Dietary Phytase: An ideal approach for a cost effective and low-polluting aquafeed

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Abstract

Global fishmeal production from wild-catch sources cannot continue to increase indefinitely; suitable alternatives have to be found for sustainable aquaculture. Plant-based aquafeed seems to be the ideal alternative to this, but has its own limitations. Plant ingredients are rich in phytic acid, which reduces the bioavailability of nutrients like minerals and protein to the fish, thereby causing aquaculture pollution. Dietary phytase treatment reduces the aquaculture pollution by improving the bioavailability of nutrients, and reduces the feed cost as evident from poultry and piggery. Phytase activity is highly dependent upon the pH of the gut. Unlike mammals, fish are either gastric or agastric, and hence, the action of dietary phytase varies from species to species. In this article, the authors attempt to summarise various effects of phytase on nutrient utilization, growth of fish and aquatic pollution.

Introduction

The aquaculture industry has been globally recognized as the fastest growing food producing industry (NACA/FAO 2000) and it will play an increasingly important role in meeting the demand for fish. Aquaculture contributes more than 19 million tonnes of fish and shellfish annually to the world's fish supply. Most of this is produced in extensive systems, particularly in China, where about 11 million tonnes of carps are produced. About 8 million tonnes of fish are produced in semi-intensive or intensive systems with the use of mixed or manufactured feeds (Heindl 2002). The growth and intensification of aquaculture has raised several issues that need to be addressed for the sustainability of this industry. One of these issues is the development of fish feed from high quality, inexpensive sources as well as methods for making the feed free from anti-nutritional factors. The aquaculture feed industry relies heavily on the use of fishmeal because of its balanced amino acid and fatty acid profile. The proportion of global fishmeal production that is being utilized for the production of fish feeds has increased substantially over the past decade. In 1989, approximately 10 percent of annual fishmeal production (Barlow 1989) was being used in fish feed, increasing to about 35 percent in 2000 and predicted to reach 44 percent by 2010. Fishmeal is produced from fish caught from the wild. Wild stocks have already reached their maximum biological limits, so any increase in fishing effort is unsustainable. Besides this, fishmeal is an expensive source of protein. The replacement of fishmeal with plant or grain by-products will become increasingly important for the development of low-cost fish feed. One of the major problems associated with the use of plant by-products in fish feed is the presence of anti-nutritional factors, like phytic acid.

Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) is the major phosphorus (P) storage compound in plant seeds and can account for up to 80 percent of total phosphorus. Soluble inorganic and cellular phosphorus (phosphorus bound in nucleic acids, phosphorylated proteins, phospholipids, phosphor-sugars) represents the remaining phosphorus. Because of the high density of negatively charged phosphate groups, phytate chelates with mineral cations like potassium (K), magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), copper (Cu) and forms poorly soluble complexes. Apart from minerals, phytate also forms complexes with proteins and amino acids. The amino group present on the side chain of the amino acids is one of functional groups involved in protein-phytate interaction, thereby decreasing the digestibility of proteins. These salts of phytic acid are known as phytins and their availability/digestibility to monogastric animals including fish is very limited due to the lack of intestinal phytase (Pointillart et al. 1987). This phytate-phosphorus is excreted into the environment and is acted upon by microorganisms that release the phosphorus, causing pollution in terms of algal growth.

Addition of microbial phytase has been reported to improve the utilization of plant phosphorus in poultry (Nernberg 1998), pig (Han et al. 1997) and fish diets (Rodehutscord et al. 1995; Li and Robinson 1997; Van Weerd et al. 1998; Forster et al. 1999; Robinson et al. 2002, Debnath 2003). Addition of microbial phytase in aquafeed increases the bio-availability of phosphorus and, hence, there is less discharge into the aquatic environment, thereby causing less pollution.

What is phytase?

Phytase is an enzyme chemically known as myo-inositol-hexaphosphate phosphohydrolase (Class 3: Hydrolases), produced either by microorganisms or present in some plant ingredients. Monogastric animals cannot produce this enzyme. Presence of phytase in some animals is of microbial origin. Microbial phytase either as a dry powder or as a liquid is available commercially. Natuphos® was the first commercially available phytase, from a genetically modified *Aspergillus niger* strain. The optimum microbial phytase activity occurs at two pH values: the highest activity being at pH 5.0-5.5 and second highest at pH 2.5 (Simons et al. 1990). One unit of phytase (FTU) is defined as the quantity of enzyme that liberates 1 micromol of inorganic phosphorus per minute from 0.0015 mol/L sodium phytate at pH 5.5 and 37°C. Von Sheuermann et al. (1988) observed the following phytase activity with different pH levels in corn and wheat (Table 1).

Phytase cannot withstand high temperature. For instance, pelleting a diet at 70°C reduces the activity by 15-25 percent (Schwarz and Hoppe 1992). The observed activity of phytase after pelleting diets at different temperatures is given in Table 2.

Table 2. Effect of pelleting temperatures on phytase activity.

	Pelleting temperature (ºC)	Phytase activity (U/kg)	Remaining activity (%)
Feed enzyme before pelleting		250	100
Meal temperature before pelleting (ºC)			
50	78	240	96
50	81	234	94
65	84	208	83
65	87	115	46

Source: Simons et al. 1990.

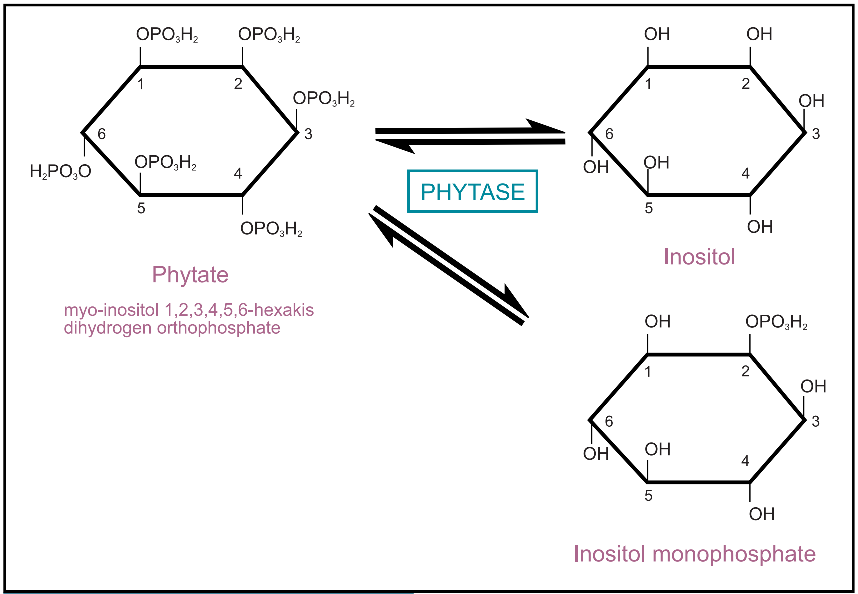

Figure 1. Action of phytase.

Mechanism of action

Phosphorous in plants normally remains in an associated form with a molecule called phytic acid (phytate). Phytic acid consists of a sugar (similar to glucose) called myo-inositol, to which phosphate (PO_4) groups are covalently linked. Phytase releases these phosphates from the inositol ring as shown in Figure 1. Release of this phosphorus depends on the pH condition of the intestine.

Table 1. Effect of phytase activity at different pH levels.

pH	Phytase activity
<1.0	Inactive
1.0	Inactive
2.0-3.0	Inactive (?)
4.0	Active
5.0	Active
6.0	Active
7.0	Inactive (?)
8.0	Inactive

(?) indicates uncertainty
Source: Von Sheuermann et al. 1988.

Effect of phytase on bioavailability of phosphorus

Addition of phytase to high phytate stripped bass diets improves the absorption and utilization of phosphorus (Hughes and Soares 1998). Dietary phytase also improves the nutritive value of canola protein concentrate and decreases phosphorus output in case of rainbow trout (Forster et al. 1999). Similar reports have been documented for different species like rainbow trout (Rodehutscord and Pfeiffer 1995), channel catfish (Li and Robinson 1997), African catfish (Van Weerd et al. 1999), common carp (Schafer et al. 1995) and *Pangasius pangasius* (Debnath 2003). Robinson et al. (2002) report that 250 units of phytase per kilogram of diet can effectively replace dicalcium phosphate supplement in the diet of channel catfish without affecting growth, feed efficiency or bone phosphorus deposition. Microbial phytase is effective in enhancing the bioavailability of phosphorus considerably, thereby reducing the faecal phosphorus output.

Effect of phytase on bioavailability of other nutrients

Phytate makes complexes with various di- and trivalent cations as well as with proteins (Wise 1980). For example, calcium-bound phytate increases chelation with trace minerals, especially zinc, to form co-precipitates that make the zinc unavailable to animals. Phytase added to diets improves the bioavailability of copper and zinc in pigs (Adeola et al. 1995) and poultry (Yi et al. 1996). Microbial phytase also improves the apparent absorption of magnesium, zinc, copper and iron in pigs. Similar results have also been reported for fish. Phytase addition increases the concentration of minerals like magnesium, phosphorus, calcium, manganese and zinc in plasma**,** bone and the whole body (Vielma et al. 1998). Channel catfish fed phytase-supplemented diets had higher concentrations of ash, calcium, phosphorus and manganese in their bones than the fish fed on a control diet (Yan and Reigh 2002). Yan and Reigh (2002) further delineated that phytase supplementation at 500 units per kilogram of diet was sufficient to improve the retention of calcium, phosphorus and manganese by catfish fed an all-plant-protein diet.

Effect of dietary phytase on protein digestibility

Phytase treatment of soy-protein concentrate was found to improve protein digestibility and retention in Atlantic salmon (Storebakken et al. 1998). Microbial phytase supplementation in the diet of *Pangasius pangasius* also increased the apparent net protein utilization (Debnath 2003). It was further concluded that apparent protein digestibility in the diets was significantly (P<0.01) improved by enzyme supplementation, while non-enzyme supplemented groups showed a low digestibility (Debnath 2003) confirming the established properties of phytate to form phytate-protein complexes that are resistant to proteolytic digestion (Cheryan 1980). In addition, phytate binds trypsin *in vitro* and thus reduces protein digestibility (Singh and Krikorian 1982). Digestibility of dry matter (Papatryphon et al. 1999) and crude protein (Storebakken et al. 1998) were also improved by dietary phytase supplementation. Many researchers have observed the negative effect of phytate on protein utilization in fish. Phytase supplementation in plant-based practical diets has been reported to increase (Vielma et al. 1998), not affect (Lanari et al. 1998) or decrease (Teskeredzic et al. 1995) protein digestibility. In poultry, phytase was reported to improve protein and amino acid utilization through breakdown of phytin-protein complexes (Kornegay 1995). In fish, the situation is somewhat ambiguous. This may be due to the presence or absence of a stomach in different fish species, as phytase activity is pH specific.

Effect of phytase on growth performance of fish

The weight gain rates and specific growth rates of Indian major carp, *Labeo rohita* were significantly decreased when phytic acid was included in diet at levels above 1 percent (Alvi 1994). Similar effects are evident on the growth performance and body composition of *Cirrhinus mrigala* fry (Usmani and Jafri 2002). It was reported that Chinook salmon, *Oncorhynchus tshawytscha*, fed semi-purified diets containing various levels of calcium, phosphorus, zinc and sodium phosphate with a high dietary phytic acid (2.58 percent) exhibit depressed growth (Richardson et al. 1985). In contrast, the growth performance increased when microbial phytase was incorporated in the diets. An increase in weight gain has been reported in channel catfish fed phytase-supplemented diets containing only plant protein or a combination of plant and animal protein sources (Jackson et al. 1996). Weight gain and feed consumption increased by 23.52 and 11.59 percent, respectively, compared to a control group. Similar performance of *P. pangasius* (Debnath 2003), African catfish *Clarias gariepinus* (Van Weerd et al. 1999) was also reported. The better performance of fish fed phytase-supplemented diets implies that either the phosphorus requirement was met along with other nutrients or that phytase has other positive effect on performance.

Effect of phytase on aquaculture pollution

The environmental impact assessment of the aquaculture industry is getting increasing attention and rigorous restrictions are being set on this industry by governments and environmentalists. Farmers engaged in freshwater aquaculture and coastal marine operations are facing increasing pressure from various organisations to control farm-discharge into the surrounding ecosystems. This discharge, particularly phosphorus loading, leads to eutrophication. The phosphorus in the feed ingredients occurs in a number of forms. It occurs in the inorganic form as well as phosphate complexes of protein, lipid and carbohydrate. These forms are available to the fish. Phosphorus present in most grain and seed by-products is generally unavailable to finfish and monogastric animals as mentioned earlier. Fish excrete phosphorus in soluble and particulate forms. The soluble forms, organic phosphorus and phosphates affect water quality directly. The particulate forms accumulate in the sludge and the phosphorus is released slowly to the water. Dissolved reactive phosphorus is usually regarded as the most important factor affecting water quality, because it is most available for phytoplankton growth. Microbial phytase supplementation in the diet of fish can overcome this problem. It makes the chelated phosphorus available to fish and hence there is less faecal excretion, thereby reducing environmental pollution.

The environmental benefits of using this enzyme in fish feed are:

- Reduced requirement of mineral supplements, thereby reducing

chances of excess inorganic phosphorus getting into the aquatic system.
- Reduced organic phosphate (phytic acid) outputs.

Use of phytase in feeds reduces or sometimes eliminates the necessity of mineral supplementation, which also decreases the cost of feeds. Although phytase was first used for environmental reasons, it has now been discovered that there are a range of other nutritional and health benefits from using these enzymes.

Conclusions

Use of plant-based feed in aquaculture is inevitable in the near future. Increasing demand for fishmeal for various sectors like livestock and poultry in addition to aquaculture, has made this commodity more expensive. It is almost impossible to include fishmeal in aqua feeds and still be able to keep the production cost low. On the other hand, plant ingredients have their own limitations due to the presence of phytate for which their inclusion level is restricted. Phytate-rich plant ingredients restrict the bioavailability of phosphorus along with other minerals thereby increasing discharge into water bodies causing algal bloom. Phytate also limits the protein availability to the fish. However, it is evident that phytase supplementation improves the bioavailability of the phosphorus and nitrogen (protein), which are the main culprits of aquaculture pollution. The increased bioavailability of nitrogen and phosphorus in the diet leads to reductions in feed costs. This is a subject that needs to be seriously researched. Though the role of phytase supplementation has been well proven and documented in poultry, its use in fish feed is less known. This is due to the pH specificity of phytase. The addition of organic acid along with phytase, especially in agastric fishes, is of special interest, and needs serious attention from researchers in aquaculture nutrition.

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