Fact sheets – comparing phytase sources for pigs and effects of superdosing phytase on growth performance of nursery and finishing pigs

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This practice tip includes fact sheets on phytase sources for pigs and effects of superdosing phytase on growth performance of nursery and finishing pigs.

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None reported.

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FACT Sheet: Comparing different phytase sources for pigs

Phytase is an enzyme that hydrolyzes phytate (or phytic acid) and consequently increases phosphorus (P) availability in feedstuffs.[1] Recently, there has been an increase in the number of phytase sources available in the market. Phytase efficiency can be influenced by factors related to the phytase itself, the animal, or the diet substrate.[2]

How to measure phytase activity

Phytase activity is expressed as the number of phytase units (FTU or FYT) per unit of feed. The standard Association of Official Agricultural Chemists (AOAC) method defines 1 phytase unit as the quantity of phytase enzyme required to liberate 1 µmol of inorganic P per minute, at pH 5.5, from an excess of 15 µmol per L of sodium phytate at 37°C.[3,4] However, 1 FTU from one source does not necessarily have the same P release as 1 FTU from another source.[1] This is because different enzymes have different optimum pH ranges, in which differentiation and in vivo estimations are not supported by the standard AOAC method.[3,4]

Analytical methods. Analytical methods to quantify phytase activity differ across laboratories. For instance, the reaction time between different methods can range from 15 to 65 minutes.[3] This is related to the fact that different phytases have different biochemical natures,[5] thus laboratories have modified the initial standard AOAC analysis method. Additionally, different analytical methods may also use different buffer solutions (eg, sodium acetate versus sodium citrate), extraction time, color reagent, and absorbance. [3]

Phytase sources and their characteristics

Table 1 shows examples of currently commercially available phytase sources and their characteristics.

Phytase sources may differ in several aspects, such as storage time or temperature, product form, coating, and activity after feed processing.

- **Storage time**. Different phytase sources will have different storage stability. In a published study,[5] one commercially available pure phytase product retained more activity over time than did two other sources. At room temperature (23°C) or less, pure products retained 91%, 85%, 78%, and 71% of their initial activity by 30, 60, 90, and 120 days of storage, respectively. Increased temperature significantly increased the rate of degradation.
- **Storage temperature**. Storage at 37°C significantly reduced phytase activity, compared to storage at 23°C.[5] Heat-stable products generally retain activity longer during storage under higher temperatures.[5]
- **Product form**. The rate of phytase degradation is more rapid in premixes containing vitamin and trace minerals than in premixes containing only vitamins,[5] whereas pure product provides the greatest recovery rate among these three product forms.
- **Coating**. Coated products had a recovery rate approximately 4%, 20%, and 39% greater than uncoated products at 30, 60, and 90 days of storage, respectively.[5] Thus, coating mitigated some of the negative effects of long storage times and high temperatures on product stability in premixes.[5]

Fast facts

Phytase sources differ in the amount of phosphorus (P) released per phytase unit. Similarly, laboratories may analyze phytase activity differently. Thus, caution must be taken when comparing phytase sources and inclusion rates.

One approach to compare different phytase sources and determine replacement rates between sources is to compare their efficacy at a particular P release value (eg, 0.10% available P release).

When phytase is included in premixes, using a coated or heat-stable product and using within 60 days of the premix manufacture date is preferred.

- **Feed processing**. Most manufacturers have heat-stable and non-heat-stable products. Pelleting feed with phytase can significantly reduce activity in non-heat-stable phytase sources, whereas heat-stable sources can withstand higher temperatures.[8-14] For instance, one study[8] observed the recovery rate of a non-heat-stable source was 11% to 27% less than that of a heat-stable source when both were subjected to the pelleting process. Post pellet application of liquid phytase is one method to retain phytase activity after thermal processing. De Jong[15] provides more detailed information on heat stability of different phytase sources.

Replacement rates for various phytase sources

Due to their different characteristics, phytase sources have different stability and P release values.[3,5] One approach for comparing different phytase sources is to compare the phytase activity needed to reach a particular available P (AvP) release value (eg, 0.10% AvP release). This allows for products to be compared on the same level of activity to determine replacement rates for each phytase source. Table 2 illustrates the number of FTUs or FYTs needed to achieve specific AvP releases from some commercially available phytase products. The effect of phytase on components of the diet beyond P is a current area of research, and at this point results are not consistent.[16] The effects of superdosing phytase on pig growth performance are summarized in a separate fact sheet.

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References

1. Jacela JY, DeRouchey JM, Tokach MD, Goodband RD, Nelssen JL, Renter DG, Dritz SS. Feed additives for swine: Fact sheets–high dietary levels of copper and zinc for young pigs, and phytase. *J Swine Health Prod*. 2010;18:87–91.

2. Dersjant-Li Y, Awati A, Schulze H, Partridge G. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *J Sci Food Agr*. 2014;95:878–896.

Table 1: Examples of currently commercially available heat-stable phytase sources and their characteristics

Trade name	Type*	Protein origin	Expression	Maximal recommended temperature (°C)†
Natuphos E G[2,6]	6	*Hafnia* sp	*Aspergillus niger*	95.0
Axtra PHY[2]	6	*Buttiauxella* spp	*Trichoderma reesei*	95.0
OptiPhos PF[2]	6	*Escherichia coli*	*Pichia pastoris*	85.0
Quantum Blue G[2]	6	*Escherichia coli*	*Trichoderma reesei*	90.5
Ronozyme Hiphos GT[2,7]	6	*Citrobacter braakii*	*Aspergillys oryzae*	95.0

* Initial carbon site of cleavage. Natuphos E G (BASF, Florham Park, New Jersey); Axtra PHY (DuPont, Wilmington, Delaware); OptiPhos PF (Huvepharma, Peachtree City, Georgia); Quantum Blue G (AB Vista, Marlborough, UK); Ronozyme Hiphos GT (DSM, Parsippany, New Jersey).

† Caution must be taken to review maximal recommended feed-processing temperatures since the products listed are more heat-stable forms intended for use with thermal processing. Note these products are all available in non-heat-stable forms.

Table 2: Examples of available P (AvP) and STTD P release and for commercially available phytase sources*

AvP release (%)	STTD release (%)†	Phytase activity (FTU or FYT/kg)				
		Axtra PHY	Natuphos E	OptiPhos	Quantum Blue	Ronozyme Hiphos
0.100	0.088	270	250	200	250	400
0.120	0.106	360	325	250	315	600
0.140	0.124	500	400	500	430	1000
0.160	0.141	750	475	565	585	1500

* Values provided here are derived or estimated from supplier's recommendation: Axtra PHY (DuPont, Wilmington, Delaware); Natuphos E (BASF, Florham Park, New Jersey); OptiPhos (Huvepharma, Peachtree City, Georgia); Quantum Blue (AB Vista, Marlborough, UK); Ronozyme Hiphos (DSM, Parsippany, New Jersey). Phytase activity is reported on the basis of company-specific activity. Readers are encouraged to consult with the supplier to ensure proper analytical methods are used.

† STTD P calculated assuming a conversion in P release due to phytase from AvP to STTD P is 88.3%, using monocalcium phosphate as reference.

P = phosphorus; 1 FTU or 1 FYT = 1 phytase unit; STTD P = standardized total tract digestible phosphorus.

3. Kerr BJ, Weber TE, Miller PS, Southern LL. Effect of phytase on apparent total tract digestibility of phosphorus in corn-soybean meal diets fed to finishing pigs. *J Anim Sci*. 2010;88:238–247.

4. AOAC. Method 2000.12: Phytase activity in feed: colorimetric enzymatic method. In: *Official Methods of Analysis of AOAC International.* 17th ed. Arlington, Virginia: Association of Official Analytical Chemists; 2001:629–630.

5. Sulabo RC, Jones CK, Tokach MD, Goodband RD, Dritz SS, Campbell DR, Ratliff BW, DeRouchey JM, Nelssen JL. Factors affecting storage stability of various commercial phytase sources. *J Anim Sci*. 2011;89:4262–4271.

6.BASF. 2015. Revealing the benefits of Natuphos E Available at `https://www.basf.com/documents/au/en/products-industries/BASF%20Book_A4_Natuphos%20E%20Booklet.pdf`. Accessed 13 January 2016.

7. European Food Safety Authority. 2012. Scientific Opinion on the safety and efficacy of Ronozyme HiPhos GT (6-phytase) as feed additive for poultry and pigs. Available at: `http://www.efsa.europa.eu/en/search/doc/2730.pdf`. Accessed 04 January 2016.

8. Slominski BA, Davie T, Nyachoti MC, Jones O. Heat stability of endogenous and microbial phytase during feed pelleting. *Livest Sci*. 2007;109:244–246.

9. Vohra A, Satyanarayana T. Purification and characterization of a thermostable and acid-stable phytase from *Pichia anomala*. *World J Microb Biot*. 2002;18:687–691.

10. Igbasan FA, Männer K, Miksch G, Borriss R, Farouk A, Simon O. Comparative studies on the in vitro properties of phytases from various microbial origins. *Arch Anim Nutr*. 2000;53:353–373.

11. Jongbloed AW, Kemme PA. Effect of pelleting mixed feeds on phytase activity and the apparent absorbability of phosphorus and calcium in pigs. *Anim Feed Sci Tech*. 1990;28:233–242.

12. Kirkpinar F, Basmacioglu H. Effects of pelleting temperature of phytase supplemented broiler feed on tibia mineralization, calcium and phosphorus content of serum and performance. *Czech J Anim Sci*. 2006;51:78–84.

13. Wyss M, Pasamontes L, Rémy R, Kohler J, Kusznir E, Gadient M, Müller F, van Loon APGM. Comparison of the thermostability properties of three acid phosphatases from molds: *Aspergillus fumigatus* phytase, *A. niger* phytase, and *A. niger* pH 2.5 acid phosphatase. *Appl Environ Microb*. 1998;64:4446–4451.

14. Timmons JR, Angel R, Harter-Dennis JM, Saylor WW, Ward NE. Evaluation of heat-stable phytases in pelleted diets fed to broilers from day zero to thirty-five during the summer months. *J Appl Poultry Res*. 2008;17:482–489.

15. De Jong J. Feed processing challenges facing the swine industry [PhD dissertation]. Kansas State University, Manhattan, Kansas. 2015:125.

16. NRC. Nonnutritive feed additives. In: *Nutrient Requirements of Swine*. 11th ed. Washington, DC: National Academy Press; 2012:165–176.

FACT Sheet: Effects of superdosing phytase on growth performance of nursery and finishing pigs

Phytase is a highly effective enzyme used to release phosphorus (P) from phytic acid. Recent reports have suggested that additional mechanisms can lead to enhanced growth response beyond the P release when high doses of phytase are fed. This has been termed "superdosing."

Fast facts

The current body of literature suggests that superdosing phytase has the potential for a greater effect on nursery-pig performance, with less evidence of its effect on finishing-pig performance, and these effects appear to be greater in average daily gain than in feed-to-gain ratio.

The relative effect of superdosing phytase appears to be greater if the levels of phosphorus, amino acids, or other nutrients are marginal in the diet.

How does superdosing phytase affect growth performance of pigs?

Nursery pigs. Increasing phytase concentrations up to 2500 phytase units (FTU) per kg of *Escherichia coli*-derived phytase[1-3] in P-adequate diets has resulted in improved growth performance. Another commercial nursery study[4] evaluated the impact of up to 3000 FTU per kg Ronozyme HiPhos (DSM, Parsippany, New Jersey) in a low-lysine diet, compared to an adequate-lysine diet with 250 FTU per kg. Average daily gain and feed efficiency were restored to levels similar to those of the adequate-lysine diet when pigs were fed low-lysine diets with 1000 FTU phytase per kg. However, in a similar study[4] conducted in university settings, a difference in growth performance was not observed. Two studies[2,5] feeding nursery pigs phytase concentrations as high as 20,000 FTU per kg resulted in higher growth rate and better feed efficiency than those of the positive-control treatment (Table 1). In these two studies,[2,5] there was a greater improvement in average daily gain than in feed:gain.

Finishing pigs. A study feeding up to 2500 FTU per kg Quantum Blue (AB Vista, Marlborough, UK) did not impact energy, crude protein, or dry matter digestibility of growing pigs.[8] Another study with growing pigs fed up to 2000 FTU per kg Quantum Blue observed linear improvements in average daily gain (ADG) and feed-to-gain ratio (F:G).[9] However, a study in a commercial finisher evaluating another phytase source observed an improvement in F:G only up to 500 FTU per kg OptiPhos (Huvepharma, Peachtree City, Geogia).[10] Additionally, a finishing-pig study in a university setting did not observe an impact of 0 versus 2000 FTU per kg from three different sources of phytase on growth performance in diets with adequate P.[11]

Variability in outcomes between studies

It is important to note that the relative effect of superdosing phytase will be greater if the concentrations of digestible P, amino acids, and other nutrients are marginal in the diet. The effect will also depend on the concentration of phytase that is already in the diet. One caution is that most superdosing studies have been performed or sponsored by the phytase manufacturers. Little peer-reviewed published data has been generated by independent third-party entities to evaluate the impact of superdosing different phytase sources in commercial diets.

Potential mechanisms of action

The mechanism of superdosing phytase remains unknown,[12] but it is most likely to be a combination of the following.

Releasing an increased amount of P. In theory, releasing P above the requirement would not bring any benefit; however, if the requirement is underestimated, marginal releases of P improve growth performance.

Improving utilization of energy, amino acids, and trace minerals. Phytate may be an anti-nutritional factor for nutrients other than P.[13,14] There is some evidence[15] that superdosing could increase utilization of energy and amino acids and digestibility of minerals. A review[12] speculated that these effects are likely to be a result of changes in threonine, cysteine, glycine, serine, proline, calcium (Ca), sodium, zinc, and iron digestibility.

Improving nutrient intake. It is suggested that superdosing improves digestible nutrient intake by stimulating intake, because phytate might be acting as an appetite suppressant. However, the literature is not clear on whether superdosing phytase increases feed intake.[6,9]

Restoration of proportional Ca:P release. Superdosing phytase may restore the digestible Ca:P ratio. It is suggested that P and Ca are not necessarily released by phytase at a 1:1 ratio.[12] Thus, this could explain the responses to high concentrations of phytase, because P would continue to be released, whereas Ca would approach maximum release.

Generating *myo*-inositol. *Myo*-inositol has a vitamin-like effect. Its deficiency is difficult to demonstrate in pigs because of endogenous synthesis, variable turnover rates, and interaction with other vitamins or nutrients.[16] As phytate is cleaved with increased levels of phytase, *myo*-inositol is released;[8] however, the literature is not clear regarding a dietary requirement for *myo*-inositol when pigs are fed typical diets.[16] *Myo*-inositol is a component of phosphoinositides and is involved in processes such as amylase secretion, insulin release, and liver glycogenolysis, among others.[16]

Interaction between phytase and P release. There is some evidence that 1500 ppm of zinc[17] (1500 g per tonne of feed) or 2000 g per ton of citric acid[18] reduces the P-releasing efficacy of phytase in young pigs or chickens. In a study in sheep, 3000 ppm of formaldehyde (3000 mg per L) applied to soybean meal and then included as 10% of the diet was reported to suppress phytate degradation.[19] Therefore, superdosing may restore available P release from inactivation of phytase when release efficacy has been compromised.

In conclusion, the current body of literature has stronger evidence supporting improvements in growth performance in nursery pigs superdosed with phytase, with less evidence for effects in finishing

Table 1: Impact of phytase activity (FTU/kg) on ADG and G:F of nursery pigs as percentages of activity in positive controls*

FTU/kg	Kies et al[5]		Zeng et al[2]	
	ADG (%)	G:F (%)	ADG (%)	G:F (%)
0	79	94	85	95
100	83	96	ND	ND
250	93	97	ND	ND
500	98	98	99	98
750	100	98	ND	ND
1000	ND	ND	100	101
1500	107	99	ND	ND
15,000	110	103	ND	ND
20,000	ND	ND	109	104

* Adapted with permission from Kies et al[5] and from Zeng et al.[2] For Kies et al,[5] the positive-control diet was formulated to meet the pigs' requirement, based on the Dutch Centraal Veevoeder Bureau (CVB, 2000).[6] For Zeng et al,[2] the positive-control diet exceeded National Research Council requirements[7] for calcium and phosphorus but was 11% below the requirement for lysine.

FTU = phytase activity/kg; ADG = average daily gain; G:F = gain-to-feed ratio; ND = not done.

pigs. However, the exact mechanism by which superdosing phytase impacts performance remains unknown. The authors recommend consulting with a nutritionist to review approaches to Ca and P issues.

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References

*1. Walk CL, Srinongkote S, Wilcock P. Evaluation of a superdose of a novel *Escherichia coli* phytase and zinc in piglets [abstract]. *J Anim Sci.* 2012;90:76.

2. Zeng ZK, Wang D, Piao XS, Li PF, Zhang HY, Shi CX, Yu SK. Effects of adding super dose phytase to the phosphorus-deficient diets of young pigs on growth performance, bone quality, minerals and amino acids digestibilities. *Asian Austral J Anim.* 2014;27:237–246.

*3. Koehler DD, Corrigan B, Elsbernd AJ, Gould SA, Holloway CL, Patience JF. Super-dosed phytase improves rate and efficiency of gain in nursery pigs [abstract]. *J Anim Sci.* 2015;93:56.

*4. Langbein KB, Goodband RD, Tokach MD, Dritz SS, DeRouchey JM, Bergstrom JR. Effects of high levels of phytase (Ronozyme HiPhos) in low-lysine diets on the growth performance of nursery pigs. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. 2013;1092:121–127.

5. Kies AK, Kemme PA, Šebek LBJ, Van Diepen JTM, Jongbloed AW. Effect of graded doses and a high dose of microbial phytase on the digestibility of various minerals in weaner pigs. *J Anim Sci.* 2006;84:1169–1175.

6. CVB. Gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. Lelystad, The Netherlands: Centraal Veevoederbureau. 2000.

7. National Research Council. Feed ingredient composition. In: *Nutrient Requirements of Swine*. 11th rev ed. Washington, DC: National Academy Press; 2012:239–242.

*8. Holloway CL, Boyd RD, Patience JF. Improving nutrient utilization through the use of superdosing of phytase in growing pig diets [abstract]. *J Anim Sci*. 2015;93:56.

*9. Wilcock P, Bradley CL, Chewning JJ, Walk CL. The effect of superdosing phytase on inositol and phytate concentration in the gastrointestinal tract and its effect on pig performance [abstract]. *J Anim Sci*. 2014;92:383.

*10. Flohr JR, Goodband RD, Tokach MD, Langbein KB, Dritz SS, DeRouchey JM, Woodworth JC. Influence of a superdose of phytase on finishing pig performance and carcass characteristics [abstract]. *J Anim Sci*. 2014;92:149.

*11. Langbein KB, Woodworth JC, Goodband RD, Tokach MD, Nelssen JL, Dritz SS, DeRouchey JM. Effects of superdosing phytase in diets with adequate phosphorus on finishing pig growth performance and carcass characteristics. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. 2013;1092:128–131.

12. Adeola O, Cowieson AJ. Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J Anim Sci.* 2011;89:3189–3218.

13. Shirley RB, Edwards HM. Graded levels of phytase past industry standards improves broiler performance. *Poultry Sci.* 2003;82:671–680.

14. Walk CL, Santos TT, Bedford MR. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broiler. *Poultry Sci.* 2014;93:1172–1177.

*15. Johnston SL, Southern LL. Effect of phytase addition on amino acid and dry matter digestibilities and growth in pigs. In: Lindberg, JE, Ogle B, eds. *Digestive Physiology of Pigs: Proc 8th Symp*, Uppsala, Sweden: CABI Publishing; 2000:326:328.

16. McDowell LR. Vitamin-like substances. In: McDowell LR, ed. *Vitamins in Animal and Human Nutrition*. 2nd ed. Ames, Iowa: Iowa State University Press; 2000:659–674.

17. Augspurger NR, Spencer JD, Webel DM, Baker DH. Pharmacological zinc levels reduce the phosphorus-releasing efficacy of phytase in young pigs and chickens. *J Anim Sci*. 2004;82:1732–1739.

18. Brenes A, Viveros A, Arija I, Centeno C, Pizarro M, Bravo C. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. *Anim Feed Sci Tech*. 2003;110:201–219.

19. Park WY, Matsui T, Konishi C, Kim SW, Yano F, Yano H. Formaldehyde treatment suppresses ruminal degradation of phytate in soyabean meal and rapeseed meal. *Brit J Nutr.* 1999;81:467–471.

* Non-refereed references.