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Effects of Storing Three Phytase Sources Over 90 Days Under High Temperature and Humidity on Phytase Stability, Growth Performance, and Bone Mineralization of Nursery Pigs[1]

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Summary

A study was performed to evaluate the effects of storing three commercially available phytase products for 90 d, simulating summer conditions on phytase stability, growth performance, and bone mineralization of nursery pigs. The phytase products [HiPhos GT (20,000 FYT/g, DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (20,000 FTU/g, Dupont, Wilmington, DE); and Quantum Blue G (40,000 FTU/g, AB Vista, Plantation, FL)] were left as pure forms or blended in a vitamin and trace mineral (VTM) premix for a 90 d period in an environmentally controlled chamber set at 85°F and 75% humidity. Sampling occurred on d 0, 30, 60, and 90 of storage. Regardless of phytase source and form (pure or VTM), there were linear decreases ($P < 0.001$) in retained phytase activities as the duration of storage increased. At the end of the 90-d storage period, the retained phytase activities ranged from 41 to 60% when phytases were in a pure form and from 38 to 54% when they were in a concentrated VTM premix. For the growth trial, a total of 300 nursery pigs (DNA 241 × 600; DNA) with an initial pen average body weight (BW) of 25.9 lb were used. Pigs were randomly allotted to pens at weaning and fed common diets for 21 d. For 4 d prior to the initiation of the trial, all pigs were fed a common diet deficient in phosphorus (0.12% available phosphorus, aP). At d 0 of the trial, the pens of pigs were randomly assigned to 1 of 8 dietary treatments in a randomized complete block design, with BW used as a blocking factor. There were 4 or 5 pigs per pen and 8 pens per treatment. Experimental diets were formulated to contain 0.12% aP (negative control, NC) or 0.27% aP (positive control, PC) supplied by an inorganic P; or the 0.12% aP diet with added phytase to provide the activity recommended by the manufacturer of each phytase source to release 0.15% aP. These diets were manufactured with each phytase source previously

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stored either in a pure form or in a VTM premix for 90 days. On d 21 of the study, one pig per pen was euthanized and the right fibula and femur were collected for percentage bone ash calculations. Overall, pigs fed the PC diet had greater ($P < 0.001$) average daily gain (ADG) compared to pigs fed Axtra Phy stored in a VTM premix for 90 days or the NC diet, with other treatments intermediate. Average daily feed intake was similar across the PC, the phytases stored for 90 days in pure forms, and the HiPhos and Quantum Blue stored for 90 d in a VTM premix, and greater ($P < 0.001$) than pigs fed the NC. Pigs fed the PC or the HiPhos stored for 90 d in a pure form had improved ($P < 0.001$) feed efficiency (F/G) compared to pigs fed the NC diet, with the remaining treatments intermediate. Final BW was similar across all added phytase treatments and the PC, which were all greater ($P < 0.001$) than the NC. Bone mineralization was greater ($P < 0.001$) for pigs fed the PC diet compared to the NC, the phytases that were stored for 90 d in a VTM premix, and the Axtra Phy and Quantum Blue stored for 90 d in a pure form, with HiPhos stored in a pure form for 90 d intermediate. In conclusion, this study indicates that regardless of phytase source and form (pure or VTM premix), phytase activity decreases as duration of storage in high temperature and high humidity conditions increases for up to 90 d. Pigs fed PC diets consistently had increased growth performance and bone mineralization compared to pigs fed the other dietary treatments. However, F/G and bone ash of pigs fed HiPhos stored for 90 d in a pure form were similar to results for pigs fed the PC diet, with pigs fed the other phytase treatments intermediate.

Introduction

Exogenous phytases are routinely included in swine diets to break down and release the phosphates from phytate phosphorus (P) found in most feed ingredients of plant origin. This is an economical way to help increase the P availability while also being beneficial for the environment as phytases help to reduce P excretion.[4] However, as with any catalytic proteins, phytases are subjected to denaturation reactions. Exposure to heat, moisture, and mechanical pressure can lead to such reactions and cause irreversible loss of phytase activity.[5,6]

Previous work has shown that the form of the product can also affect efficacy of phytase.[7] Sulabo et al.[7] demonstrated that a pure phytase source stored in ambient temperatures greater than 73.4°F with high humidity is detrimental to the stability of this enzyme. Moreover, the authors also observed loss of phytase activity as duration of storage increased up to 360 d when the enzyme was blended in a vitamin and trace mineral (VTM) premix. This suggests that not only heat and humidity, but also interactions with components of VTM premixes can increase the degradation of phytase.

The value of a phytase product relies on its ability to increase the amount of P available to the pig, which is dependent on its efficacy and stability. However, phytase activity is

[4]Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. *Anim. Feed Sci. Technol.* 135:1–41.

[5]Iyer, P. V., and L. Ananthanarayan. 2008. Enzyme stability and stabilization—Aqueous and non-aqueous environment. *Process Biochem*. 43:1019–1032.

[6]Ward, N. E. 2002. Phytase stability may be improved by new technology. *Feedstuffs* 74:11–13.

[7]Sulabo, R.C., Jones, C.K., Tokach, M.D., Goodband, R.D., Dritz, S.S., Campbell, D.R., Ratliff, B.W., DeRouchey, J.M. and Nelssen, J.L., 2011. Factors affecting storage stability of various commercial phytase sources. *Journal of animal science*, 89(12), pp.4262-4271.

usually determined at the time it is manufactured, not at the time of use. Therefore, the objective of this study was to determine the effects of a 90-d storage period under high temperature and high humidity conditions for three commercially available phytase products stored in pure forms or in a VTM premix on phytase stability, and on growth performance, and bone mineralization of nursery pigs.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The storage part of the study was conducted at the Bioprocessing and Industrial Value Added Program Building at Kansas State University, and the growth part of the study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS.

Three commercially available phytases were used in this experiment: HiPhos GT (20,000 FYT/g, DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (20,000 FTU/g, Dupont, Wilmington, DE); and Quantum Blue G (40,000 FTU/g, AB Vista, Plantation, FL). The three phytase sources were included as part of corn-soybean meal-based swine diets. The amount added for each phytase product was determined such that including 0.15% VTM premix in the diet would provide the activity of phytase recommended by the manufacturer to release 0.15% available P (1,000 FYT/kg feed for HiPhos, 651 FTU/kg feed for Axtra Phy, and 500 FTU/kg feed for Quantum Blue). Thus, the inclusion rate of each phytase product per ton of feed was 0.1000, 0.0651, and 0.0250 lb of HiPhos, Axtra Phy, and Quantum Blue, respectively.

Each phytase product was added to a concentrated phytase-free VTM premix (Table 1) to create 20-lb batches by mixing for 5 min in a paddle mixer. The phytase-free VTM premix, the three pure phytase products, and the three batches of VTM premix with each phytase were bagged into single-lined paper bags. They were stored for 90 d in an environmentally-controlled chamber set at 85°F and 75% humidity. A total of six samples from each bag were taken on d 0, 30, 60, and 90 of storage, except for the phytase-free VTM premix, which was only sampled on d 90. Before sampling, each bag was mixed to ensure that a representative sample was collected. Each sample was assigned with a code so as not to be identified and sent immediately after collection to the Technical Marketing Analytical Services of DSM Nutritional Products Inc. (Belvidere, NJ) for phytase analysis using a slight modification of AOAC official method 2000.12.[8,9,10] Results were sent back to K-State with the assigned codes, which were linked to the product source for statistical analysis.

For the growth study, 300 nursery pigs (241 × 600; DNA, Columbus, NE) were used in a 21-d trial. Pigs were weaned at approximately 21 d of age, and allotted to pens of 4 or 5 pigs according to initial BW and gender upon entry in the nursery. Each pen was

[8]Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int. 77:760–764.
[9]Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, and W. A. C. Somer. 2001. Determination of phytase activity in feed by a colorimetric enzymatic method: Collaborative interlaboratory study. J. AOAC Int. 84:629–633.
[10]AOAC. 2000. Official Methods of Analysis of AOAC International. 17th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

equipped with a 4-hole, dry self-feeder and a cup waterer to provide *ad libitum* access to feed and water. At weaning, pigs were fed a common pelleted phase 1 diet and a common meal phase 2 diet for 21 d. Four days prior to the initiation of the trial, all pigs were fed a common diet deficient in phosphorus (0.12% aP). At d 0 of the trial (initial pen average BW of 25.9 lb), the pens of pigs were randomly assigned to 1 of 8 dietary treatments in a randomized complete block design. There were 8 replicate pens per treatment and BW was used as the blocking factor.

The eight experimental treatments consisted of: a negative control (NC); a positive control (PC); HiPhos; Axtra Phy, or Quantum Blue stored for 90 d in a pure form; and HiPhos, Axtra Phy, or Quantum Blue stored for 90 d in a VTM premix form. The NC and PC diets were formulated with the inclusion of 0.15% phytase-free VTM premix. They were formulated to 0.12 and 0.27% aP, respectively, achieved with the inclusion of inorganic P provided by monocalcium phosphate. The remaining treatments were formulated to 0.27% aP, which were achieved with 0.12% aP from inorganic P and 0.15% aP released from each phytase product. Two VTM premixes were made for each phytase source. One VTM premix was made at the beginning of the study prior to storage, and one VTM premix was with the pure products after 90 d of storage. The same amount of pure product was added to each VTM within phytase source. The amount added for each phytase product was determined to provide the activity of phytase recommended by the manufacturer to release 0.15% aP (1000 FYT/kg feed HiPhos, 651 FTU/kg feed Axtra Phy, and 500 FTU/kg feed Quantum Blue).

All experimental diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. A total of 3 samples of corn, soybean meal, and monocalcium phosphate used in the diets were analyzed for P in duplicate (Ward Laboratories, Inc., Kearney, NE). The average of the six lab results for each ingredient was used for diet formulation. Dietary treatments were derived from 8, 1-ton basal batches (Table 4). After manufacturing, each basal batch was bagged off into 8 separate tons. For each experimental diet, a subset of bags (50 lb each) from each batch of the basal diet was added to the mixer along with treatment-specific ingredients to achieve the final dietary treatments (Table 5). Dietary treatments were fed in meal form for 21 d. Pens of pigs were weighed and feed disappearance was recorded on d 0, 7, 14, and 21 to determine ADG, ADFI, and F/G.

Diet samples were taken from 6 feeders per dietary treatment in the first and third weeks of the trial. These samples were combined to create a composite sample in the first and third weeks of the trial, and 4 subsamples of the composite samples from each diet were immediately sent to the Technical Marketing Analytical Services of DSM Nutritional Products Inc. (Belvidere, NJ) and New Jersey Feed Laboratory Inc. (Trenton, NJ) for phytase analysis. The remaining of the composite samples were stored at -20°C. Subsequently, subsamples were sent to Ward Laboratories, Inc. (Kearney, NE) and analyzed for dry matter, crude protein, ether extract, calcium, and phosphorus.

On d 21 of the study, pigs with body weights closest to the average pen weights were selected and euthanized via captive bolt. The right fibula and femur were removed from euthanized pigs to determine percentage bone ash criteria. Bones were individually placed in a plastic bag with a permanent identification tag within the bag and stored at

-20°C until analysis. On the day of processing, bones were autoclaved for one hour at 121°C. Femurs and fibulas were cleaned of extraneous soft tissue and placed in a 105°C drying oven for 7 d to determine the dry weight. Bones were then ashed in a muffle furnace at 600°C for 24 h to determine the total ash weight and percentage ash. Ash is expressed as a percentage of dried bone weight.

The study consisted of a randomized complete block design, with pen as the experimental unit and BW as the blocking factor. Statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC). Least square means were calculated for each response variable. When treatment was a significant source of variation, differences were determined by using the preplanned pairwise comparisons (PDIFF option of SAS) using the Tukey-Kramer adjustment. In addition, a non-orthogonal contrast was built to compare storing phytases in the pure form with storage in VTM premixes. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Storage Period

The calculated and analyzed phytase activities of the samples on d 0, 30, 60, and 90 of storage are shown in Table 2. Based on the AOAC assay, analyzed phytase activities of HiPhos, Axtra Phy, and Quantum Blue in pure forms were 115, 77, and 94% of their minimum guaranteed phytase activity according to their labels on d 0 prior to storage, respectively. When the phytases were mixed in a VTM premix, the analyzed phytase activities were 109, 89, and 103% of their minimum guaranteed phytase activity according to their labels on d 0 prior to storage for HiPhos, Axtra Phy, and Quantum Blue, respectively. Regardless of phytase source and form (pure or VTM), there were linear decreases ($P < 0.001$) in phytase activity as the duration of storage increased up to d 90. The retained activities ranged from 41 to 60% when phytases were stored in a pure form for 90 d, and from 38 to 54% when they were stored in a concentrated VTM premix for 90 d.

The calculated and analyzed phytase activities of the feed samples collected on the first or third week of the growth trial are shown in Table 3. As expected, the NC and PC treatments had phytase lower than the detection limit of the assay. The analyzed phytase activity of feed samples in the first and third week of the growth trial of HiPhos, Axtra Phy, and Quantum Blue treatments stored for 90 d as pure products ranged between 76 to 80, 94 to 72, and 51 to 53% of their calculated activity to release 0.15% aP based on the label minimum guaranteed activity on d 0 prior to storage, respectively. When they were stored for 90 d in a VTM premix, the analyzed phytase activity in the first and third week of the growth trial ranged from 89 to 73, 84 to 71, and 60 to 55% of their calculated phytase activity to release 0.15% aP based on the label minimum guaranteed activity on d 0 prior to storage for HiPhos, Axtra Phy, and Quantum Blue, respectively. These ratios were greater than expected considering the retained activity after the 90 d duration of storage. However, analysis of phytase in final diets is typically more difficult and variable than phytase activity analysis in concentrated products; therefore, we assume the difference is due to analytical variation or difficulties with analyzing the phytase in a feed matrix.

Growth Trial Period

Diet analysis of dry matter, crude protein, fat, and P (Table 6) showed that values were reasonably consistent with formulated estimates. Average values of analyzed calcium concentrations were slightly greater than the formulated values; however, they followed similar patterns as the designed treatment structure.

Overall, pigs fed the PC diet had greater ADG compared to pigs fed Axtra Phy stored for 90 d in a VTM premix or the NC diet, with other treatments intermediate (Table 7, $P < 0.001$). Average daily feed intake was similar across the PC, the phytases stored for 90 d in pure forms, and the HiPhos and Quantum Blue stored for 90 d in a VTM premix form, and greater than the NC ($P < 0.001$). Pigs fed the PC or the HiPhos stored for 90 d in a pure form had improved F/G compared to pigs fed the NC diet, with the remaining treatments intermediate ($P < 0.001$). Final BW was similar for pigs fed the PC diet or all added phytase treatments and greater than the NC ($P < 0.001$).

Percentage ash of femur samples was greater for pigs fed the PC diet compared to the NC, Quantum Blue stored for 90 d in a pure form, and Axtra Phy and Quantum Blue stored for 90 d in a VTM premix, with other treatments intermediate ($P < 0.001$). For fibula samples, pigs fed the PC diet had greater percentage ash compared to Quantum Blue stored for 90 d in a VTM premix and the NC diets, with all the remaining treatments intermediate ($P < 0.001$). Because there was no evidence of a bone type by treatment interaction ($P = 0.548$), the main effect of bone ash was evaluated. Bone mineralization was greater for pigs fed the PC diet compared to the NC, the three phytases stored for 90 d in a VTM premix, and Axtra Phy and Quantum Blue stored for 90 d in a pure form, with HiPhos stored for 90 d in a pure form intermediate ($P < 0.001$).

A specific preplanned, non-orthogonal contrast was completed to compare storage of phytases in pure form versus storage in VTM premixes (Table 7). No evidence of significant differences ($P > 0.10$) was observed when comparing the average of the three phytases sources stored for 90 d in pure form to the average of the three phytase sources stored for 90 d in VTM for any response criteria.

In conclusion, this study indicates that regardless of phytase source and form (pure or VTM), phytase activity decreases as duration of storage in high temperature and humidity conditions (85°F and 75% humidity) increase for up to 90 d. Pigs fed PC diets consistently had increased growth performance and bone mineralization compared to pigs fed the other dietary treatments. However, F/G and bone ash of pigs fed HiPhos stored for 90 d in a pure form were similar to results for pigs fed the PC diet, with the other phytase treatments intermediate.

Table 1. Composition of the phytase-free vitamin and trace mineral (VTM) premix used in the study[1]

Item	Added per lb of VTM premix
Vitamin	
Vitamin A, IU	3,024,000
Vitamin D_3, IU	604,800
Vitamin E, IU	30,240
Vitamin K, mg	1,210
Riboflavin, mg	3,024
Niacin, mg	13,608
Pantothenic acid, mg	9,979
Cobalamin, mg	14
Folic acid, mg	907
Thiamine, mg	907
Pyridoxine, mg	1,210
Biotin	91
Trace mineral	
Copper ($CuSO_4$), mg	4,536
Iodine [$Ca(IO_3)^2$], mg	303
Iron ($FeSO_4$), mg	45,359
Manganese (MnO^2), mg	16,633
Selenium (selenium yeast), mg	91
Zinc ($ZnSO_4$), mg	33,248

[1]The amount added for each phytase product was determined such that including 0.15% premix in the diet would provide the phytase recommended by their respective manufacturers to release 0.15% aP [(1000 FYT/kg feed HiPhos, DSM Nutritional Products, Parsippany, NJ); (651 FTU/kg feed Axtra Phy, Dupont, Wilmington, DE); and (500 FTU/kg feed Quantum Blue, AB Vista, Plantation, FL)].

Table 2. Calculated and analyzed phytase composition of samples at d 0, 30, 60, and 90 of storage[1]

	Phytase composition									
	Minimum guaranteed	AOAC analysis, PU/g				AOAC ratio[4]				Probability, $P <$
Item[2]	PU[3]/g	d 0	d 30	d 60	d 90	d 0	d 30	d 60	d 90	Linear time
Pure product										
HiPhos[5]	20,000	22,940	21,807	16,037	12,098	1.15	1.09	0.80	0.60	0.001
Axtra Phy[6]	20,000	15,524	11,768	11,051	8,272	0.77	0.59	0.55	0.41	0.001
Quantum Blue[7]	40,000	37,592	29,176	27,046	22,443	0.94	0.73	0.68	0.56	0.001
VTM premix										
HiPhos[5]	666	728	519	454	332	1.09	0.78	0.68	0.50	0.001
Axtra Phy[6]	434	388	254	282	166	0.89	0.59	0.65	0.38	0.001
Quantum Blue[7]	333	344	216	310	179	1.03	0.65	0.93	0.54	0.001

[1]Values represent averages of 6 replicates. The AOAC analysis were performed at the DSM Nutritional Products Laboratory (Belvidere, NJ).
[2]The VTM premix without phytase was sampled and analyzed for phytase activity on d 90 and found to be free of phytase.
[3]PU = phytase units. Minimum guaranteed PU according to the label of each phytase source.
[4]Ratio of average AOAC analyzed values to calculated values.
[5]DSM Nutritional Products, Parsippany, NJ.
[6]Dupont, Wilmington, DE.
[7]AB Vista, Plantation, FL.

Table 3. Calculated and analyzed phytase composition of feed samples at first and third week of the growth trial period[1]

	Phytase composition				
	Calculated	AOAC analysis, PU/kg		AOAC ratio[3]	
Item	PU[2]/kg feed	First week	Third week	First week	Third week
Negative control[4]	0	<50	<50	---	---
Positive control[5]	0	<50	<50	---	---
Pure product[6]					
HiPhos[7]	1,000	759	769	0.76	0.80
Axtra Phy[8]	651	613	474	0.94	0.72
Quantum Blue[9]	500	257	267	0.51	0.53
VTM premix[10]					
HiPhos[7]	1,000	890	727	0.89	0.73
Axtra Phy[8]	651	548	459	0.84	0.71
Quantum Blue[9]	500	300	275	0.60	0.55

[1]Dietary samples were collected in the first and third week of the growth trial, and values represent averages of 8 replicates. The AOAC analyses were performed at the DSM Nutritional Products Laboratory (Belvidere, NJ) and at the New Jersey Feed Laboratory Inc. (Trenton, NJ).
[2]PU = phytase units. Calculated values represent the amount of PU of each phytase source needed to release 0.15% aP based on their label minimum guaranteed phytase activity on d 0 prior to storage.
[3]Ratio of average AOAC analyzed values to calculated values.
[4]The negative control diet was formulated to 0.12% aP provided by monocalcium phosphate.
[5]The positive control diet was formulated to 0.27% aP provided by monocalcium phosphate.
[6]The three sources of phytase (Hiphos, Axtra Phy, and Quantum Blue) were added to the diets to release 0.15% aP. They were stored in a pure form for 90 days in an environmental chamber (85°F and 70% humidity) before diet manufacturing.
[7]DSM Nutritional Products, Parsippany, NJ.
[8]Dupont, Wilmington, DE.
[9]AB Vista, Plantation, FL.
[10]The three sources of phytase (Hiphos, Axtra Phy, and Quantum Blue) were added to the diets to release 0.15% aP. They were mixed in a phytase-free VTM premix and stored for 90 days in an environmental chamber (85°F and 70% humidity) before diet manufacturing.

Table 4. Diet composition of basal diet (as-fed basis)[1]

Item	Basal diet
Ingredient, %	
Corn[2]	61.22
Soybean meal, 46.5% crude protein[2]	36.37
Calcium carbonate	1.04
Monocalcium phosphate, 21%[2]	0.19
Sodium chloride	0.65
L-Lysine-HCl	0.29
DL-Methionine	0.14
L-Threonine	0.10
Total	100.00
Calculated analysis	
Standardized ileal digestible (SID) amino acids	
Lysine	1.30
Isoleucine:lysine	64
Leucine:lysine	128
Methionine:lysine	34
Methionine and cysteine:lysine	58
Threonine:lysine	62
Tryptophan:lysine	19.1
Valine:lysine	69
Total lysine, %	1.46
Metabolizable energy, kcal/lb	1,497
Net energy, kcal/lb	1,095
SID lysine:ME,[3] g/Mcal	3.94
Crude protein, %	22.8
Calcium, %	0.54
Phosphorus, %	0.47
Available phosphorus, %	0.12

[1]The basal batch was used as the major ingredient within each experimental diet.
[2]A total of 3 samples of corn, soybean meal, and monocalcium phosphate were analyzed for P concentration in duplicate (Ward Laboratories, Inc., Kearney, NE). The average of the six lab results for each ingredient was used for diet formulation, which corresponded to 0.31, 0.66, and 20.54% for corn, soybean meal, and monocalcium phosphate, respectively.
[3]ME = metabolizable energy.

Table 5. Diet composition of experimental diets (as-fed basis)

Item	Negative control	Positive control	Stored in pure form[1]			Stored in VTM premix form[2]		
			Hiphos	Axtra Phy	Quantum Blue	HiPhos	Axtra Phy	Quantum Blue
Ingredient, %								
Basal diet	98.95	98.97	98.95	98.95	98.95	98.95	98.95	98.95
Calcium carbonate	---	0.15	---	---	---	---	---	---
Monocalcium phosphate	---	0.73	---	---	---	---	---	---
Sand[3]	0.90	---	0.90	0.90	0.90	0.90	0.90	0.90
VTM premix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis								
Calcium, %	0.54	0.72	0.54	0.54	0.54	0.54	0.54	0.54
Phosphorus, %	0.47	0.62	0.47	0.47	0.47	0.47	0.47	0.47
Available phosphorus, %	0.12	0.27	0.27	0.27	0.27	0.27	0.27	0.27

[1]The three sources of phytase (Hiphos, Axtra Phy, and Quantum Blue) were stored in a pure form for 90 days in an environmental chamber (85°F and 70% humidity) before diet manufacturing.
[2]The three sources of phytase (Hiphos, Axtra Phy, and Quantum Blue) were mixed in a phytase-free VTM premix and stored for 90 days in an environmental chamber (85°F and 70% humidity) before diet manufacturing.
[3]Sand was used to equalize inclusion rates of experimental ingredients.
[4]The negative and positive control diets were formulated with a phytase-free VTM premix. For the other treatments, the amount added for each phytase product was determined such that including 0.15% VTM premix in the diet would provide the activity of phytase recommended by the manufacturer to release 0.15% available P (1000 FYT/kg feed HiPhos, 651 FTU/kg feed Axtra Phy, and 500 FTU/kg feed Quantum Blue).

Table 6. Chemical analysis of experimental diets (as-fed-basis)[1]

Item	Negative control	Positive control	Stored in VTM premix form			Stored in pure form		
			HiPhos	Quantum Blue	Axtra Phy	Hiphos	Quantum Blue	Axtra Phy
Dry matter	90.02	89.77	90.08	89.64	89.90	90.06	89.92	89.98
Crude protein	23.20	23.80	23.35	23.10	23.65	22.75	23.40	22.90
Ether extract	2.55	2.40	2.60	2.30	2.45	2.15	2.50	2.25
Ash	5.30	5.31	5.31	5.42	4.90	5.21	5.23	5.76
Calcium	0.62	0.66	0.61	0.63	0.54	0.66	0.64	0.63
Phosphorus	0.46	0.57	0.45	0.44	0.46	0.47	0.47	0.46

[1]A representative sample of each diet was collected from 6 feeders, homogenized, and submitted to Ward Laboratories, Inc., Kearney, NE, for chemical analysis.

Table 7. Effects of phytase when stored in a concentrated VTM premix or as a pure product on growth performance and bone mineralization of nursery pigs[1]

			Stored in pure form[5]			Stored in VTM form[6]				Probability, [7] *P* =	
Item[2]	Negative control[3]	Positive control[4]	HiPhos	Quantum Blue	Axtra Phy	HiPhos	Quantum Blue	Axtra Phy	SEM	Overall[8]	Stored in VTM vs Pure[9]
d 0 to 21											
ADG, lb	1.07^{c}	1.42^{a}	1.41^{a,b}	1.29^{a,b}	1.38^{a,b}	1.35^{a,b}	1.33^{a,b}	1.27^{b}	0.052	<0.001	0.106
ADFI, lb	1.91^{b}	2.18^{a}	2.17^{a}	2.15^{a}	2.23^{a}	2.13^{a}	2.24^{a}	2.12^{a,b}	0.091	<0.001	0.660
F/G	1.80^{a}	1.54^{c}	1.54^{c}	1.66^{a,b,c}	1.63^{b,c}	1.58^{b,c}	1.68^{a,b}	1.67^{a,b,c}	0.031	<0.001	0.155
Body weight, lb											
d 0	25.9	25.9	25.9	25.9	25.9	25.9	25.9	25.9	1.45	0.989	0.987
d 21	49.0^{b}	55.7^{a}	55.6^{a}	53.0^{a}	54.7^{a}	54.2^{a}	53.9^{a}	53.4^{a}	2.33	<0.001	0.360
Bone ash, %											
Femur	37.7^{c}	47.4^{a}	44.8^{a,b}	43.5^{b}	44.5^{a,b}	45.2^{a,b}	42.0^{b}	43.2^{b}	0.85	<0.001	0.275
Fibula	39.0^{c}	46.4^{a}	44.3^{a,b}	42.2^{a,b,c}	42.0^{a,b,c}	43.0^{a,b,c}	40.6^{b,c}	42.5^{a,b,c}	0.96	<0.001	0.305
Femur + Fibula[10]	38.4^{d}	46.9^{a}	44.6^{a,b}	42.8^{b,c}	43.3^{b,c}	44.1^{b}	41.3^{c}	42.8^{b,c}	0.64	<0.001	0.125

[1]A total of 300 pigs (DNA, 241 × 600, initial pen average body weight 25.9 lb) were used in a 21-d growth study with 4 or 5 pigs per pen, and 8 pens per treatment. All pigs were fed a diet deficient in phosphorus (0.12% aP) for 4 days prior to the initiation of the trial.

[2]ADG = average daily gain. ADFI = average daily feed intake. F/G = feed-to-gain ratio.

[3]The negative control diet was formulated to 0.12% aP provided by monocalcium phosphate.

[4]The positive control diet was formulated to 0.27% aP provided by monocalcium phosphate.

[5]The three sources of phytase (Hiphos, Axtra Phy, and Quantum Blue) were added to the diet in order to release 0.15% aP for a 0.15% premix inclusion in the diet. They were stored for 90 days in a pure form in an environmental chamber (85°F and 70% humidity) before diet manufacturing.

[6]The three sources of phytase (Hiphos, Axtra Phy, and Quantum Blue) were added to the diet in order to release 0.15% aP for a 0.15% premix inclusion in the diet. They were stored for 90 days in a VTM premix form in an environmental chamber (85°F and 70% humidity) before diet manufacturing.

[7]The interaction term between phytase source (HiPhos, Axtra Phy, Quantum Blue) and storage form (VTM premix and pure product) was tested; however, no significant interactions were observed for any response criteria.

[8]All possible pairwise comparisons were protected by the Tukey-Kramer adjustment. Different superscripts within a column differ.

[9]This contrast compared the average of the three phytase sources stored for 90 in pure form to the average of the three phytase sources stored for 90 d in VTM premix.

[10]The interaction term between dietary treatment and bone type (femur or fibula) was tested, but the interaction was not statistically significant. Thus, bone means were combined for the analysis.