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The Phosphorus Requirement of Broiler Chicks as Affected by Dietary Calcium and Vitamin D and Phytase Supplementation

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Abstract: Two experiments determined the quantitative relationships between dietary calcium, NPP (Nonphytate Phosphorus), vitamin D and phytase in diets for broiler chicks to 20 days of age. In experiment one, the NPP requirement was approximately 0.40% when the diet contained 0.90% calcium and 3000 ICU of vitamin D/kg. This was based on a linear response in chick weight, feed intake, bone ash and blood calcium and phosphorus to increasing dietary NPP. When the diet contained 0.70% calcium, bone ash and blood phosphorus had significant responses to added dietary NPP but other parameters had no significant responses. The NPP requirement was approximately 0.30% of the diet. Data from these experiments indicate the calcium: NPP ratio is approximately 2.25. Adding phytase at 773 or 1545 units per kg of diet gave responses of approximately 0.05% NPP per 773 units. In experiment two, vitamin D additions were 500, 2,500 or 12,500 ICU kg^{-1} of diet that contained 0.80% calcium. Between 500 and 2,500 ICU kg^{-1}, the response was equal to approximately 0.013% NPP per 1000 ICU kg^{-1}. Between 2,500 and 12,500 ICU kg^{-1} diet, the response was equal to approximately 0.003% NPP per 1000 ICU. The addition of 537 units of phytase kg^{-1} to a diet with 0.20% NPP caused a response equivalent to 0.05-0.07% NPP. Increasing the phytase to 1074 units per kg of diet gave no additional response. A discussion of the results suggests the importance of the solubility product and the common ion effect for explaining results in calcium and phosphorus nutrition.

Key words: Calcium, phosphorus, vitamin D, phytase, solubility product

INTRODUCTION

Phosphorus that is part of poultry feed is divided into two parts by poultry. One is retained in products such as live poultry and eggs and is usually removed from the poultry farm in the product. The remaining phosphorus passes through the chicken and is part of the manure. The amount of manure that can be applied to land is affected by its phosphorus content. A balance must be achieved between the amount of phosphorus applied to the land and the amount that will be removed by a crop.

Because phosphorus from land that enters water can cause deleterious effects on the aquatic environment, it is important to minimize the phosphorus content of poultry manure (Carpenter *et al*., 1998). Phytase is an enzyme that is added to feeds to improve the digestion of phytate phosphorus and decrease the phosphorus content of manure (Yan *et al*., 2001; Plumstead *et al*., 2008). An accurate assessment of the enzyme's activity is important for improving phosphorus balance. To receive benefit from the enzyme and decrease phosphorus in the manure, total phosphorus in the diet should be decreased so that all of the phosphorus that is released from phytate is used to satisfy the NPP requirement of the animal. If the activity of phytase is overstated, phosphorus from the diet will not meet requirements, causing poultry to become deficient and decreasing performance.

The calcium content of the diet is known to affect the utilization of dietary phosphorus. One explanation is that increasing calcium decreases phytase activity thus decreasing the use of phytate phosphorus (Applegate *et al*., 2003; Tamim *et al*., 2004). Another variable that can affect phosphorus utilization is the dietary content of vitamin D or its metabolites (Biehl *et al*., 1998; Edwards, 1993; Mitchell and Edwards, 1996). The efficacies that have been reported vary considerably, based on the metabolite and amounts added to the diet.

The goal of this research was to quantify effects of various components of a broiler diet that affect the amount of NPP that is required. Accurate information is needed about these interactions to make the best possible choices for computer feed formulation. These include calcium content, vitamin D content and phytase addition.

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MATERIALS AND METHODS

Experiment one was to determine to what extent the calcium content of the diet affected the requirement for NPP in broiler chicks. A secondary objective was to determine if the effectiveness of phytase was altered by the two calcium levels. The two calcium concentrations were 0.70 and 0.90% of the diet (Table 1). To develop a response curve for NPP, diets were calculated to contain 0.25, 0.30, 0.35 and 0.40% NPP. Diets were analyzed for nutrient content using a gravimetric procedure for total phosphorus (AOAC, 2006, Official Method 966.01) and the FAAS procedure (AOAC, 2006, Official Method 975.03B) for calcium. Two additional diets for each calcium treatment included a planned supplement of 500 or 1000 phytase units per kg (Natuphos 5000) to a diet that had 0.20% NPP. Diets with phytase had a lower NPP than the standard curve so that phytase activity could be more accurately measured. The assumption was that phytase would release enough NPP so that chick responses would be above those for the 0.25% NPP diet.

Ross 708 chicks of both sexes were used for the experiment. They were randomly distributed to pens in battery brooders, six per pen. Two replicate pens of chicks were fed each diet in three different trials, spaced approximately 1 month apart. Standard management practices were approved by the institutional animal care committee. Chicks were fed and watered *ad libitum*. They were weighed at 20 days and feed consumption was determined.

Table 1: Composition (%) of the diets

Composition	0.20% NPP, 0.70% Ca	0.40% NPP, 0.70% Ca	0.20% NPP, 0.90% Ca	0.40% NPP 0.90% Ca
Ingredient				
Corn	57.90	57.45	57.37	56.92
Soybean meal (48%)	32.70	32.70	32.70	32.70
Corn gluten meal	3.00	3.00	3.00	3.00
Soybean oil	3.00	3.00	3.00	3.00
Dicalcium phosphate	0.41	1.48	0.41	1.48
Limestone	1.34	0.72	1.87	1.25
Salt	0.50	0.50	0.50	0.50
Methionine	0.15	0.15	0.15	0.15
Vitamin and TM mix[1]	1.00	1.00	1.00	1.00
Phytase premix	+	-	+	-
Calculated nutrients				
NPP (%)	0.20	0.40	0.20	0.40
Total P (%)	0.41	0.65	0.41	0.65
Calcium (%)	0.70	0.70	0.90	0.90
Protein (%)	22.00	22.00	22.00	22.00
Met+Cys (%)	0.90	0.90	0.90	0.90
Lys (%)	1.16	1.16	1.16	1.16
Analyzed minerals				
Total P (%)	0.44	0.65	0.45	0.63
Calcium (%)	0.71	0.74	0.93	0.89

[1]The vitamin and Trace Mineral (TM) premix provided the following per kg of diet: retinyl palmitate, 3,000 IU; cholecalciferol, 3000 ICU; DL-alpha-tocopherol, 10 IU; menadione sodium bisulfite, 1 mg; thiamin. 1.8 mg; riboflavin, 3.6 mg; niacin, 25.0 mg; pantothenic acid, 10.0 mg; pyridoxine, 3.5 mg; folacin, 0.5 mg; biotin, 0.15 mg; vitamin B_{12}, 0.01 mg; choline, 500 mg; ethoxyquin, 50 mg; copper, 8 mg; iron, 80 mg; manganese, 60 mg; selenium, 0.1 mg and zinc, 40 mg

At 20 days, a male and female that appeared closest to average size for the pen were selected. A blood sample was collected by heart puncture and delivered into a heparinized tube. After centrifugation, plasma was analyzed for calcium and phosphorus content (Cobas 6000 analyzer, Roche, Indianapolis, IN 46250). Chicks were killed by cervical dislocation. The left tibia of each chick was removed and cleaned to expose the proximal end. A razor blade was used to make a longitudinal cut so that the length of the growth plate, including the primary spongiosa, could be measured. The right tibia was removed and cleaned for drying and ashing.

Statistical analyses were done with pen as the experimental unit, providing 72 experimental observations. For mineral values in blood, values of the 2 chicks were averaged for a pen mean. The same was done for bone ash and growth plate measurements. Significant treatment differences were determined by using the General Linear Model of SAS Institute (1996). When significant differences were found, equations describing a linear NPP response for each parameter were calculated for each dietary calcium concentration.

Experiment Two investigated the ability of vitamin D to improve the utilization of NPP. Ross 708 chicks were again used in battery pens, six chicks per pen with four replicate pens per treatment. The experimental design was a factorial with three concentrations of vitamin D and four concentrations of NPP. Vitamin D was included at 500, 2,500 and 12,500 ICU kg^{-1}. NPP was included at 0.200, 0.233, 0.267 and 0.300% of the diet. All diets contained 0.80% calcium. In addition, phytase was added at a planned concentration of 600 units kg^{-1} to diets with 0.20% NPP for each of the three vitamin D concentrations and at 1200 units kg^{-1} for diets with 2,500 and 12,500 ICU kg^{-1} of vitamin D.

At 20 days of age, chick weight and feed consumption were determined. A male and female from each pen were selected to provide the left tibia for measurements. Dried tibia weight, percentage ash in the tibia and ash relative to body weight were measured. Results were compared using the General Linear Model of SAS Institute (1996).

RESULTS

Determined values for total phosphorus and calcium were close to calculated values (Table 1). For phytase activity, the determined values were somewhat higher than the calculated values. For all of the parameters that were examined, dietary NPP and dietary calcium caused significant responses (Table 2). Further statistical

Table 2: Chick growth, bone measurements and blood minerals as affected by dietary components (Experiment one)

Dietary treatments							Blood (mg dL^{-1})	
NPP (%)	Phytase (U kg^{-1})	Ca (%)	Ck Wt. (g)	Feed/Chick (g)	GP (mm)	Bone Ash (%)	CA	P
0.25	0	0.70	633.00	778.00	6.50	37.50	11.80	4.40
0.30	0	0.70	656.00	812.00	5.80	41.10	11.30	5.70
0.35	0	0.70	645.00	796.00	5.20	40.00	11.10	7.10
0.40	0	0.70	675.00	834.00	4.60	41.10	11.10	7.90
0.20	773	0.70	558.00	691.00	10.00	34.20	13.00	3.10
0.20	1545	0.70	639.00	788.00	6.00	37.30	12.50	3.40
0.25	0	0.90	537.00	718.00	8.00	35.60	13.40	3.40
0.30	0	0.90	602.00	761.00	7.10	37.00	13.40	4.60
0.35	0	0.90	637.00	809.00	6.00	39.80	12.20	5.50
0.40	0	0.90	663.00	836.00	5.30	40.30	11.30	7.40
0.20	773	0.90	539.00	675.00	9.20	35.40	13.40	3.90
0.20	1545	0.90	594.00	763.00	7.20	36.90	13.30	3.80
SEM	-	-	47.00	55.00	1.50	1.82	0.70	0.60
Prob.	NPP	-	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Calcium	-	<0.01	0.16	<0.01	<0.01	<0.01	<0.01
NPP x calcium		-	0.02	0.02	0.37	0.14	0.57	0.57

[1]Based on manufacturer's analysis of phytase premix

examination of the results by using linear equations (Table 3) showed that with 0.70% dietary calcium and 0.25% dietary NPP, chick weight and feed/chick were close to a maximum: Adding more NPP to the diet caused no significant increase. When diets contained 0.90% calcium, chick weight and feed intake increased in a linear manner in response to added dietary NPP.

Growth plate length and bone ash percentage also showed significant responses to dietary calcium and NPP content (Table 2 and 3). Growth plate length decreased in response to increasing NPP but bone ash percentage increased. Dietary calcium content affected both of these parameters.

Blood concentrations of calcium and phosphorus were altered by the dietary concentrations of calcium and phosphorus (Table 2 and 3). When dietary calcium was 0.70%, blood calcium was not affected by dietary NPP however, blood phosphorus showed a significant increase with increasing dietary NPP. When dietary calcium was 0.90%, blood calcium decreased in a linear manner with increasing dietary NPP and blood phosphorus increased in a linear manner.

For the 0.70% calcium diet, adding phytase at 773 units kg^{-1} of diet to the 0.20% NPP diet produced results that were inferior to those from the 0.25% NPP diet (Table 2) indicating this amount of phytase was equivalent to <0.05% NPP. When phytase was added to the 0.20% NPP diet to provide 1545 units kg^{-1} of diet, results were comparable to those from the 0.25% NPP diet indicating this amount of phytase was equivalent to approximately 0.05% NPP.

For the 0.90% calcium and 0.20% NPP diet, adding phytase at 773 units kg^{-1} produced results that were comparable to those from 0.25% NPP. When 1545 units of phytase were added per kg of diet, results were similar to those from the 0.30% NPP diet. At a calcium level of 0.90%, 773 and 1545 units kg^{-1} were equivalent to 0.05 and 0.10% NPP, respectively. The calcium content of the diet affected the response to NPP content of the diet (Table 2). Chicks fed 0.70% calcium had numerically superior results for all of the criteria at a particular NPP concentration of the diet. For chick weight, bone ash, blood calcium, blood phosphorus and growth plate length, the 0.70% calcium diet produced significantly better results than the 0.90% calcium diet.

Table 3: Linear equations to describe response curves to dietary NPP content at two dietary calcium concentrations (Experiment one)

Calcium (%)	Calcium (%)
0.7	0.9
Chick weight	Chick weight
p = 0.40	Intercept = 0.34 (p<0.01)
	NPP = 0.83 (p<0.01)
Feed/chick	Feed/chick
p = 0.46	Intercept = 0.51 (p<0.01)
	NPP = 0.86 (p<0.01)
Growth plate length	Growth plate length
Intercept = 8.93 (p<0.01)	Intercept = 12.58 (p<0.01)
NPP = -11.33 (p<0.01)	NPP = -18.33 (p<0.01)
Bone ash	Bone ash
Intercept = 33.40 (p<0.01)	Intercept = 27.06 (p<0.01)
NPP = 20.03 (p<0.01)	NPP = 34.13 (p<0.01)
Blood calcium	Blood calcium
p = 0.13	Intercept = 17.56 (p<0.01)
	NPP = -15.37 (p<0.01)
Blood phosphorus	Blood phosphorus
Intercept = -1.46 (p = 0.24)	Intercept = -3.17 (p<0.01)
NPP = 23.90 (p<0.01)	NPP = 25.80 (p<0.01)

In Experiment two, added dietary NPP caused significant improvements in males for chick weight, feed intake and all of the bone parameters (Table 4). For females, vitamin D concentrations caused significant improvements in all of the parameters except dried tibias. Higher concentrations of vitamin D elevated the measured parameters when 0.200% NPP was fed for the response curve. For example, chicks fed 0.200% NPP and 12,

Table 4: Effects of dietary vitamin D and NPP on chick performance and bone measurements (experiment two)

					Male			Female		
Vit. D (ICU kg^{-1})	NPP (%)	Phyt. (U kg^{-1})	Ck Wt. (g)	Fd Int. (g)	Bone (g)	Ash (%)	Ash (100 BW mg^{-1})	Bone (g)	Ash (mg)	Ash (100 B Mg^{-1})
500	0.2	-	386.000	598.000	1.28	25.0	79.90	1.47	26.00	78.90
	0.233	-	521.000	665.000	1.75	30.0	96.70	1.79	30.00	87.90
	0.267	-	600.000	797.000	2.19	33.0	108.40	1.86	36.00	96.30
	0.3	-	594.000	811.000	1.97	34.0	108.20	1.97	34.00	110.40
	0.2	537	573.000	734.000	1.84	31.0	96.50	1.74	34.00	103.30
2,500	0.2	-	495.000	655.000	1.66	29.0	95.60	1.58	30.00	88.30
	0.233	-	566.000	742.000	1.83	30.0	88.80	1.67	30.00	92.20
	0.267	-	595.000	792.000	2.00	33.0	100.90	1.82	34.00	106.40
	0.3	-	600.000	798.000	2.16	35.0	114.50	1.91	35.00	113.30
	0.2	537	591.000	780.000	2.03	34.0	107.60	1.77	33.00	98.30
	0.2	1074	550.000	736.000	1.67	33.0	99.80	1.78	36.00	106.10
12,500	0.2	-	580.000	752.000	1.93	31.0	98.10	1.68	34.00	105.10
	0.233	-	605.000	785.000	2.07	32.0	104.40	1.73	35.00	104.80
	0.267	-	636.000	827.000	2.27	35.0	109.80	1.88	36.00	110.70
	0.3	-	635.000	826.000	2.27	35.0	117.60	1.94	36.00	109.20
	0.2	537	615.000	785.000	2.11	34.0	113.30	1.93	34.00	106.30
	0.2	1074	597.000	774.000	2.14	37.0	119.30	1.84	35.00	107.90
SEM	-	-	-	-	0.28	1.9	9.58	0.16	2.00	8.73
Prob.	NPP	-	<0.010	<0.010	<0.01	<0.01	<0.01	0.01	<0.01	<0.01
	Vitamin D	-	<0.010	<0.010	<0.01	<0.01	0.02	0.34	<0.01	<0.01
	Vit. D x NPP	-	0.270	0.120	0.05	0.12	0.15	0.37	0.01	0.09

[1]Based on manufacturer's analysis of phytase premix

500 ICU kg^{-1} had a body weight of 580 g. For chicks that were fed 2, 500 ICU kg^{-1}, 580 g was equivalent to approximately 0.25% NPP. For chicks that were fed 500 ICU kg^{-1}, 580 g was equal to approximately 0.26% NPP. As a result, higher vitamin D concentrations compressed the NPP response curve. If body weight is again used as the parameter, the maximum response to 0.10% NPP was 214 g for chicks fed 500 ICU kg^{-1}, 109 g for chicks fed 2, 500 ICU kg^{-1} and 58 g for chicks fed 12, 500 ICU kg^{-1}. A similar compression of the NPP response curve was evident for other parameters when vitamin D concentrations in the diet were increased.

A phosphorus equivalence can be calculated from the vitamin D supplementation. If one assumes a linear response to weight gain between 500 and 2, 500 ICU kg^{-1} then a weight gain of 521 g can be achieved with 500 ICU of vitamin D kg^{-1} and 0.233% NPP or with 2, 500 ICU of vitamin D and 0.208% NPP therefore, an increase of 2000 ICU of vitamin D was equivalent to approximately 0.025% NPP, 0.013% NPP per 1000 ICU. If the same calculations to equalize weight gain to 580 g are completed with the interval between 2, 500 and 12,500 kg^{-1}, this can be achieved with a diet of 2,500 ICU kg^{-1} and 0.234% NPP or 12, 500 ICU and 0.200% NPP. At this vitamin D interval each 1000 ICU per kg increase is equivalent to 0.003% NPP.

When 537 units of phytase were added to a diet with 500 ICU, parameter responses were between those for 0.233 and 0.267% NPP for growth, feed intake and bone parameters for males. An approximate equivalence was 0.05% NPP. For females, the responses suggested a similar value except that ash 100 g^{-1} of body weight suggested a higher efficacy for phytase. When 537 units of phytase were added to a diet with 2, 500 ICU, the responses in parameters suggested a higher phytase efficacy of approximately 0.06% NPP. An additional dose of 537 units of phytase to 1034 units, provided no additional response. When 537 units of phytase were added to a diet that contained 12, 500 ICU, responses in parameters were again between those for 0.233 and 0.267% NPP. An approximate equivalence was 0.05% NPP. When 1074 units of phytase were added, responses were more variable than in other phytase treatments. Overall, the data suggest little if any, improvement over the treatment with 537 units of phytase kg^{-1} when 1074 units were added.

DISCUSSION

Decisions about the amount of NPP to include in poultry diets need to be based mostly on the concentrations that will support normal growth and bone mineral content. These will generally also provide the lowest feed cost per unit of animal produced. In the case of phosphorus, the amount of phosphorus in the manure may be a secondary factor to consider because of its possible detrimental effect on the environment.

The NPP requirement can be affected by several components of the diet. One is the calcium content. The NRC (1994) requirement for starting broiler chickens is listed as 0.45% when the calcium content is 1.0% for a ratio of 2.22. Other research indicates this ratio is in the range of 2.2-2.5 (Plumstead *et al*., 2008; Mitchell and Edwards, 1996). Results from the present research are also

in that range. It appears that the most important factor that determines NPP requirement is the calcium content of the diet that is chosen and then the appropriate NPP concentration can be based on the ratio. Results from the present research show that production and bone parameters are just as good with starter diets that contain 0.70% calcium as with those that contain 0.90% calcium. Most of the research related to calcium, phosphorus, vitamin D and phytase has used a calcium:total phosphorus ratio using 1.4 as the ideal ratio (Wu *et al*., 2004; Qian *et al*., 1997). Table 5 shows how a constant Ca:total P ratio of 1.4 in finisher diets does not result in a constant ratio of Ca:NPP (Wu *et al*., 2004). Supplemental phosphorus comes from phosphates or animal byproducts, both sources supplying only NPP. The committee that wrote Nutrient Requirements of Poultry (NRC, 1994) apparently thought that NPP was a better way to express phosphorus requirements of poultry because no requirements are listed for total phosphorus. The Ca:NPP ratio may more correctly generalize outcomes than Ca:total P so that not as much research is needed for each specific situation. A rationale for this ratio can be explained by the dissociation into ions before absorption. If dicalcium phosphate is used as an example, the dissociation is:

$$CaHPO_4 \text{ - - - } \rightarrow Ca^{2+} + HPO_4^{2-}$$

Approximately 0.2 g of dicalcium phosphate dissolve in 1 L of water for a solubility product (K_{sp}) of 1×10^{-7} (Brown *et al*., 2009). In a more acidic environment which is created by the proventriculus, the solubility is increased many times. The dissociation of dicalcium phosphate is decreased when a common ion is supplied by another compound. In the digestive system, most of the common ion, calcium is supplied by limestone. The result is that the K_{sp} remains at 1×10^{-7} but calcium from limestone depresses the ionization of dicalcium phosphate.

Application of the concept of solubility product suggests explanations for other observations related to calcium and phosphorus nutrition. One is the observation that a high calcium:phosphorus ratio decreases the utilization of phosphorus. This has been explained as the effect of calcium decreasing the activity of phytase in the intestine (Applegate *et al*., 2003; Tamim *et al*., 2004) thus decreasing the production of NPP. Other research showed that less than half of the effect of increasing dietary calcium could be explained by its effect on phytase activity (Plumstead *et al*., 2008). In that experiment, calcium was fed at 0.47, 0.70, 0.91 or 1.16% of the diet. From Table 4 of their research, it can be calculated that increasing dietary calcium from 0.47-1.16 decreased the apparent prececal absorption of phosphorus from 0.378-0.295% of dry matter intake. In contrast, the apparent prececal absorption of phytate phosphorus decreased from 0.047-0.010% therefore, less than half of the decrease in phosphorus absorption can be attributed to a negative effect of calcium on phytase activity. A possible explanation for the decreased absorption of phosphorus is that high calcium content exerted its effect through the common ion effect thus decreasing the NPP available for absorption.

Table 5: Ratios are different when calculating calcium:total P or calcium:NPP ratios[1]

Ca (diet (%))	Total p (diet (%))	NPP (diet (%))	Ratios	
			Ca:total p	Ca:NPP
0.67	0.48	0.20	1.40	3.35
0.75	0.54	0.26	1.40	2.88
0.83	0.60	0.32	1.40	2.59
0.91	0.66	0.38	1.40	2.34

[1]Data taken from Wu *et al*. (2004)

An *in vitro* effect of calcium concentration on phytase activity has also been reported (Applegate *et al*., 2003; Tamim *et al*., 2004). A different explanation for this observation is possible, based on solubility product. The usual procedure for determining phytase activity uses the formation of a complex of molybdate, vanadate and soluble phosphorus (Chen, 1996). The vanadomolybdophosphor complex is then measured spectrophotometrically. Phytase premixes have little soluble phosphorus but analysis of a feed sample for phytase activity requires a correction for soluble phosphorus that is present. Phytase yields more soluble phosphorus by hydrolyzing it from phytic acid. When more calcium is present in the phytase assay solution, it would be expected that more calcium phosphates would be formed which converts phosphorus to an insoluble form. Even though normal phytase activity released phosphorus from phytic acid, it might be trapped in calcium phosphate instead of forming the complex needed to detect soluble phosphate.

It is known that adding vitamin D or its metabolites to diets decreases the severity of a phosphorus deficiency (Biehl *et al*., 1998; Edwards, 1993; Mitchell and Edwards, 1996). It was postulated that vitamin D compounds do this by stimulating intestinal transport of phosphate (Wasserman and Taylor, 1973). Data from the present experiment offer a different explanation. If commercial diets contain 2, 500 ICU of vitamin D per kg, calculations in the results section suggest that the addition of each 1000 ICU of vitamin D is equivalent to 0.003% NPP. Other research assigned a value of 2800 ICU kg^{-1} as equal to 0.026% NPP. Assuming the correct phosphorus equivalency of vitamin D lies between these values, the effect of vitamin D on phosphorus utilization is relatively

small. It is accepted that vitamin D promotes the absorption of calcium. If higher dietary concentrations of vitamin D promote more absorption of calcium from the digestive system, the solubility product suggests that phosphorus will follow, resulting in more phosphorus absorption. Based on the Ca:NPP ratio of 2.25, the absorption of 0.02% calcium will cause the absorption of 0.01% NPP.

The phosphorus equivalency of phytases used in this experiment was less than generally indicated by phytase manufacturers. Depending on the product, 500 or 600 phytase units per kg of diet are generally indicated as equal to 0.10% NPP. Early research reported that the concentration of phytase per kg needed to equal 0.1% NPP was 652 units for turkeys (Qian *et al*., 1996) and 785 units for broilers from 1-21 days (Yi *et al*., 1996). In their research, phosphorus was calculated as total phosphorus which probably confounds the effects of phytase and decreasing Ca:total P ratio (Table 5). Additional research indicated 500 units per kg diet were equal to 0.064% NPP (Angel *et al*., 2001, 2005) and 800 units were equal to 0.09% NPP for bone ash (Yan *et al*., 2001). The present research indicates that 500 or 600 units of phytase per kg have a phosphorus equivalency of 0.05-0.06% NPP. Doubling the phytase content provided additional NPP in the case of one brand of phytase but gave no additional benefit from the other brand.

CONCLUSION

The fact that several components of the diet can affect phosphorus utilization makes research more difficult. Responses to phosphorus status are fairly sensitive which necessitates an appropriate response curve. One research report concluded that it is impossible to assign one phosphorus equivalency value for all phytases and all situations (Driver *et al*., 2005). While that summary is probably correct, research will continue to look for ways to decrease phosphorus added to poultry feeds. Information and discussion from the present research should be useful for working toward that objective.

REFERENCES

AOAC, 2006. The Official Methods of Analysis of AOAC International. 18th Edn., AOAC International, Arlington, USA.

Angel, R., A.S. Dhandu, T.J. Applegate and M. Christman, 2001. Phosphorus sparing effect of phytase, 25-hydroxycholecalciferol and citric acid when fed to broiler chicks. Poult. Sci., 80: 133-134.

Angel, R., W.W. Saylor, A.S. Dhandu, W. Powers and T.J. Applegate, 2005. Effects of dietary phosphorus, phytase and 25-hydroxycholecalciferol on performance of broiler chickens grown in floor pens. Poult. Sci., 84: 1031-1044.

Applegate, T.J., R. Angel and H.L. Classen, 2003. Effect of dietary calcium, 25-hydroxycholecalciferol or bird strain on small intestinal phytase activity in broiler chickens. Poult. Sci., 82: 1140-1148.

Biehl, R.R., D.H. Baker and H.F. DeLuca, 1998. Activity of various hydroxylated vitamin D3 analogs for improving phosphorus utilization in chicks receiving diets adequate in vitamin D3. Br. Poult. Sci., 39: 408-412.

Brown, T.L., H.E. LeMay, Jr. B.E. Bursten, C.J. Murphy and P. Woodward, 2009. Chemistry. 11th Edn., Pearson/Prentice Hall, Upper Saddle River, NJ., pp: 741-745.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley and V.H. Smith, 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl., 8: 559-568.

Chen, J., 1996. Natuphos Phytase Analytical Methods. In: Phytase in Animal Nutrition and Waste Management: A BASF Reference Manual, Coehlo, M.B. and E.T. Kornegay (Eds.). 2nd Edn., BASF Corporation, New Jersey, pp: 649-654.

Driver, J.P., G.M. Pesti, R.I. Bakalli and H.M. Edwards, 2005. Effects of calcium and nonphytate phosphorus concentrations on phytase efficacy in broiler chicks. Poult. Sci., 84: 1406-1417.

Edwards, Jr. H.M., 1993. Dietary 1, 25-dihydroxy-cholecalciferol supplementation increases natural phytate phosphorous utilization in chickens. J. Nutr., 123: 567-577.

Mitchell, R.D. and H.M. Jr. Edwards, 1996. Effects of phytase and 1,25 dihydroxycholecalciferol on phytate utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult. Sci., 75: 95-110.

NRC, 1994. Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC., USA., ISBN-13: 9780309048927, Pages: 155.

Plumstead, P.W., A.B. Leytem, R.O. Maguire, J.W. Spears, P. Kwanyuen and J. Brake, 2008. Interaction of calcium and phytate in broiler diets: 1. Effects on apparent prececal digestibility and retention of phosphorus. Poult. Sci., 87: 449-458.

Qian, H., E.T. Kornegay and D.M. Denbow, 1996. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium to phosphorus ratios and phosphorus levels. Poult. Sci., 75: 69-81.

Qian, H., E.T. Kornegay and D.M. Denbow, 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol and the calcium: Total phosphorus ratio in broiler diets. Poult. Sci., 76: 37-46.

SAS Institute, 1996. SAS User's Guide: Statistics. Version 7.0, SAS Inst. Inc., Cary, NC., USA.

Tamim, N.M., R. Angel and M. Christman, 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci., 83: 1358-1367.

Wasserman, R.H. and A.N. Taylor, 1973. Intestinal absorption of phosphate in the chick: Efficacy of vitamin D3 and other parameters. J. Nutr., 103: 586-599.

Wu, Y.B., V. Ravindran, W.H. Hendriks, P.C.H. Morel and J. Pierce, 2004. Evaluation of a microbial phytase, produced by solid state fermentation, in broiler diets II. Influence on phytate hydrolysis, apparent metabolizable energy and nutrient utilization. J. Applied Poult. Res., 13: 561-569.

Yan, F., J.H. Kersey and P.W. Waldroup, 2001. Phosphorus requirements of broiler chickens three to six weeks of age as influenced by phytase supplementation. Poult. Sci., 80: 455-459.

Yi, Z., E.T. Kornegay, V. Ravindran and D.M. Denbow, 1996. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalence values for phytase. Poult. Sci., 75: 240-249.