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Phytase: Basics of Enzyme Function

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Introduction

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 in plants is found in seeds mainly as a component of a molecule called phytin. Phytin-phosphorus 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 phosphorus available to the animal. Phytase is the only recognized enzyme that can initiate the release of phosphate from phytin (IUB, 1979).

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 as the key to unlocking specific end-products (Figure 1). The enzyme and substrate are 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. Enzymes typically have ideal conditions (temperatures, pH, etc.) where they function more readily. As an example, plant phytases work better at

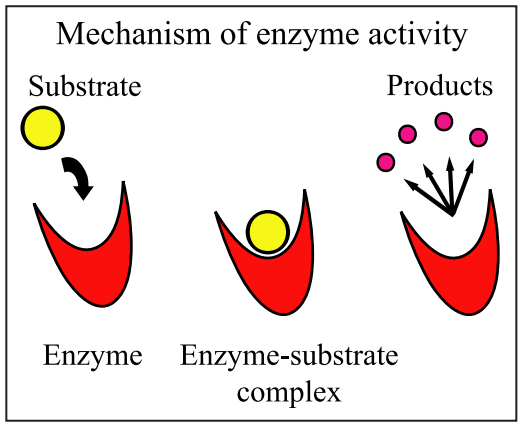

Figure 1: Diagram of how an enzyme speeds release of a product. Phytase (the enzyme) attaches to phytin (the substrate) to help in release of phosphorus and different inositol phosphates (other products).

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, it must be in proximity to the substrate, and the substrate cannot 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 cannot 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).

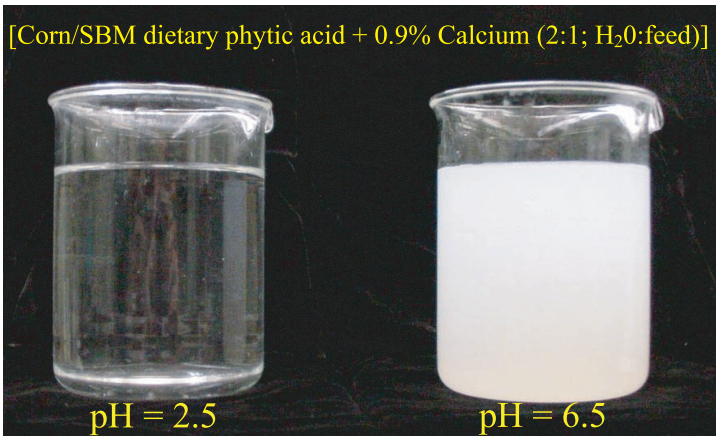

Figure 2: Demonstration of what occurs to phytin-Ca complex in the stomach (pH 2.5) and small intestine (pH 6.5). At the higher pH, phytase cannot work as easily on the substrate phytin because the subtstrate is precipitated.

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-phosphorus within a given feedstuff is variable, but typically averages 72 and 60 percent of total seed phosphorus 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 substantially 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 phosphorus contained in the phytin molecule partially or completely unavailable to the animal.

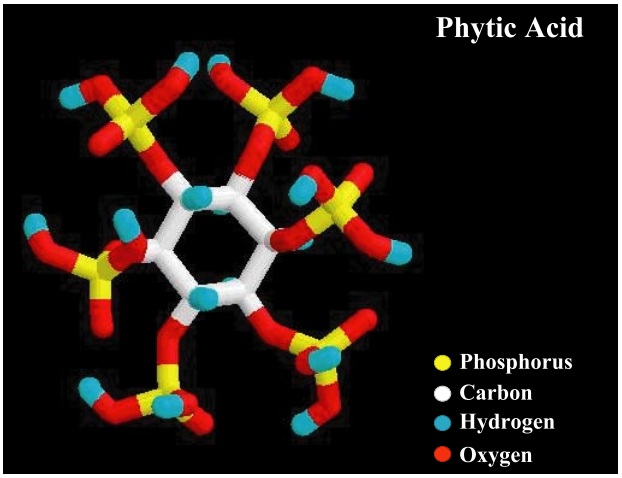

Figure 3. Phytic acid, the predominate storage form of phosphorus in mature seeds (figure courtesy of W. Schmidt – USDA/ARS).

Phytin in feedstuffs is relatively heat stable. Pelleting does not appear to affect phytin content greatly (Skoglund et al., 1997). These researchers found that pelleting at 81C reduced phytin content in a mixed rapeseed, barley, and pea diet by 7 percent. O'Dell (1962) found, however, that nearly 88 percent 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 plants. 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 phosphorus.

Phytin phosphorus content in grains can be variable (Table 1). Factors influencing this variability are still unknown, but soil and environmental factors may affect this content (Cossa et al., 1997, Raboy and Dickinson, 1993). Raboy and Dickinson (1993) reported that the magnitude of the effect of soil phosphorus on soybean seed phytic acid was variety specific. Regardless of variety, phytic acid content increased as soil phosphorus availability increased. Non-phytic acid phosphorus levels in the seed, however, did not change.

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 three position and plant phytase the phosphate at the six 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). The hydrolytic action of phytase on phytin-phosphorus has been known for some time (Dox and Golden, 1911); however, large-scale, commercial production of phytase has occurred only in the 1990's (Wodzinski and Ullah, 1996).

Enzyme Activity

One unit of phytase is defined as the amount of enzyme required to liberate one 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 in the above conditions does not necessarily translate into the same amount of phosphorus 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 phosphorus 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 phosphorus made available.

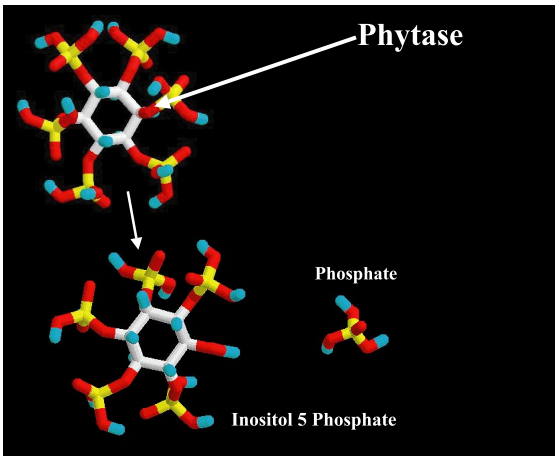

Figure 4. Diagram of release of phosphate from phytin by the enzyme, phytase. (figure courtesy of W. Schmidt – USDA/ARS).

TABLE 1. Phytin-phosphorus (PP) content of feed ingredients as a percent of total phosphorus (TP) [2]Nelson et al. (1968) and [3]Cossa et al. (1997), [4]Barrier-Guillot et al. (1996a)

Ingredient	Number of Samples	PP, % (SD)	PP, % of TP
[1]Soy beans *G max* *G soja*	24 24	0.41 (0.22) 0.56 (0.18)	69.5 72.7
SBM (50% protein)	20	0.37 (0.03)	71
[2]SBM (44% protein)	3	0.38	58
[2]Corn	10	0.17 (0.02)	66
[3]Corn	54	0.27 (0.24)	86
[2]Corn Gluten Meal	1	0.36	62
[2]Milo	11	0.21 (0.03)	68
[4]Wheat	56	0.218 (.035)	60
[2]Wheat	2	0.18	67
[2]Wheat Middlings	1	0.35	74

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 activity 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 cannot 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 that are not pelleted) diets 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 phosphorus retention. When at least 1000 U/kg of fungal phytase is included in corn/SBM-based diets of pigs, phosphorus retention was increased from 52 to 64% (Kornegay, 1999). Similarly, phosphorus 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.

Summary

The enzyme phytase is a novel and cost effective tool in poultry and swine diets that improves phosphorus utilization from phytin, the storage form of phosphorus in feedstuffs. As phosphorus retention is still far below a hypothetical maximum of 100%, considerable room for improvement in phytin-phosphorus release and overall phosphorus retention by poultry and swine still exists.

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