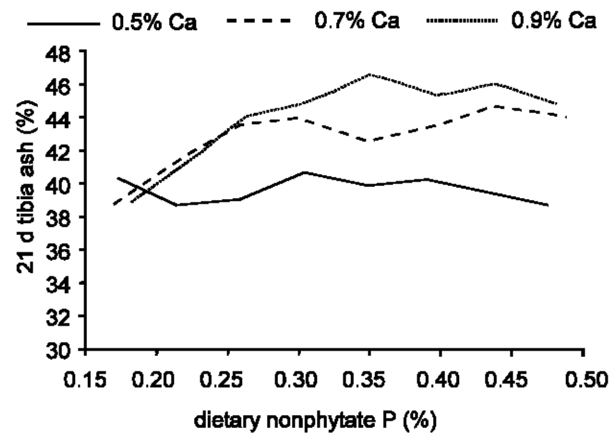

Fig. 6: Effects of dietary nonphytate P level on tibia ash content of 21-d-old broilers fed diets with three levels of Ca in the presence of phytase

weight with phytase supplementation were not accurate. The reason was that even the lowest nPP level supported quite high body weight and there was no increasing trend in the data.

When diets did not contain phytase, the inflection points for maximum tibia ash were 0.30, 0.33, and 0.44% nPP at the Ca level of 0.5, 0.7, and 0.9%, respectively. When phytase was added to the diets, the inflection points were reduced to 0.20, 0.24, and 0.27% nPP at 0.5, 0.7, and 0.9% Ca, respectively. The inflection points for maximum tibia ash increased with the increase in dietary Ca level regardless of phytase supplementation and the values of tibia ash percentage at inflection points also increased with the increase in Ca levels, which again indicated that 0.7% dietary Ca was not adequate to support optimum tibia ash, regardless of phytase supplementation. When the diet contained sufficient amount of Ca (0.9%), the inflection point of 0.44% nPP was in good agreement of NRC (1994) recommended level of 0.45% nPP. The reduction of 0.17% nPP in the inflection point at 0.9% Ca due to phytase supplementation (from 0.44% to 0.27%) was higher than the generally accepted value of 0.10% from the literature. (Kornegay *et al.,* 1996; Huff *et al.,* 1998; Waldroup *et al.,* 2000). One possible explaination was thatthe analyzed levels of phytase in the diets, 1298 ± 257 units/kg, were higher than the commonly used recommendation level of 600 to 800 units/kg in the broiler diets. It has been reported that phytase levels exceeding the industry recommendation level continued to liberate more P and improve perfomance and bone characteristics when the diet was deficient in P (Shirley and Edwards, 2003).

The nPP levels required for optimum feed conversion and livability were not well estimated by nonlinear regression analysis. Based on the results of factorial analysis, an nPP level sufficient to support optimum body weight or tibia ash was adequate for optimum feed conversion and minimum mortality.

Excreta phosphorus content: The excreta P content as influenced by dietary levels of nPP, Ca and phytase supplementation was shown in Table 5. Dietary Ca level and phytase supplementation had minimum effects on excreta P content. The greatest effect on excreta P percentage was dietary nPP level; excreta P content consistently increased with the increase in dietary nPP. The excreta P content of birds fed diets with 0.45% nPP, which is the NRC (1994) recommended level of nPP and is very close to the current industry level (Agri Stats Inc., Fort Wayne IN 46818.), and 0.9% Ca contained 1.11% total P on dry basis. Results of the regression analysis discussed previously demonstrated that the nPP need for maximum tibia ash can be reduced to 0.27% with phytase supplementation at the Ca level of 0.9%. The excreta samples of birds fed diets with 0.30% nPP and 0.9% Ca contained only 0.78% total P. Thus, compared with birds fed NRC (1994) recommended level of nPP, phytase supplementation could reduce excreta P content by 30% while still maintaining optimum growth performance and tibia ash percentage. This is in agreement with the findings of most published studies that phytase supplementation can reduce excreta P excretion by about 20 to 50% (Simons *et al.*, 1990; Kiiskinen *et al.*, 1994; Kornegay *et al.*, 1996; Yi *et al.*, 1996; Hirabayashi *et al.*, 1998).

In conclusion, data from this research showed that regardless of phytase supplementation, 0.7% dietary Ca was sufficient to support optimum growth performance, and 0.9% Ca was required to maximize tibia ash. The primary function of phytase in the test diets was to release P from phytate molecule, and phytase supplementation had minimum effect in releasing Ca. No reduction in Ca should be implemented when phytase was supplemented in the broiler diet. Responses (body weight and tibia ash) to phytase supplementation in broilers were significantly affected by dietary Ca level as well as dietary nPP level. When evaluating the P sparing effect of phytase using body weight or tibia ash as criteria, a proper amount of Ca should be provided to ensure chickens respond to the P released by phytase. Excreta P excretion of broilers could be markedly reduced by phytase supplementation while maintaining optimum performance and tibia bone ash content.

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[3]Analyses conducted by Agricultural Diagnostic Laboratory, University of Arkansas, Fayetteville AR using inductively coupled plasma-atomic spectroscopy following HNO_3 digestion.

[4]Natuphos, BASF Corporation, Mt. Olive, NJ 07828. One unit of phytase activity is defined as the quantity required to producer 1µmol of inorganic P/min from 5.1 mmol/L of sodium phytate at a pH of 505 and a water bath temperature of 37^{o}C

[5]Cobb 500. Cobb-Vantress, Inc., Siloam Spring, AR 72761