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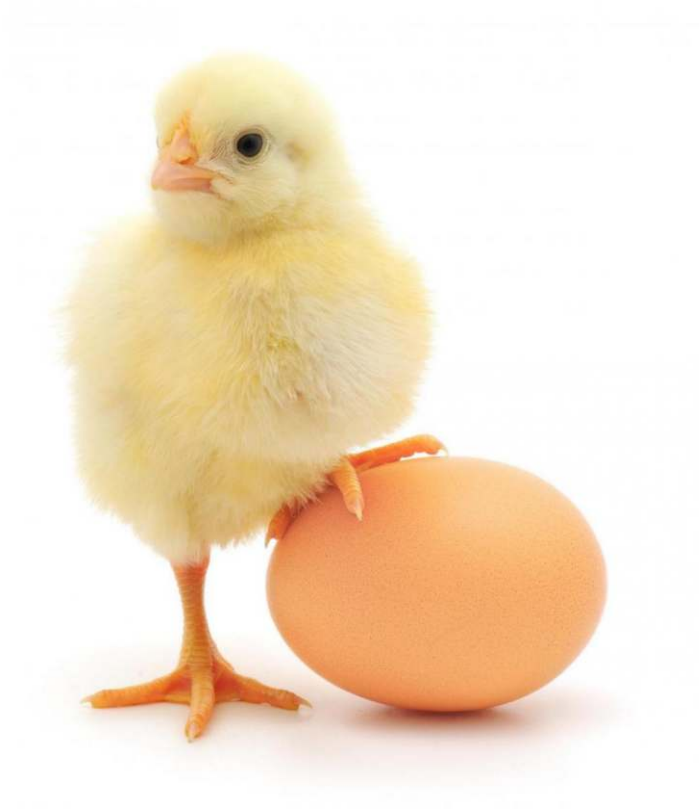

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Nonphytate Phosphorus Requirement and Phosphorus Excretion of Broiler Chicks Fed Diets Composed of Normal or High Available Phosphate Corn as Influenced by Phytase Supplementation and Vitamin D Source[1]

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Abstract: A study was conducted to evaluate the ability of young broiler chicks (0-3 wk) to utilize the P provided by a high available phosphate corn (HAPC) in comparison with yellow dent corn (YDC) and to determine the extent to which microbial phytase supplementation and use of 25-hydroxycholecalciferol (25-OH-D_3) in the diet could reduce the requirements for P and subsequently reduce P excretion. Diets were prepared using either YDC or HAPC, which contained about the same total P but differed in phytate-bound P content. Within each corn type, diets were fortified with either vitamin D_3 or 25-OH-D_3 at the rate of 68.9 µg/kg diet. Treatment diets were prepared by varying the amount of dicalcium phosphate and ground limestone, and ranged from 0.09 to 0.50% nonphytate P (nPP) for YDC diets and 0.18 to 0.50% nPP for HAPC diets. Sublots of each diet were supplemented with 1000 units/kg phytase. Each diet was fed to four replicate pens of six male broilers of a commercial strain from 1 to 21 d of age. After factorial analysis, nonlinear regression analysis was conducted to estimate a nPP level sufficient for maximum body weight gain or tibia ash percentage for each corn type as influenced by phytase supplementation or use of 25-OH-D_3. For broilers fed YDC diets, the estimations for maximum tibia ash were 0.40, 0.35, 0.32, and 0.27% nPP for diets supplemented with D_3, D_3 + phytase, 25-OH-D_3, and 25-OH-D_3 + phytase respectively. For broilers fed HAPC diets, substitution of D_3 with 25-OH-D_3 had no significant effect on tibia ash percentage and the inflection points for maximum tibia ash were 0.39 and 0.33% with and without phytase supplementation respectively. These nPP levels were sufficient to support body weight, feed conversion, or livability. The nPP in HAPC was equivalent in bioavailability to the P from dicalcium phosphate. In the absence of phytase, dry feces of broiler chicks fed YDC diets at the NRC (1994) recommended level of 0.45% nPP contained 1.19% P, whereas at the above inflection points, the fecal P content was 1.06, 1.11, 0.98, and 0.78% for chicks fed YDC diets supplemented with D_3, D_3 + plus phytase, 25-OH-D_3, and 25-OH-D_3 + phytase respectively. For chicks fed HAPC diets at the inflection points, the fecal P content was 0.84 and 0.68% with and without phytase supplementation respectively. Thus fecal P output can be reduced significantly while maintaining optimum live performance and bone mineralization by supplementation with microbial phytase, addition of 25-OH-D_3, use of HAPC, reduction in dietary nPP level, or combinations of the above.

Key words: Phytase, 25 hydroxycholecalciferol, high available phosphate corn, fecal P

Introduction

P is an essential mineral for growing broilers. Because of the demands for adequate skeletal development of the rapidly growing bird and the deleterious consequences of P deficiencies on productivity, it is necessary to provide an adequate margin of safety for this mineral in broiler diets. However, due to the relative expense of this nutrient and growing concerns about the effects of excreted P on eutrophication of surface waters (Sharpley, 1999), there is great interest in providing adequate P while reducing P excretion.

A considerable amount of the P in poultry diets is in the form of phytate P, an organically bound form of the mineral (O'Dell *et al.*, 1972; Raboy, 1990). Chickens are lacking or limited in endogenous phytase that is necessary for breakdown of the molecule and subsequent release of P for absorption. Nelson *et al.* (1968; 1971) demonstrated that addition of exogenous phytase enzyme to broiler diets was an effective way of improving the availability of phytate-bound P. Supplementation of poultry diets with commercially available exogenous phytase enzyme has been shown to be an effective approach to reducing overall dietary P levels by increasing the ability of the chick to utilize a portion of the phytate-bound P (Simons *et al.*, 1990; Broz *et al.*, 1994; Kornegay *et al.*, 1996; Mitchell and Edwards, 1996a, 1996b; Qian *et al.*, 1996; 1997; Aksakal and Bilal, 2002; Rutherfurd *et al.*, 2002, 2004a, 2004b).

Another approach to reducing dietary P levels and minimizing P in the excreta is to develop feedstuffs with modified levels of phytate-bound P. A corn mutation with low phytate P and high available P content has been developed by USDA (Gerbasi *et al.*, 1993; Raboy and Gerbasi, 1996). This hybrid, designated as "high available phosphate corn" (HAPC) contains approximately 0.27% total P, of which 0.17% is

Table 1: Nutrient profile of normal yellow dent corn (YDC) and high available phosphate corn (HAPC) on as fed basis[1]

Nutrient	YDC	HAPC
ME (kcal/kg)	3596.2	3582.67
Dry matter, %	88.13	86.61
Crude protein, %	8.88	8.8
Crude fiber, %	1.9	1.9
Ash, %	1.01	1.2
Crude fat, %	3.79	3.76
Ca, %	0.008	0.009
Total P, %	0.23	0.27
Phytate P, %	0.2	0.097
Nonphytate P, %	0.03	0.173
Alanine, %	0.61	0.61
Arginine, %	0.31	0.33
Aspartic acid, %	0.58	0.59
Cystine, %	0.15	0.17
Glutamic acid, %	1.57	1.56
Glycine, %	0.29	0.3
Histidine, %	0.26	0.27
Isoleucine, %	0.28	0.28
Leucine, %	1.06	1.03
Lysine, %	0.21	0.22
Methionine, %	0.13	0.15
Phenylalanine, %	0.41	0.4
Proline, %	0.75	0.81
Serine, %	0.4	0.41
Threonine, %	0.3	0.3
Tyrosine, %	0.16	0.16
Tryptophan, %	0.05	0.05
Valine, %	0.38	0.39

[1] Provided by Pioneer Hi-Bred International, Inc., Johnson IA 50131.

estimated to be available to the chicken. In contrast, a normal corn hybrid contains similar level of total P, but only 0.03% available P. Substitution of the normal corn with the low-phytate corn would therefore reduce the amount of phytate-bound P in the diet and consequently reduce the amount of P excreted in the litter, which has been successfully demonstrated in broiler diets (Huff *et al.*, 1998; Waldroup *et al.*, 2000).

Another factor that may influence P excretion by broilers is the source of vitamin D used in the diet. Recent research indicates that P utilization by the chick may be improved by increasing dietary levels of cholecalciferol, or by utilizing some vitamin D derivatives, such as 1,25-dihydroxycholecalciferol or 25-hydroxycholecolciferol (25-OH-D$_3$). Studies suggest a synergistic interaction between phytase supplementation and the utilization of the vitamin D derivatives (Biehl *et al.*, 1995; Mitchell and Edwards, 1996a, 1996b). These vitamin D derivatives have been shown to enhance intestinal phytase or to act additively with microbial phytase to improve P utilization in chick diets (Edwards, 1993; Roberson and Edwards, 1994; Biehl *et al.*, 1995; Mitchell and Edwards, 1996a, 1996b). However, the reported studies deal primarily with compounds that are not commercially available (1α-hydroxycholecalciferol and 1, 25-dihydroxycholecalciferol). Some studies with 25-OH-D$_3$, a commercial available vitamin D derivative, suggest activity equal to or greater than that of cholecalciferol (McNaughton *et al.*, 1997; Yarger *et al.* 1995; Edwards, 2002; Ledwaba and Roberson, 2003; Bar *et al.*, 2003; Fritts and Waldroup, 2003).

A study was undertaken to evaluate the ability of the young broiler chicken to utilize the P provided by HAPC and normal dent corn, and to determine the extent to which supplementation with exogenous phytase enzyme or 25-OH-D$_3$ could reduce the demands for dietary P and subsequently reduce P excretion.

Materials and Methods

Diets were formulated with either normal yellow dent corn (YDC) or HAPC, using nutrient composition values provided by the corn breeder (Table 1). Diets were formulated to meet or exceed 105% of the minimum amino acid allowances recommended by NRC (1994) with 0.50% nonphytate P (nPP) and 1.00% Ca. Composition of the diets was shown in Table 2. Removal of the supplemental dicalcium phosphate and limestone from the YDC diet resulted in a diet with 0.09% nPP and 0.35% total P with 0.10% Ca. Removal of the supplemental dicalcium phosphates and limestone from the HAPC diet resulted in a diet with 0.18% nPP and 0.37% total P with 0.09% Ca. Thus, broiler diets based on HAPC contain approximately 27% less phytate-bound P than YDC-based diets.

A large quantity of the respective YDC and HAPC basal diets, exclusive of the dicalcium phosphate and limestone, were prepared and divided into two sublots. One sublot of each basal diet was fortified with the formulated levels of dicalcium phosphate and limestone to provide 0.50% nPP and 1.00% Ca; while the second sublot was supplemented only with sufficient limestone to provide a total of 1.00% Ca. Washed sand was used as inert filler. Therefore, for each corn type, a low-P and a high-P basal diet were prepared, each with 1.00% Ca. These two lots within each corn type were further subdivided and fortified with either vitamin D$_3$ or 25-OH-D$_3$ at the rate of 68.9µg/kg. The basal lots were then analyzed for total P and Ca content[3] to verify proper mixing.

Based upon the results of the analysis, the low-P and high-P basal diets within each corn type and within each vitamin D source were blended in appropriate amounts to provide a series of diets ranging in nPP content from the lowest unsupplemented amount (0.09% for the normal corn diets and 0.18% for the HAPC diets) to 0.50% for both corn types. Increments of 0.05% nPP were used for both corn types. The resulting experimental diets were analyzed for total P and Ca content to verify proper mixing.

Within each corn-type series, diets were fed with and without supplementation with 1000 units/kg phytase enzyme[4]. The enzyme was sprayed on to the mixed feed;

Table 2: Composition (g.kg) of broiler starter diets formulated with normal yellow dent corn (YDC) or high available phosphate corn (HAPC)

Ingredient	YDC	HAPC
Normal yellow dent corn	619.74	0
High available phosphate corn	0	626.95
Soybean meal (47.5% CP)	309.57	305.48
Poultry oil	24.72	23.85
L-Threonine	0.52	0.58
L-Lysine HCl (98%)	0.92	0.98
DL-Methionine	3.27	3.06
Broiler vitamins[1]	2	2
Trace mineral mix[2]	1	1
Salt	4.53	4.54
Variable[3]	33.73	31.56
TOTAL	1000	1000
Calculated analysis		
ME, kcal/kg	3,200.00	3,200.00
Crude protein, %	21.11	21.6
Ca, %	0.1	0.1
Total P, %	0.35	0.37
Nonphytate P, %	0.09	0.18
Sodium, %	0.2	0.2
Chloride, %	0.34	0.35
Methionine, %	0.64	0.63
Lysine, %	1.2	1.19
TSAA, %	0.98	0.98

[1] Provides per kg of diet: 8,800 IU vitamin A; 20 IU vitamin E; 0.015 mg vitamin B12; 8 mg riboflavin; 50 mg niacin; 15 mg pantothenic acid; 465 mg choline; 2 mg vitamin K; 1 mg folic acid; 2 mg thiamin; 2.5 mg pyridoxine; 0.1 mg d-biotin; 125 mg ethoxyquin; 0.1 mg Se.

[2] 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.

[3] Variable amounts of dicalcium phosphate, limestone, or sand.

one part of enzyme was mixed with ten parts of distilled water and slowly applied to the feed in a mixer. Samples of the mixed feeds were assayed for phytase content to verify adequate mixing.

The combination of two types of corn, fed at variable P levels, with and without phytase supplementation, and two different vitamin D sources, resulted in a total of 68 dietary treatments. Each of these treatment diets was fed to four replicate pens of six male chicks of a commercial strain[5]. The chicks 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 battery brooders with raised wire floors and 24 hr fluorescent illumination. They were offered test diets in mash form and tap water for *ad libitum* consumption from day-old to 21 d of age. Care and management of the birds followed recommended guidelines (FASS, 1999).

Body weight of birds was obtained at day-old and at 21 d of age. Feed consumption during the test period was determined. Mortality was checked twice daily; weight of birds that died was used to adjust feed conversion ratio (FCR; grams of feed required per gram of gain). At 14 d, excreta were collected on aluminum foil for a 24 hr period; the excreta were frozen, freeze-dried, and analyzed for total P content. At 21 d, two birds per pen, representative of the mean body weight, were killed by CO_2 inhalation and the right tibia removed for bone ash determination (AOAC, 1990).

Pen means served as the experimental unit for statistical analysis. Data were subjected to analysis of variance as a factorial arrangement of treatments. Main effects of corn type, nPP level, phytase supplementation, and vitamin D source, as well as all two-way, three-way and four-way interactions were evaluated. In order to have a balanced analysis, nPP level of 0.09% for normal corn diets was not considered in the factorial analysis; nPP level of 0.15% for YDC diets and nPP level of 0.18% for HAPC diets were considered equivalent. 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 among or between 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, nonlinear regression analysis was conducted to estimate the inflection points of selected variables using the PROC NLIN procedure of SAS (SAS Institute Inc., 1991), incorporating the SAS macro of Robbins (1986).

Results and Discussion

Crude protein, Ca, total P content of the basal diets prior to preparation of low and high-P diets within each corn type were in agreement with calculated values (Table 2). There was good agreement between calculated and analyzed total P and Ca for the final treatment diets. Total P levels were generally within ±0.02% of the calculated levels (data not shown). The results of phytase assays indicated that the phytase supplemented feed contained the activity commensurate with expected values. Mean phytase content of the supplemented feeds was 1283 ± 142 FTU/kg..

Factorial analysis: A summary of statistical analysis for body weight, feed conversion ratio, and percentage tibia ash is shown in Table 3. Body weights of chicks were significantly affected by main effects of nPP level, phytase supplementation and the interaction between nPP level and phytase supplementation (Table 4). Regardless of corn type and vitamin D source, phytase supplementation significantly increased body weight at dietary nPP level of 0.25% or lower; however addition of phytase had no effects when nPP level was 0.30% or higher, accounting for the significant nPP level × phytase interaction. There were no differences in body weight of chicks fed two types of corn. The body weights of birds fed diets supplemented with vitamin D_3 was not significantly different from those of birds fed diets with 25-OH-D_3 added.

Table 3: Results of statistical analysis for performance parameters

Source of variation	Body weight		Feed conversion		Mortality	
	Prob > F	SEM	Prob > F	SEM	Prob > F	SEM
Corn type (Corn)	0.7393	4.5	0.803	0.015	0.3403	0.165
P level (P)	0.0001	9.54	0.1026	0.034	0.0001	0.348
Corn ×P	0.1921	14.43	0.9953	0.05	0.2073	0.474
Phytase (Phy)	0.0001	4.5	0.0014	0.015	0.0001	0.163
Corn×Phy	0.1308	6.37	0.0814	0.022	0.0132	0.235
P×Phy	0.0001	13.49	0.1219	0.05	0.0001	0.492
Corn×P×Phy	0.1671	20.4	0.4199	0.071	0.8003	0.745
Vitamin D source (Vit D)	0.6067	4.5	0.4201	0.014	0.01	0.163
Corn×Vit D	0.498	6.37	0.3702	0.021	0.0007	0.235
P×Vit D	0.3323	13.49	0.8404	0.049	0.5894	0.492
Corn×P×Vit D	0.5258	20.4	0.0533	0.071	0.1131	0.745
Phy×Vit D	0.513	6.44	0.4797	0.021	0.4202	0.232
Corn×Phy×Vit D	0.8434	9.19	0.266	0.031	0.0608	0.335
P×Phy×Vit D	0.8463	19.08	0.272	0.071	0.607	0.696
Corn×P×Phy×Vit D	0.5002	28.86	0.3689	0.11	0.5351	1.053

Table 4: Body weight (g) of male broilers fed normal corn or high available phosphate corn as influenced by levels of nonphytate P (nPP), phytase supplementation and sources of vitamin D2

nPP (%)	Without phytase[1]			With phytase			Mean		
	D_3	25-OH -D_3	Mean	D_3	25-OH -D_3	Mean	D_3	25-OH-D_3	Mean
Normal corn									
0.15	375	343	359	580	540	560	477	441	459
0.2	464	503	483	580	618	599	522	561	541
0.25	547	588	567	615	605	610	581	596	589
0.3	562	583	573	620	602	611	591	593	592
0.35	621	525	573	638	630	634	629	577	603
0.4	597	625	611	604	600	602	600	612	606
0.45	579	627	603	583	600	591	581	613	597
0.5	596	598	597	605	593	599	600	596	598
Mean	542	549	546	603	598	601	573	574	573
High available phosphate corn									
0.18	435	442	439	575	523	549	505	483	494
0.2	469	463	466	586	541	563	527	502	515
0.25	560	525	542	592	571	581	576	548	562
0.3	588	574	581	594	585	590	591	579	585
0.35	587	588	587	590	570	580	588	579	584
0.4	569	604	586	634	626	630	601	615	608
0.45	629	616	623	572	624	598	601	620	610
0.5	608	596	602	611	631	621	609	613	611
Mean	556	551	553	594	584	589	575	567	571
Mean of corns									
0.15/0.18	405	393	399^{g}	577	531	554^{e}	491	462	477^{l}
0.2	466	483	475^{f}	583	580	581^{abcd}	524	531	528^{k}
0.25	554	556	555^{de}	603	588	596^{abc}	578	572	575^{j}
0.3	575	578	577^{cde}	607	594	600^{abc}	591	586	588^{ij}
0.35	604	556	580^{bcd}	614	600	607^{abc}	609	578	593^{ij}
0.4	583	614	598^{abc}	619	613	616^{a}	601	614	607^{i}
0.45	604	621	613^{ab}	577	612	595^{abc}	591	617	604^{i}
0.5	602	597	599^{abc}	608	612	610^{abc}	605	604	605^{i}
Mean	549	550	549^{y}	599	591	595^{x}	574	570	

a-g, i-l, x-y, means within comparison with common superscripts do not differ significantly (P<0.05).

[1] With or without addition of 1000 units /kg of phytase. [2] Addition of vitamin D3 or 25-OH-D3 at 68.9 mg/kg diet.

Phytase supplementation significantly improved feed conversion ratio by chicks (Table 5). No significant difference in feed conversion ratio was noted for birds fed the two types of corn. Feed conversion ratio was not affected by source of vitamin D either.

Tibia ash was significantly affected by main effects of nPP level, phytase supplementation, vitamin D source, and the interactions between nPP level and phytase, corn and phytase, and corn and vitamin D source (Table 6). Phytase supplementation significantly increased tibia ash when added to diets low in P and had no effects at higher dietary nPP levels, accounting for phytase × nPP

Table 5: Feed conversion (g feed/g gain) by male broilers fed normal corn or high available phosphate corn as influenced by levels of nonphytate P (nPP), phytase supplementation and sources of vitamin D2

nPP (%)	Without phytase[1]			With phytase			Mean		
	D_3	25-OH -D_3	Mean	D_3	25-OH -D_3	Mean	D_3	25-OH-D_3	Mean
0.15	1.778	1.633	1.706	1.439	1.487	1.463	1.609	1.56	1.584
0.2	1.609	1.543	1.576	1.408	1.334	1.371	1.508	1.438	1.473
0.25	1.662	1.479	1.57	1.585	1.508	1.547	1.623	1.494	1.559
0.3	1.516	1.488	1.502	1.506	1.525	1.515	1.511	1.506	1.509
0.35	1.377	1.593	1.485	1.386	1.554	1.47	1.382	1.574	1.478
0.4	1.549	1.547	1.548	1.473	1.433	1.543	1.511	1.49	1.501
0.45	1.506	1.459	1.483	1.441	1.427	1.434	1.474	1.443	1.458
0.5	1.468	1.777	1.623	1.451	1.336	1.394	1.46	1.557	1.508
Mean	1.558	1.565	1.562	1.461	1.451	1.456	1.51	1.508	1.509
0.18	1.711	1.602	1.657	1.437	1.618	1.527	1.574	1.61	1.592
0.2	1.496	1.446	1.471	1.354	1.643	1.498	1.425	1.545	1.485
0.25	1.495	1.617	1.556	1.461	1.466	1.463	1.478	1.541	1.509
0.3	1.42	1.589	1.505	1.482	1.56	1.521	1.451	1.574	1.513
0.35	1.442	1.482	1.462	1.559	1.471	1.515	1.501	1.477	1.489
0.4	1.548	1.602	1.575	1.324	1.457	1.391	1.436	1.53	1.483
0.45	1.449	1.354	1.401	1.435	1.522	1.478	1.442	1.438	1.44
0.5	1.603	1.452	1.527	1.552	1.464	1.508	1.577	1.458	1.517
Mean	1.52	1.518	1.519	1.45	1.525	1.488	1.485	1.522	1.503
0.15/0.18	1.745	1.618	1.681	1.438	1.552	1.495	1.591	1.585	1.588
0.2	1.552	1.495	1.523	1.381	1.489	1.435	1.466	1.492	1.479
0.25	1.578	1.548	1.563	1.523	1.487	1.505	1.551	1.517	1.534
0.3	1.468	1.538	1.503	1.494	1.542	1.518	1.481	1.54	1.511
0.35	1.41	1.538	1.474	1.473	1.513	1.493	1.441	1.525	1.483
0.4	1.549	1.575	1.562	1.399	1.445	1.422	1.474	1.51	1.492
0.45	1.478	1.406	1.442	1.438	1.474	1.456	1.458	1.44	1.449
0.5	1.535	1.615	1.575	1.502	1.4	1.451	1.519	1.507	1.513
Mean	1.539	1.541	1.540^{a}	1.456	1.488	1.472^{b}	1.498	1.515	

a-b means in rows with common superscripts do not differ significantly ($P<0.05$).

[1]With or without addition of 1000 units /kg of phytase. [2]Addition of vitamin D3 or 25-OH-D3 at 68.9 mg/kg diet.

level interaction. Tibia ash content of chicks fed both types of corn was increased by phytase supplementation; however, the improvement was greater in chicks fed YDC diets, accounting for corn × phytase interaction. Chicks fed YDC diets with the presence of 25-OH-D_3 had higher tibia ash percentage than chicks fed YDC diets supplemented with D_3; however for chicks fed HAPC diets, vitamin D source did not result in a significant difference in tibia ash content. High mortality was encountered only on the test diets made of YDC with 0.09 and 0.15% nPP without phytase supplementation, due to the deficiency of P. There was no significant difference in mortality among the rest of the treatments, which indicated that in the absence of phytase supplementation in YDC diets, a nPP level of 0.20% (0.11% supplemental P) was required to minimize mortality of chicks raised in battery brooders; whereas for HAPC diets, the lowest unsupplemented level (0.18%) was adequate. With phytase supplementation no supplemental inorganic phosphate was needed to support livability regardless of the corn source (Data not shown).

There were no differences in body weight, feed conversion ratio, and percentage tibia ash of chicks fed two types of corn. The YDC used in this study was an isogenic normal phytate counterpart to the HAPC, grow at the same location during the same year, with virtually identical analyzed nutrient contents except P. No difference in performance was expected when the YDC and HAPC diets were formulated to contain the same amount of nutrients including nPP level. However, more nPP in the HAPC diets was from corn itself and less nPP was from dicalcium phosphate, compared with YDC diets. The results of the current study indicated that the availability of nPP in HAPC was basically the same from that in dicalcium phosphate. This agrees with the findings of Waldroup *et al.* (2000), who reported that the nPP in the HAPC is equivalent in biological value to commercial dicalcium phosphate in broiler chicks. Some studies with HAPC or low phytate corns also showed that the P from HAPC was more available that the P from the normal corn (Cromwell *et al.*, 1998; Ertl *et al.*, 1998; Li *et al.*, 2000). Cromwell *et al.* (1998) reported that the P in the low phytic acid corns was approximately five times as bioavailable as the P in normal corn for growing chicks with bioavailability of P being 10% for the normal corn and 45% or 52% for the low phytic acid corns (bioavailability of the P in monosodium phosphate

Table 6: Tibia ash percentage of male broilers fed normal corn or high available phosphate corn as influenced by levels of nonphytate P (nPP), phytase supplementation and sources of vitamin D[2]

nPP (%)	Without phytase[1]			With phytase			Mean		
	D_3	25-OH -D_3	Mean	D_3	25-OH -D_3	Mean	D_3	25-OH-D_3	Mean
Normal corn									
0.15	30.49	31.04	30.77	37.74	37.26	37.5	34.12	34.15	34.13
0.2	32.46	34.64	33.55	39.91	39.76	39.83	36.18	37.2	36.69
0.25	34.55	37.91	36.23	40.72	42.84	41.78	37.63	40.37	39
0.3	36.29	41.93	39.11	41.51	42.18	41.85	38.9	42.05	40.48
0.35	40.95	42.29	41.62	43.47	44.49	43.98	42.21	43.39	42.8
0.4	41.85	42.46	42.15	43.51	44.43	43.97	42.68	43.44	43.06
0.45	43.11	43.95	43.53	43.39	44.43	43.91	43.25	44.19	43.72
0.5	41.38	43.07	42.23	42.25	43.37	42.81	41.82	43.22	42.52
Mean	37.64	39.66	38.65l	41.56	42.34	41.95^{j}	39.60^{p}	41.00^{o}	40.3
High available phosphate corn									
0.18	33.92	31.98	32.95	38.8	37.56	38.18	36.36	34.77	35.57
0.2	33.74	33.89	33.82	39.72	39.61	39.66	36.73	36.75	36.74
0.25	38.45	36.81	37.63	39.52	40.51	40.02	38.99	38.66	38.83
0.3	41.29	39.5	40.39	42.64	41.16	41.9	41.97	40.33	41.15
0.35	41.68	40.42	41.05	42.35	43.28	42.82	42.02	41.85	41.94
0.4	41.55	43.92	42.74	43.55	43.53	43.54	42.55	43.72	43.14
0.45	42.8	43.25	43.03	42.59	44.23	43.41	42.69	43.74	43.22
0.5	43.94	44.02	43.98	43.39	43.08	43.24	43.66	43.55	43.61
Mean	39.67	39.22	39.45^{k}	41.57	41.62	41.60^{j}	40.62o	40.42^{o}	40.52
Mean of corns									
0.15/0.18	32.21	31.51	31.88^{i}	38.27	37.41	37.84^{g}	35.24	34.46	34.85^{w}
0.2	33.1	34.27	33.68^{h}	39.81	39.68	39.75^{f}	36.46	36.97	36.72^{v}
0.25	36.5	37.36	36.93^{g}	40.12	41.67	40.90^{ef}	38.31	39.52	38.91^{u}
0.3	38.79	40.71	39.75^{f}	42.08	41.67	41.87^{cde}	40.43	41.19	40.81^{t}
0.35	41.32	41.36	41.34^{de}	42.91	43.89	43.40^{ab}	42.11	42.62	42.37^{s}
0.4	41.7	43.19	42.44^{bcd}	43.53	43.98	43.75^{a}	42.61	43.58	43.10^{rs}
0.45	42.95	43.6	43.28^{ab}	42.99	44.33	43.66^{ab}	42.97	43.97	43.47^{r}
0.5	42.66	43.55	43.10^{abc}	42.82	43.22	43.02^{abc}	42.74	43.39	43.06^{rs}
Mean	38.65	39.44	39.05^{y}	41.57	41.98	41.77^{x}	40.11^{n}	40.71^{m}	

a-i, j-l, m-n, o-p, r-w, x-y means within comparison with common superscripts do not differ significantly ($P<0.05$).

[1] With or without addition of 1000 units /kg of phytase. [2] Addition of vitamin D3 or 25-OH-D3 at 68.9 mg/kg diet.

= 100%). Li *et al.* (2000) concluded that the P in the low phytate corn was more available than the P in normal corn for broilers. They also used an *in vitro* digestion procedure to determine the percentage of total P released and reported that 65% of total P was released in the low phytate corn and only 23% released in the normal corn.

A significant corn × phytase interaction was noted in percentage tibia ash with greater response to phytase for YDC diets than for HAPC diets. This should be expected since YDC contained 50% more phytate P than HAPC (0.20 vs 0.10%), which resulted in 37% more phytate-bound P, available to be released by phytase, in YDC diets compared to HAPC diets (0.26 vs 0.19%). Waldroup *et al.* (2000) reported that the increase in body weight gain and tibia ash of broiler chicks fed YDC diets by phytase supplementation was greater than those of birds fed HAPC diets. Kornegay and Denbow (1999) found the magnitude of the responses (body weight gains, toe and tibia ash percentage, P retention) to supplemental phytase in the low phytate corn diets was about 63% of those obtained when phytase was added to normal corn diets in young turkeys.

Supplementation of 25-OH-D_3 resulted in a higher percentage tibia ash of chicks fed YDC diets compared with vitamin D_3; however, it had no effect when added to HAPC diets. Difference in phytate-bound P content is the primary difference between YDC and HAPC diets. The results indicated that 25-OH-D_3 had the potential to increase P utilization, most probably via increasing phytate P utilization. Limited research has been done to study the effects of 25-OH-D_3 on P utilization and the results have not been consistent. Applegate *et al.* (2000) reported that 25-OH-D_3 increased apparent ileal phytate P hydrolysis of young broilers. Roberson (1999) did not observe any effects of 25-OH-D_3 on phytate P utilization in young chicks. Applegate *et al.* (2003) failed to demonstrate the ability of 25-OH-D_3 to increase ileal phytate P hydrolysis, although they did observe a 2.7% increase in tibia ash of broiler chickens. Edwards (2002) compared the efficacy of vitamin D derivatives for stimulating phytate utilization in broilers and found that 1,25-$(OH)_2$-D_3 and 1 α –OH-D_3 were consistently effective in increasing phytate P utilization, whereas the effect of 25-OH-D_3 was smaller and less consistent. Whether 25-OH-D_3 has any effect on intestinal phytase activity is not

Table 7: Results of nonlinear regression analysis to estimate the nonphytate P requirement of male broilers fed normal yellow dent corn (YDC) or high available phosphate corn (HAPC) diets as influenced by phytase supplementation and vitamin D source

Corn type	Added Phytase[1]	Vitamin D source[2]	Value at inflection	Inflection point[3]	Asymptotic standard error	Asymptotic 95% confidence interval
YDC	No	----	614	0.283	0.016	0.244-0.322
YDC	Yes	----	607	0.211	0.023	0.155-0.266
HAPC	No	----	596	0.287	0.017	0.246-0.327
HAPC	Yes	----	595	0.246	0.035	0.159-0.332
Tibia ash, %						
YDC	No	D3	42.11	0.395	0.02	0.345-0.445
YDC	No	25-OH-D3	43.24	0.321	0.009	0.299-0.344
YDC	Yes	D3	43.06	0.35	0.031	0.271-0.429
YDC	Yes	25-OH-D3	43.3	0.269	0.02	0.219-0.318
HAPC	No	----	43.26	0.387	0.015	0.348-0.427
HAPC	Yes	----	42.75	0.325	0.022	-0.116

clear. Applegate *et al.* (2000; 2003) reported that intestinal phytase was not affected by 25-OH-D_3 and suggested that the increase in phytate P utilization was probably due to an increase in intestinal Ca or P absorption.

Regression analysis: Results of nonlinear regression analysis to estimate the nPP requirement for optimum body weight and tibia ash content of chicks as influenced by corn type, vitamin D source, and phytase supplementation were shown in Table 7. The requirement was established as the inflection point of the one-slope regression model (Robbins, 1986; Yu and Morris, 1999). Because vitamin D source had no effects on body weight, four estimates were made for body weight to include YDC and HAPC diets with and without phytase supplementation using the mean of D_3 and 25-OH-D_3 results. The 25-OH-D_3 supplementation increased tibia ash of chicks fed YDC diets; therefore, to obtain inflection points of tibia ash for YDC diets, four estimates were made to include vitamin D_3 and 25-OH-D_3 with and without phytase supplementation. Because no significant difference in tibia ash of chicks fed HAPC diets due to vitamin D source was noted, to obtain inflection points of tibia ash for HAPC diets, only two estimates were made to include with and without phytase supplementation using the mean of D_3 and 25-OH-D_3 results.

Without phytase supplementation, the inflection point for body weight was 0.283±0.016% nPP for chicks fed YDC diets, 0.287±0.017% nPP for chicks fed HAPC diets. When phytase was added to the diets, inflection points for body weight of 0.211±0.023% and 0.246±0.035% nPP were obtained for chicks fed YDC and HAPC diets, respectively. For chicks fed YDC diets without phytase supplementation, tibia ash percentage was optimized with 0.395±0.020% by use of D_3 and 0.321±0.009% by use of 25-OH-D_3 in the diet. When phytase was added to diets containing YDC, inflection points for tibia ash were reduced to 0.350±0.031% and 0.269 ± 0.020 % nPP by use of D_3 and 25-OH-D_3, respectively. For chicks fed HAPC diets, percentage tibia ash was maximized with 0.387±0.015% nPP without phytase added. With phytase present in the HAPC diet, 0.325±0.022% nPP was required to maximize tibia ash. An nPP level needed to optimize feed conversion ratio or minimize mortality was not well estimated by nonlinear regression. Based upon the results of factorial analysis, an nPP level required to maximize body weight or percentage tibia ash was sufficient to optimize feed conversion ratio or minimize mortality.

The greatest need of chicks for nPP was for maximizing tibia ash, followed by the need to maximize body weight. An nPP level needed for optimum feed conversion ratio or minimum mortality was much less than a nPP level required for maximum body weight or tibia ash. When diets were supplemented with vitamin D_3 and in the absence of phytase, the estimated nPP requirements for maximum tibia ash were 0.395±0.020% for chicks fed YDC diets and 0.387±0.015% for chicks fed HAPC diets. These values again showed that the nPP in HAPC was biologically equivalent to the P in dicalcium phosphate. These values are lower than suggested by NRC (1994). The results are in agreement with Waldroup *et al.* (2000), who reported that in the absence of phytase, the requirement for maximum tibia ash was 0.39% and 0.37% for YDC and HAPC diets, respectively. Substitution of vitamin D_3 with 25-OH-D_3 alone in the YDC diets reduced the inflection point of tibia ash by about 0.07%, whereas phytase supplementation to YDC diets alone reduced the inflection point of tibia ash by 0.05%. The combination of phytase supplementation and 25-OH-D_3 further reduced the nPP required to maximize tibia ash by 0.13%. It appeared that the effects of phytase and 25-OH-D_3 on increasing P availability and reduce P requirements were additive, indicating a different mechanism of action. Mitchell and Edwards (1996a) reported that dietary phytase (600 unit/kg) or 1,25-$(OH)_2$-D_3 (5µg/kg) can replace 0.1% inorganic P for criteria such as body weight, bone ash, and plasma P, and the combination of the two can replace 0.2% inorganic P in young broiler chickens. Mitchell and

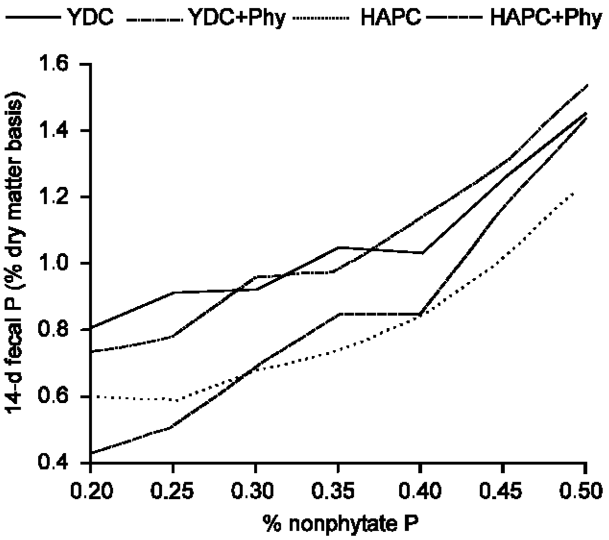

Fig. 1: Excreta P content (% dry matter basis) of broilers fed diets with yellow dent corn (YDC) or high available phosphate corn (HAPC) with and without phytase supplementation of 1000 units/kg phytase (Phy) in diets varying levels of nonphytate P

Edwards (1996b) demonstrated that phytase and 1, 25-$(OH)_2$-D_3 had synergistic effects on improving the birds' ability to utilize phytate P. They suggested that 1, 25-$(OH)_2$-D_3 increase phytate P utilization through stimulating uptake of the P released by phytase, or through increasing intestinal phytase activity.

Fecal P content: Fecal P contents of chicks fed YDC and HAPC diets with and without phytase supplementation at varying levels of dietary nPP were shown in Fig. 1. The mean of D_3 and 25-OH-D_3 was used in the figure since vitamin D source had little effect on fecal P content. Regardless of corn type and phytase supplementation, fecal P content increased gradually with increasing dietary nPP until an nPP level sufficient to maximize tibia ash was reached when fecal P content rose sharply. The percentage P in dry feces of chicks fed HAPC diets was markedly lower than that of chicks fed YDC diets, regardless of dietary nPP level or phytase supplementation.

Dried feces of chicks fed YDC diets with 0.45% nPP, the NRC recommended level, supplemented with vitamin D_3 in the absence of phytase contained 1.19% P. Fecal P content of chicks fed YDC diets at the inflection points for maximum tibia ash was 1.06% for D_3 supplementation without phytase, 1.11% for 25-OH-D_3 supplementation without phytase, 0.98% for D_3 and phytase supplementation, and 0.78% for 25-OH-D_3 and phytase supplementation. For chicks fed HAPC diets, the fecal P at inflection points for tibia ash was 0.84% without phytase and 0.68% with phytase supplementation. Therefore, use of 25-OH-D_3 and phytase in the YDC diets reduced fecal P content by 6.72 and 17.6% and the combination reduced fecal P by 34%. The dry fecal P of chicks fed HAPC diets at the inflection points for tibia ash contained 29.4% less P compared with that of chicks fed YDC diets with NRC (1994) recommended level of nPP. The use of HAPC combined with phytase supplementation at the inflection point for tibia ash decreased fecal P content by 43%. This is in agreement with Waldroup *et al.* (2000) who reported a 47% reduction in fecal P content by the combined use of HAPC and phytase in conjunction with reduced dietary P levels in broiler chicks.

In conclusion, the results of the current study showed the potential contributions of phytase, 25-OH-D_3, and HAPC, when used alone or in combination, to reducing dietary P level and therefore reducing fecal P excretion while still maintaining optimum growth performance and bone mineralization.

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[3] Agricultural Diagnostic Services Laboratory, University of Arkansas, Fayetteville, AR 72701

[4] Natuphos, BASF Corporation, Mt. Olive, NJ 07828). 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 water bath temperature of 37°C

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