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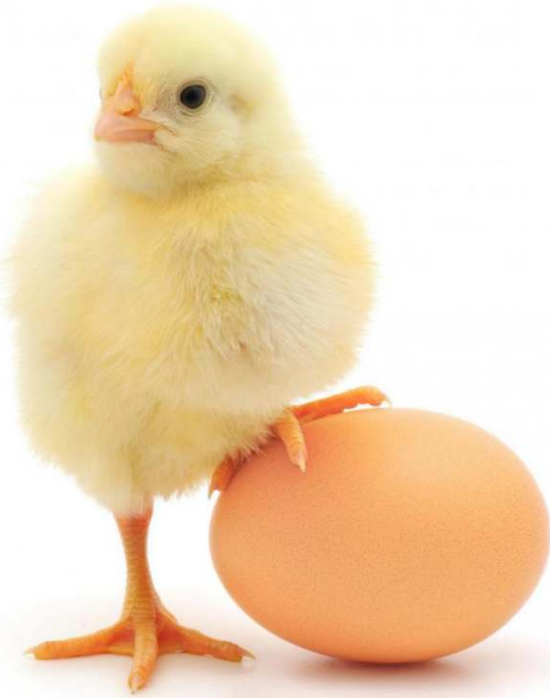

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Effects of Co-administration of Phytase and NSPase on Broiler Performance and Bone Ash

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Abstract: An experiment was conducted to evaluate the effect of varying levels of phytase with and without NSPase inclusion on broiler performance when supplemented in corn/soy bean meal diets low in available phosphorus. The objective was to determine if NSPase inclusion enhances phytase activity in relation to growth parameters and bone ash in broilers reared in batteries through 14 days of age. Four diets with selected available phosphorus levels of 0.15, 0.20%, 0.25% and 0.30% were included in the experimental design to develop a dose response curve to calculate phosphorus release from experimental treatments. An addition six treatments were included that included three levels of phytase (150, 200 and 250 FTU/kg) with and without NSPase inclusion in a diet containing 0.15% available phosphorus. Evaluated parameters included body weight, feed conversion ratio and bone ash percentage. Body weight and bone ash percentage were positively influence with increases in available phosphorus levels. Phytase inclusion positively influenced growth performance and bone ash percentage. Broilers fed the 200 and 250 FTU/kg phytase inclusion levels outperformed the broilers fed the 150 FTU/kg inclusion level. Addition of NSPase with 150 FTU/kg phytase resulted in increased broiler body weight as compared to the 150 FTU/kg phytase diet alone. Using regression equations determined from dose response treatments for body weight, bone ash (mg) and bone ash percentage, NSPase inclusion increase phosphorus release at the 150 FTU/kg level from a 0.06% to 0.09%. These data indicate that NSPase inclusion may increase phytase effectiveness when co-administered during early stages of growth.

Key words: Phosphorus, phytase, NSPase, bone ash, broiler

INTRODUCTION

Phosphorus (P) is an essential nutrient required for proper bone development and for efficient poultry production (Nelson *et al*., 1971; Waldroup, 1999; Selle and Ravindran, 2007) and the failure to meet a bird's requirement of P can lead to many detrimental factors, including reduced bird performance, increased leg disorders and increased bird mortality (Waldroup, 1999; Powell *et al*., 2008). To avoid these consequences, many integrators have included a safety margin when formulating P concentrations in broiler diets to include a level of P slightly higher than the requirements for bird growth (Waldroup, 1999). This strategy of over supplementing P in poultry diet formulations was utilized until the cost of inorganic phosphate sources increased and awareness of the environmental concerns about excessive P concentrations in poultry litter motivated producers to consider alternative strategies that could reduce the total P concentrations in broiler diets (Ravindran *et al*., 1995).

The main strategy utilized to decrease the levels of P required in broiler diets is the use of phytase enzymes, which hydrolyze the phosphate ester bond found in phytate (Rao *et al*., 2009) which can account for as much as 70% of the P found in cereal grains. The use of phytase in broiler diets has shown to increase bird performance and improve bone mineralization (Simons *et al*., 1990; Sebastian *et al*., 1998). The polyanionic form of phytate can chelate with cations like calcium, magnesium, copper and iron (Rao *et al*., 2009; Kumar *et al*., 2010) forming insoluble salts that can decrease absorption of these nutrients. For this reason, phytate is often considered to be an anti-nutritive compound because the binding of these nutrients results in being partially or completely unavailable for digestion by the chicken (Applegate and Angel, 2004; Rao *et al*., 2009).

Another anti-nutritive factor found in cereal grains used in broiler diets include the presence of Non-Starch Polysaccharides (NSP), which are fibrous material found in plant cell walls. Chickens lack the digestive capacity of ruminant animals and the presence of NSP in the diet increases intestinal viscosity resulting in decreased digestibility of the diet (Bedford and Morgan, 1996). The presence of high levels of NSP in broiler diets have shown to reduce bird performance by decreasing body weights, increasing feed conversions, increasing litter moisture and increasing the incidence of pasty vents (Bedford and Morgan, 1996; Lazaro *et al*., 2003; Lee *et*

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al., 2003a; Meng and Slominski, 2005; Gao *et al*., 2007). To alleviate the negative effects of NSP, enzymes (carbohydrase) have been utilized to help increase digestibility of high fiber broiler diets. These enzymes function to degrade NSP present in the diet by breaking the fiber chains in the cell walls into smaller fragments (Wyatt *et al*., 2008). By breaking down the cell wall of grains, carbohydrase inclusion has shown to decrease intestinal viscosity and increase digestibility and performance (Choct *et al*., 1999; Kocher *et al*., 2002; Gracia *et al*., 2003; Lee *et al*., 2003b).

Previous research has investigated whether co-administration of phytase and carbohydrase enzymes can enhance activity of each enzyme. Ravindran *et al*. (1999) and Juanpere *et al*. (2005) witnessed increased Apparent Metabolizable Energy (AME) and digestibility with the inclusion of phytase and carbohydrases. Therefore, the objective of the current research was to determine if the co-administration of a cocktail enzyme preparation (NSPase) could enhance phytase activity when supplemented in low P diets.

MATERIALS AND METHODS

The experimental design consisted of 10 experimental treatments with 6 replicates per treatment each consisting of 12 male broilers per replicate, for a total of 720 Cobb 500 male chicks raised in battery brooders for 14 days. A corn and soybean meal based basal mash diet was used which contained a calculated total P level of 0.41%, available P level of 0.15% and a calcium level of 0.20% (Table 1). Mono-calcium phosphate, limestone, enzymes, were added to the basal diet to achieve the desired available P level, 0.80% calcium and enzyme inclusion rates required for each individual treatment. Four treatments (1-4) were utilized to develop a dose response curve for observed parameters to calculate the increase of bioavailability of P associated with phytase inclusion. The available P concentrations of these diets were 0.15%, 0.20%, 0.25% and 0.30%. The remaining six treatments included three levels of phytase (150, 200 and 250 FTU/kg) with and without NSPase inclusion in a diet containing 0.15% available P. Information from the manufacturer of the phytase enzyme[1] indicated a targeted increase in P bioavailability of 0.06%, 0.08% and 0.10% with the three selected of inclusion of 150, 200 and 250 FTU/kg. The NSPase[2] was included at a rate of 0.25 lb/ton. Animal care was provided in accordance with an approved protocol by the Institutional Animal Care and Use Committee (IACUC). Broiler chicks were provided age appropriate supplemental heat and given access to dietary treatment and water *ad libitum*. Battery pens contained two stainless steel feeders and one water container for each replicate of broilers.

Body weights and feed consumption were determined on days 7 and at the termination of the trial on d 14.

Table 1: Ingredient profile and nutrient concentration of a low phosphorus starter basal diet fed to broilers through 14 d of age. Inorganic phosphorus was added to the basal diet to create four treatments of available phosphorus concentrations between 0.15% and 0.30% (Experiment 1)

Ingredient profile	%
Corn	63.90
Soybean meal (48%)	33.57
Sodium chloride	0.52
A/V fat blend	0.92
L-lysine HCl	0.18
DL-methionine (99%)	0.23
Vitamins[1]	0.25
Minerals[2]	0.05
Coban 90[3]	0.05
Mono-calcium PO_4	0.12
Nutrient concentration	
Protein (%)	22.00
Metabolizable energy (kcal/kg)	3054.00
Methionine (%)	0.56
Total sulfur amino acids (%)	0.92
Lysine (%)	1.30
Threonine (%)	0.82
Tryptophan (%)	0.26
Calcium	0.20
Sodium	0.22
Total phosphorus	0.41
Available phosphorus	0.15

[1]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3]Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria nectarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria mivati* and *Eimeria maxima*

Right tibias were removed from all broilers for bone ash determination. Tibias were cleaned of any adhering tissue, dried for 24 hrs at 105°C and ashed at 600°C for 24 hrs to determine ash weight.

Statistical analysis: Data (body weight, mortality corrected feed conversion ratio and bone ash) were analyzed via a one-way ANOVA using the General Linear Model in SPSS V 18.0. Means were deemed significantly different at $p \leq 0.05$ and separated using Duncan's Multiple Range Test. Regression analysis was conducted on dose response treatments (TRT 1-4) and determined equation used to calculate increase in P-bioavailability associated with enzyme inclusion (TRT 5-10).

Table 2: Body weight, feed consumption and mortality corrected feed conversion±SE of broilers fed selected levels of available phosphorus, multiple phytase inclusion rates and NSPase inclusion

				Body Wt		Feed Cons.	Feed conversion		
TRT	Avail. P (%)	Phytase[1] (FTU/kg)	NSpase[2]	Day 7 (g)	Day 1-14	Day 1-14 (g)	Day 1-7	Day 8-14	Day 1-14
1	0.15	--	--	110.0±2.2[d]	214.4±7.6[e]	17.51±0.69[f]	1.43±0.03[ab]	2.02±0.08[ab]	1.69±0.04[ab]
2	0.20	--	--	109.7±1.7[d]	222.8±4.7[e]	19.12±0.51[f]	1.47±0.05[a]	2.32±0.35[a]	1.84±0.13[a]
3	0.25	--	--	124.1±2.7[ab]	286.1±4.8[b]	23.09±0.12[b]	1.33±0.06[bc]	1.38±0.05[c]	1.36±0.04[cd]
4	0.30	--	--	128.2±1.9[a]	312.7±5.6[a]	25.72±0.58[a]	1.32±0.02[bc]	1.48±0.08[c]	1.42±0.06[cd]
5	0.15	150	--	117.6±1.0[c]	244.5±8.9[d]	19.55±0.87[de]	1.28±0.04[c]	1.75±0.08[bc]	1.53±0.04[bc]
6	0.15	200	--	123.0±1.8[bc]	276.6±2.7[bc]	22.94±0.68[bc]	1.37±0.04[abc]	1.37±0.08[c]	1.37±0.06[cd]
7	0.15	250	--	121.4±1.2[bc]	280.3±6.5[bc]	22.46±0.57[bc]	1.32±0.02[bc]	1.32±0.05[c]	1.32±0.03[d]
8	0.15	150	+	117.8±1.2[c]	264.7±1.7[c]	21.12±0.52[cd]	1.36±0.03[abc]	1.71±0.17[bc]	1.54±0.07[bc]
9	0.15	200	+	118.5±1.6[c]	268.6±5.6[bc]	21.84±0.65[bc]	1.33±0.02[bc]	1.57±0.12[c]	1.47±0.07[cd]
10	0.15	250	+	121.5±1.6[bc]	269.3±7.6[bc]	22.10±0.42[bc]	1.30±0.03[c]	1.46±0.07[c]	1.39±0.05[cd]

[a-f]Means within columns with different superscripts differ significantly at $p<0.05$.

[1]Optiphos® 2000 PF-Enzyvia LLC., Sheridan, IN. Low phytase is 150 FTU/kg, Mid is 200 FTU/kg and High is 250 FTU/kg

[2]Enspira G1-Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram beta-Glucanase, 22 units/gram beta-Mannanase and 7 units/gram alpha-Galactosidase. Inclusion of 0.25 lb/ton. Avail. = Available

RESULTS

Increasing the level of available P in the dose response treatment groups (TRT 1-4) improved broiler performance parameters (Table 2). Increasing the available P from 0.15-0.20% did not improve performance parameters; however subsequent increases of 0.05% in available P improved evaluated parameters. The inclusion of phytase (TRT 5-10) enhanced growth parameters as early as d 7 (Table 2) when compared to TRT 1, which had the same level of available P. The medium and high levels (200 and 250 FTU/kg) of phytase inclusion resulted in increased body weight similar to TRT 3, indicating that these levels of phytase were capable of producing growth response of a diet containing 0.1% more available P. NSPase inclusion (TRT 8-10) did not result in any improvements in day 7 Body Weight (BW) as compared to the similar levels of phytase alone treatment groups.

Day 14 BW (Table 2) was similar to day 7 data, with all phytase treatment groups exhibiting increased BW as compared to TRT 1. The 200 and 250 FTU/kg inclusion levels of phytase resulted in BW similar to TRT 3. The inclusion of NSPase with the low level of phytase (TRT 8) resulted in an increase in BW as compared to the 150 FTU/kg phytase inclusion alone (TRT 5) while no improvements were observed with the inclusion of NSPase in the two higher levels of phytase.

Similar to BW, feed consumption (g/bird/day) also increased with increasing P levels and the inclusion of phytase in the diet. The low level of phytase inclusion (TRT 5) increased feed consumption as compared to TRT 1 and 2 while increasing the inclusion rate to 200 and 250 FTU/kg resulted in a feed consumption rate similar to TRT 3. The inclusion of NSPase with the low level of phytase (TRT 8) resulted in an increase ($p<0.05$) in feed consumption as compared to the 150 FTU/kg phytase alone treatment (TRT 5). Increases in feed consumption were not observed with NSPase inclusion at the two higher levels of phytase. Mortality corrected Feed Conversion Ratio (FCR) (day 1-14) was improved ($p<0.05$) as the level of available P increased from 0.20% to 0.30%. Similar FCR were observed between the two lowest levels of available P as well as the two highest level of available P (Table 2). Phytase inclusion improved FCR. Improvements were observed as phytase level increased from 150 FTU/kg to 250 FTU/kg. The inclusion of NSPase with phytase (TRT 8-10) did not result in improvements in FCR when compared to the phytase alone treatment groups.

Bone mineralization data was similar for both parameters evaluated (bone ash weight and bone ash%) (Table 3). Increases in bone ash weight and percentage were observed as the level of available P increased in dose response treatments (TRT 1-4). The inclusion of phytase increased bone ash weight and percent compared to TRT 1. The low level of phytase (150 FTU/kg) resulted in bone data similar to TRT 2, which had a 0.05% higher level of available P. The inclusion of 200 and 250 FTU/kg of phytase increased bone mineralization parameters to a level comparable to TRT 3, suggesting that these levels of phytase inclusion were capable of producing bone ash weight and percent of a diet containing 0.1% more available P. The bone mineralization data confirms the manufacturer's recommendations that 250 FTU/kg of phytase is capable of increasing phosphorus bioavailability by 0.10%. The inclusion of NSPase with the low level of phytase (150 FTU/kg) increased ($p<0.05$) observed bone ash and percent to a level comparable to the two higher levels of phytase inclusion. The inclusion of NSPase with the two higher levels of phytase (200 and 250 FTU/kg) did not increase observed bone ash and percent. To calculate the increase in bioavailability of P from the inclusion of the three levels of phytase inclusion with and without NSPase inclusion, regression analysis was performed to develop equations based on the observed body

Table 3: Tibia ash weight and percentage ± SE of broilers fed selected levels of available phosphorus, multiple phytase inclusion rates and NSPase inclusion

TRT	Available P (%)	Phytase[1] (FTU/kg)	NSPase[2]	Ash (mg)	Ash (%)
1	0.15	--	--	127.02±7.3[e]	33.59±0.78[e]
2	0.20	--	--	144.93±6.5[de]	35.35±0.33[de]
3	0.25	--	--	193.64±9.0[b]	38.63±0.77[b]
4	0.30	--	--	225.57±11.9[a]	40.54±0.86[a]
5	0.15	150	--	157.44±7.9[cd]	35.96±0.80[cd]
6	0.15	200	--	183.67±3.8[b]	38.16±0.43[b]
7	0.15	250	--	179.68±5.2[b]	37.79±0.34[bc]
8	0.15	150	+	171.66±3.7[bc]	37.21±0.46[bcd]
9	0.15	200	+	173.95±4.8[bc]	36.94±0.58[bcd]
10	0.15	250	+	178.71±3.8[bc]	37.62±0.73[bc]

[a-e]Means within columns with different superscripts differ significantly at $p<0.05$.

[1]Optiphos® 2000 PF-Enzyvia LLC., Sheridan, IN. Low phytase is 150 FTU/kg, Mid is 200 FTU/kg and High is 250 FTU/kg.

[2]Enspira G1-Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram beta-Glucanase, 22 units/gram beta-Mannanase and 7 units/gram alpha-Galactosidase. Inclusion rate of 0.25 lb/ton

Table 4: Calculated increase in P bioavailability (%) due to inclusion of phytase and NSPase enzymes. Calculations were based on regression equations of day 14 body weight (equation 1), bone ash weight (equation 2) and ash percentage (equation 3) of male broilers fed selected levels of available phosphorus (aP)

TRT#	Phytase level[1]	NSPase[2]	Day 14 BW (g)[3]	Ash (mg)[4]	Ash (%)[5]
5	150	_	0.064	0.063	0.065
6	200	_	0.105	0.095	0.104
7	250	_	0.114	0.091	0.098
8	150	+	0.094	0.082	0.090
9	200	+	0.097	0.083	0.780
10	250	+	0.097	0.091	0.096

[1]Optiphos® 2000 PF-Enzyvia LLC., Sheridan, IN.

[2]Enspira G1-Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram beta-Glucanase, 22 units/gram beta-Mannanase and 7 units/gram alpha-Galactosidase. Inclusion rate of 0.25 lb/ton.

[3]Calculated using regression analysis comparing inorganic phosphate consumption and observed body weight at day 14 (g). ($y = 187.28x + 195.51$)

[4]Calculated using regression analysis comparing inorganic phosphate consumption and observed bone ash weight (mg). ($y = 181.88x + 111.13$)

[5]Calculated using regression analysis comparing inorganic phosphate consumption and observed bone ash (%). ($y = 12.552x + 32.773$)

weight at day 14 (Fig. 1), bone ash weight (Fig. 2) and bone ash percentage (Fig. 3) as a function of inorganic P consumed. Individual data points represent observed parameters for each replicate pen. Following regression analysis, the equations were used to determine the amount of inorganic P that would need to be consumed to achieve the observed effect of each phytase inclusion rate. The determined equations for each evaluated parameters were $y = 187.28x + 195.51$ (Equation 1, Day 14 BW in grams), $y = 181.88x + 111.13$ (Equation 2, Bone Ash in mg) and $y = 12.552x + 32.773$ (Equation 3, bone ash%).

The calculated increase in bioavailability of P was determined for each of the treatment groups (TRT 5-10) in which the equations previously discussed were utilized to determine the P release resulting from the phytase inclusion rate for the evaluated parameter (Table 4). The inclusion of the levels of phytase alone and with NSPase were capable of releasing the 0.06%, 0.09% and 0.10% available P in the low, mid and high level of phytase inclusion in the diet. Regression analysis determined that NSPase inclusion increased P bioavailability from 0.06 to 0.09% at the 150 FTU/kg inclusion rate of phytase. No other increases in P bioavailability were observed with the inclusion of NSPase in the diets with higher levels of phytase (200 and 250 FTU/kg).

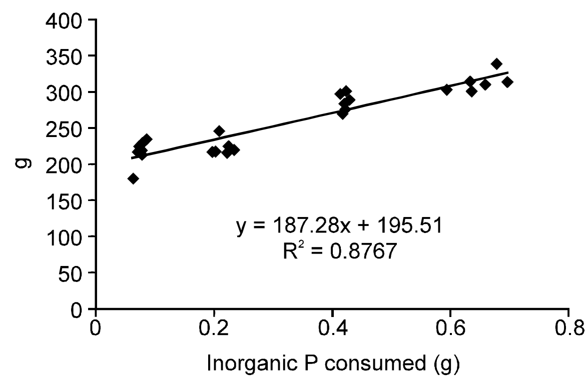

Fig. 1: Regression analysis depicting the relationship between inorganic phosphorus consumption and the observed average body weight of male broilers at 14 days of age fed diets varying in available phosphorus concentration

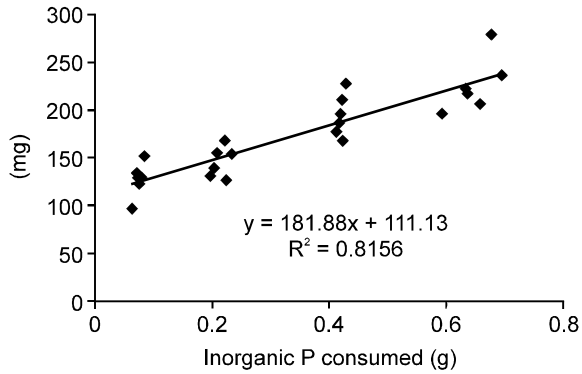

Fig. 2: Regression analysis depicting the relationship between inorganic phosphorus consumption and the observed bone ash weight (mg) of male broilers fed diets varying in available phosphorus concentration

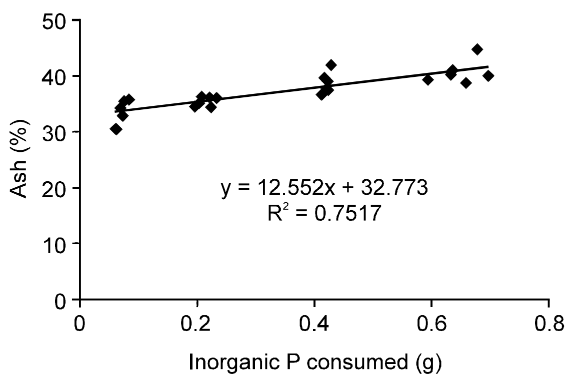

Fig. 3: Regression analysis depicting the relationship between inorganic phosphorus consumption and the determined tibia ash percentage observed from male broilers fed diets varying in available phosphorus concentrations

DISCUSSION

Reducing levels of available P in the diet reduced growth performance and bone mineralization in both experiments. It is recognized that decreasing the level of available P in diets will result in decreases in bird performance and reduced bone mineralization. Simons *et al.* (1990) witnessed lower bird weights, increased FCR and increased mortality when broilers were fed lower levels of available P. Yan *et al.* (2001) also observed that increasing the available P level resulted in increased tibia ash weight and body weight.

The inclusion of dietary phytase enzymes can assist in increasing the bioavailability of P and improve broiler performance while also decreasing the level of inorganic phosphorus required in the diet (Simons *et al.*, 1990; Waldroup, 1999; Waldroup *et al.*, 2000; Yan *et al.*, 2001; Powell *et al.*, 2008). The inclusion of the three different phytase levels resulted in an increase in P bioavailability leading to improvements in broiler performance and bone mineralization as compared to the dose response treatments (TRT 1 and 2) that contained equivalent or higher levels of available P concentrations.

These data illustrate that phytase inclusion in P deficient diets have the ability to improve broiler performance and bone ash. Enhanced growth parameters and bone mineralization associated with multiple commercial phytase products have been previously reported. Yan *et al.* (2001) reported increases in body weight and tibia ash and improvements in feed conversion with Natuphos® in broiler diets. Dilger *et al.* (2004) observed increased body weight gain and feed intake during the starter and grower period with the inclusion of varying levels of Phyzyme® XP as compared to a control diet with the same level of available P. The higher inclusion rates of phytase in the current trial resulted in increased bone ash percentage, body weight and decreased FCR as compared to the lower level of inclusion. Simons *et al.* (1990) also witnessed similar results in that increasing phytase levels from 250-1500 resulted in decreased FCR and increased body weights when included in diets fed through 24 days of age.

FCR was decreased in treatment groups with phytase inclusion as compared to TRT 1, which had the same level of available P. The two higher levels of phytase inclusion (200 and 250 FTU/kg) had a similar FCR as treatments containing 0.10% and 0.15% more available P suggesting that phytase inclusion was capable of increasing P bioavailability by up to 0.15%. Simons *et al.* (1990) witnessed similar results with inclusion of 500 FTU of phytase in a diet containing 4.5 g/kg of P had similar body weights and feed conversion as a diet with 6 g/kg of P feed through day 24 of age. Waldroup *et al.* (2000) reported the inclusion of 800 FTU/kg of Natuphos® with a diet containing 0.15% available P resulted in a feed conversion similar to a diet containing 0.25% available P.

Regression analysis was conducted on observed parameters to quantify the increase in P bioavailability from the inclusion of the three levels of phytase with and without NSPase inclusion. Equations were determined based on the observed body weight at day 14 (Fig. 1), bone ash (mg) (Fig. 2) and bone ash percentage (Fig. 3) as a function of inorganic P consumption. Derived equations were used to calculate P bioavailability by calculating the amount of inorganic P needed to achieve the observed effect and subtracting the actual amount of inorganic P consumed by broilers receiving phytase. Calculation confirm the inclusions of the three levels of phytase (TRT 5-7) were capable of meeting or exceeding the expected available P sparing percentages of 0.06%, 0.08% and 0.10% with 150, 200 and 250 FTU/kg inclusion rates, respectively. Previous research has estimated varying amounts of phytate-bound P that can be released from broiler diets, with variations depending on the level of phytase used, type of diet and the stage of growth that is evaluated. Nelson *et al*. (1971) indicated that between 50-100% of phytate-bound P in a corn soy diet is capable of hydrolyzation by use of varying levels of phytase. Waldroup *et al*. (2000) suggested that the inclusion of 800 FTU of Natuphos® was capable of releasing approximately 50% of the phytate P from the diet.

The addition of NSPase to a diet containing the low level of phytase (150 FTU/kg) in this experiment increased broiler performance parameters and bone ash to a level comparable to the higher phytase treatment groups (200 and 250 FTU/kg). Regression analysis determined that NSPase inclusion increased P bioavailability from 0.06% to 0.09% with 150 FTU/kg phytase inclusion rate indicating that reduced levels of phytase in conjunction with a cocktail NSPase preparation can be used to achieve desired effects in young broilers as compared to higher levels when fed alone.

The improvements in bird performance resulting from co-administration of phytase and NSPase agree with Cowieson and Adeola (2005) in that the inclusion of both a phytase and cocktail energy enzyme could enhance growth as compared to diets that were individually supplemented with enzymes. Francesch and Geraert (2009) observed an increase in feed consumption and a reduction in FCR in male broilers through 43 days of age, when an enzyme complex containing carbohydrases and phytase was added to reduced energy diets. Tiwari *et al*. (2010) determined that the improvements in bird performance associated with the co-administration seemed to be largely due to phytase and broilers benefited most from the enzymes at an early age. However, other published reports do not indicate a significant effect associated with co-administration. Olukosi *et al*. (2010) reported the inclusion of phytase and carbohydrase did not produce greater benefit than the use of phytase alone. Ravindran *et al*. (1999) witnessed that the inclusion of xylanase with phytase did not result in an improvement in bird performance and Ghorbani *et al*. (2009) reported no significant improvements resulting from co-administration of enzymes on carcass composition.

These data indicate that inclusion of a cocktail NSPase preparation can improve phytase activity in young broilers allowing for reduction in phytase inclusion rates. However, recently published literature indicates inconsistent results of co-administration of phytase and carbohydrases in corn-soy based diets. Therefore, further research is needed to accurately determine the effectiveness of different cocktail carbohydrases with multiple phytases under multiple inclusion levels to determine the timeline allowable for reduced phytase inclusion levels while achieving the desired performance and bone mineralization effects.

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[1]Optiphos® 2000 PF- Enzyvia LLC., Sheridan, IN.

[2]Enspira G1- Enzyvia LLC., Sheridan, IN. Contains 300 units/gram Xylanase, 220 units/gram beta-Glucanase, 22 units/gram beta-Mannanase and 7 units/gram alpha-Galactosidase. Inclusion of 0.25 lb/ton.