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Phytase and Other Phosphorus Reducing Feed Ingredients

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Introduction

This fact sheet has been developed to support the implementation of the Natural Resources Conservation Service Feed Management 592 Practice Standard. The Feed Management 592 Practice Standard was adopted by NRCS in 2003 as another tool to assist with addressing resource concerns on livestock and poultry operations. Feed management can assist with reducing the import of nutrients to the farm and reduce the excretion of nutrients in manure.

The primary constituents of diets for poultry and swine are plant-based ingredients which come primarily from the seeds of plants. Most of the stored phosphorus (P) in plants is found in seeds mainly as a component of a molecule called phytin. Phytin-P is poorly available to poultry and swine, and this availability varies both within and among ingredients. The enzyme ***phytase*** releases phosphate groups from ***phytin*** potentially making this released P available to the animal, thereby reducing P excreted from poultry and swine by 15 to 30%. Phytase is the only recognized enzyme that can initiate the release of phosphate from phytin (IUB, 1979).

Most commercially available phytases are of fungal or of bacterial gene origin, but expressed for production purposes in yeasts. Because of the nature of where they were derived, the efficacy in the animal can be considerably different. When diets are formulated with phytases, a certain amount of inorganic P should be removed from the diet. If it is not, the soluble portion of the P in the diet will increase as a result of the additional inorganic P not needed by the animal. The amount of inorganic P removed from the diet formulation will depend, in how much P is being fed over and above the requirement of the animal (see Phosphorus Requirements Factsheets).

As the fungal phytases have been on the market for a longer period of time, more is known about how much inorganic P can be removed from the diet. Typical removal amounts of P for 500 units of phytase / kg diet can vary from 0.06% to 0.10% for broilers, turkeys, and swine. The laying hen usually uses a lower amount (~ 300 units of phytase / kg diet) with similar bio-efficacy. In swine, typically higher levels (500-1000 U/kg) are used in the nursery with 250 to 1000 used in grow/finish and sow diets. The newer yeast (E. coli-based) phytases can have greater efficacy in the animal with similar dietary inclusion.

Phytase – Specifics on Enzyme Function in the Non-Ruminant

Enzymes are proteins or protein-based substances that speed up or catalyze chemical reactions. For example, an enzyme in saliva (amylase) helps break down starch in the mouth. Enzymes are very unique, in that they are highly selective for substrate (substance or molecules they act upon) and for the end products they produce. An analogy of how an enzyme functions would be that of the key unlocking specific end-products (Figure 1). The enzyme and substrate are

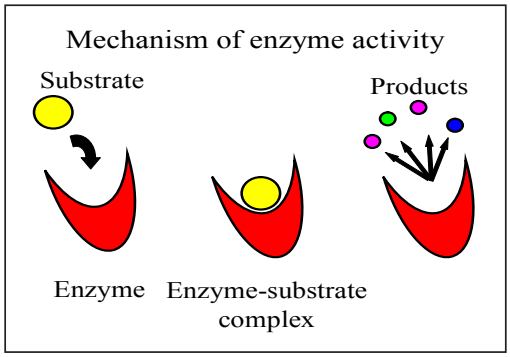

Figure 1. Diagram of how an enzyme speeds release of a product. Phytase (the enzyme) attaches to phytin (the substrate) to help in release of P and different inositol phosphates (other products).

configured uniquely as locks and the keys that open them.

Since enzymes are proteins, they are susceptible to possible denaturation or destruction by digestive enzymes or anything that can change their structure, such as temperatures during feed pelletization. Enzymes typically have ideal conditions (temperatures, pH, etc.) where they function more readily. As an example, plant phytases work better at 45 to 60° C (113 to 140° F) whereas microbial phytases work more readily at wider temperature ranges (35 to 63° C; 95 to 145° F) (Wodzinski and Ullah, 1996).

Additionally, for an enzyme to work effectively, it must be in proximity to the substrate and the substrate can not have the site of action blocked. In certain regions of the gastro-intestinal tract (small intestine), phytin can react readily with other compounds (such as Ca, Fe, Cu, Zn, and others) and precipitate out of solution such that the phytase enzyme can not act on this precipitated substrate. In other areas of the gastro-intestinal tract (stomach in pigs and proventriculus and gizzard in poultry), phytin is more soluble and can more readily be acted upon by the phytase enzyme (Figure 2).

Phytin

Phosphorus is predominately stored in mature seeds as a mineral complex known as phytin. The molecule in its uncomplexed-state is referred to as phytic acid (Figure 3). Phytin-P within a given feedstuff is variable, but typically averages 72 and 60 % of total seed P in corn and soybean meal (SBM), respectively, the two predominant feed ingredients in poultry and swine diets in the U.S. (Ravindran *et al*., 1995). Phytic acid is highly reactive and readily forms complexes with Ca, Fe, Mg, Cu, Zn, carbohydrates, and proteins.

These complexes are substan-tially less soluble in the small intestine and, therefore, less likely to interact with phytase (Figure 2; Angel *et al*., 2002). For this reason, phytin is often considered to be an anti-nutrient because of its ability to bind with other nutrients rendering those nutrients as well as the P contained in the phytin molecule partially or completely unavailable to the animal.

Phytin in feedstuffs is relatively heat stable. Pelleting does not appear to affect phytin content greatly. Skoglund et al. (1997) found that pelleting at 81° C reduced phytin content in a mixed rapeseed, barley and pea diet by 7%. O'Dell (1962) found,

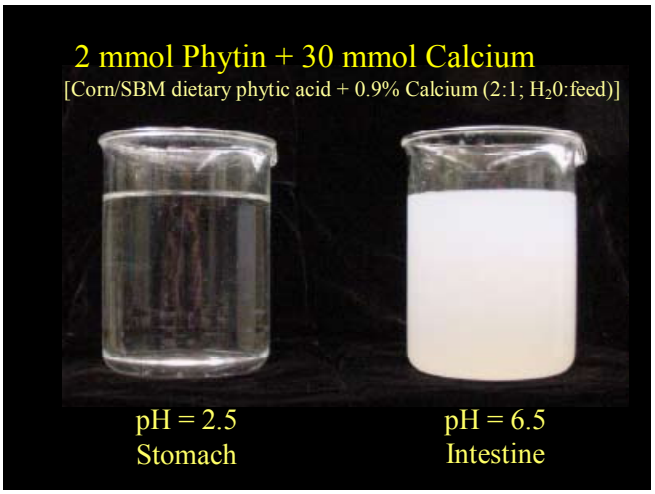

Figure 2. Demonstration of what occurs to phytin-Ca complex in the stomach (pH 2.5) and small intestine (pH 6.5). At the higher pH, phytase can not work as easily on the substrate phytin because the substrate is precipitated.

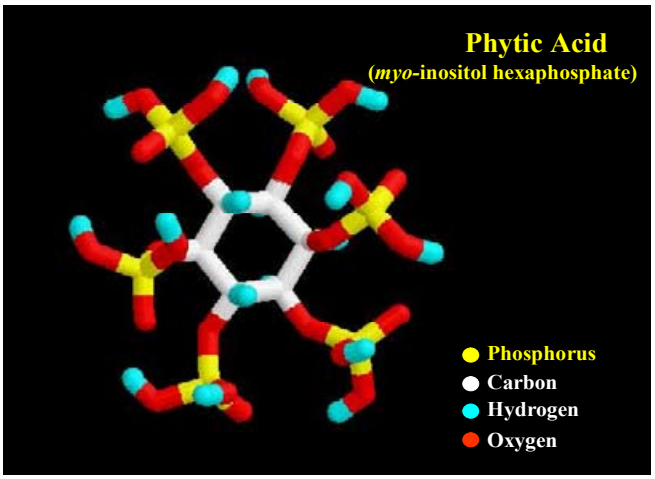

Figure 3. Phytic acid, the predominate storage form of P in mature seeds (figure courtesy of W. Schmidt – USDA/ARS).

however, that nearly 88% of phytin in soybeans could be degraded if autoclaved at 115° C (239° F) for four hours.

The location of phytin within seeds differs among different plant seeds. Ninety percent of the phytin in corn is found in the germ portion of the kernel, while in wheat and rice most of the phytin is in the aleurone layers of the kernel and the outer bran (O'Dell *et al*., 1976). In most oilseeds and grain legumes, the phytin is associated with protein and concentrated within sub-cellular inclusions called globoids that are distributed throughout the kernel; however, in soybean seeds, there appears to be no specific location for phytin (Ravindran *et al*., 1995). The location specificity within certain grains can be potentially exploited such

that by-products of the grain can be produced that contain minimal quantities of phytin P.

Phytase

The International Union of Biochemistry (1979) recognizes two general classes of phytases, 3-phytase and 6-phytase based on the location of the phosphate group, within the phytin molecule, that is hydrolyzed first. Microbial or fungal phytases typically hydrolyze the phosphate at the 3 position and plant phytase the phosphate at the 6 position of the phytin molecule. After releasing the first phosphate group, the five remaining phosphate groups can be sequentially released from phytin by phytase and non-specific acid phosphatases, which are present in large quantities in the digestive tract (Maenz and Classen, 1998). Phytin P, however, may or may not be completely hydrolyzed. The hydrolytic action of phytase on phytin-P has been known for some time (Dox and Golden, 1911), however, large-scale, commercial production of phytase has occurred only since the 1990s (Wodzinski and Ullah, 1996).

Enzyme Activity

One unit of phytase is defined as the amount of enzyme required to liberate 1 µmol of orthophosphate from phytin per minute at pH 5.5 and 37° C (Zyla et al., 1995). Phytase assays, however, may differ among suppliers. Similarly, as enzyme characteristics differ among phytases, therefore, a unit of activity under the above conditions does not necessarily translate into the same amount of P released within the animal. This last point is key, because it is not the commercially misused term "efficacy" that is important when considering commercial phytases, but the amount of P liberated by the phytase at the manufacturers recommended inclusion level for the specific dietary ingredients and nutrient levels being used. This ultimately would translate to a cost of the phytase per unit of P made available to the animal.

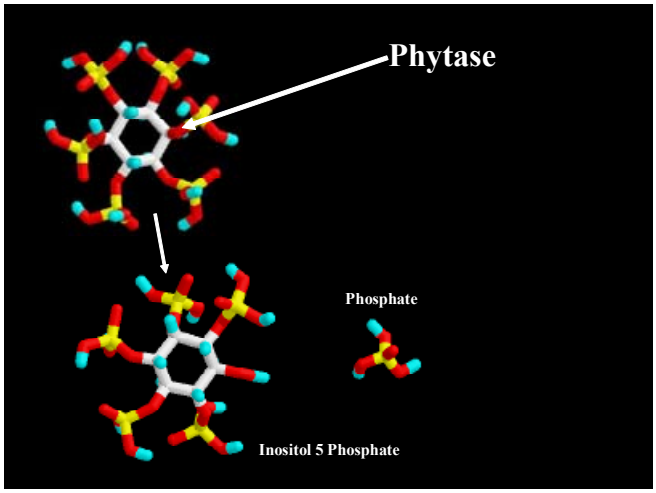

Figure 4. Diagram of release of phosphate from phytin by the enzyme, phytase. (figure courtesy of W. Schmidt – USDA/ARS).

Phytase in Plants

Some feedstuffs contain considerable phytase activity (wheat, wheat bran, rye, barley), whereas others have little or no phytase activity (corn, oats, sorghum, and oilseeds) (Eeckhout, and de Paepe, 1994). Phytase activities in grains, such as wheat, have a very high correlation with overall P retention in both pigs and broilers when diets are fed in mash form (i.e. diets that are not pelleted) (Barrier-Guillot *et al.*, 1996). Within wheat samples, the phytase activity can be highly variable (915 to 1581 U/kg; Eeckhout and de Paepe, 1994). Much of this variation can be explained through cultivar differences (Barrier-Guillot *et al.*, 1996) and possibly through grain storage time and conditions. Because of this high variability of phytase activity in feedstuffs it can not be counted on as a consistent source in most commercial poultry and swine operations.

Optimal temperature ranges of plant phytases are from 45 to 60° C (113 to 140° F) (Wodzinski and Ullah, 1996). Plant phytases, however, may be partially or totally inactivated by over-heating or high steam-pelleting temperatures (Ravindran *et al.*, 1995). Phillippy (1999) also demonstrated that wheat phytase lost substantial activity when incubated with pepsin, a proteolytic digestive enzyme. Temperature stability of plant phytases, therefore, is not good and therefore, is a primary drawback when diets are pelleted. Producers that feed mash diets (diets that are not pelleted) may find some benefit from plant phytases but must consider the high inherent variability.

Phytase from Bacteria, Fungi, and Yeasts

Inclusion of fungal phytase in diets for poultry and swine has resulted in considerable improvement in reducing P excretion by the animal. When at least 1000 U/kg of fungal phytase is included in corn/SBM-based diets of pigs, P retention increased from 52 to 64% (Kornegay, 1999). Similarly, P retention by broilers was improved from 50 to 60% by supplementing diets with a fungal phytase (Kornegay et al., 1996; Simons et al., 1990). Efficacy of phytase supplementation, however, is dependent on microbial source, form of the enzyme (coated, size of the particle, etc.),

temperature, and pH optima of the enzyme, diet mineral concentration (Ca, Fe, Mg, Cu, and Zn), ingredients used in the diet, diet manufacturing methodology, form of the diet (pelleted, mash, or liquid), location of addition of phytase (post-pelleting or mixer), type and level of vitamin D metabolites, disease status of the animal, and other factors (Ravindran et al., 1995).

Commercial phytases are typically produced using recombinant DNA technology. For example, a bacterial phytase gene has recently been inserted into yeast for commercial production. Recent gene insertion technology has greatly improved functional use of phytases by improving their thermostability, pH specificity, and resistance to break-down by other digestive enzymes in the animal.

Phytase Efficacy – How Much Inorganic Phosphorus Should be Removed?

Deciding what P concentration to formulate to is difficult due to a lack of clarity on what the P requirements are and the amount of phytin P liberated with supplemental phytase. In summarizing three experiments (11 to 21 d or 12 to 22 d of age), Angel *et al.* (2002) noted a range of values to obtain a 0.1% sparing effect of non-phytin P (nPP) from 781 to 1413 U phytase/kg diet with a fungal phytase source. In fact, when the dietary Ca was fixed at 0.7%, the additional nPP spared with 500 U phytase/kg diet averaged 0.065% (as calculated from additional toe ash obtained in comparison with graded concentrations of monocalcium phosphate). In turkeys, the additional nPP spared appears to be somewhat higher. In summarizing 4 experiments, the additional nPP spared when 500 to 600 U fungal phytase / kg diet averaged 0.091% when calculated from tibia ash and 0.088% when calculated from toe ash (Angel *et al.* 2001; Applegate *et al.*, 2003).

The difference in the amount of P liberated in research trials with an analyzed 500 to 600 U phytase / kg diet and the 0.1% P that is recommended by the phytase suppliers at the same inclusion rate can easily be explained through product safety margins. As such, most phytases will contain substantial (and frequently variable) safety margins to account for product shelf-life and animal functionality. As such, the safety margins will push the analyzed phytase concentration well into the 781 to 1413 U/kg range where Angel *et al.* (2002) noted at least 0.1% P spared.

The second generation of phytase products (E. coli-derived phytase expressed in yeast) have entered the market place, and they can have substantially greater efficacies than the *Aspergillus* and *Peniophora* derived phytases (Applegate *et al*., 2003).

The price of feed phosphates as well as meat and bone meals has increased considerably. This has led nutritionists to look at the possibility of using phytase enzymes at concentrations above those recommended by the manufacturer. There is limited data available regarding the efficacy of the different phytases at concentrations above those suggested by the manufacturer.

In studies with chicks, Augspurger and Baker (2004) reported the impact of using different phytases at concentrations ranging from 500 to 10,000 U/kg and Shirley and Edwards (2003) tested a fungal phytase at levels between 94 and 12,000 U/kg. Shirley and Edwards(2003) reported that at 12,000 U of phytase/kg 95% of the phytate-P disappeared but when calculating the amount of P retained per each additional 100 U of phytase the highest efficiency (increased phosphorus retention per additional 100 U phytase/kg) was achieved at 750 U/kg with the fungal phytase they tested. At inclusion levels beyond 750 U phytase/kg these researchers found diminishing returns, however, there is still a large impact on P utilization up to 1500 U phytase/kg. Similarly from the data presented by Augspurger and Baker (2004), one can calculate the amount of gain or tibia ash gained with each additional 500 U of phytase per kg. Likewise, there are diminishing returns past an inclusion of 500 U/kg regardless of phytase. There is still a relatively large impact up to 1000 U/kg but then the efficiency per 500 U/kg inclusions drops markedly specially with the E. coli phytase they tested. It is difficult though, with the information available currently, to give a good estimate of P released when phytases are used beyond their recommended levels.

In summarizing swine studies, Knowlton et al. (2004) noted an average improvement in P digestibility by 17 %-units when 500 U/kg of a fungal phytase was fed. Feeding up to 1000 U/kg resulted in a less rapid improvement in P digestibility, but still was improved up to 25%-unit, after which little improvements in P digestibility occurred.

Other Feed Ingredients that can Lower Manure Phosphorus

New plant genotypes are being developed that contain lower levels of phytin P, such as, the new low phytin P corn (LPA; high available P). These new genotypes contain the same level of total P as normal corn varieties. In LPA corn only 35% of the total P is phytate P versus 75 to 80% in other corn varieties. Chick and pig studies have shown that the P in LPA corn and soybean meal is more available (Cromwell et al., 2000a,b; Spencer et al., 2000a; Waldroup et al., 2000) and when used in combination with phytase can reduce litter P from broilers by 58% (Applegate et al., 2003b) and P excretion from pigs by 37% (Spencer et al., 2000b). Other key ingredients are currently being selected for high availability of P. Soybean phytic acid content could be reduced (Raboy and Dickinson, 1993) with a concomitant decrease in phytin P from 70% to 24% of total P through genetic selection (Raboy et al., 1985).

Dehulled, degermed corn (DDC) is a product of the corn dry milling industry. Phytin-P is deposited in protein bodies of the aleurone (10 percent) and scutellum (90 %) of corn grain (O'Dell et al., 1972). Removal of the germ during dry-milling, therefore removes a large portion of the indigestible portion of phytin-P located in the scutellum. Removal of the fibrous and phytin P rich portions from the corn kernel by dry milling makes DDC a compelling feedstuff for non-ruminant diets. Diets formulated with DDC instead a portion of corn grain results in 20% less P being excreted by broilers and 30 to 35% less in swine. However, due to it's fine particle size it can greatly impact stomach ulcers in swine.

Some lactobacillus-based pro-biotics have been shown to improve growth and feed conversion in poultry. Research by Angel et al. (2005) indicates that P retention was 22% higher and N retention was 10% higher in birds fed a low nutrient diet supplemented with a probiotic versus birds fed a control diet. The addition of the probiotic to the low nutrient diet allowed broilers to grow as well as those fed a control diet in part because they were more efficient in retaining nutrients.

Another feed additive that can reduce P excretion is vitamin D_3 metabolites. Not only does vitamin D_3 stimulate P transport mechanisms in the intestine but it also appears to enhance the activity of supplemental phytase (Mohammed et al, 1991). Vitamin D as well as its metabolites, 25-hydroxycholecalciferol and 1,25-dehydroxycholecalciferol (Edwards, 1993; Mitchell and Edwards, 1996) have been shown to enhance the efficacy of supplemental phytase.

Summary

The enzyme phytase is a novel and cost effective tool in poultry and swine diets that improves P utilization from phytin, the storage form of P in feedstuffs. Typical removal amounts of P for 500 units of phytase / kg diet can vary from 0.06% to 0.10% for broilers, turkeys, and swine. As a result, P excretion can be reduced by 15 to 30%. As P retention compared to P provided through the diet is still far below a hypothetical maximum of 100%, considerable room for improvement in phytin-P release and overall P retention by poultry and swine still exists.

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Project Information

Detailed information about training and certification in Feed Management can be obtained from Joe Harrison, Project Leader, jhharrison@wsu.edu, or Becca White, Project Manager, rawhite@wsu.edu.

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