Practice tip

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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Phytase is an enzyme that hydrolyzes phytate (or phytic acid) and consequently increases phosphorus (P) availability in feedstuffs. 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.

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 Analytical 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. However, 1 FTU from one source does not necessarily have the same P release as 1 FTU from another source. 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.

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. This is related to the fact that different phytases have different biochemical natures, thus laboratories have modified the initial standard AOAC analysis method. Additionally, different analytical methods may also use different buffer solutions (e.g., sodium acetate versus sodium citrate), extraction time, color reagent, and absorbance.

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

- **Storage time.** Different phytase sources will have different storage stability. In a published study, 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. Heat-stable products generally retain activity longer during storage under higher temperatures.
- **Product form.** The rate of phytase degradation is more rapid in premixes containing vitamin and trace minerals than in premixes containing only vitamins, 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. Thus, coating mitigated some of the negative effects of long storage times and high temperatures on product stability in premixes.

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 (e.g., 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. For instance, one study 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 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. One approach for comparing different phytase sources is to compare the phytase activity needed to reach a particular available P (AvP) release value (e.g., 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. The effects of superdosing phytase on pig growth performance are summarized in a separate fact sheet.

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References
Examples of currently commercially available heat-stable phytase sources and their characteristics

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Type*</th>
<th>Protein origin</th>
<th>Expression</th>
<th>Maximal recommended temperature (°C)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natuphos E G</td>
<td>6</td>
<td>Hafnia sp</td>
<td>Aspergillus niger</td>
<td>95.0</td>
</tr>
<tr>
<td>Axtra PHY²</td>
<td>6</td>
<td>Butiauxella sp</td>
<td>Trichoderma reesei</td>
<td>95.0</td>
</tr>
<tr>
<td>OptiPhos PF²</td>
<td>6</td>
<td>Escherichia coli</td>
<td>Pichia pastoris</td>
<td>85.0</td>
</tr>
<tr>
<td>Quantum Blue G²</td>
<td>6</td>
<td>Escherichia coli</td>
<td>Trichoderma reesei</td>
<td>90.5</td>
</tr>
<tr>
<td>Ronozyme Hiphos GT²,⁷</td>
<td>6</td>
<td>Citrobacter braakii</td>
<td>Aspergillus oryzae</td>
<td>95.0</td>
</tr>
</tbody>
</table>

* 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.

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

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

* Values provided here are derived or estimated from supplier’s recommendation: Aextra PHY (DuPont, Wilmington, Delaware); Natuphos E (BASF, Florham Park, New Jersey); OptiPhos (Huvepharma, Peachtree City, Georgia); Quantum Blue G (AB Vista, Marlborough, UK); Ronozyme Hiphos GT (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.

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

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\(^5\),\(^6\) 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, Georgia).\(^10\) 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 digestive 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 digestive 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;\(^16\) 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*

<table>
<thead>
<tr>
<th>FTU/kg</th>
<th>Kies et al5</th>
<th>Zeng et al2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADG (%)</td>
<td>G:F (%)</td>
</tr>
<tr>
<td>0</td>
<td>79</td>
<td>94</td>
</tr>
<tr>
<td>100</td>
<td>83</td>
<td>96</td>
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<tr>
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<td>500</td>
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<td>750</td>
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<td>1000</td>
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<td>1500</td>
<td>107</td>
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<tr>
<td>15,000</td>
<td>110</td>
<td>103</td>
</tr>
<tr>
<td>20,000</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Adapted with permission from Kies et al5 and from Zeng et al2 For Kies et al, the positive-control diet was formulated to meet the pigs’ requirement, based on the Dutch Centraal Veevoeder Bureau (CVB, 2000). For Zeng et al2 the positive-control diet exceeded National Research Council requirements 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.

Acknowledgement

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References

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 Univ. Contribution no. 16-053-J from the Kansas Agricultural Experiment Station, Manhattan, KS 66506-0210.*

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