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Effects of Various Phytase Concentrations in Diets with Low-phytate Corn on Broiler Chick Performance and Nutrient Use

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Abstract: Research indicates a reduction in phosphorus content of broiler excreta with the supplementation of phytase to corn-soybean meal based diets or with the use of Low Phytate Corn (LPC). This study examined how broiler chicks are impacted by phytase supplementation to a LPC [0.136% Phytate Phosphorus (PP) by analysis] based diet. Different dietary inclusion levels from a 2,500 or a 5,000 FYT/g of *Peniophora lycii* phytase product were used as experimental treatments. Following a six day pretest, 576 broiler chicks were randomly assigned to one of 12 dietary treatments, using eight diet replicates and six birds per cage. Live performance, tibia ash, mineral digestibility and apparent metabolizable energy were determined. The percent of phosphorus that may have been spared due to each phytase treatment was calculated using response curves created with increasing levels of monocalcium phosphate. Results demonstrated that phytase supplementation to a LPC based diet had a positive impact on broiler chick growth. However, this impact is expressed to a lesser extent than would be expected for normal corn. In addition, phytase efficacy did not vary based on the concentration of phytase product, likely due to adequate mix uniformity.

Key words: Phytase, low phytate corn, concentration and mixer uniformity

Introduction

The poultry industry and other livestock operations are facing growing concerns about the land application of manure contaminating surface waters (Sharpley, 1999). Manure fertilizer for crops provides necessary, but often more than required nutrients for crop growth. The surplus nutrients, phosphorus being of greatest concern, may leach into watersheds and contribute to eutrophication (Grattan *et al*., 1998; Sharpley, 1999).

Phosphorus is an essential mineral for broiler chicken metabolism and skeletal development. However, two-thirds of the phosphorus provided in typical broiler diet ingredients such as corn and soybean is bound to phytic acid (Nelson, 1967; Sohail and Roland, 1999). Phytate phosphorus is either unavailable or poorly utilized by monogastric animals due to insufficient quantities of endogenous phytase enzyme that aids in digestion of the phytic acid complex (Waldroup *et al*., 2000; Angel *et al*., 2002). Phytic acid can also act as an anti-nutrient due to the ability of the complex to bind starch, proteins and trace minerals, such as phosphorus, zinc, iron, calcium and magnesium (Kornegay, 1999; Camden *et al*., 2001; Radcliffe, 2002).

The addition of phytate-degrading enzymes can improve the nutritional value of plant-based foods by enhancing nutrient digestibility through phytate hydrolysis during digestion in the gut (Yi and Kornegay, 1996; Konietzny and Greiner, 2002). Research has shown that the supplementation of exogenous phytase to broiler diets is an effective means for increasing the availability of phosphorus to the bird and reducing phosphorus excretion by liberating phytate bound phosphorus (Nelson, 1967; Jongbloed and Kemme, 1990; Kornegay *et al*., 1995; Waldroup *et al*., 2000; Angel, 2002). Results of phytase dosage efficacy have been inconsistent, in part this may be due to variable PP content of the diet (Kornegay, 1999; Angel, 2002; Radcliffe, 2002) or variable uniformity of phytase distribution in the diet (Johnston and Southern, 2000; Angel *et al*., 2002).

The sparing ability of phytase on inorganic phosphorus is a common determination of phytase efficacy. The amount of inorganic phosphorus spared with the addition of phytase is termed the phosphorus sparing effect. Phytase supplementation is measured in phytase units (FYT). One phytase unit is defined as the quantity of phytase that generates one micromole of inorganic phosphorus from 5.1 mmol/L of sodium phytate at pH of 5.5 and 37°C (Johnston and Southern, 2000; McMullen, 2001). Typically, phytase manufacturers claim that 0.1% phosphorus sparing is obtainable with the addition of 300-500 FYT/kg of diet. In contrast, past literature reports that 0.1% phosphorus sparing has been achieved with a range of 781-1413 FYT/kg of diet (Angel *et al*., 2002). These large discrepancies in efficacy could be a result of variation in corn and soybean PP content or mix uniformity of the feed (Johnston and Southern, 2000).

Given that young chicks only consume a few grams of feed each day, it is necessary to provide all essential nutrients, in the proper quantity (Beumer, 1991). Utilizing different concentrations of phytase may alter growth and performance due to uniformity of mix. The addition of a more concentrated product leaves more space available

in the diet for other ingredients, but it is more difficult to ensure that all ingredients are adequately dispersed. Commercial recommendations for mixer Coefficient of Variation (CV) are less than 10% but a CV of 30% has been reported to not be detrimental to performance (Johnston and Southern, 2000). Thus, phytase efficacy and performance data may vary due to inadequate mixer uniformity.

Many studies analyze total phosphorus of the diet, but few include information on PP content and phytase analysis (Angel *et al.*, 2002). In several studies that focused on similar corn-soybean meal diets, phytase supplementation, total phosphorus and calculated PP results of toe ash were largely varied (Kornegay *et al.*, 1995; Qian *et al.*, 1997; Camden *et al.*, 2001). More specifically, nonphytate phosphorus (nPP) was calculated to be 0.27-0.28% with 500-600 FYT/kg and toe ash results ranged from 11.6-12.7%. These differences are noteworthy because toe ash is comparable to tibia ash and both are highly sensitive indicators of phosphorus levels in the broiler chick (Angel *et al.*, 2002). Inconsistencies in metabolizable energy may also be due to variations in PP content of feed. With the addition of phytase to broiler diets, variations in metabolizable energy from no change (Biehl and Baker, 1997) up to a 5.5% increase have been reported (Camden *et al.*, 2001). Data are still limited concerning the variability in PP content of feed ingredients (Applegate and Angel, 2003). Phytate phosphorus analysis of ingredients will allow for improved diet formulation (Angel *et al.*, 2002).

Phytate content of corn can be manipulated to increase phosphorus utilization via the use of genetic engineering for a homozygous lpa1-1 gene (Raboy and Gerbasi, 1996). This hybrid corn, known as Low Phytate Corn (LPC) or High Available Phosphorus Corn (HAPC) has enhanced phosphorus availability to animals due to low PP levels and increased nPP (Huff *et al.*, 1998; Li *et al.*, 2000). Low phytate corn and normal yellow dent corn contain similar total phosphorus contents, however, PP and nPP distribution vary. Normal corn has been reported to contain 0.03% nPP. In contrast, LPC contains about 0.17% nPP (Huff *et al.*, 1998; Waldroup *et al.*, 2000; Yan *et al.*, 2001).

Li *et al.* (2000) demonstrated that phosphorus in LPC is more available than phosphorus in normal corn and that a reduction in phytate content with LPC does not compromise nutritional value. Results have also indicated that phosphorus excretion could be substantially reduced by substituting LPC for normal corn in the diet (Li *et al.*, 2000). The combined effects of LPC and phytase supplementation result in marked reduction in phosphorus excretion without compromising broiler chick performance (Huff *et al.*, 1998; Waldroup *et al.*, 2000; Yan *et al.*, 2001).

In an attempt to determine appropriate levels of phytase supplementation in LPC-soybean meal based diets, the current study explored the effects of varying phytase concentrations of two different commercial phytase products mixed as adequately as possible on broiler performance and nutrient use.

Materials and Methods

Diet formulation: Experimental LPC-soybean based mash diets were formulated to meet or exceed NRC (1994) recommendations for all nutrients except calcium and phosphorous. Formulations were adjusted to determine the efficacy of phytase in liberating phosphorus, calcium and energy from phytic acid. All dietary treatments used LPC containing 0.23% total phosphorus, 0.14% PP and by difference 0.09% nPP. Birds consumed a NRC (1994) based pre-test diet formulated with 1% calcium and 0.45% nPP from 1-5 d of age. A series of diets, designated as the standard curve, were created to assist in determining phosphorus-sparing effect. Diets utilized for the standard curve contained 0.8% calcium and varying levels of nPP (0.23% (the control), 0.28%, 0.33% and 0.38%). Nonphytate phosphorus was adjusted using monocalcium phosphate, ground limestone and cellulose. The activities of exogenous phytase in diets were varied by adding different levels of commercial phytase products to the 0.23% nPP control diet (Table 1).

Feed manufacture: Two dry *Peniophora lycii* phytase products were used, each containing a different concentration of the enzyme (Product 1 and Product 2). Product 1 contained 2,500 FYT/g and was added to diets in concentrations of 250, 500, 750, 1,000 and 2,000 FYT/kg. Product 2, contained 5,000 FYT/g and was added to diets in concentrations of 250, 500 and 750 FYT/kg.

Due to the small inclusions of enzyme, it was essential to ensure adequate homogeneity in the mixer by determining an appropriate mix time to produce a minimal CV. All diets were mixed in a single screw vertical mixer[1]. Mixer coefficient of variation was determined by mixing 907 kg batch of ground corn and salt for varying lengths of time. To mimic enzyme inclusion, salt was added at 0.01% of the test batch. Ten samples were analyzed from each batch at each test time with Quantab titrators[2] for chloride analysis. Testing procedure followed those of McCoy (1994).

Four 907 kg basal diet batches were formulated (0.23%nPP, 0.38%nPP, a 0.23%nPP diet with Product 1 at 2,000 FYT/kg, a 0.23%nPP diet with Product 2 at 750 FYT/kg) and assayed for phytase[3], total phosphorus[3] and PP[4] to ensure appropriate mix and formulation. High Performance Liquid Chromatography was used to determine phytate phosphorus with post column detection. The four basal diets were then blended in small rations and in different proportions to create eight additional experimental diets (0.28% nPP, 0.33% nPP,

Table 1: Basal diets utilized for composition of experimental treatments in phytase study

Ingredient	0.23%n PP Diet	0.38%n PP Diet	Product 1[A] @ 2000 FYT/Kg	Product 2[B] @ 750 FYT/Kg
Low phytate corn[CD]	56.21	56.21	56.21	56.21
Soybean meal (44%)	31.08	31.08	31.08	31.08
Corn Gluten Meal (60%)	5	5	5	5
Soybean oil	4.26	4.26	4.26	4.26
Limestone[D]	1.7	1.36	1.7	1.7
Cellulose[D]	0.46	0	0.4	0.44
Salt	0.44	0.44	0.44	0.44
L-Lysine Hcl	0.09	0.09	0.09	0.09
DL-Methionine	0.18	0.18	0.18	0.18
Mono Calcium Phosphate[D]	0.34	1.13	0.34	0.34
NB 3000 Vitamin Premix[E]	0.25	0.25	0.25	0.25
Phytase	0	0	0.05	0.01
Calculated Composition				
ME (Kcal/Kg)	3200	3200	3200	3200
Crude protein (%)	21.95	21.95	21.95	21.95
Methionine+Cystine (%)	0.9	0.9	0.9	0.9
Lysine (%)	1.1	1.1	1.1	1.1
Crude fat (%)	6.86	6.85	6.86	6.86
Calcium (%)	0.8	0.8	0.8	0.8
Nonphytate P (%)	0.23	0.38	0.23	0.23
Analyzed Composition				
Phosphorus (%)	0.43	0.56	0.41	0.42
Phytate Phosphorus (%)	0.207	0.217	0.217	0.215
Calcium (%)	0.79	0.78	0.77	0.78
Phytase (FYT/kg)	50	58	1813	742

[A]Product 1 contains 2,500 FYT/g, [B]Product 2 contains 5,000 FYT/g, [C]Analyses of LPC: Total phosphorus, 0.23%; Phytate Phosphorus, 0.14%, [D]Particle sizes of corn=697.5µm, limestone=169.5µm, cellulose=185.5µm, MonoCalPhos=789µm, [E]Supplied per kilogram of diet: manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B_6, 1.38 mg; niacin, 27.56 mg; panthothenic acid, 6.61 mg; thiamine, 2.20 mg; menadione, 0.83 mg; vitamin B_{12}, 0.01 mg; vitamine E, 16.53 IU; vitamin D_3, 2,133 ICU; vitamin A, 7,716 IU

Product 1 @ 250, 500, 750 and 1,000 FYT/kg and Product 2 @ 250, 500 FYT/kg). Feed samples from all diets were analyzed for total phosphorus[3,5], soluble phosphorus[3], calcium[5] and gross energy[6].

Broiler performance: Following a 5 d adaptation period, 576 Ross 308×Ross 344 straight run broilers[7] were randomly assigned to one of 12 dietary treatments. The experiment was conducted as a randomized complete block design, from 6-21 d. Treatments were replicated 8 times using a pen of 6 birds as an experimental unit. Birds were housed in raised wire brooding cages in a cross-ventilated negative pressure room. Mash feed, supplied in external troughs and water, supplied through nipple drinkers was provided *ad libitum*. Nipple drinkers were adjusted by visual inspection to appropriate height for chicks (Lott *et al*., 2001). Live weight gain was determined by difference in chick weights from 6-21 days of age. Total feed consumption was calculated and mortality weights were recorded throughout the experiment. Feed conversion was calculated as the feed intake to weight gain ratio and included mortality weight. On day 21, birds were euthanized and right tibias were extracted from all birds and pooled by pen for tibia ash determination of dry fat-free bone. Tibias were dried for 48 h at 110°C. After drying, bones were defatted with diethyl ether by the Soxhlet extraction method (AOAC, 1990). Dry defatted bones were ashed in an oven at 550°C for 12 h (AOAC, 1990). Tibia ash percentage was calculated using the percent of the dry fat-free bone weight remaining as ash.

Energy and mineral utilization: Apparent Metabolizable Energy (AME) was estimated during the 18 to 21 d period. Total excreta collection began after an 18-h fast. Feed intake and total excreta per pen were measured. Excreta were dried for 48 h at 65°C (Namkung and Leeson, 1999) and ground using a Thomas-Wiley Mill, Model 4[8]. Gross energy via adiabatic bomb calorimetry[6] was determined for feed and excreta to calculate AME. Feed and excreta samples were analyzed for total phosphorus[3,5], soluble phosphorus[3] and calcium[5] by Inductively Coupled Plasma Atomic Emissions Spectrophotometer[5]. Digestible P and digestible Ca were calculated by percentage differences between feed and excreta.

Statistical analysis: Statistical analysis was performed with the general linear model program of SAS[9]. The analysis of variance probability values are presented for the overall 12-treatment comparison. If significance differences were determined ($P<0.05$) then Fischer's

Table 2: Performance variables calculated for 6 – 21 d broilers

	Bird LWG[A] (kg)	Pen FI[B] (kg)	FC[C] (kg/kg)	Mortality[D] (%)	Tibia Ash[E] (%)
0.23 calc. nPP	0.3297^{e}	3.393^{e}	1.817^{a}	8.33	28.02^{e}
0.28 calc. nPP	0.4275^{b}	4.117^{b}	1.611^{cde}	0	31.40^{cd}
0.33 calc. nPP	0.4785^{a}	4.427^{a}	1.560^{de}	2.08	34.16^{ab}
0.38 calc. nPP	0.4927^{a}	4.459^{a}	1.509^{de}	0	35.56^{a}
Product 1[F] @ 250 FYT/kg	0.3764^{d}	3.808^{cd}	1.727^{ab}	4.17	29.43^{de}
Product 1 @ 500 FYT/kg	0.3853^{cd}	3.750^{cd}	1.625^{bcd}	0	31.11^{cd}
Product 1 @ 750 FYT/kg	0.3962^{bcd}	3.822^{cd}	1.629^{bcd}	2.08	30.21^{de}
Product 1 @ 1000 FYT/kg	0.4080^{bcd}	3.802^{cd}	1.591^{cde}	4.17	30.83^{cd}
Product 1 @ 2000 FYT/kg	0.4216^{bc}	4.130^{b}	1.634^{bcd}	0	32.58^{bc}
Product 2[G] @ 250 FYT/kg	0.3861^{cd}	3.602^{de}	1.681^{bc}	8.33	29.32^{de}
Product 2 @ 500 FYT/kg	0.3922^{bcd}	3.873^{c}	1.667^{bcd}	2.08	29.80^{de}
Product 2 @ 750 FYT/kg	0.4199^{bc}	3.924^{bc}	1.642^{bcd}	6.25	30.06^{de}
ANOVA P-value	0.0001	0.0001	0.0003	0.0653	0.0001
LSD[H]	0.038	0.226	0.115	--	2.32
Standard curve- linear effect p-value	0.0001	0.0001	0.0001	0.0158	0.0001
Product 1 linear effect p-value	0.0309	0.4255	0.0010	0.8549	0.5978
Product 1 quadratic effect p-value	0.1967	0.0925	0.0022	0.694	0.9648
Product 2 linear effect p-value	0.7840	0.1626	0.9907	0.2001	0.6074
Product 2 quadratic effect p-value	0.6500	0.2965	0.9340	0.217	0.7747
	---------- Product 1 vs. Product 2 (2 products×3 levels-factorial arrangement)[I] ----------				
Product p-value	0.2796	0.9132	0.9303	0.1192	0.3343
Level p-value	0.1953	0.0826	0.2472	0.1590	0.2502
Product×Level p-value	0.8348	0.0556	0.5942	0.9033	0.5886

[A]Live Weight Gain, [B]Feed Intake, [C]Feed Conversion, [D]mortality, [E]Dry, Defatted right tibia, [F]Product 1 contains 2,500 FYT/g, [G]Product 2 contains 5,000 FYT/g, [H]Fischer's least significant difference, [I]Activity levels include only 250, 500, and 750 FYT/kg

Table 3: Phosphorus sparing effects (%) calculated from standard curve linear regression using monocalcium phosphate

Treatment	Derived Calc P (%) LWG[A]	LWG% P sparing effect[B]	Derived Calc P (%) FC[C]	FC % P sparing effect[B]	Derived Calc P (%) Ash[D]	Ash % P sparing effect[B]
Product 1[E] @ 250 FYT/kg	0.253	0.023	0.252	0.022	0.249	0.019
Product 1 @ 500 FYT/kg	0.262	0.032	0.305	0.075	0.282	0.052
Product 1 @ 750 FYT/kg	0.271	0.042	0.303	0.073	0.264	0.034
Product 1 @ 1000 FYT/kg	0.282	0.053	0.322	0.092	0.276	0.046
Product 1 @ 2000 FYT/kg	0.295	0.065	0.300	0.070	0.311	0.081
Product 2[F] @ 250 FYT/kg	0.262	0.032	0.276	0.046	0.247	0.017
Product 2 @ 500 FYT/kg	0.268	0.038	0.283	0.053	0.256	0.026
Product 2 @ 750 FYT/kg	0.293	0.064	0.296	0.066	0.261	0.031

[A]Calculated P values derived from the linear Regression of LWG for the standard curve (LWG-0.1028)/1.07951 = calc. P, r^2=0.6825
[B]Sparing effect based on Monocalcium P
[C]Calculated P values derived from the linear Regression of FC for the standard curve (FC-2.2192)/-1.95051=calc. P, r^2=0.4534
[D]Calculated P values derived from the linear Regression of Tibia Ash for the standard curve (Ash%-16.7975)/50.77848 = calc. P, r^2=0.4935.
[E]Product 1 contains 2,500 FYT/g.
[F]Product 2 contains 5,000 FYT/g

Least Significant Difference Test was utilized for multiple comparisons of treatment means (α = 0.05). Four diets, increasing in monocalcium phosphate, consisting of the standard curve were evaluated for linearity. Experimental diets containing phytase were compared to the control diet (0.23 nPP%, without phytase). The linear equation derived from the standard curve and control diet reference was used to calculate phosphorus sparing effects (Table 3). Diets containing each phytase product were evaluated across increasing levels for linear and quadratic effects. The three treatments containing Product 2 were compared with the three corresponding treatments of Product 1 based on enzyme activity level. Probability values are presented for the product type and enzyme activity level main effects as well as the interaction.

Results

Feed manufacture: Single screw vertical mix time was determined to be optimal at 40 min. Replicated measures of mixer CV, with 0.01% salt inclusion, did not exceed 18%. Analysis of diets indicated total phytase, total phosphorus and calcium within expected calculated values (Table 1).

Broiler performance: Live weight gain increased in all experimental treatments compared to the control diet ($P<0.05$, Table 2). Increasing levels of Product 1 had a significant linear effect on LWG ($P = 0.0309$). Increasing levels of Product 2 did not affect LWG ($P>0.05$). Live weight gain produced from similar enzyme activities of Product 1 and Product 2 were not different ($P = 0.2796$). Feed Intake (FI) was greater compared to the control diet

Table 4: Mineral Digestibility and AME (Day 18-to-Day 21)

	Digestible Ca (%)	Digestible P (%)	Excreta Water Soluble P g kg^{-1}	Apparent Metabolizable Energy [standard deviation]
0.23 calc. nPP	67.1^{c}	71.3	1.93^{e}	3452^{c} [183]
0.28 calc. nPP	75.5^{ab}	75.7	2.80^{c}	3680^{a} [94]
0.33 calc. nPP	77.5^{ab}	76.7	3.44^{b}	3588^{ab} [247]
0.38 calc. nPP	81.2^{a}	77.4	4.16^{a}	3643^{ab} [241]
Product 1^{A} 250 FYT/kg	71.7^{bc}	74.8	2.58^{cd}	3573^{b} [269]
Product 1 500 FYT/kg	72.2^{bc}	77.2	1.86^{e}	3595^{ab} [140]
Product 1 750 FYT/kg	77.1^{ab}	81.5	1.93^{e}	3691^{a} [126]
Product 1 1000 FYT/kg	76.0^{ab}	80.5	2.14d^{e}	3644^{ab} [96]
Product 1 2000 FYT/kg	75.1^{b}	79.3	2.34^{cde}	3639^{ab} [176]
Product 2^{B} 250 FYT/kg	71.6^{bc}	75.8	2.11^{de}	3607^{ab} [103]
Product 2 500 FYT/kg	73.4^{b}	79.2	2.24^{de}	3665^{ab} [137]
Product 2 750 FYT/kg	73.9^{b}	77.8	1.95^{e}	3610^{ab} [150]
ANOVA p-value	0.0040	0.0708	0.0001	0.0147
LSD^{C}	6.03	--	0.49	116
Standard curve-linear effect p-value	0.0092	0.2177	0.0001	0.1502
Product 1 linear effect p-value	0.2349	0.0843	0.0585	0.2474
Product 1 quadratic effect p-value	0.2879	0.1173	0.0466	0.2969
Product 2 linear effect p-value	0.7863	0.3394	0.4184	0.3350
Product 2 quadratic effect p-value	0.8488	0.3801	0.3654	0.3330
Product p-value	0.6534	0.8444	0.8724	0.7966
Level p-value	0.1214	0.0579	0.0399	0.2811
Product× Level p-value	0.4759	0.2333	0.0393	0.1287

^{A}Product 1 at 2,500 FYT/g, ^{B}Product 2 at 5,000 FYT/g, ^{C}Fischer's least significant difference value, ^{D}Levels include only 250, 500, and 750 FYT/kg

for all treatments other than Product 2 at 250 FYT/kg (P<0.05, Table 2). Feed intake did not differ between similar enzyme activities of Product 1 and Product 2 (P = 0.9132). Feed conversion improved with the addition of enzyme in all treatments except Product 1 at 250 FYT/kg (P>0.05, Table 2). Increasing levels of Product 1 resulted in a linear decrease in FC (P = 0.0010). This trend was not demonstrated for Product 2 (P = 0.9907). However, similar enzyme activities of Product 1 and 2 were not different in FC (P = 0.9303). Product 1 at 1000 FYT/kg numerically had the lowest FC of all diets containing phytase and highest FC phosphorus sparing effect (Table 2 and 3).

The percent of tibia ash for Product 1 at 500, 1,000 and 2,000 FYT/kg increased compared to the control diet (P<0.05, Table 2). Tibia ash produced from birds fed Product 2 did not increase compared to the control diet. Product 1 and Product 2 were not different with respect to tibia ash produced from birds fed similar enzyme activities (P = 0.3343). Treatments did not affect mortality (Table 2).

Phosphorus sparing: Phosphorus sparing values were calculated for LWG, FC and tibia ash (Table 3). The greatest numerical sparing effects for LWG, FC and tibia ash were associated with Product 1 used at 2,000 FYT/kg, Product 1 used at 1,000 FYT/kg and Product 1 used at 2,000 FYT/kg, respectively.

Mineral and energy utilization: The addition of phytase increased Ca digestibility from the control diet in treatments greater than 500 FYT/kg for Product 1 and 250 FYT/kg for Product 2 (P<0.05, Table 4). Phosphorus digestibility showed a strong trend towards improvement for Product 1 and 2 with increasing enzyme activity levels of 250, 500 and 750 FYT/kg (P = 0.0579). Calcium and phosphorus digestibility were not different when Product 1 and Product 2 contained similar activity levels (P = 0.6534 and 0.8444, respectively). The standard curve did not illustrate a linear increase in phosphorus digestibility as the level of monocalcium phosphate was increased (P = 0.2177); however, calcium digestibility did increase (P = 0.0092).

Excreta water soluble phosphorus content increased as monocalcium phosphate increased in the standard curve diets (P = 0.0001). For standard curve diets, the lowest excreta water soluble phosphorus content was associated with the 0.23 calculated nPP control diet. All phytase additions with the exception of Product 1 at 250 FYT/kg produced similar excreta water soluble phosphorus as the control. Increasing enzyme activity levels of 250, 500 and 750 FYT/kg demonstrated decreased excreta water soluble phosphorus (P = 0.0399). However, no differences between Products 1 and 2 at similar enzyme activities were detected (P = 0.8724).

Apparent metabolizable energy increased in all experimental treatments containing phytase compared to the control diet (P<0.05, Table 5). The effect on AME was similar for Product 1 and Product 2 at similar enzyme activities (P = 0.7966).

Discussion

No differences between Product 1 and 2 at similar enzyme activities could be detected for any measured variable. The greatest phosphorus sparing effects were observed for birds fed Product 1 at 1,000 and 2,000 FYT/kg; activity levels not tested with Product 2. The lack of difference between Product 1 and 2 may be due to care taken to ensure proper mix uniformity. A CV of less than 10% has become the accepted degree of variation that separates uniform from non-uniform feed mixes (McCoy *et al.*, 1994; Johnston and Southern, 2000). Coefficient of variation up to 20% has been reported to be adequate for maximum growth performance in broiler chicks with diets containing a 0.03-0.04% marker inclusion (McCoy *et al.*, 1994). The current study utilized a more concentrated ingredient (Product 2 at 0.01% of the diet). A mixer CV of 18% was the lowest CV achievable with the single screw vertical mixer and 0.01% inclusion. Based on results, this CV was adequate. However, commercial feed mills may not exercise similar mixing caution when including phytase products of high concentration but low volume.

Efficacy of phosphorus has been reported to increase with the addition of phytase to LPC, but not as much as reported with normal yellow dent corn (Waldroup *et al.*, 2000). Low phytate corn contains less phytate bound phosphorus for the phytase to liberate; therefore, reduced efficacy is expected (Radcliffe, 2000; Kornegay, 1999; Angel *et al.*, 2002).

Phosphorus sparing in this experiment was not as effective as commercial claims of 0.1% with 300-500 FYT/kg. At 500 FYT/kg in Product 1, a 0.052% phosphorus sparing effect was found based on tibia ash. These results were below observations reported by Applegate and Angel (2003) of 0.065% phosphorus sparing with 500 FYT/kg based on tibia ash and normal corn. Feed conversion was the most affected variable for phosphorus sparing with 0.092% at 1,000 FYT/kg in Product 1. Feed conversion and tibia ash are common indices for the determination of phytase efficacy and often the most sensitive for comparing phosphorus sparing (Applegate and Angel, 2003).

The addition of phytase in both products at greater than 500 FYT/kg improved all measured variables, other than tibia ash. Tibia ash efficacy was not consistent, resulting in improvement for Product 1 only at 500, 1,000 and 2,000 FYT/kg compared to the control diet. Product 2 did not improve tibia ash compared to the control. In theory, phosphorus stores in bone would increase with phytase inclusion due to increased liberation of dietary phosphorus. However, tibia ash did not coincide with LWG and FC results suggesting that caution should be used when evaluating phytase efficacy based on tibia ash alone.

Although a reduction of total phosphorus is often emphasized in literature, water-soluble phosphorus has the greatest environmental implications on eutrophication (Miles *et al.*, 2003). The combination of LPC and phytase in the diet has been reported to reduce water-soluble phosphorus in litter compared with normal corn (Miles *et al.*, 2003). Applegate and Angel (2003) reported that, with correct phytase inclusion, both total phosphorus and water-soluble phosphorus decrease. However, they also found that incorrect application of phytase and an insufficient decrease in dietary total phosphorus will result in no change in excreta phosphorus and an increase in water-soluble phosphorus. In the current study, similar trends were demonstrated with the standard curve diets. As monocalcium phosphorus increased, digestible phosphorus was not significantly improved and excreta water soluble phosphorus was increased. In addition, when phytase was added to the control diet, excreta water soluble phosphorus was typically low (similar to the control) and trends towards improved phosphorus digestibility were observed.

Apparent metabolizable energy was markedly increased with phytase supplementation at all levels. Increases in AME up to 6% have been reported (Ravindran, 1999; Camden *et al.*, 2001). The addition of phytase to diets may have liberated phosphorus as well as energy substrates bound to phytic acid. Increased energy utilization with added phytase is in part due to increased protein (Camden *et al.*, 2001) and starch digestibility (Ravindran, 1999). In addition, past research has speculated that calcium-phytate complexes with fatty acids, forming metallic soap in the gut lumen that decrease fat utilization (Leeson, 1993; Ravindran, 1999; Ravindran *et al.*, 2000).

Analysis of phytate level of corn is important when utilizing phytase in research or commercial practice to ensure appropriate enzyme use. Inappropriate diet formulation is costly and may result in performance decrements and/or environmental burdens. Low phytate corn may alter enzyme efficacy results. If the PP content of corn is not known, preconceived advantages of phytase use may not occur. Inclusion of phytase to diets that have been analyzed for PP content allows for a more accurate diet formulation to achieve desired results. The addition of concentrated products, such as phytase, requires uniform mixing to ensure appropriate enzyme dispersion for maximal chick performance.

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