

Phytase in Swine Diets

Phytate is the primary storage of phosphorus in feedstuffs of plant origin. However, phytate-bound phosphorus is mostly unavailable to pigs, with digestibility in the range of 20 to 30%. Phytase is an enzyme that acts on phytate to release phosphorus in a form available to pigs. Phytate also forms complexes with protein and minerals, preventing nutrient absorption. The strategic use of phytase in swine diets to improve phosphorus digestibility and reduce the antinutritional effects of phytate is discussed in this fact sheet.

Phytate

Phytic acid is the primary storage of phosphorus in plants, typically in the form of phytate and contributing to 60 to 80% of phosphorus in feedstuffs of plant origin (Eeckhout and De Paepe, 1994). Phytate consists of an inositol bound to six phosphates and contains approximately 28% phosphorus. Corn-soybean meal-based swine diets typically contain 1% phytate or 0.28% phytate-bound phosphorus, but the level varies with the ingredients in the diet.

Phytate is considered an antinutritional factor for swine because it reduces digestibility of phosphorus, energy, and other nutrients in pigs. The antinutritional effect of phytate on phosphorus availability is a consequence of pigs not being able to effectively release phosphorus from phytate. Phytate becomes negatively charged in the digestive tract of pigs, which confers phytate the capacity to form stable complexes with protein and minerals like calcium, zinc, and iron in the digestive tract, preventing nutrient absorption (Woyengo and Nyachoti, 2013). Therefore, the degradation of phytate in the upper part of the digestive tract is essential to improve phosphorus availability and eliminate the antinutritional effects of phytate.

Phytase

Phytase is an enzyme that catalyzes the release of phosphorus from phytate. The sources of phytase with respect of swine nutrition are: endogenous phytase produced in the small intestine, microbial phytase produced in the large intestine, intrinsic plant phytase derived from feedstuffs, and exogenous microbial phytase added to the diet (Humer et al., 2015). The endogenous phytase activity is negligible in swine and the intrinsic phytase activity in feedstuffs is variable, with corn and soybean typically containing minor phytase activity (Eeckhout and De Paepe, 1994). Consequently, only 20 to 30% of phosphorus bound to phytate is released by the action of these phytase sources (Adeola and Cowieson, 2011).

The addition of exogenous microbial phytase to swine diets is a common practice to efficiently and economically enhance phosphorus release from phytate (Selle and Ravindran, 2008). The effects of exogenous microbial phytase follow a curve of diminishing returns, with most of the beneficial effects generated within the dose of phytase necessary to destroy 30 to 40% of the dietary phytate and proportionately lower effects thereafter (Cowieson et al., 2017).

Exogenous microbial phytases are typically derived from bacteria or fungi, such as *Escherichia coli*, *Aspergillus niger*, *Peniophora lycii*, and *Buttiauxella* spp. (Selle and Ravindran, 2008). These microbial phytases are divided into 3- and 6-phytases according to site of action on phytate, and into first or new generation depending on generation of development. All commercially available microbial phytases for swine are classified as acidic phytases, with optimal activity at pH of 2.5 to 5.5 (Humer et al., 2015). **Table 1** presents the characteristics of some of the current commercial phytase sources for swine.

Phytase activity

Phytase activity is expressed as phytase units (FTUs or FYTs). One FTU is officially the amount of phytase required to liberate 1 mmol of inorganic phosphate per minute from 0.0051 mol/L sodium phytate at pH 5.5 and temperature of 37°C (AOAC, 2000).

Table 1. Characteristics of some of the currently commercially available phytase sources for swine

| Trade name | Supplier | Type ¹ | Protein origin | Expression | Optimal pH ² | Maximal temperature ³ |
|---------------------|-------------|-------------------|----------------------------|---------------------------|-------------------------|----------------------------------|
| Axtra® PHY GOLD | IFF/Danisco | 6-phytase | <i>Buttiauxella</i> spp. | <i>Trichoderma reesei</i> | 3.0 | 203°F |
| GralNzyme | Agrivida | 6-phytase | <i>Escherichia coli</i> | Corn | 3.5 - 5.0 | 185°F |
| Natuphos® E G | BASF | 6-phytase | Hafnia sp. | <i>Aspergillus niger</i> | 2.0 - 5.5 | 203°F |
| OptiPhos® CT | Huvepharma | 6-phytase | <i>Escherichia coli</i> | <i>Pichia pastoris</i> | 3.5 - 5.0 | 185°F |
| Quantum® Blue G | AB Vista | 6-phytase | <i>Escherichia coli</i> | <i>Trichoderma reesei</i> | 3.5 - 5.0 | 194°F |
| Ronozyme® Hiphos GT | DSM | 6-phytase | <i>Citrobacter braakii</i> | <i>Aspergillus oryzae</i> | 3.0 - 4.5 | 203°F |
| Smizyme TS G5 | Origination | 6-phytase | <i>Escherichia coli</i> | <i>Pichia pastoris</i> | 3.5 - 5.0 | 203°F |

¹Initial site of action of phytase on phytate.

²Based on *in vitro* assays. Adapted from Dersjant-Li et al. (2014).

³Maximal recommended temperature for heat-stable forms of the products only.

Phytase efficacy

The efficacy of phytase varies with phytase characteristics, which are determined based on phytase origin (bacterial or fungal phytase), phytase generation (first or new generation), and site of action of phytase on phytate (3- or 6-phytase, referring to the initial carbon site of hydrolysis on phytate). The most important characteristics influencing phytase efficacy include activity in the upper digestive tract, affinity to phytate, and resistance to degradation.

Characteristics influencing phytase efficacy

- ◆ **Activity in the upper digestive tract:** The degradation of phytate in the upper part of the digestive tract (stomach and upper small intestine) is essential to improve phosphorus availability and eliminate the antinutritional effects of phytate (Dersjant-Li et al., 2014). The optimal pH range of phytase provides an indication of phytase activity in the upper part of the digestive tract. The pH in the pigs' empty stomach is normally 2.0 to 2.5 and gradually increases to 3.5 to 4.0 with feed, whereas the pH in the pig's upper small intestine is around 4.0 to 6.0 (Pagano et al., 2007). The optimal pH for phytase activity typically varies over a range of 2.5 to 5.5.
- ◆ **Affinity to phytate:** The most effective phytases have great affinity to phytate and are able to target phytate at low concentration and from many feedstuff sources (Dersjant-Li et al., 2014).

- ◆ **Resistance to degradation:** As phytase is a protein that can be degraded by enzymes in the digestive tract, the most effective phytases are resistant to degradation by enzymes in the digestive tract (Dersjant-Li et al., 2014).

Dietary factors influencing phytase efficacy

Beyond the phytase characteristics, several factors influence the efficacy of phytase, including the amount of phytate in the diet, the amount of phytase added to the diet, and diet formulation. Although it is not clear to which extent diet formulation affects phytase efficacy, it is important to understand the dietary factors that influence the activity of phytase.

- ◆ **Feedstuffs:** There is considerable variation in the susceptibility of phytate to phytase depending on feedstuff. Also, the amount of intrinsic phytase varies with feedstuff, with wheat containing more intrinsic phytase than corn, for example (Selle and Ravindran, 2008).
- ◆ **Ratio of phytase to phytate:** The ideal ratio of phytase to phytate allows for maximum release of phosphorus from phytate. However, in most of the cases, either phytase or phytate levels are limiting. When phytase is the limiting factor, the release of phosphorus improves with addition of more phytase. When phytate is the limiting factor, the release of phosphorus occurs until all phytate is depleted by phytase but does not improve with further addition of phytase (Cowieson et al., 2016).

- ◆ **Inorganic sources of calcium and phosphorus:** The use of high concentrations of inorganic sources of calcium and phosphorus interfere in phytase efficacy. Sources such as limestone and monocalcium phosphate have the potential to increase gut pH, which affects phytase activity and reduces phytate solubility (Dersjant-Li et al., 2014).
- ◆ **Calcium level and Ca:P ratio:** Diets formulated with high calcium levels and wide calcium:phosphorus ratios will lower phytase efficacy. Calcium forms a complex with phytate which reduces phytate susceptibility to phytase activity (Selle et al., 2009).
- ◆ **Pharmacological levels of zinc:** Diets formulated with pharmacological levels of zinc have lower phytase efficacy. Similar to calcium, zinc forms a complex with phytate which reduces phytate susceptibility to phytase activity (Selle and Ravindran, 2008).

Phytase stability

The stability of phytase under storage and during feed processing determines the ultimate value of phytase as much as its efficacy. There are many factors influencing phytase stability, including thermostability, coating, storage form, storage temperature, storage duration, and feed processing (Table 2).

Thermostability and coating

Phytase is susceptible to denaturation by excessive temperature during storage and feed processing. Phytase thermostability can be achieved through coating application to provide protection to phytase or through genetic modification to make phytase intrinsically thermostable. Heat-stable phytase is able to withstand high temperatures under storage and application of heat during pelleting compared to non-heat-stable phytase (Slominski et al., 2007).

Moreover, coating also provides protection to phytase against environmental insults. Coated phytase is able to counteract some of the adverse effects of premix components, high storage temperature, and long storage duration, compared to uncoated phytase (Sulabo et al., 2011).

Storage form

Phytase can be stored in pure form or in a mixture with vitamins or vitamin and trace minerals. Phytase activity is lost to greater extent in premixes containing vitamins and trace minerals than in premixes containing only vitamins (Sulabo et al., 2011; De Jong et al., 2016). The interaction of phytase with premix components seems to affect phytase stability, with inorganic trace minerals appointed as the most likely components to interact with phytase (Shurson et al., 2011). Storage of phytase in pure form is the best means to optimize phytase stability and minimize loss of phytase activity during storage (Sulabo et al., 2011; De Jong et al., 2016).

Storage temperature

Phytase is exposed to varied temperatures and humidity during storage depending on location and season. Storage under conditions of high temperature and humidity, i.e. at 99°F and 75% humidity, considerably reduces phytase activity (Yang et al., 2007; Sulabo et al., 2011). Freeze storage at -4°F also reduces phytase activity (De Jong et al., 2016). In general, storage at room temperature (73°F) or 39 to 73°F at low humidity is ideal to optimize phytase stability and maximize phytase activity during storage (Sulabo et al., 2011; De Jong et al., 2016).

Storage duration

Phytase is stored for varying lengths of time depending on inclusion rate and feed mill volume. Phytase activity gradually decreases with an increase in storage duration, but both storage form and storage temperature influence the rate of degradation during storage (Sulabo et al., 2011; De Jong et al., 2016). In general, storage of phytase for less than 90 to 120 d in pure form or less than 60 d in a premix optimizes phytase stability (De Jong et al., 2016).

Feed processing

The most commonly adopted feed process in swine diets that affects phytase stability is pelleting. Pelleting conditions vary depending on equipment and diet, but normally consist of conditioning temperatures ranging from 149 to 203°F. Phytase activity gradually decreases with an increase in conditioning temperature above 149°F, even with use of heat-stable phytase (De Jong et al., 2017). Alternatively, post-pelleting application of liquid phytase onto pellets is one strategy to maintain phytase stability (Gonçalves et al., 2016).

Table 2. Recommendations to improve phytase stability

| Factor | Recommendation |
|---------------------|--|
| Thermostability | Use heat-stable phytase if pelleting feed or excessive temperature during storage |
| Coating | Use coated phytase if mixed in a premix or long storage duration (more than 60 to 90 d) |
| Storage form | Store in pure form |
| Storage temperature | Store at room temperature or 39 to 73°F at low humidity |
| Storage duration | Store for less than 90 to 120 d in pure form or less than 60 d in a premix |
| Feed processing | Use heat-stable phytase if pelleting feed or post-pelleting application of liquid phytase onto cool pellets. Test phytase activity using conventional processing conditions. |

Extra-phosphoric effects of phytase

The effects of phytase beyond phosphorus release are termed 'extra-phosphoric' effects of phytase. The primary effect of phytase is the improvement of phosphorus availability through the release of phosphorus from phytate. However, phytate also forms stable complexes with proteins and minerals like calcium, zinc, and iron in the digestive tract and prevents nutrient absorption (Woyengo and Nyachoti, 2013). Thus, the extra-phosphoric effects of phytase are related to the improvement of digestibility of energy, amino acids, and minerals through the dissociation of such complexes (Selle and Ravindran, 2008).

The extra-phosphoric effects of phytase provide economic advantages in diet formulation and enhance the value of dietary phytase. The matrix values for calcium release in a digestible calcium basis seems to be similar to the digestible phosphorus release. However, the assignment of matrix values for other minerals and amino acids should be adopted with caution (Cowieson et al., 2017), as the effects are more variable and have not been fully elucidated (Adeola and Cowieson, 2011).

Particularly in the case of amino acids, there is evidence to support the use of amino acids matrix values, but because the effects are not as obvious or consistent it is appropriate to use a more conservative approach (Cowieson et al., 2017).

The use of phytase above conventional levels (500 to 1,000 FTU/kg) seems to have the potential to improve growth performance beyond what is expected with adequate phosphorus levels (Zeng et al., 2014). The exact mode of action of high phytase levels remains unknown, but it is believed to be related to extra-phosphoric effects due to greater degradation of phytate (Adeola and Cowieson, 2011; Cowieson et al., 2011). The greater degradation of phytate removes most of the antinutritional effects of phytate, further improving digestibility of energy, amino acids, and minerals (Selle and Ravindran, 2008). Moreover, the complete degradation of phytate releases *myo*-inositol, a vitamin-like compound with many metabolic functions (Laird et al., 2018; Moran et al., 2018).

The use of high levels of phytase appears to have potential for a greater effect on nursery pig performance (Zeng et al., 2014; Gourley et al., 2018; Laird et al., 2018), with less evidence for effect on grow-finish pig performance (Holloway et al., 2016; Miller et al., 2016; She et al., 2018). Moreover, the effects of high phytase levels appear to be greater if the levels of

phosphorus, calcium, and other minerals are marginal in the diet (Zeng et al., 2014; Miller et al., 2016; Laird et al., 2018). Also, it has been suggested that the effects of high phytase levels follow a curve of diminishing returns, with most of the beneficial effects generated within the dose of phytase necessary to destroy 30 to 40% of the dietary phytate and proportionately lower effects thereafter (Cowieson et al., 2017).

Comparison of phytase sources

Several phytase sources are commercially available for use in swine diets. Because of differences in phytase characteristics and variation in recommended levels for similar phosphorus release among products, there is an interest to be able to effectively compare phytase sources (Jones et al., 2010). An approach for comparing different phytase sources is to compare the phytase activity needed to reach a particular available phosphorus or standardized total tract digestible phosphorus release value. This allows for products to be compared on the same level of activity to determine replacement rates for each phytase source (Gonçalves et al., 2016). **Table 3** presents the aP and STTD P release values for comparison of some of the current commercial phytase sources. To provide consistent information to swine producers, data has been summarized and a [calculator \(KSU Phytase Calculator\)](#) has been developed to provide recommendations for release of phosphorous in swine diets.

Data used in this tool has been provided by phytase suppliers and all release values are [based on bone mineralization measures](#) including bone ash, dual energy x-ray absorptiometry (DEXA) scans, and/or bone phosphorous analysis when available in the data.

It is recognized that phytase provides growth benefits beyond bone mineralization. However, the goal of this tool is to provide release estimates for phytase sources based on estimates of bone mineralization.

Moreover, analytical techniques used to determine phosphorus release values are variable among commercial phytase manufacturers (Jacela et al., 2010). Because of this, the amount of phosphorus released per unit of phytase differ between phytase products. The standard method is the AOAC assay (AOAC, 2000), but some phytase suppliers modify this method according to different phytase characteristics.

An important concept with this calculator is that 100% of the phytate P is not digestible even when adequate phytase is available. Thus, the expected release of phosphorous is dependent upon the amount of phytate P within the diet. The exact proportion of phytate P that can be release and become available to the pig is not fully information currently available, the calculator uses a default

understood. To incorporate this concept with the best

of 70% to provide an estimate of the potential amount of the dietary phytate P that can be released if adequate phytase is available.

The phytase level used within all calculations was based on assayed concentration of phytase source, and phytase units are reported using manufacturer assay.

Table 3. KSU Phytase Calculator – Phytase Inclusion Table

| Digestible phosphorus (STTD) release, % ² | Available phosphorus release, % | Phytase units/kg to provide specific level of phosphorous release ¹ | | | | | | |
|--|---------------------------------|--|--------------------|-----------------|--------------------------|-----------------------|---------------------|----------------------------|
| | | Phytase units reported using manufacturer assay | | | | | | |
| | | Extra PHY GOLD IFF/Danisco | Grainzyme Agrivida | Natuphos E BASF | Optiphos Plus Huvepharma | Quantum Blue AB Vista | Ronozyme HiPhos DSM | Smizyme TS G5 Origination |
| 0.06 | 0.07 | 130 | 500 | 190 | 180 | 140 | 200 | 160 |
| 0.07 | 0.08 | 160 | 620 | 230 | 220 | 170 | 260 | 210 |
| 0.08 | 0.09 | 190 | 750 | 270 | 270 | 210 | 320 | 270 |
| 0.09 | 0.10 | 220 | 900 | 320 | 330 | 250 | 400 | 350 |
| 0.10 | 0.11 | 260 | 1070 | 370 | 400 | 290 | 490 | 470 |
| 0.11 | 0.13 | 300 | 1260 | 430 | 480 | 350 | 620 | 630 |
| 0.12 | 0.14 | 340 | 1490 | 500 | 590 | 410 | 770 | 890 |
| 0.13 | 0.15 | 390 | 1770 | 580 | 710 | 480 | 990 | 1380 |
| 0.14 | 0.16 | 450 | 2100 | 660 | 880 | 580 | 1310 | > Max release ³ |
| 0.15 | 0.17 | 510 | 2500 | 770 | 1100 | 690 | 1820 | > Max release ³ |
| 0.16 | 0.18 | 590 | 3000 | 880 | 1410 | 830 | 2000 | > Max release ³ |
| Max phytase dose ⁴ | | 2000 | 4000 | 1000 | 1500 | 2000 | 2000 | 1500 |

¹ Data is summarized from manufacturer provided data reporting measures of bone mineralization.

² Phosphorus release values can only be obtained if adequate phytate P is available in the diet.

³ Bone mineralization data not available for this level of P release.

⁴ Max phytase dose where bone mineralization data is available

Phytase product inclusion

The amount of phytase product that needs to be included in swine diets to achieve the dietary phytase level goal can be calculated by the following:

Dietary phytase level goal, FTU/kg ÷ Phytase product concentration, FTU/g = Phytase product inclusion, g/kg

Phytase product inclusion, g/kg × 907.2 kg/ton = Phytase product inclusion, g/ton of complete diet

Phytase product inclusion, g/ton ÷ 453.6 g/lb = Phytase product inclusion, lb/ton of complete diet

For example, consider the goal is to achieve 200 FTU/kg of phytase in the diet and the phytase product contains 2500 FTU/g. Using the calculations described above, the amount of phytase product that needs to be included in the diet is 0.16 lb/ton, as follows:

200 FTU/kg ÷ 2500 FTU/g = 0.08 g/kg phytase product inclusion

0.08 g/kg × 907.2 = 72.6 g/ton phytase product inclusion

72.6 g/ton ÷ 453.6 g/lb = 0.16 lb/ton phytase product inclusion

Phytase calculator

A phytase calculator has been developed to determine the levels of dietary phytase for some of the commercially available phytase sources and the amount of phytase product to be included in swine diets ([KSU Phytase Calculator](#)).

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