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Xylanase supplementation in fish feed

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Summary/recommendation:

Plant ingredients have so far been the most cost efficient alternative for fish in the aquafeed industry. This has increased the dietary content of indigestible carbohydrates, including non-starch polysaccharides (NSP) which dilute the energy concentration of the feeds and reduces digestibility/bioavailability of nutrients. Enzymes that hydrolyse NSP (NSP-enzymes) for better digestibility and growth performance have been introduced in the swine and poultry industry, and have also been studied in some fish species. Xylanase, an NSP-enzyme that hydrolyse cell wall components in plant, efficiently reduce NSP content in plant ingredient. Xylanase are found in yeasts, fungi and bacteria that are present naturally in the gut of some herbivorous fish species, such as carp and tilapia. This enzyme is commercially available alone or in multi-enzyme complexes for use in pre-processing of ingredients/diets or supplementation of diets during feed production. Dietary supplementation of xylanase have improved protein utilization and growth in several fish experiment. In addition some studies have reported increased endogenous enzyme production or prebiotic effects on the intestinal microbiota, which may lead to improved growth and health. To our knowledge, no peer-review publications exist on the use of xylanase as a supplement in feeds for Atlantic salmon.

Summary/recommendation in Norwegian:

Fôringredienser fra planter har så langt vært det mest kostnadseffektive alternative til fisk i fiskefôr. Dette har i midlertidig økt innholdet av ufordøyelige polysakkarider som reduserer energiinnholdet i fôret og reduserer fordøyeligheten av næringsstoffer. Tilførsel i fôret av enzymer som bryter ned polysakkarider for økt proteinfordøyelighet og vekst har vært studert og er i bruk i produksjonen av svin og fjørfe, og nå også studert i fisk. Xylanase, ett naturlig enzym som bryter ned polysakkarider i plantespisende fisk, er kommersielt tilgjengelig som enzym-preparat alene eller i kombinasjon med andre enzym. Flere studier har vist at tilførsel av xylanase i fôr øker proteinfordøyelighet og vekst hos fisk fôret planteingredienser. I tillegg er det rapportert om økt produksjon av endogene enzymer og prebiotiske-effekter på tarmfloraen for en bedre vekst og helse hos fisk gitt xylanase. Tilførsel av xylanase i fôret til Atlantisk laks er ikke dokumentert. Bruk av xylanase i fôr til laks må dermed studeres for økt utnyttelse av polysakkarider fra planteingredienser.

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1 Challenges for fish feeds into a new era

Aquaculture production accounts for almost half of the world total fish supply. Capture fisheries and aquaculture supplied the world with 154 million tons fish in 2011 of which 128 million tons were used as human food (FAO, 2012). In 2008, 53 % of the world´s wild fish stocks were fully exploited, 28 % were overexploited, 3 % depleted, 1 % recovered from depletion, and the remaining 15 % were underexploited or moderately exploited (FAO, 2010). 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. Plant ingredients have so far been the most cost efficient alternative, and feeds for Norwegian farmed salmon have changed from a marine based diet (90 % marine ingredients) to a plant based diet (30 % marine ingredients) (Ytrestøyl et al., 2014). The major high protein plant ingredients in Norwegian salmon diets are soy protein concentrate (24 %) and wheat/wheat gluten (17 %) (Ytrestøyl et al., 2014), but increased use of other plant ingredients have to be considered for further growth in the aquaculture production.

1.1 Dietary carbohydrate utilization by fish

The most important challenges with plant products as protein sources in feeds for carnivorous fish are: low level of protein, high level of carbohydrates, unfavourable amino acid profile and content of other nutrients, and the presence of anti-nutritional factors (Gatlin et al., 2007; Sørensen et al., 2010). Unfavourable amino acid composition and imbalanced nutrient composition can be balanced by combining ingredients of different origin and use of additives such as amino acids, vitamins and minerals (Sørensen et al., 2010). 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). Fish do not have endogenous enzymes for hydrolysis of the linked monomers of hexoses and pentoses in NSP, e.g. β-glucanases and β-xylanases. 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. Numerous studies have however recently shown beneficial effect from hydrolysed products from NSP, so called prebiotics, on fish growth and health (reviewed by Ringø et al. (2010)). These could either be included in the feeds as prebiotics or indirectly given to the fish by adding exogenous enzymes in the feeds that hydrolyse NSP (Stone, 2003; Sinha et al., 2011).

1.2 Supplementation of prebiotics in fish feed

The term "prebiotics" are used for non-digestible components that are metabolised by health-promoting bacteria. These bacteria are considered beneficial to the health and growth of the host by decreasing the presence of intestinal pathogens and/or changing the production of health related bacterial metabolites from prebiotics (Roberfroid, 1993; Ringø et al., 2010). Prebiotics are in this sense energy sources for beneficial bacteria, and diversity and abundance of these bacteria could in theory be modulated through dietary supplement of specific prebiotics. However, some prebiotics (e.g. β-glucans) could also stimulate the innate immune system directly through ligand-receptor interaction with immune cells (Brown et al., 2002), and as such stimulate the immune system.

Prebiotic carbohydrates can be classified according to molecular size as monosaccharides, oligosaccharides and polysaccharides, but are often available commercially as oligosaccharides of glactose, fructose, glucose, xylose and/or mannose (Ringø et al., 2010; Ganguly et al., 2013).

Today, oral administration of fermentable prebiotics seems most promising in terms of a positive influence on the composition and activity of the indigenous microbiota of the gastrointestinal system. However, extensive research has been performed the last decade on oral administration of health promoting bacteria (Probiotics; e.g. *Lactobacillus*), but high costs, potential environmental impact, food safety issues and challenges regarding incorporation in modern extruded fish feed (i.e. low survival of the bacteria through extrusion) have limited the commercial implementation of probiotics (Ringø et al., 2010). Prebiotics on the other hand, pose no safety issue and are easily included in extruded feeds. An alternative to the direct supplementation of prebiotics in feeds could be addition of exogenous enzymes that may hydrolyse polysaccharides to prebiotic components in the intestine, in order for beneficial microbiota to be established. The high content of unutilised NSP in commercial diets for carnivorous fish species makes this an attractive approach. As probiotics, however, enzymes have particular requirements that may increase costs and limit the implementation and commercial use in extruded fish feed.

1.3 Supplementation of exogenous enzymes in fish feed

Exogenous enzymes have been proven to improve nutritional value of feed and decrease environmental pollution (Kolkovski et al., 1993; Bedford, 1995). Addition of exogenous phytase in fish diets resulted in increased utilization of phytate phosphorus, other trace elements and protein, and decreased discharge of phosphorus into the water (Lanari et al., 1998; reviewed by Kumar et al., 2012). In addition, several fish experiments have been performed with diets supplemented with pancreatic enzymes such as proteases (Carter et al., 1992, 1994; Drew et al., 2005; Lin et al., 2007; reviewed by Castillo and Gatlin, 2015). The authors observed improved growth, feed efficiency and protein digestibility by supplementation of exogenous enzymes to plant protein diets to fish.

Recently, an increased focus has been into research on NSP enzymes in fish feeds. These have been studied and utilized in swine and poultry industry for some time (reviewed by 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). Supplementations of these have also shown to improve protein utilization and growth in fish (Ai et al., 2007; Jiang et al., 2014).

Since commercial salmon feeds are extruded at high temperature, exogenous enzymes to be added pre-extrusion have to be modified to tolerate high temperature. The alternatives are to pre-process the plant ingredients prior to extrusion or to add the enzymes to the pellet after extrusion (Stone, 2003). Pre-processing studies with plant ingredient and NSP-enzymes have to our knowledge, not improved digestibility in salmonids (Denstadli et al., 2011). Pre-processing will lower NSP content in the ingredient/diet, however high content of NSP products in the diets may cause diarrhoea (Sinha et al., 2011) so the maximum levels at which these compounds can be included in the diets should be considered in future studies. Supplementation of NSP-enzymes in experimental diets to fish have been done either by a cold feed production process (e.g. cold pelleting) or by adding the enzymes at the stage of oil coating of the pellets (Ai et al., 2007; Dalsgaard et al., 2012; Jiang et al., 2014). Both methods avoid the heat treatment that could otherwise inactivate the enzymes. However, implementation of these experimental methods in large-scale production of fish feed is not necessarily feasible, and efforts should be made to develop protocols for implementing inclusion of exogenous enzymes in large-scale feed production. When the exogenous enzymes are meant to work after consumption by the fish, the liberation of the enzyme from the feed matrix throughout the gastrointestinal system has to be studied. Low pH and proteases in the stomach could also lower the activity of exogenous enzymes added to the feeds. The balance between the need to protect the enzymes from inactivation and the need for contact with substrates should be considered when selecting enzyme carriers.

2 Xylanase supplementation in fish feed

Xylanase is a class of enzymes that degrades linear polysaccharides, and breaks drown 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).

2.1 Source of xylanase

Xylanases are naturally produced in numerous yeasts, fungi and bacteria (Goswami and Pathak, 2003). Fungal mono-component xylanases produced by a genetically modified strain of *Aspergillus oryzae are commercially sold and used as feed additive for poultry and pigs (EFSA Journal for* Ronozyme®WX*, 2012), but have also been tested on fish.* In addition, there are a numerous commercial products available with xylanase, used pharmacologically or as food additives (Goswami and Pthak, 2003).

Gut-associated yeasts that produce xylanases are found in some freshwater fish species as a microbial symbiont (Gatesoupe, 2007). Banerjee and Ghosh (2014) characterized yeasts in six different freshwater carp species and in Nile tilapia (*Oreochromis niloticus*). They found 2 – 9 yeast strains per species, of which the yeast strains CMH6A and ONF19B in Indian major carp (*Cirrhinus mrigala*) and in Nile tilapia, respectively, could produce xylanase. Xylanases are thus available in some herbivorous fish species, and further studies should be performed to understand their function for NSP digestibility and utilization in these species.

2.2 Xylanase inclusion in fish feeds

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. The enzymes were added in the moist feed mass, and no heat was used in the feed production. Dalsgaard et al. (2012) observed no significant effects on protein digestibility and growth in 70 – 110 g rainbow trout (Oncorhynchus mykiss) by using 67 mg/kg of Ronozyme®WX added by coating after extrusion in diets with either soybean meal (344 g/kg), sunflower meal (246 g/kg) or rapeseed meal (264 g/kg). However, they observed a positive effect on the apparent digestibility of lipids when supplementing xylanase to diets containing rapeseed meal. Increased lipid digestibility has also been observed in piglets fed a diet supplemented with xylanase (Velhjen et al., 2007). In the same study reduced faecal loss of bile acid was observed and this was explained by reduced viscosity caused by the hydrolysis of NSP.

Xylanase (6000 U/g) from Huvepharma (Belgium) have been tested at increased inclusion levels (220 – 2470 U/kg diet) in diets high in soybean meal (150 g/kg), rice gluten meal (105 g/kg), cotton seed meal (150 g/kg), rapeseed meal (100 g/kg) and wheat middling (311 g/kg) to juvenile (8 g) Jian carp (*Cyprinus carpio* var. Jian). The feed was made moist with a hand-noodle maker and dried by a fan (Jiang et al., 2014). Increased inclusion up to 1480 U of xylanase per kg diet improved growth, feed and protein efficiency, while higher inclusion of xylanase gave comparable results to fish fed the diet with the lowest content of xylanase.

There are also available commercial enzyme complexes where xylanase is present in combination with other enzymes such as proteases and other NSP enzymes. These have been 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. Signor et al. (2010) observed no significantly effect on survival and growth in Nile tilapia fingerlings fed diets supplemented with 0, 0.033, 0.066 and 0.099 % of an enzymatic complex with amylase, protease, cellulose, lipase, pectinase, xylanase, β-glucanase and phytase, but feed consumption and feed conversion were improved. Kazerani and 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. 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.

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 grinded 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.

The multi-enzyme complex Natuzyme® and Hemicell® (Bioprotein, Australia) are two enzyme supplements that contain protease, lipase and several NSP-enzymes. These two enzyme complex improved growth and feed efficiencies in 60 – 80 g Caspian salmon (*Salmo trutta caspius*) when incorporated together at 0.5 g/kg trout diet (Zamini et al., 2014).

Natugrain-blend® (a commercial enzyme supplement produced by BASF in liquid form that contains a mixture of β-glucanase and β-xylanase with an activity at 36.000 – 44.000 endo-xylanase units/ml) included at 300 mg/kg diet, improved dry matter and energy digestibility in 112 g Nile tilapia (Tachibana et al., 2010). However, Stone et al. (2003) did not see any significant effect on digestibility of nutrients when adding 75 – 300 µl/kg Natugrain-blend® in diets containing 30 % wheat or 30 % dehulled lupin to 40 g silver perch (*Bidyanus bidyanus*). They suggested intolerance to high content of NSP digestive product to be the reason for the lack of effect of Natugrain-blend® inclusion on nutrient digestibility. Xylanase inclusion at high levels would thus reverse the positive effects of the enzymes, due to overproduction of hydrolysis products from NSP, such as xylooligosaccharides and xylose. These compounds may cause osmotic diarrhoea that would affect fish microbiota, digestion and fish performance (Sinha et al., 2011; Jiang et al., 2014). The optimal inclusion level may vary between species and life stages, so tests should be carried out with different dosage levels to find optimum inclusion (Jiang et al., 2014). The overview of results also suggests that the effects of exogenous enzymes may depend on the amounts and composition of plant feedstuffs in the diet formulation.

2.3 Effect of xylanase supplementation in fish feed

The main effects of xylanase supplementation in fish feed seem to be increased protein utilization and growth (Ai et al., 2007; Jiang et al., 2014). Whether these effects are due to reduced anti-nutritional effects of NSP for increased utilization of protein alone or in combination with increased hydrolysis of protein-carbohydrate complexes are unknown. Similar enzymes have also been reported to increase utilization of other nutrients as lipid, starch and carbohydrates in general (Castillo and Gatlin, 2015). In the opposite direction, there are at least one study on piglets showing reduced endogenous enzyme production with supplementation of NSP enzymes (Li et al., 2004), possibly explained by reduced viscosity of the intestinal content (Ikegami et al., 1990). However, Jiang et al. (2014) showed positive change in intestinal morphology and increased activity of hepatopancreatic and intestinal enzymes in juvenile Jian carp fed diets supplemented with intermediary levels of xylanase. In addition increased activity of the enzymes located in the intestinal brush-border section was observed, indicating increased ability to absorb nutrients. Positive effects of xylanase on endogenous enzyme production have also been shown for tilapia (Lin et al., 2007). Finding the right inclusion level of xylanase could be the key to optimal effects of the enzyme on intestinal physiology and health.

Inclusion of xylanase have also been reported to give a prebiotic effect on the microbiota by the hydrolysis of NSP, which again stimulates the production of beneficial bacteria such as *Lactobacillus*, and reduce the number of harmful bacteria like *Aeromonas* and *E.coli*. Juvenile Jian Carp fed low (220 IU/kg) and high (2470 IU/kg) content of xylanase in the diets showed comparable microbiota, while fish fed an intermediate level of xylanase (1480 IU/kg) showed a significant improved microbiota with a higher abundances of beneficial bacteria (Jiang et al., 2014). In addition have xylanase shown to give general health benefits through lower visceral and liver ratio to the body, increase protein content and reduce lipid of whole body and liver (Lin et al., 2007).

The increased nutrient utilisation and growth seen after supplementation of xylanases in some studies are most likely due to a complex improvement of nutrient digestion by morphological changes (e.g. increased absorptive surface), physiological changes in hydrolysis and absorption, and changes in microbiota.

3 Concluding remarks

Dietary supplementation of xylanase has improved fish performance in several fish experiments. However, in many experiments xylanase is supplied to the diet as part of an enzyme complex with other NSP-enzymes, and a clear effect of xylanase cannot be demonstrated in these experiments. Supplementation of xylanases that have affinity for both soluble and insoluble NSP will be more effective and should be studied in fish. In addition, more knowledge about the substrate/enzyme interaction is needed in order to understand the effect of enzyme supplementation to diets with plant ingredients.

To our knowledge there are no publications describing feeding experiments in which Atlantic salmon have been fed diets supplemented with xylanase. Atlantic salmon are today fed high quality plant protein concentrates with a relatively moderate NSP content. However, new plant protein ingredients with higher NSP content have to be considered for continued growth in the salmon industry.

Xylanase catalyse the hydrolysis of NSP, other polysaccharides and protein-carbohydrate complexes for better protein digestibility and fish performance. Increased protein availability as an effect of xylanase supplementation gives a protein sparing affect that could be utilised in the feed formulation of diets. Weather these effects are similar in Atlantic salmon are unknown and should be further studied. Xylanase could be supplemented for pre-processing of ingredients/diets or during feed production. Supplementation of xylanase will increase the content of hydrolysed polysaccharides compounds depending on ingredient/dietary composition and amount of enzyme supplied. Studies have to be performed to understand the intestinal tolerance of these compounds in Atlantic salmon regarding utilization, and triggering of diarrhoea and intestinal inflammation. In addition, the temperatures Atlantic salmon are reared in are relatively low compared to the fish species where supplementation of xylanase has been studied so far. Close attention should therefore be made to elucidate the activity of these enzymes through pre-processing, feed-production, storage of feed and throughout the gastrointestinal system to conclude what is the best approach for use of xylanase to increase dietary digestibility and utilization of polysaccharides for better fish performance in Atlantic salmon.

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