# **RESEARCH** BRIEF

# A Stability Comparison of Three Phytase Sources and Effects on Growth Performance and Bone Mineralization in Nursery Pigs<sup>1</sup>

# **HiPhos**<sup>™</sup>

# Objective

To evaluate three heat-stable phytase products, each stored as their original concentrates and in a nursery vitamin-trace mineral (VTM) premix, in high temperature and humidity conditions during 90 days prior to feed manufacturing; and to compare the retention of their expected activities and dietary phosphorus value using a laboratory phytase assay and pig growth responses during a 21-day feeding period.

### **Key study results**

- Overall, pigs fed the Positive Control (PC) diet had greater (P < 0.001) average daily gain (ADG) compared to pigs fed Phytase B stored in the VTM premix for 90 days or the Negative Control (NC) diet (0.15% less available phosphorus (P) than PC).
- Average daily feed intake (ADFI) was similar for pigs fed the PC, the diets using phytases stored for 90 days in their concentrated forms, and the HiPhos<sup>TM</sup> and Phytase A phytases that had been stored in a VTM premix; which all had greater (P < 0.001) ADFI than pigs fed the NC.
- Pigs fed the PC or the diet using HiPhos stored 90 days in concentrated form had improved (P < 0.001) F/G compared to pigs fed the NC diet.
- Final body weight (BW) was similar across all 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, and for phytases A and B in concentrated form and in premix; but bone mineralization of pigs fed HiPhos stored 90 days in concentrated form was intermediate. See Table 3.

## Background

**PHYTASES ANALYZED:** Three commercially available phytases were used in this experiment: HiPhos GT (20,000 FYT/g, DSM Nutritional Products); Phytase A (40,000 FTU/g); and Phytase B (20,000 FTU/g). Each phytase product was added to a concentrated, phytase-free, nursery VTM premix. 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, 500 FTU/kg feed for Phytase A, and 651 FTU/kg feed for Phytase B). The phytase-free VTM premix. the three pure phytase products, and the three batches of VTM premix with each phytase were stored for 90 days in an environmentally controlled chamber set at 85°F and 75% humidity. Six samples from each were taken for analysis on days 0, 30, 60, and 90 of storage.

**PERFORMANCE:** After the 90 days of storage, a total of 300 nursery pigs (DNA 241 × 600; DNA) with an initial average BW of 25.9 lbs. were used to evaluate the effects of the phytase storage on growth over a 21-day period. Pens of pigs were assigned to one of eight dietary treatments in a randomized complete block design with BW used as a blocking factor. There were four or five pigs per pen and eight pens per treatment. Experimental treatment diets consisted of NC (0.12% aP) or PC (0.27% aP) using inorganic P, or the NC diet with phytase for each source added to provide the claimed activity recommended by the manufacturer of each phytase to release 0.15% aP.

**MEASUREMENT:** Growth trial data were analyzed using the GLIMMIX procedure of SAS with pen as the experimental unit. 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) and the Tukey-Kramer adjustment. In addition, a non-orthogonal contrast was built to compare storing phytases in the pure, concentrated form with storage in VTM premixes. Results were considered significant at  $P \le 0.05$  and marginally significant at  $0.05 < P \le 0.10$ .



Phytase activity										
ltem <sup>2</sup>	Minimum	AOAC analysis, PU/g					AOAC	Probability, P <		
	guaranteed PU³/g	d 0	d 30	d 60	d 90	d 0	d 30	d 60	d 90	Linear time
Pure product										
HiPhos	20,000	22,940	21,807	16,037	12,098	1.15	1.09	0.80	0.60	0.001
Phytase A	40,000	37,592	29,176	27,046	22,443	0.94	0.73	0.68	0.56	0.001
Phytase B	20,000	15,524	11,768	11,051	8,272	0.77	0.59	0.55	0.41	0.001
VTM premix										
HiPhos	666	728	519	454	332	1.09	0.78	0.68	0.50	0.001
Phytase A	333	344	216	310	179	1.03	0.65	0.93	0.54	0.001
Phytase B	434	388	254	282	166	0.89	0.59	0.65	0.38	0.001

#### Table 1. Calculated and analyzed phytase activity composition of samples at d 0, 30, 60, and 90 of storage.<sup>1</sup>

Values represent averages of 6 replicates. The AOAC phytase activity analysis were performed at the DSM Nutritional Products Laboratory (Belvidere, NJ). <sup>2</sup>The VTM premix without phytase was sampled and analyzed for phytase activity on d 90 and found to be free of phytase. <sup>3</sup>PU = phytase units. Minimum guaranteed PU according to the label of each phytase source.

<sup>4</sup>Ratio of average AOAC analyzed values to calculated values.

Table 2. Calculated and analyzed phytase activity composition of feed samples at first and third week of the growth trial period<sup>1</sup>

Phytase activity								
11	Calculated	AOAC anal	ysis, PU/kg	AOAC ratio <sup>3</sup>				
ltem	PU <sup>2</sup> /kg feed	First week	Third week	First week	Third week			
Negative control <sup>4</sup>	0	<50	<50	—	—			
Positive control	0	<50	<50	_	_			
Pure product								
HiPhos	1,000	759	769	0.76	0.80			
Phytase A	500	257	267	0.51	0.53			
Phytase B	651	613	474	0.94	0.72			
VTM premix <sup>7</sup>								
HiPhos	1,000	890	727	0.89	0.73			
Phytase A	A 500 30		275	0.60	0.55			
Phytase B	651	548	459	0.84	0.71			

Dietary samples were collected in the first and third week of the growth trial, and values represent averages of 8 replicates. The AOAC phytase activity analyses were preformed at the DSM Nutritional Products Laboratory (Belvidere, NJ) and at the New Jersey Feed Laboratory Inc. (Trenton, NJ). <sup>2</sup>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

<sup>2</sup>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.
 <sup>3</sup>Ratio of average AOAC analyzed values to calculated values.
 <sup>4</sup>The negative control diet was formulated to 0.12% aP provided by monocalcium phosphate.
 <sup>4</sup>The Positive control diet was formulated to 0.27% aP provided by monocalcium phosphate.
 <sup>4</sup>The Positive control diet was formulated to 0.27% aP provided by monocalcium phosphate.
 <sup>4</sup>The Positive control diet was formulated to 0.27% aP provided by monocalcium phosphate.
 <sup>4</sup>The rostive control diet was formulated to 0.27% aP provided to monocalcium phosphate.
 <sup>4</sup>The rostive control diet was formulated to 0.27% aP provided to monocalcium phosphate.
 <sup>4</sup>The rostive control diet was formulated to 0.27% aP provided to monocalcium phosphate.
 <sup>4</sup>The rostive control diet was formulated to 0.27% aP provided to monocalcium phosphate.
 <sup>4</sup>The rostive control diet was formulated to 0.27% aP provided to monocalcium phosphate.
 <sup>4</sup>The rostive control diet was formulated to 0.27% aP provided to manufacturing.
 <sup>4</sup>The three sources of phytase (Hiphos, Phytase A and Phytase B) 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 3. Effects of phytase when stored in a concentrated VTM premix or as a pure product on growth performance and bone mineralization of nursery pigs1

ltem <sup>2</sup>	Negative control <sup>3</sup>	Positive control⁴	Stored in pure form <sup>5</sup>			Stored in VTM form <sup>6</sup>				Probability, <sup>7</sup> P =	
			HiPhos	Phytase A	Phytase B	HiPhos	Phytase A	Phytase B	SEM	Overall <sup>8</sup>	Stored in VTM vs Pure <sup>9</sup>
d 0 to 21											
ADG, lbs.	1.07 <sup>c</sup>	1.42ª	1.41 <sup>a,b</sup>	1.29 <sup>a,b</sup>	1.38 <sup>a,b</sup>	1.35 <sup>a,b</sup>	1.33 <sup>a,b</sup>	1.27 <sup>b</sup>	0.052	<0.001	0.106
ADFI, lbs.	1.91 <sup>b</sup>	2.18ª	2.17ª	2.15ª	2.23ª	2.13ª	2.24ª	2.12 <sup>a,b</sup>	0.091	<0.001	0.660
F/G	1.80ª	1.54 <sup>c</sup>	1.54 <sup>c</sup>	1.66 <sup>a,b,c</sup>	1.63 <sup>b,c</sup>	1.58 <sup>b,c</sup>	1.68 <sup>a,b</sup>	1.67 <sup>a,b,c</sup>	0.031	<0.001	0.155
Body weight, lbs.											
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 <sup>b</sup>	55.7ª	55.6ª	53.0ª	54.7ª	54.2ª	53.9ª	53.4ª	2.33	<0.001	0.360
Bone ash, %											
Femur	37.7 <sup>c</sup>	47.4ª	44.8 <sup>a,b</sup>	43.5 <sup>b</sup>	44.5 <sup>a,b</sup>	45.2 <sup>a,b</sup>	42.0 <sup>b</sup>	43.2 <sup>b</sup>	0.85	<0.001	0.275
Fibula	39.0 <sup>c</sup>	46.4ª	44.3 <sup>a,b</sup>	42.2 <sup>a,b,c</sup>	42.0 <sup>a,b,c</sup>	43.0 <sup>a,b,c</sup>	40.6 <sup>b,c</sup>	42.5 <sup>a,b,c</sup>	0.96	<0.001	0.305
Femur + Fibula <sup>10</sup>	38.4 <sup>d</sup>	46.9ª	44.6 <sup>a,b</sup>	42.8 <sup>b,c</sup>	43.3 <sup>b,c</sup>	44.1 <sup>b</sup>	41.3 <sup>c</sup>	42.8 <sup>b,c</sup>	0.64	<0.001	0.125

<sup>1</sup>A total of 300 pigs (DNA, 241 x 600, initial pen average body weight 25.91b) were used in a 21-d growth study with 4 to 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.
 <sup>2</sup>ADG = average daily gain. ADFI = average daily feed intake. F/G = feed-to-gain ratio.
 <sup>2</sup>The negative control diet was formulated to 0.12% aP provided by monocalcium phosphate.
 <sup>2</sup>The treative control diet was formulated to 0.27% aP provided by monocalcium phosphate.
 <sup>2</sup>The treative set (Hiphos, Pytase A, Pytase B) 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.
 <sup>3</sup>The treates (Liphos, P) 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.

The three sources of phytase (Hiphos, Pytase A, Phytase B) 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

\*The three sources of phytase (Hiphos, Pytase A, Phytase B) 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? diet manufacturing.
\*The interaction term between phytase sources (Hiphos, Pytase A, Phytase B) and storage form (VTM premix and pure product) was tested: however, no significant interactions were observed for any response criteria.
\*All possible pairwise comparisons were protected by the Turkey-Kramer adjustment. Different superscripts within a column differ.
\*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.

### **Key points**

- This study indicates that phytase activity decreases as the duration of storage in high temperature and high humidity conditions increase.
  - Decay in phytase activity may be increased when a concentrated phytase product is included in a VTM premix that is stored in stressful conditions.
- Pigs fed the PC diet or the NC diet containing HiPhos added in its concentrated form (20,000 FYT/g HiPhos GT) had the best overall growth performance and bone mineralization when compared to pigs fed the other dietary treatments.

#### Implications

Using a phytase in formulation without accounting for potential losses in activity can reduce growth performance as much as 5 to 8%

Inadequate P and associated reductions in average daily gain and feed efficiency of 5 to 8% throughout an entire growing and finishing period can result in financial losses of as much as \$5 to \$7 per pig.

Reductions in bone mineralization that can occur with reduced phytase activity may result in further economic losses.



Vier, C., Menegat, M., Gourley, K. M., Dritz, S. S., Tokach, M., Bergstrom, J., Goodband, R., DeRouchey, J., and Woodworth, J. 2018. 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. Kansas Agricultural Experiment Station Research Reports: Vol. 4: Iss. 9. https://doi.org/10.4148/2378-5977.7662.

HiPhos™ is a trademark of DSM Nutritional Products. DSM10-0628



#### **BRIGHT SCIENCE. BRIGHTER LIVING.™**