Phytases in fish nutrition

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Abstract: In the past, one of the basic components of fish feed was fish meal and other meals of animal origin. Thanks to a good amino acid profile, fish meal is one of the most advantageous sources of protein. Due to the reduced availability and relatively high price of fish meal and restrictions of the use of other feeds of animal origin, there have been efforts to replace these with alternative components of plant origin. However, plant components in fish feed carry limitations due to anti-nutrients. One of these is phytic acid, a phytate binding phosphorus. Phosphorus is essential for plants and animals. It is a component of nucleic acids and plays a role in the metabolism of lipids, saccharides and proteins. Phytate cannot be used by monogastric animals and fish because their digestive tracts lack the enzyme phytase needed to separate phosphorus from the phytic acid molecule. Undigested phosphorus excreted into water further contributes to the eutrophication. The ideal solution for increasing the digestibility of phosphorus from phytate is to add a phytase enzyme to compound feeds for fish. Phytases are commonly found in nature. Plants contain endogenous phytase which helps the plant grow during germination. Microbial phytases are generally more active than endogenous phytases. Phytase activity is affected by temperature and pH. Most phytases exhibit the greatest activity between a pH of 2.5–5.5. Fish without a stomach are not able to actively utilize phytase additives. A solution may be to acidify compound feeds with organic acids. Another limiting factor is the temperature in which phytases act. The maximum temperature at which industrially produced phytases are active is 46–60 °C. During the production of compound feeds by extrusion, this limit tends to be exceeded, resulting in denaturation of the enzyme. The addition of phytase and organic acids and the use of proper technological processes when manufacturing fish compound feeds may be a solution in the effort to increase the use of phytate phosphorus in feed and thus reduce water pollution.

Key Words: phytate, phytase, carp, cereals

INTRODUCTION

Aquaculture is one of the fastest-growing food industries. Fish feed in intensive aquaculture is widely based on fish meal (New and Wijkström 2002, Baruah et al. 2004). Thanks to a good amino acid profile, fish meal is the best source of protein rich in n-6 and n-3 fatty acids. However, limited supply, high price and the uncertain future availability of fish meal are reasons why fish meal is being replaced by cheaper and more available products. Another reason for restricting the use of fish meal is its high phosphorus content ranging from 24.6 to 32 g (Jirásek et al. 2005). Feed manufacturers are trying to incorporate plant products into compound feeds in various combinations that ensure a suitable balance of amino acids. The most commonly used plant component for fish feeds is soybean. Soybeans have a high nitrogen content, high production yields, and are available year-round (Kumar et al. 2011). Other cereal products are also used such as corn, rapeseed, peas and even lupine. However, plant components in feed carry limitations with respect to their utility for animals because they contain antinutrients. Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients. One of these is phytate, or phytic acid, which binds phosphorus (Papatryphon et al. 1999). Plant seeds stores 50–80% of all phosphorus in the form of phytate. Unfortunately, such phosphorus cannot be utilized by monogastric animals and fish which lack the enzyme phytase needed to separate phosphorus from phytate molecules. Undigested phosphorus is then excreted into the water environment, contributing to its eutrophication. A natural source of phosphorus that can be utilized by fish and monogastric animals comes from phosphate minerals.

However, this source is not renewable, which leads to the idea of using the phosphorus from plant products in a more efficient way (Kumar et al. 2011). Adding phytase enzymes to compound feeds is the ideal approach for increasing the digestibility of phosphorus from phytate. Phytases are commonly found in nature. Endogenous phytase is responsible for the growth of plants during germination. In ruminants, they are found in the rumen and intestinal microflora as microbial phytase. Microbial phytases are more active than endogenous phytases and hence produced and used as additives to compound feeds. Microbial phytases are already a common additive to feeds for pigs and poultry (Cao et al. 2007).

Phosphorus

Phosphorus is important for plants and animals. It serves an important function by providing energy for cells in the form of ADP and ATP. It is also a component of nucleic acids (Jelínek and Koudelka 2003) and plays a role in the metabolism of saccharides, lipids, proteins and amino acids in the body (Leeson and Summers 2001). It is also responsible for the mineralization of bones and teeth, and is also contained in the skin. The ideal amount of phosphorus in fish feed ranges from 0.3 to 0.9% depending on the species and breeding system (Halver and Hardy 2002).

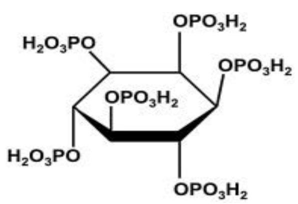
Fish meal made of the bones and muscle tissue of fish is added to compound feeds for fish as a good source of organic phosphorus. Fish meal contains sources of quality saturated and unsaturated fatty acids and an ideal balance of amino acids. Due to limited availability and high price, fish meal is being replaced with plant components (Malý 2015). Lundová (2014) states that the digestibility of phosphorus from fish meal in laying hens is up to 100 %. In salmonids, the digestibility reaches 70–80%, however, in common carp (*Cyprinus carpio* L.) the digestibility is only 25% (Jirásek et al. 2005). Another possibility for adding phosphorus to feeds is monocalcium phosphate. This component achieves digestibility in carp of up to 94%, but the high price makes it uneconomical for fish compound feeds in aquaculture (Singh 2008).

By replacing fish meal with plant components, we can achieve quality levels of protein and amino acids in feed. Plant components are also another source of phosphorus in fish feeds. Some of the most commonly used plant components are cereals, pulses and extracted meals. Phosphorus is stored in plant products in the form of phytic acid, which provides a supply of phosphorus for plant growth during germination. Phytic acid is poorly digested by fish. Jirásek et al. (2005) report that the digestibility of phytate phosphorus in carp ranges from 8 to 35%, and in rainbow trout (*Oncorhynchus mykiss*) only 0–18%.

Phytic acid

Phytate is created during the maturation of plant seeds and grains. It is an ester of a cyclic, six-carbon hydrocarbon called myo-inositol to which six groups of phosphoric acid residues are attached (Fig. 1). Phytate is a storehouse of phosphorus in grains and seeds. In live animals, phytate has been described as an "anti-nutritional factor and indigestible nutrient" (Swick and Ivey 1992). The six phosphoric acid residues bound to myo-inositol can further bind to various types of cations such as calcium, magnesium, zinc, iron and potassium to form insoluble salts. These complicated complexes, also known as phytins, reduce the utilization of these indispensable minerals in feed (Papatryphon et al. 1999). Cao et al. (2007) report that phytate can also combine proteins and vitamins as insoluble complexes, which then reduces their use, activity and digestibility. Phytate and protein complexes tend to be less affected by proteolytic enzymes such as pepsin and amylase because they are inhibited by the phytate. Phytate also affects the intensity of growth and the feed conversion ratio in commonly farmed fish species such as common carp, tilapia or rainbow trout. It can be generally said that phytate is not an anti-nutrient factor only with respect to the digestibility of phosphorus, but it may also reduce the digestibility of many feed components such as minerals, proteins and even vitamins and enzymes.

Figure 1 Structural formula of phytic acid



The phytate concentration in plants differs significantly. In most cereals, oilseeds and grains phytate comprises 0.7–2% (Adeola and Sands 2003). The most poorly digestible phytate is found in the outer shells and bran, while the most easily digestible is found in the seed germ (Singh 2008). Table 1 shows selected plant components and their phytic acid content. One way how to make the phytate phosphorus available to fish is to add microbial phytases to compound feeds. Positive experience with the use of phytase additives in compound feeds for rainbow trout was reported as early as the 1990s (Halver and Hardy 2002).

Table 1 Content of phytic acid in grains (Schlemmer et al. 2009)

Cereals	Phytic acid (g/100 g)	Legumes	Phytic acid (g/100 g)	Oilseeds	Phytic acid (g/100 g)
Wheat	0.39–1.35	Kidney beans	0.61–2.38	Soybeans	1.0–2.22
Wheat bran	2.1–7.3	Peas	0.22–1.22	Soy concent.	10.7
Barley	0.38–1.16	Chickpeas	0.28–1.60	Linseed	2.15–3.69
Oat	0.42–1.16	Lentils	0.27–1.51	Rapeseed	1.44–5.36
Triticale	0.50–1.89			Rapeseed concent.	5.3–7.5
Maize	0.72–2.22			Sunflower meal	3.9–4.3

Phytases

The chemical name of phytase is myo-inositol-(1,2,3,4,5,6)-hexaphosphate-phosphohydrolase. This enzyme hydrolyses phytic acid into inositol and phosphate acid residues to form free inorganic phosphorus along with a chain of esters (Baruah et al. 2004). Phytase is contained in plant components as endogenous phytase or is created by microorganisms as the so-called microbial phytase. In monogastrics and fish, the presence of microorganisms in the intestinal mucosa is scarce, so they are not able to produce the enzyme. Industrially produced phytases are obtained from genetically modified microorganisms. These are primarily bacteria, yeasts and fungi. Phytase activity is reported in FTU units (Simons et al. 1990).

Phytases are divided into two categories depending on the place where hydrolysis of the phytate molecule begins. These two kinds of phytases are the 3-phytase (myo-inositol hexaphosphate 3-phosphohydrolase) and the 6-phytase (myo-inositol hexaphosphate 6-phosphohydrolase). In the 3-phytase group, hydrolysis begins at the third atom of carbon, and in the 6-phytase group, hydrolysis begins at the sixth carbon atom of the myo-inositol hexaphosphate ring (Cao et al. 2007). Most microbial phytases are classified in the 3-phytase group, while plant (endogenous) phytases are generally 6-phytases (Nayini and Markakis 1986). Phytases are further classified into acidic or alkaline phytases according to the optimal pH of the environment in which they are active. Most microbial phytases and industrially produced phytases belong to the acidic group and degrade phytate at a pH around 5. On the other hand, plant phytases are generally alkaline and exhibit the greatest activity at a pH around 8 (Kumar et al. 2011). Phytase activity depends on the temperature and pH of the environment.

Temperature

Phytases are very sensitive to temperature. Most phytases exhibit the greatest activity in a temperature range of 40 to 60°C (Table 3). During the manufacture of granulated and extruded feeds for fish, mixtures are often heated to over 100°C. In such cases, the enzyme protein is denatured, making the phytase enzyme unusable (Cao et al. 2007). For this reason, it is important to choose a suitable type of phytase that is more resistant to the effects of high temperature, and to choose a suitable manner of applying the enzyme to the feed. Cheng and Hardy (2003) report that during the production of feeds by extrusion there was a significant reduction in the availability of phosphorus and other minerals, possibly caused by the denaturing of the endogenous phytase protein of plant components of the mixture. Cao et al. (2007) report that one of the ways to reduce phytate in plant components is to treat them before using in compound feeds. Soaking or fermentation under suitable conditions (temperature and pH) can enhance the activity of endogenous phytase. Another way to avoid destruction of the enzyme by high temperatures is to pre-treat the feed with an enzyme application, so-called dephytinization. However, effects of this process are quite variable. The most suitable enzyme application in common carp (Schaefer and Koppe 1995) and in rainbow trout (Vielma et al. 1998) was found to be the application of the enzyme on the surface of already manufactured pellets. The enzyme can be applied in both liquid and powdered form, where the medium is an inorganic salt. Another possibility is to use special types of enzymes, such as Phyzyme 10,000 TPT, the thermal stability of which exceeds 95% (Maly et al. 2017)

The most important factor is modification of the feed production process to avoid heating of the enzyme above the critical point.

pH value

The optimal value for phytase activity is a pH of 2.5–5.5. Higher activity occurs at a pH of 5.5. Salmonids, which have a digestive system with a stomach, meet these conditions. Carp, which do not have a stomach, have problems. The pH value of its digestive tract ranges from 6.5 to 8.4 (Ji 1999). Fish with stomachs, such as trout, have suitable conditions in their gastrointestinal tracts for phytate hydrolysis by microbial phytase. Increased utilization of phytase by fish without stomachs can be obtained by adding organic acids to compound feeds. Most commonly used acids are citric, fumaric, formic or lactic acid. Increasing the acidity of feed can increase phytase activity and thus increase the digestibility of phosphorus and minerals and improve the production parameters of farming (Baruah et al. 2005).

Plant phytases

The greatest phytase activity is found in cereals (rye, barley, triticale and wheat) and in secondary cereal products, while in pulses the activity is lower (Kumar et al. 2011). In dry seeds and grains phytase is inactive. It is activated during germination when phosphorus is released and becomes available for plant growth. However, this depends on the type and age of plant, humidity and seed storage conditions (Singh 2008). The optimum pH value for the action of endogenous phytases is 4–7.5 and the optimal temperature of the environment is 40–60 °C. It may generally be said that endogenous plant phytases are more sensitive to the effects of higher temperatures as well as to the pH values (Simons et al. 1990). Table 2 below gives an overview of certain plant components, the activity of their endogenous phytase and the proportion of phytate phosphorus to total phosphorus.

Table 2 Activity of endogenous phytase of plant components and share of phytate phosphorus (Kumar et al. 2011)

Ingredients	Phytase activity (FTU/kg)	Phytate P / total P	Ingredients	Phytase activity (FTU/kg)	Phytate P / total P
Wheat	503	74.9	Wheat bran	2173	76.3
Barley	348	67.3	Soybean meal	42	68.4
Oat	38	86.4	Sunflower meal	<10	82.8
Soybeans	40	55.5	Canola meal	5	76.4
Peas	58	48.4			
Lupins	<10	52			

Table 3 Selected microorganisms that produce phytase and the characteristics of their phytases (Cao et al. 2007)

Source	Phytase activity (FTU/g)	pH optimum	°C optimum
*Aspergillus niger**	50–103	5.0–5.5	55–58
*Aspergillus terreus**	142–196	5.0–5.5	70
*Peniophora lycii**	1080	5.5	58
*Escherichia coli***	811–1800	4.5	55–60
*Citrobacter braakii***	3457	4	50
*B. amyoliquefaciens***	20	7.0–8.0	70
*Candida krusei****	1210	4.6	40

*Legend: * fungi, ** bacteria, *** yeasts*

Microbial phytases

The most commonly used industrially manufactured phytases are produced by filamentous fungi (*Aspergillus*, *Peniophora*, *Cladosporium*), bacteria (*Bacillus*, *Lactobacillus, Streptococcus*) and yeasts (*Hansenula*, *Scheanniomyces*) (Malý 2013).

Phytases from filamentous fungi exhibit greater resistance to high temperatures but tend to be more active in the lower part of the pH spectrum. Phytases produced by bacteria, especially *E. coli*, are less resistant to environmental temperature than those from filamentous fungi. Considering the diverse pro-perties of phytases, Table 3 gives an overview of some species of microorganisms and the charac-teristics of the phytases they produce.

CONCLUSION

Aquaculture is increasingly using plant components as a source of protein, especially due to their low price and good availability. The availability of fish meal continues to decrease, making the use of plant components in fish farming inevitable. Plant components contain anti-nutrients, such as phytic acid, which decreases the availability of phosphorus stored in plants along with other minerals. Considering the environmental conditions of the digestive tract in fish, especially the pH, the temperatures during the production of compound feeds, and the great anatomical and physiological variability of individual fish species, the use of phytases in fish feeds is still in the research phase. Especially in the case of carp fish, which do not have favourable conditions in their digestive tract for phytase to function, phytase additives without further feed modification are ineffective. By adding organic acids to feed and using proper technological processes, it is possible to create a suitable environment for phytase activity, even when farming common carp. In livestock, phytases are already commonly used as a feed additive. In fish farming, phytases have great potential considering the ever-increasing pressure on fish producers. In particular, by improving farming from an ecological perspective, but also from an economic perspective. The use of phytases improves the feed conversion rate and thus reduces costs for fish production.

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