An Evidential Review on Potential Benefits of Enzymes in Aqua Feed Industry

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ABSTRACT

Enzymes are basically a type of protein in biological systems. They are generally used as catalysts in order to catalyse the rate of reaction. Feeding these enzymes in the aquaculture sector has some nutritional advances. Application of enzymes reducing the effects of anti-nutritional factors, improves the dietary energy resulting in better performance of fish/shrimps. Feed enzymes in the form of granules help enzymes to stay for longer time durations and are suitable for pelletisation process. Efficiency of feeds needs to be at maximum for economical operations. There are various kinds of enzymes which include phytase, xylanase, cellulase, lipase, protease, amylase and many more which can increase the nutrient availability, nutrient absorption during digestion, increase the rate of fish growth and assist survival of fish in early stages of life. In addition, it makes the feeds more economical. Enzyme application may give a solution of high larval mortality of aquatic animals. Feeding larvae with enzymes would be beneficial. Enzymes play a significant role in formulating cost effective, high quality and eco-friendly aqua feeds. At present, the use of enzymes in aqua feeds can reduce use of fishmeal which ultimately reduces the cost of fish production. This may help to reduce the demand for fishmeal from the aquaculture sector in coming years.

Keywords
Enzyme, Fish meal, Phytase, Protease, Xylanase, Plant based ingredients

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Introduction

The United Nations Food and Agriculture Organization (FAO) projected that world population will increase from the current 7.5 billion to 9.1 billion by 2050 (FAO, 2009). A significant increase in food production will be required to feed this population growth and the FAO in its report on “How to feed the world in 2050” estimated that food production in developing countries will need to double (FAO, 2009). Human diets are also shifting to more meat and dairy foods. However, the FAO data showed that world per capita meat consumption is increasing only for chicken and fish. As the conversion of feed to edible meat from fish is the most efficient for all animals farmed for meat, aquaculture is potentially the most viable source of future protein to meet global needs.

Economical fish and shrimp production requires maximum nutritional efficiency from feed. The main issue in aquaculture revolves
around fish meal reduction in feeds and fish oil substitution in high energy diets. By 2030, aquaculture production will contribute 62% or 93.6 million tonnes to global seafood production (The World Bank, 2013). The pattern of FM use has shifted nearly exclusively to aqua feed production from livestock (Hardy 2010). Aquaculture consumed 3.72 million tonnes or 60.8% of total FM produced (Tacon et al., 2011) and 0.78 million tonnes (73.8%) of global fish oil (FO) in 2008 (FAO 2012), at the expense of the livestock sectors which have continued to reduce their usage of these marine commodities. By 2012, aquaculture’s fish meal consumption rose to 68% while FO usage remained the same (74%) (Tacon and Metian, 2015). Despite efforts to improve fish meal availability and quality, global fish meal production has remained static (5 – 7 million tonnes) year over year due to fully/over-exploited fisheries while the production of cereal grains and oil seeds are trending upwards at 2.9 billion and 574.1 million tonnes respectively (USDA 2015). Further growth in the aquaculture production can therefore not depend on an increase in the catch volume of wild fish, but must rely on a further increase in the use of alternative feed resources. The main source of plant based protein aquatic feed includes soybean meal, corn gluten meal, sunflower meal, canola/rapeseed meal, peas and lupins. Soybean meal having highest proportion of plant protein in fish diets owing to high yield, relatively high crude protein content and easy and round the year availability. Nutritionists are investigating the ways of utilising proteins of plants origin, since they are cheaper, readily available, and easily accessible than animal protein sources. Plant ingredients have so far been the most cost efficient alternative, and cite an example, feeds for Norwegian farmed salmon have changed from a marine based diet (90 % marine ingredients) to a plant based diet (30 % marine ingredients) (Ytrestoyl et al., 2014). The major high protein plant ingredients in Norwegian salmon diets are soy protein concentrate (24 %) and wheat/wheat gluten (17 %) (Ytrestoyl et al., 2014), but increased use of other plant ingredients have to be considered for further growth in the aquaculture production.

**Challenges with plant-based ingredients**

The most important challenges with plant products as protein sources in feeds for fishes particularly for carnivorous fish are: low level of protein, low digestibility, high level of carbohydrates, adverse digestibility, other nutrients and the presence of anti nutritional factors (Gatlin et al., 2007; Sorensen et al., 2010). Poor amino acid composition and unfair nutrient composition can be balanced by combining ingredients of different origin and use of additives such as amino acids, vitamins and minerals. (Sorensen et al., 2010).

**Lower digestibility**

Nutrient digestibility of plant-based ingredients is a critical component in determining the potential of raw feedstuffs for inclusion in fish feed. Digestibility refers the amount of the nutrients/energy in the ingested feed that is not excreted by the animal (NRC 2011). It is essential for optimising inclusion levels and minimising resource waste.

Compared to FM, plant-based ingredients have relatively lower digestibility. This is due to structural components (cellulose, hemicellulose etc.) and metabolites (ANFs) which interfere with the animal’s digestive metabolism, lowering dietary nutrients absorption. Consequently, the nutritive value of a feedstuff also includes its nutrient and energy bioavailability (Altan and Korkut, 2011).
Anti-nutritional Factors (ANFs)

Plants commonly synthesize metabolites of low and high molecular weight called antinutritional factors as a defence mechanism against herbivores (Khokar and Apenten, 2003). ANFs are classified as endogenous compounds found in all plant-based ingredients which may negatively influence feed intake, nutrient digestibility and utilisation, growth, affect the function of internal organs and alter disease resistance (Krogdahl et al., 2010). They include, but are not limited to, phytases, Protease Inhibitors (PIs), Non-Starch Polysaccharides (NSPs) (cellulose and hemicellulose), saponins, tannins, haemagglutinins or lectins, gossypols and cyanogenic glycosides (Soetan and Oyewole, 2009). The structure and chemical composition, specifically heat-sensitivity, of ANFs can determine which physical or chemical processes may be effective in reducing their biological effects in animals (Khokar and Apenten, 2003). ANFs can be removed or inactivated by selective breeding, genetic modification, heat treatment or extraction (extrusion, pelleting, alcohol extraction), or through supplementation (enzyme, mineral, etc.) (Krogdahl et al., 2010) (Table 1).

Phytate binds naturally occurring plant P making it unavailable to monogastrics and impairs mineral absorption; NSP (soluble and insoluble) interferes with digestive processes limiting nutrient uptake while PIs depress the digestion of protein, hindering amino acid absorption (Krogdahl et al., 2010).

Phytate-phosphorus

Phytate is the primary storage form of P in many plants accounting for 0.4 – 6.4% by weight and 60 – 90% of total P (Khokar and Apenten, 2003). Phytate consists of an inositol group, hexahydrocyclohexane in a chair configuration with six phosphate ester bonds (Haros et al., 2005; Kumar et al., 2012).

Phytate can strongly chelate with cations such as calcium, magnesium, zinc, copper, iron and potassium to form insoluble salts. This adversely affects the absorption and digestion of these minerals in fish (Papatryphon et al., 1999). Around 50% to 80% of the total phosphorous content in plant seeds is stored in the form of phytate (Ravindran et al., 1995). Phosphorus in this form is generally not bioavailable to monogastric animals (human, dogs, pigs, birds) and also to agastic animals because they lack the intestinal digestive enzyme, phytase, required to separate phosphorous from the phytate molecule (Jackson et al., 1996). As a consequence of low digestibility of phytate by fish, most of the phytate-P ends up being excreted into the water and may cause algal bloom pollution (Baruah et al., 2004). Moreover phytate can also integrate with cation groups on protein, amino acids, starch and lipids in feedstuff reducing the digestibility of these nutrients in fish, poultry and pig. Phytate depresses protein and amino acid digestibility and utilisation efficiency in fish and other higher animals. The concentration of phytate and phytase in the feedstuffs varies considerably. Phytate constitute between 0.7% and 2% of most cereal grains and oilseeds (Adeola and Sands, 2003). In general plant derived fish feed ingredients such as soybean meal, rapeseed meal, and sesame meal contain 1.0-1.5%, 5.0–7.5% and 2.4% phytate respectively (Francis et al., 2001) (Fig. 1).

Non-starch polysaccharides

Dietary fibre is the portion of plant nutrient containing lignin and polysaccharides (cellulose and hemicellulose) (McDonald et al., 2002; NRC, 2011). NSPs are, hemicellulose, a complex group of polysaccharides (with the exception of starch)
containing several hundred linked monomers of hexoses and pentoses (Sinha et al., 2011). The main constituents are rhamnose, arabinose, xylose, glucose, galactose, mannose, glucuronic acid and galacturonic acid. Arabinoxylans (the arabinose and xylose fractions) make up 60 – 70% of the endosperm wall in most cereals with the exception of rice and barley where the percentages are 40% and 20% respectively.

Soybean meal the most highly utilised plant-based ingredient, contains significant amounts of NSPs (Ogunkoya et al., 2006). Raw soya beans contain approximately 200 g kg\(^{-1}\) NSP (Refstie and Svihus, 1999) and cereals 100 – 200 g kg\(^{-1}\) of NSPs in soluble and insoluble forms (Castanon et al., 1997). RB contains approximately 20 – 25% NSP which consist of equal portions of cellulose and arabinoxylans (Choc, 1997). Arabinoxylans are also the major NSP in maize (NRC, 2011) (Fig. 2).

Unlike the structure of starch, NSPs are composed of different monomers linked by \(\beta\)-glycosidic bonds. The digestion of starch is facilitated by \(\alpha\)-amylase, \(\alpha\)-glucosidase and oligo-1,6-glucosidase, specialized enzymes for hydrolysing \(\alpha\)-glycosidic bonds (Sinha et al., 2011).

In herbivores and some omnivores, the activities of these enzymes range from high to medium, negating the need for exogenous additives. Monogastrics, however, do not produce enzymes such as \(\beta\)-xylanase or \(\beta\)-glucanase that can hydrolyse the bonds found in NSPs (Sinha et al., 2011).

**Protease Inhibitors (PIs)**

One of the main limitations of using high inclusions of plant-based feedstuff is their comparatively low quality protein content (López et al., 1999). The presence of PIs reduce the activities of proteolytic digestive enzymes (\textit{i.e.} protease). Proteases are enzymes that catalyse the hydrolytic cleavage of specific peptide bonds in their target proteins (Habib and Fazili, 2007). PIs are therefore proteins that form complexes with specific proteases (\textit{e.g.} trypsin, chymotrypsin, etc) and suppress their activity along the GI trace (Krogdahl et al., 2010). In essence Protease Inhibitors are natural anti-metabolic proteins which interfere with the digestive processes and protein utilization, similar to the effects seen with phytate (Alarcon et al., 1999).

Protease Inhibitors are found in nearly all plants accounting for 1-10% of total protein and are abundant in storage organs such as seeds and tubers (Wait et al., 2009). PIs represent 6% of the protein present in soybean and despite the efficiency of processing, residual levels may remain (Mikie et al., 2009). Although some PIs are heat-labile and can be eliminated using thermal treatments (\textit{i.e.} pelleting), some researches argue that technological treatments do not always guarantee elimination of trypsin inhibitor (a type of serine protease inhibitor) in feeds (Lopez et al., 1999). However, other studies have confirmed that heat treatment typically used in the extrusion process (>120\(^\circ\) C) for fish feed may be sufficient to inactive most of the trypsin inhibitor activity in untreated SBM (Romarheim et al., 2005).

**Enzymes**

Enzymes are basically a type of protein in biological systems. They are generally used as catalysts in order to catalyze the rate of reaction. Enzymes catalyze the reaction to convert complex substances into absorbable substances. The catalysis reaction is very specific to substrates. Feeding these enzymes in the aquaculture sector has some nutritional advances. Since last few years and will also aid in reducing the effects of anti-nutritional factors, improve the dietary energy resulting in better performance of fish/shrimps.
Sources of enzymes

Enzymes are produced in all the living organisms right from simple unicellular organisms to complex higher forms of life. Various microorganisms are involved in enzyme production including bacteria from Bacillus group, fungus from Aspergillus groups and yeasts. There are few microbes in the digestive tract of animals which are potent in production of proteolytic enzymes and cellulose. Incorporation of live microbes in feed can produce enzymes. Microbial fermentation technique is widely used in large scale commercial applications.

Feed enzymes

Stability of enzymes is important in order to incorporate them in feed. Heat stability is an important parameter to be considered. Feed enzymes in the form of granules help enzymes to stay for longer time durations and are suitable for pelletization process. Efficiency of feeds needs to be at maximum for economical operations. There are various kinds of enzymes which include phytase, xylanase, cellulase, lipase, protease, amylase and many more which can increase the nutrient availability, nutrient absorption during digestion, increase the rate of fish growth and assist survival of fish in early stages of life. In addition, it makes the feeds more economical. Enzyme application may give a solution of high larval mortality of aquatic animals. Feeding larvae with enzymes would be beneficial.

Advantages of feed enzymes

Aid in improvement of digestion and absorption of nutrients such as fat and proteins
Improves metabolizable energy of diet
Lead to increased feed intake, gain in weight
Improves digestibility of nutrients
Reduces production of ammonia

Phytases

Phosphatases are a diverse group of enzymes that catalyse the hydrolysis of phosphomonoester bonds of various phosphate esters. Phytases are a sub-group of phosphatases with specificity for hydrolysing phytate into phosphoric acid and myo-inositol phosphate (Haros et al., 2005), with complete hydrolysis yielding one molecule of inositol and six molecules of inorganic phosphate (Makhode, 2008). This action reduces the chelation capacity of phytate (Kumar et al., 2012).

Phytase activity was first detected many decades ago in rice bran (Suzuki et al., 1997). Warden and Schaible (1962) are the earlier to verify that exogenous phytase improve phytate-P use and bone mineralization in poultry. However, before 1990s, the application of phytase has mainly been confined to poultry and swine to improve utilization of plant P. Initial commercial phytase, Natuphos was created from Aspergillus niger and was released in market in 1991 (Selle and Ravindran, 2007).

Following the prologue of commercial phytase, more emphasis were given to evaluating the effects of supplemental phytase on nutrient utilization and growth of common aquaculture species such as rainbow trout (Forster et al., 1999), common carp (Cyprinus carpio L.) (Schaffer et al., 1995), channel catfish (Ictalurus punctatus) Li and Robinson, 1997), African catfish (Clarias gariepinus) (Van Weerd et al., 1999). Atlantic salmon (Salmo salar) (Storebakken et al., 2000), stripped bass (Morone saxatilis) (Papatryphon et al., 1999), and Nile tilapia (Oreochromis niloticus) (Liebert and Portz, 2005).
Phytase application in aquaculture

Enhancement in phosphorus bioavailability

Various scientists around the world reported a positive effect of phytase supplementation on total P availability in fish. The following table 3 shows that the bioavailability of P when phytase is added in the feed ingredients for different fishes.

It was seen that exogenous phytase was substantially efficient in enhancing the bioavailability of P and thus reducing the amount of faecal-P. Supplementation of phytase in fish feed reduces the phosphate load in water from fish and ultimately prevents phosphate induced algal bloom contamination. Any reduction in P excreted by fish and other animals is of benefit to both the environment and sustainable production.

Enhancement of bioavailability of other nutrients and minerals

The concentration of minerals in plasma, bone and whole body will be increased by the addition of phytase in fish feeds (Jackson et al., 1996; Van Weerd et al., 1999; Papatryphon and Soares, 2001; Debnath et al., 2005; Liebert and Portz, 2005). Supplementation of phytase at a level of 1000 FTU/kg diet was sufficient to enhance Ca, Mg and Mn content of bone in channel catfish, and addition of phytase at a level of 8000 FTU/kg feed significantly increased the bioavailability of naturally occurring Zn from feed (Yan and Reigh, 2002). Phytase supplementation in rainbow trout increased the apparent absorption of Ca, Mg, Cu, Fe, Sr and Zn in low-ash soybean meal diet (Sugiura et al., 2001). Baruah et al., (2005) conducted an experiment on rohu fingerlings and found that Phytase-supplemented groups in general recorded significantly (p < 0.05) higher percentage of bone ash and also higher concentration of bone Ca and P compared with the non-supplemented group. These results were similar to those observed for rohu (Baruah et al., 2005), common carp (Schafer et al., 1995), and other fish species (Storebakken et al., 1998; Papatryphon et al., 1999; Yan and Reigh, 2002; Debnath et al., 2005b; Liebert and Portz, 2005). From these studies it can be concluded that bone ash and bone P are sensitive indicators of the P status in fish. This is because the P requirement for maximum bone mineralization is greater than maximum body weight gain. Insufficient P intake leads to the mobilization of P from the bone and transfer to soft tissues and metabolic processes (Baeverfjord et al., 1998). Phytase supplementation results increment in bone ash in fish feed that is an indication of the increased mineral bioavailability in fishes (Baruah et al., 2005; Debnath et al., 2005).

Phytase supplementation also enhances digestibility of minerals which are bound to phytate. Addition of phytase in a semi-purified diet containing 50% soybean meal in rainbow trout significantly improved the apparent digestibility of Zn (Cheng et al., 2004). Moreover, dietary phytase have been shown to increase the apparent availability of protein, ash, Ca, Cu, Mg, Fe, Sr and Zn in low ash diets while little effect in high ash diets (Sugiura et al., 2001).

Cheng and Hardy (2004) reported that graded level of phytase inclusion in the rainbow trout diet did not affect body composition; whereas, it was effective in releasing most minerals and trace mineral. This result showed that supplementation of trace minerals in rainbow trout diets can be reduced when phytase is added in the diet. Schafer et al., (1995) observed that P excretion was lower by 30% on feeding a diet supplemented with phytase compared to a diet supplemented with mono calcium phosphate.
Enhancement of protein and amino acid digestibility

Nonselectively phytase binds with proteins and inhibits the activities of pepsin, trypsin and alpha-amylase (Liener, 1994) as well as to decrease protein digestibility. De-phytinization of dietary phytate by exogenous phytase accounts for increased protein utilisation in common carp (Schafer et al., 1995), Atlantic salmon (Storebakken et al., 1998; Sugiura et al., 1998), Seabass (Olive-Teles et al., 1998), Tilapia (Heindl, 2002) and pangus (Debnath et al., 2005b) by corrupting the pre-formed phytate–protein complex. Forster et al., (1999) assessed the potential of using dietary phytase to improve the nutritive value of canola protein concentrate diets for rainbow trout. Similarly, chemical and enzymatic processing of canola meal efficiently lowered most of the anti-nutritional factors in rainbow trout. The digestibility and nutritional value of expeller and solvent-extracted Australian canola meals when included in the diets of juvenile red seabream (Pagrus auratus) was comparable to those of the fishmeal (Glencross et al., 2004). 6.6% phytase supplementation of 500 FTU/kg diet improves digestibility of crude protein in Crucian carp (Lie et al., 1999).

Phytase supplementation in expelled soybean diet of rainbow trout increased ADC of amino acid significantly compared to raw soybean but had no significant effect when added in extruded soyabean (Cheng and Hardy, 2003). Spraying soybean meal-based diets with phytase improves protein digestibilities in rainbow trout (Vielma et al., 2004). Phytase supplemented diet in pangus increased apparent net protein utilisation (Debnath et al., 2005) and apparent protein digestibility and were significantly (p<0.01) higher at a minimum supplement of 500 FTU/kg or higher in contrast to diet without phytase. There is discrepancy among authors for the positive impact of phytase on protein and amino acid bioavailability. Research conducted on rainbow trout by Predergast et al., (1994) and Teskeredzic et al., (1995) showed that pre-treatment of rapeseed protein concentrate with the enzyme phytase did not improve the protein utilisation by rainbow trout. Similarly, no positive effect of phytase on protein digestibility could be noted in rainbow trout (Lanari et al., 1998), Atlantic salmon (Storebakken et al., 1998) and striped bass (Papatryphon et al., 1999).

Similarly Riche et al., (2001) reported that Nile tilapia offered diet with and without phytase showed no difference in protein utilisation, and also concluded that the available methionine and lysine decreased with increasing incorporation of phytase pre-treated soybean meal. Phytase addition in poultry, pigs and swine diets also showed conflicting results as observed for fish. The probable reason for the neutral and/or negative interaction of phytase and amino acids is that removal of phytate may increase the efficiency of other anti-nutritional factors and protect amino acids from degradation, or decrease leaching of water soluble components (Cao et al., 2007). More research is needed to obtain a better insight into the mechanisms for the phytase-protein interaction and availability of proteins and amino acids.

Enhancement of growth performance

Supplementation of phytase-containing diets neutralises the negative effects of phytate and increases growth in fish. Positive impact of phytase on growth of fish has been reported by a number of authors: Jackson et al., (1996) in channel catfish, Vanweerd et al., (1999) in African catfish, Papatryphon and Soares (2001) in striped seabass, Vielma et al., (2000) in rainbow trout, Liebert and Portz (2005) in tilapia, Debnath et al., (2005) in pangus. Nwanna et al., (2005), in common carp and Baruah et al., (2007a) in rohu. These authors have demonstrated phytase hydrolysis in
plant-based diets by phytase and improvement of fish growth and mineralization. Diet containing 250 FTU phytase per kg increases the feed intake and increases the weight than the control diet containing no phytase (Li and Robinson, 1997). Increase in weight gain from 243 to 459% in rainbow trout fed soybean meal-based diets with phytase and phosphorous supplementation (Vielma et al., 2004). Similar results were reports in salmonids (Sugiura et al., 2001). Nwanna and Schwarz (2007), Nwanna et al., (2007) found better growth observed in common carp fed a diet (incubated plant feed ingredients) containing phytase than another diet (without incubated plant feed ingredients) with and without phytase. This is probable because incubation process reduce phytate content of feed improve phosphorous and mineral usage as compared to untreated diet. The optimal growth of Nile Tilapia is achieved by phytase supplementation at 750-1250 FTU/kg in plant-based diets (Liebert and Portz, 2005). Addition of phytase at 1500 FTU/kg diet in contrast to no inclusion of phytase enhanced the weight gain of rainbow trout (Vielma et al., 2001). No considerable effect of phytase supplementation was noticed on performance of large sized rainbow trout fed diet supplemented with phytase at 1000 FTU/kg (Vielma et al., 2000) (Fig. 3).

No effect on growth performance, protein digestibility, energy retention on phytase addition in the diet of sea bass (Olivia-Teles et al., 1998). Forster et al., (1999) and Sajjadi and Carter (2004) did not report any improvement in the growth of rainbow trout and Atlantic salmon when fed with canola protein concentrate incorporated with phytase. Similarly Masumoto et al., (2001) and Yoo et al., (2005) reported no effect of dietary phytase on weight gain of Japanese flounder and Korean rockfish (Sebastes schlegeli). The discrepancy in above findings may be associated with differences in their diet composition and also with different rearing conditions, (Baruah et al., 2007). Supplementing exogenous microbial phytase in feed ration exhort an enhancement in growth rate and performance which could be attributed to various factors, in individual and combine form namely better bio-availability of phosphorous (Rodehutscord and Pfeffer 1995; Vielma et al., 2000; Baruah et al., 2007) and minerals (Vielma et al., 2004; Debnath et al., 2005b), improved protein digestibility (Vielma et al., 2004; Debnath et al., 2005a; Liebert and Portz 2005; Baruah et al., 2007a) and increased absorption of nutrients owing to well functioning of the pyloric caeca region of the intestine (NRC, 1993).

**Reduction in pollution from aquaculture operation**

Discharge of high levels of soluble P from fish culture systems into open water environment stimulate phytoplankton growth, resulting in wide fluctuations in dissolved oxygen concentrations (Li et al., 2004). Many studies have reported a clear effect of phytase supplementation in reducing P excretion from fish. Total phosphorous effluent was significantly lowered when fish cultured with a diet enriched with phytase (200 FTU/kg) (Ai et al., 2007). Similarly, soybean meal based diets supplemented with phytase decreased the excretion of phosphorous from red sea bream and maximum reduction was reported at 2000 FTU/kg feed (Biswas et al., 2007b). Comparable results were observed in rainbow trout (Sugiura et al., 2001). Faecal waste of P in rainbow trout was reduced by phytase supplementation in soybean protein concentrate diet (Vielma et al., 1998) and a significant decrease was noticed when practical feed supplemented with phytase at a level of 2000 FTU/kg was fed (Vielma et al., 2001). Phosphorus concentration in faecal matter was reduced when trout were fed a diet with phytase supplemented at 500 and 1000 FTU/kg compared to non-supplemented feed (Verlhac et al., 2007). Soybean based phytase
supplemented diet considerably lower excretion of phosphorus compared to the fishmeal diet fed to Atlantic salmon (Storebakken et al., 2000). Phosphorus content of faeces was also reduced in Atlantic salmon fed a phytase supplemented diet (Sajjadi and Carter, 2004). Microbial phytase supplementation in the diets of juvenile catfish reduced the excretion of faecal phosphorous about 60% (Li and Robinson, 1997). Many studies suggest potential environmental benefits to the extent of 30% to 40% reduction in P excretion (Omogbenigun et al., 2003)

**NSP-enzymes**

A greater concern is the high content of indigestible carbohydrates such as non-starch polysaccharides (NSP) which dilute the dietary energy and protein concentration and reduce feed digestibility, content of anti-nutritional factors that affects fish health, nutrient utilization and growth, and reduced digestibility/bioavailability of nutrients due to extensive processing (Stone, 2003; Sørensen et al., 2010). Therefore, processing are used to increase the protein content and reduce the level of NSP in plant ingredients used in feeds for carnivorous fish like Atlantic salmon (Salmo salar). Genetical selection and optimization of growing conditions can also be used to optimize nutrient content of plants.

Non-starch polysaccharides (NSPs) can be water soluble or insoluble. Soluble NSPs such as arabinoxylans swell and form viscous gels when hydrated in the intestine, thus preventing secreted enzymes from reaching digestible substrates, and impeding digested nutrients from migrating to the gut wall for absorption. Insoluble NSPs such as cellulose and lignin induce a “cage” effect, and nutrients are trapped within the folds of the NSP molecules. Ronozyme®WX (xylanase) works to reduce the viscosity of NSP gels, and breaks down insoluble NSPs as well as improving assimilation of digested peptides and fats.

Numerous studies have however recently shown beneficial effect from hydrolysed products from NSP, so called prebiotics, on fish growth and health (reviewed by Ringo et al., 2010). These could either be included in the feeds as prebiotics or indirectly given to the fish by adding exogenous enzymes in fish diets that hydrolyse NSP (Stone, 2003; Sinha et al., 2011). In recent times, many researchers focussing on NSP enzymes in fish feeds. These have been studied and utilised in swine and poultry industry for several time (Khattak et al., 2006). NSP-enzymes include glucanases, pentosanases, cellulosases and xylanases. These enzymes hydrolyze NSP to products available for bacteria as prebiotics or for the fish as digestible nutrients (Sinha et al., 2011). Supplementation of these have also shown to improve protein utilization and growth in fish (Ai et al., 2007; Jiang et al., 2014) (Table 4).

Xylanase is a class of enzymes that degrades linear polysaccharides, and breaks down hemicelluloses that are the major component of the cell wall from plant (Ganguly et al., 2013). This enzyme have proven to be especially efficient in maize-soy-based diet to broilers where the enzyme disrupts the plant cell wall that allows water hydration and entering of endogenous enzyme to act for a better digestion of starch and proteins (Sinha et al., 2011). Xylanases are naturally produced in numerous yeasts, fungi and bacteria (Goswami and Pathak, 2003). Ronozyme®WX (1000 U xylanase/g) from DSM Nutritional Products (Switzerland) has been used in several fish experiments. Ai et al., (2007) showed that Japanese seabass (Lateolabrax japonicas) at 6 g fed a diet of plant protein as soybean meal (170 g/kg), rapeseed meal (100 g/kg) and peanut meal (100 g/kg), improved growth and protein utilization, by inclusion of 800 mg/kg diet of Ronozyme®WX. There are also available commercial enzyme complexes where xylanase is present in combination with other
enzymes like proteases and NSP enzymes. These were tested in several fish studies with variable results. Tilapia fed diets with soybean meal (170 g/kg), rapeseed meal (170 g/kg) and cottonseed meal showed improved growth, feed conversion and endogenous enzyme activities with increased inclusion (0, 1 and 1.5 g/kg) of a commercial enzyme complex (Yingheng Biotechnology, China) with xylanase (1600 U/g), protease and β-glucanase (Lin et al., 2007). The ingredients was mixed and cold pelleted through an experimental feed mill. Shahsavai (2011) showed that common carp (30 – 50 g) fed diets with wheat bran (340 g/kg), soybean meal (150 g/kg) and cottonseed meal (140 g/kg) supplemented with 1, 2 and 3 g/kg diet of an enzyme complex (Endofeed W, GNC Bioferm, Canada), with xylanase (≥1200 IU/g), β-glucanase, cellulase and hemicellulase had no effect on feed conversion and growth. Farmazyme® (Famavet, Turkey) a multi enzyme complex containing fungal xylanase, glucanase and other enzymes have shown to improve growth and protein content in 46 g African Catfish (Claris gariepinus) (Yildirim and Turan 2010). The enzyme complex was mixed with water and a pulverized trout diet at 0, 0.25, 0.5 and 0.75 g/kg diet, and ground with a 2 mm die plate. Growth and protein content was significantly improved at level of the enzyme complex above 0.5 mg/kg diet. As mention earlier, however, some of these herbivorous freshwater species have naturally occurring enzyme producing yeasts in their gut, which improve the carbohydrate digestibility. Therefore, supplementation of enzymes may perhaps have larger effects on carnivorous fish species.

Proteases

Digestibility of protein and amino acids in alternative ingredients of plant and animal origin can be improved by adding protease enzyme to feeds. ProAct protease (DSM, Switzerland) is at the moment the best solution for improving protein digestibility available to the feed industry. Experiments using an in vitro poultry gut model show significant improvements in ingredient digestibility when ProAct is provided on top of endogenous digestive enzymes, and results are not expected to be different with fish. The adoption of protease by the aquafeed industry is just beginning, so there is not much information available on the benefits of protease.

However, Dalsgaard et al., (2012) were able to show a significant improvement in apparent digestibility of soy (34% inclusion level in the feed) and a significant decrease in solid N waste excretion when protease alone or protease combined with xylanase was added to rainbow trout feed. Plant ingredients such as soy, rapeseed and canola contain trypsin inhibitors that stop trypsin from cutting protein into peptides before further digestion by other proteases in the intestine.

ProAct has been shown to digest trypsin inhibitor proteins, thus improving digestive function; It is less specific in selecting active sites on proteins for digestion than trypsin, hence it actually accelerates the initial stages of protein breakdown. In an experiment with tilapia with three different protein levels and three different dosages of ProAct enzyme (Verlhac and Diaz, 2012), apparent protein digestibility was improved from 2–4% in a 31% crude protein (CP) diet, and from 3-8% for 28 and 26% CP diets (Table 2), suggesting that in feeds with lower quality protein the benefit of using protease may be greater. Protease, then, has a lot of potential to improve digestibility of all types of protein ingredients, and will assist nutritionists in formulating feeds that are more digestible and less polluting, while at the same time offering the possibility of choosing less expensive ingredients to control formulation costs.
Table 1 Processing steps for removal/inactivation of ANFs (Nwanna 2007)

| Anti-Nutrient         | Heat Sensitivity | Extraction | Other Treatment |
|-----------------------|------------------|------------|-----------------|
| Phytic Acid           | No               | No         | Phytase         |
| Arabinoyxylans (NSP)  | No               | No         | Xylanase        |
| Protease inhibitors   | Yes              | No         | Protease        |
| Hemagglutinin         | Yes              | No         | No              |
| Saponin               | No               | Yes        | No              |
| Phytoestrogen         | No               | Yes        | No              |

Table 2 List of anti-nutrients in plant sources

| Antinutrients                  | Chemical name                                                                 | Plant source                                  | Source                                                                 |
|--------------------------------|-------------------------------------------------------------------------------|-----------------------------------------------|------------------------------------------------------------------------|
| Phytic acid or Phytate-P       | Myoinositol 1,2,3,4,5,6-hexakisdihydrogen phosphate                           | Cereal and legumes                            | (Khokar and Apenten, 2003)                                            |
| Non-Starch Polysaccharides     | e.g. Arabinoyxylans (arabinose and xylose)                                   | Cereals (wheat, rye, barley, rice, sorghum)   | (Sinha et al., 2011)                                                  |
| Protease Inhibitors            | e.g. Trypsin inhibitor                                                       | Most plants particularly legumes and cereals | (Francis et al., 2001; Krogdahl et al., 2010)                          |

Table 3 Total P and phytate-P in common plant-based ingredients. Source: Kumar et al., (2012) and Ravindran et al., (1994)

| Ingredients | Total P (g kg-1) | Phytate-P (g kg-1) | Proportion of Phytate-P in Total P (%) |
|-------------|------------------|--------------------|----------------------------------------|
| Maize       | 2.40             | 2.05               | 85.4                                   |
| Corn        | 2.50             | 1.70               | 73.0                                   |
| Rice        | 1.20             | 0.80               | 65.0                                   |
| RB          | 17.51            | 15.83              | 90.2                                   |
| Soya bean   | 5.55             | 3.08               | 55.5                                   |
| SBM         | 6.66             | 4.53               | 68.3                                   |
| Cassava     | 1.60             | 0.40               | 25.0                                   |
**Table 4** NSP comparison of major plant-based ingredients (in g kg⁻¹), NRC (2011)

| Ingredients   | Total NSP | Arabinoxylans<sup>a</sup> | Other fractions<sup>b</sup> |
|---------------|-----------|----------------------------|-----------------------------|
| Rice Bran     | 218       | 85                         | 133                         |
| Corn          | 81        | 52                         | 29                          |
| Maize         | 97        | 52                         | 45                          |
| Soya beans    | 192       | 47                         | 145                         |
| SBM           | 196       | 42                         | 154                         |

**Table 5** Commercially available microbial phytases (Sources: Hou, 2001; Srefan et al., 2005; Cao et al., 2007)

| Company          | Country       | Phytase Source | Production Strain | Trademark        |
|------------------|---------------|----------------|-------------------|------------------|
| AB Enzymes       | Germany       | *Aspergillus awamari* | *Trichoderma reesei* | Finase           |
| Aiko Biotechnology | Finland     | *A.oryzoe*      | *A.oryzoe*         | SP, TP, SF       |
| Alltech          | USA           | *A.niger*       | *A.niger*          | Allzyme Phytase  |
| BASF             | Germany       | *A.niger*       | *A.niger*          | Natuphos         |
| Biozyme          | USA           | *A.oryzoe*      | *A.oryzoe*         | AMAFERM          |
| DSM              | USA           | *P.Lyci*        | *A.oryzoe*         | Bio-feed phytase |
| Fermic           | Mexico        | *A.oryzoe*      | *A.oryzoe*         | Phyzme           |
| Finnfeeds        | Finland       | *A.awamari*     | *T.reesei*         | Avizyme          |
| International    | USA           | *Penicillium simplicissimum* | *Penicillium funiculosum* | Rovabio         |
| Genencor         | USA           | *Penicillium simplicissimum* | *Penicillium funiculosum* | Rovabio         |
| Roal             | Finland       | *A. Awamari*    | *T.reesei*         | Finase           |
| Novozymes        | Denmark       | *A.oryzoe*      | *A.oryzoe*         | Ronozyme         |
### Table 6

| References                          | Fish Feed ingredients (plant protein sources)                                                                 | P availability (%) without phytase | Phytase dose FTU Kg diet | P availability (%) with phytase |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------|------------------------------------|--------------------------|---------------------------------|
| **Rainbow trout** *(Oncorhynchus mykiss)* | Canola meal, solvent extracted soybean meal, full fat soybean, peanut meal, corn gluten meal, cotton seed meal, canola meal, Barley, wheat | 4.8;(-13.4)8.4; 22.1; 30.7; NA     | 3.8 x 10^6               | 46.2; 46.6; 64.4; 75.6; 76.8; 56.3 |
| Riche and Brown (1996)              |                                                                                                              |                                    |                          |                                 |
| Cherg and Hardy (2002)              | Canola meal, Barley, Wheat                                                                                  | 12.2; 79.4; 61.6                   | 500                      | 41.8; 82.7; 64.6                |
| Cherg and Hardy (2003)              | Raw soybean, expelled soybean; Extruded full fat soybean                                                    | 21.2; NA; 12.5                     | 750; 200; (200, 400, 600, 800, 1000) | NA; 31.7; (81.3, 92.2, 89.7, 95.2, 93.9,) |
| Vielma *et al.*, (2006)             | Rapeseed meal, soybean meal; corn gluten meal, sunflower meal;                                               | (-1.0); 48.3; 61; (-0.9); 45.0; 65.2 | 750                      | 53.8; 85.2, 118; 45.7, 72; 846 |
| Verlac *et al.*, (2007)             | Soy protein concentrate; Pea meal, Faba bean meal                                                             | 29.9; 74.1; 47.8                   | 750                      | 46.9; 80.3; 69.9                |
| **Nile Tillapia** *(Oreochromis niloticus)* |                                                                                                              |                                    |                          |                                 |
| Verlac *et al.*, (2007)             | Soybean meal. Palm kernel cake, rice bran, corn, cassava                                                     | 47.9, 25.5; 35.; 23.6; 72.4         | 750                      | 76.9; 50.4; 59.5; 58.3; 92.6    |
| **Sea bass** *(Dicentrarchus labrax)* |                                                                                                              |                                    |                          |                                 |
| Papatryphon and Soares (2001)       | Isolated soy protein; soybean meal, corn gluten mean, wheat middings                                        | 48; 59; 52; (-10)                  | 1000                     | 74; 87; 70; 11                  |

**Fig.1** Chemical structure of phytate-phosphorus showing its chair-like conformation. Source: Adeola and Sands, (2003)
Fig. 2 Chemical structure of arabinoxylan [Source: Sinha et al., (2011)]

Fig. 3 Hydrolysis of phytate by phytase. Source: Kumar et al., (2012)
Benefits of combining enzymes

The cooperativity of enzymes to degrade feedstuff and their interactions require much research. The benefits of combining phytases and xylanases have been demonstrated to some extent in broilers (Bedford, 2000). Several enzyme companies (Novozyme/Royal DSM, Altech, Ameco-Bio & Cp., Canada Bio-Systems Inc etc.) are now producing enzyme cocktails to improve, even further, the efficiencies of feed utilisation, particularly those with high inclusions of plant-based ingredients, and the synergistic benefits for animal performances. Combining enzymes may provide additional benefits, in that, different enzymes act in different location along the GI tract and target different substrates (Walk, 2009).

Considerable effects of multi-enzyme supplementation on ADC of DM, CP, nitrogen free extract (NFE), P and GE in SBM-based diets fed to rainbow trout (Ogunkoya et al., 2006). Using a similar commercial enzyme complex, higher FI was recorded with tilapia fed diets containing 0.15 g kg⁻¹ but no difference were observed in protein, lipid and GE ADCs between treatments (0, 0.15 and 1.0 g kg⁻¹) (Lin et al., 2007). Khalafalla et al., (2010) also showed the addition of Amecozyme in diets at 0.5% and 1.0% enhanced the growth performance of O. niloticus fingerlings. Similarly, a cocktail containing protease, xylanase, glucanase, lipase, amylase and cellulase was used to supplement five grain diets fed to tilapia which improved fish performance, nutrient digestibility, carcass characteristics and faecal recovery (Soltan, 2009) (Table 5).

Economic benefits of supplementation

The use of enzymes must sufficiently demonstrate substantial improvements in feed conversion or product quality to cover any adjustments in formula cost resulting in higher profit margin (Chesson, 1993). In other words, they must somehow improve upon least-cost formulation by lowering input cost while maximizing outputs in terms of animal performance, health and cost to produce one unit of animal protein. Economic benefits of using phytase are by far more straight forward than those of xylanases and proteases. Phytase delivers direct cost benefit by replacing the need for inorganic phosphate (Bedford, 2000). The benefits of reducing P load and feed formulation cost are clear, and as a result phytase is now considered a standard feed additive. Though most enzyme studies acknowledge supplementation-related formula cost savings, rarely are these figures published for reference (Table 6).

Enzyme research for the future

With the increasing use of more plant ingredients such as rice bran, wheat bran, copra meal, and palm oil milling byproducts in aqua feeds, there is merit in improving digestion of plant cell walls to unlock valuable nutrients trapped inside cells. Cell walls of cereals (wheat, corn, barley, rice) are mainly made of arabinoxylans and β-glucans, whereas oilseed crops (soy, canola, rapeseed, sunflower) are mainly xylolglucans and pectins. Feed enzymes that digest cellulose, xylans, glucans, mannans and pectins are now widely used in livestock and poultry feeds, but have yet to be applied to aqua feeds. Adding phytase and xylanase together with protease improves protein utilization the most, with phytase reducing phytic acid-protein interactions, and xylanase improving protein, peptide and amino acid migration in the intestine in feeds containing large quantities of NSPs. Research is focussed to investigate combination of enzymes to further improve the feed efficiency of fishes.

In conclusion, flourishing and sustainable aquaculture depends on efficiently viable and environmentally responsible aqua feeds. Feed
is the major working cost involved in intensive farming of aquatic organisms. The major feed ingredient, fishmeal, is expensive and there is increasing competition with other livestock industries for the available supply. Hence, researchers are focussing to find alternatives to fishmeal. Substitution of fishmeal with plant proteins supplemented with feed enzymes is an effective alternative in aqua feeds. Enzymes play a significant role in formulating cost effective, high quality and eco-friendly aqua feeds. At present, the use of enzymes in aqua feeds can reduce use of fishmeal which ultimately reduces the cost fish production. This may help to reduce the demand for fishmeal from the aquaculture sector in coming years.

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