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Review

A review of the use of prebiotic in aquaculture for fish and shrimp

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It has been documented in a number of food animals that gastrointestinal bacteria play important roles in affecting the nutrition and health of the host organism. Thus, various means of altering the intestinal bacteria to achieve favorable effects such as better resistance to pathogens, enhancing growth and immune stimulation of the host organism have been investigated in various fish and shrimp. In this respect, probiotics and prebiotics are used in farm animal and for aquaculture, although the probiotic approach has been extensively used and advocated, viability after ingestion is difficult to guarantee and almost impossible to prove. The prebiotic concept dictates that non viable dietary components fortify certain components of the intestinal flora. This concept has the advantage that survival of the ingested ingredient through the upper gastrointestinal tract is not a prerequisite because it is indigenous bacterial genera that are targeted. Despite some positive effects prebiotic supplements on fish and crustaceans have been published however it seems such information for aquatic organism is inadequate. This paper will give short review of recent studies in which the effects of various prebiotics have been evaluated for potential application in the aquacultural production of fish and shrimp.

Key word: Prebiotic, probiotic, inulin, oligofructose, bifidobacteria.

INTRODUCTION

The world aquaculture activities shows a rapid increase (18% per year) in production and (17.8% per year) of the aquaculture business since 1997 to 2008 (FAO, 2007).

Increasing the surface of production or intensification of production are the means of increasing production. In intensive method, decrease of water quality, increase of stress, decrease of food quality, increase bacterial, viral or parasite infections can suppress this growth.

For a long time, the most common method for dealing with the occurrence of bacterial infections in aquaculture, was the administration of antibiotics. However, aquaculture faces serious problems due to various adverse effects of these drugs such as accumulation in the tissue, immunosuppression, development of antibiotic resistant bacteria and destruction of environmental microbial flora.

In the other hand, to use antibiotic or vaccine for fish is expensive and in many farms unavailable, therefore probiotics and prebiotic are two field commanding that recently have received considerable attention.

There are several definition for probiotics and prebiotic. The term, probiotic, simply means “for life”, originating from the Greek words “pro” and “bios” (Gismondo et al., 1999). The most widely quoted definition was made by Fuller (1989). He defined a probiotic as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance”.

The main purpose of using probiotic and prebiotic maybe are the use of this material to improve the health of their host and increasing growth rate. The application of probiotic and prebiotic in aquaculture have shown positive results, but insufficient evaluation of biological influence of bacteria in natural environment and cost of the material are the restriction of probiotics and prebiotics at this time.

Many beneficial effects such as competition with pathogens for nutrients or for adhesion sites and stimulation of the immune system may be expected from probiotics (Gatesoupe, 1999).

The prebiotics have several advantages, but the main advantage of prebiotics over probiotics is that they are

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natural feed ingredients. Their incorporation in the diet does not require particular precautions and their authorization as feed additives may be more easily obtained, in spite of some concerns about their safety and efficacy. Originally, prebiotics were chosen to stimulate bifidobacteria and lactobacilli in human microbiota (Gatesoupe, 2005).

Prebiotic, unlike probiotic, is not an organism and has less influence in natural environment. Based on definition of Gibson and Roberfroid (1995), prebiotics are a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health.

There are several food mainly carbohydrates use as prebiotic nowadays, but for a food can be classified as a probiotic, must have some characteristics. Gibson et al. (2004) noted a food ingredient that most fulfill the following criteria: resistance to gastric acidity, hydrolysis by digestive enzymes and gastrointestinal absorption, fermentation by intestinal microflora and selective stimulation of the growth and or the activity of intestinal bacteria associated with health.

In different studies since 1999, many substances have been investigated as prebiotic. Based on the study of Mahious and Ollevier (2005), Fooks et al. (1999), and Gibson et al. (2004), any foodstuff that reaches the colon, e.g. non-digestible carbohydrates, some peptides and proteins, as well as certain lipids, is a candidate prebiotic. Certain non-digestible carbohydrates seem authentic prebiotics. They include resistant inulin and oligofructose, transgalactooligosaccharides (TOS), lactulose, isomalto oligosaccharides (IMO), lactosucrose, xylo-oligosaccharides (XOS), soyabean oligosaccharides and gluco-oligosaccharides. From *in vivo* and *in vitro* studies, inulin and oligofructose, TOS and lactulose are presently classified as prebiotics. IMO, lactosucrose, XOS, soyabean oligosaccharides and glucooligosaccharides are not considered as functional ingredients since they do not fulfill all criteria for classification as prebiotics. Prebiotics are selectively fermented by Bifidobacteria, Lactobacillus and Bacteroides.

Inclusion of prebiotic in the diet has been reported to increase the uptake of glucose (Breves et al., 2001) and bioavailability of trace elements (Bongers and van den Heuvel, 2003).

In later use of prebiotics, they have the binding capacity therefore increasing the absorption of mineral such as calcium, magnesium and iron; these minerals, are not absorbed in the small intestine and so reach the colon, where they are released from the carbohydrate matrix and absorbed.

Bongers and van den Heuvel (2003), explained this enhancing effect of prebiotics on mineral absorption, the osmotic effect with the exchange of protons and possible decrease in proteins such as calcium-binding protein which may increase the availability of trace elements in the small intestine, acidification of the colonic content due to fermentation and production of short chain fatty acids (SCFA), formation of calcium and magnesium salts of these acids, and hypertrophy of the colon wall. Prebiotics also may alter the fermentation products of the gastrointestinal (GI) track (Smiricky -Tjardes et al., 2003; Gibson et al., 1995). The prebiotic may have many other properties and some of them are explained by scientists. Prebiotic may have the role of supplying energy for the host. Prebiotics are selectively fermented by probiotic bacteria e.g. *Bifidobacteria*, *Lactobacillus* and *Bacteroides* to produce short chain fatty acids (acetate, butyrate, propionate) and lactate. It has been demonstrated that short chain fatty acids are absorbed through the intestinal epithelium, thus becoming an energy source for the host, whereas lactate enters the liver and is used as precursor for gluconeogenesis (Smiricky-Tjardes et al., 2003; Gibson et al., 1995; Burr et al., 2005). Reducing the pH of the colon resulting from the production of SCFA is another prebiotic properties. Lower pH values inhibit the growth of certain pathogenic bacterial species while stimulating the growth of the bifidobacteria and other lactic acid species (Mussatto and Mancilha, 2007).

PREBIOTIC IN AQUACULTURE

Prebiotic may have the role of increasing growth rate, improve immune system as well as change the community of bacterial in gastrointestinal track. Many scientists have worked to optimize the dosage of supplementary prebiotic in feed to achieve better growth rate and survival. In the following some results are discussed.

Effects of prebiotics on growth parameters

Feed costs account for over 50% of the variable costs in most aquaculture operations, therefore applying the best feeding strategy can have a significant impact on optimizing profit, which is the primary goal of commercial aquaculture.

Also, if more fish are able to resist disease and survive until they are of marketable size, the subsequent cost of medication and overall production costs would be reduced drastically.

Reports about effect of prebiotic on growth parameters in fish are inconclusive. Supplementation of Beluga's (*Huso huso)* diet with 1, 2 and 3% inulin showed negative relationship between some performance indices including weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), energy retention (ER), feed efficiency (FE), protein retention (PR) and supplementation level of inulin. Also growth parameters in fish fed inulin was lower than control group (Akrami et al., In press). The reduction of some growth parameter in treatment groups may be due to affecting some other parameters in experimental place of work or the condition of fish itself and not by the versus effect of inulin, but we may conclude that at least the inulin had no positive effect in growth rate of *Huso huso* young fishes.

In using prebiotic such as mannanoligosaccharide, fructooligosaccharide and galactooligosaccharide, the use of Atlantic salmon fish meal-based diet supplemented with 10 g kg^{-1} of these prebiotics did not showed effects on growth and digestibility (Grisdale-Helland et al., 2008). In using commercial prebiotic Grobiotic®-AE, feed efficiency was significantly improved when using a 7-week diet was supplemented with 10 - 20 g kg^{-1} of this commercial food on hybrid striped bass, but the growth was not significant (Li and Gatlin, 2004).

The growth of the fish my increase by using supplementary prebiotic in feed. In a 3-week trial, Refstie et al. (2006) found that Atlantic salmon fed with a fish meal-based diet supplemented with 75 g kg^{-1} inulin had increased relative mass of the gastrointestinal tract, but the absorptive capacity of the fish was not affected.

A diet containing 20 g kg^{-1} oligofructose, a fructooligosaccharide (FOS) produced by partial enzymatic hydrolysis of inulin by hot water extraction of chicory roots, resulted in increased growth of turbot larvae, but 20 g kg^{-1} inulin itself had no effect on growth (Mahious et al., 2006). Growth, feed efficiency and survival were improved in two experiments with rainbow trout that were fed a diet containing 2 g kg^{-1} mannanoligosaccharide compared with those fed the basal diet (Staykov et al., 2007; Grisdale-Helland et al., 2008)

The body composition may be affected by dietary prebiotic however in rainbow trout and hybrid tilapia, the body protein concentration has been reported to increase as the level of MOS was increased in the diet from 1.5 to 4.5 g kg^{-1} (Genc et al., 2007b; Yilmaz et al., 2007). In contrast, supplementing the diet with 10 g kg^{-1} MOS or GOS resulted in a decrease in the protein concentration in the body of the salmon in the present trial, in line with the results of Genc et al. (2007a), in which *Penaeus semisulcatus* (de Haan, 1844) were fed a diet containing 4.5 g kg^{-1} MOS. Genc et al. (2007a) speculated that the lower body protein concentration in shrimp fed the MOS diet may have been the result of lower amino acid utilization and diet digestibility (Grisdale-Helland et al. 2008). Dietary supplementation with inulin didn't affect the body composition of juvenile Beluga (Akrami et al. In press).

As a result, despite the works of different farmers and researcher, the intake of prebiotic is primarily dependent on the types of ingredients used in diet formulation and will therefore vary widely among species and diets. Considerations in supplementing prebiotics in fish diets have been arisen to some extent. The type of prebiotic to supplement, specific animal characteristics (species, age, stage of production) and type of diet are important considerations. In addition, practical formulations and economic considerations should be carefully considered.

Effects of prebiotics on immune system

The use of different chemotherapies is advisable to avoid bacterial infection of fish. Also, using several antibiotics are used to treat bacterial infection, however the recent techniques have increased drug-resistant bacteria in fish. Whilst vaccination is the method of choice over antibiotic treatments for the control of many fish diseases, vaccines for others are unavailable or, at best, in the early stages of their development. In recent years in the aquaculture industry, increasing consideration has been given to alternative strategies for disease control as adjuncts to vaccination and as a potential route to the reduction in the widespread use of antibiotics. Prebiotics is one group of these alternative strategies that their health promoting effects has been proven by many studies in human and terrestrial animals (Cerezuela et al. 2008; Gibson, 1999; Niness, 1999; Kelly-Quagliana et al., 1998; Cooper, 1995; Causey et al., 1998).

Bailey et al. (1991), reported that prebiotics can modify the GI tract microbial community to enhance non specific immuno responses. The prebiotic fermentation in the caeco-colon by the bacteria there existent cause a significant modification of the colonic microflora, because these oligosaccharides serve as substrate for growth and proliferation of anaerobic bacteria, mainly the bifidobacteria, which inhibit the growth of putrefactive and pathogenic bacteria present in the caeco-colon (Mussatto and Mancilha, 2007). They produce substances that stimulate the immune system, thus, enhancing the host's protection against infections.

The use of Grobiotic®-AE have shown improved survival rate of hybrid stripped bass challenged with live streptococcus marinum and Mycobacterium marinum (Li and Galin, 2005). In trials with rainbow trout (Staykov et al., 2007), common carp (Staykov et al., 2005) and Jian carp (Zhou and Li, 2004), the non-specific immune system was positively affected when the diet was supplemented with MOS (Staykov et al., 2007). Torrecillas et al. (2007) reported dietary incorporation of MOS at 0.4% activated sea bass' immune system and increased its resistance to a bacterial infection directly inoculated in the gut, one of the main sites of infection in fish.

In a 6-week trial, Li et al. (2007) found that short chain FOS supplementation at concentrations from 0.025 to 0.800% by weight, enhanced hemocyte respiratory burst (which is one measure of non-specific immunity) of Pacific white shrimp *Litopenaeus vannamei* cultured in a recirculation system. Dietary supplementation of Grobiotic®-A improved survival of pacific white shrimp cultured in low-salinity (2 ppt) water. In addition, a fresh water challenge also showed similar improvement in survival of shrimp fed Grobiotic®-A, although the mechanism(s) for enhanced survival under low-salinity conditions, have not been identified (Gatlin et al., 2006).

Prebiotics can modify the GI tract microbial community to enhance non specific immuno responses (Bailey et al., 1991). Prebiotics also offer one rational approach to the probiotic concept, e.g. reduction of gut pH through SCFA formation; Secretion of antimicrobial substances; blocking of adhesion sites; attenuation of virulence; blocking of toxin receptor sites; immune stimulation; competition for nutrients, and suppression of toxin production (Gibson, 1999; Fooks et al., 1999).

Effects of prebiotics on gastrointestinal track microbial community

In the gastrointestinal track, the bacterial community is affected by the substances and vice verse. On the other hand, there are positive and/or interaction between the bacterial and substance in gastrointestinal track. Flickinger et al. (2003), explained these phenomenon such a way that, the GI tract of invertebrates and vertebrates provide habitat for a diverse ecosystem of microorganisms. The colonic microflora is of crucial importance to any consideration of the role of feed ingredients in health and disease because many physiological effects of such compounds influence their activities. Prebiotic oligosaccharides such as inulin and oligofructose are fermented in the colon where they promote the growth of bacterial populations associated with a healthy, well-functioning colon. This selective stimulation occurs because oligosaccharides are readily fermented by beneficial types of colonic bacteria and are not used effectively by potentially pathogenic bacterial species.

In general, we may divide the bacteria in two groups. Some bacteria are hazardous and the others are beneficial for fish. Due to activity of the first group, the hazard effect or toxin material may be produced. As Flickinger et al. (2003) explained, a number of these bacteria are pathogenic whereas health-promoting, or pathogen suppressing, properties have been attributed to particular bacteria (e.g., *Bifidobacterium, Lactobacillus*). A number of adverse consequences result from toxic metabolites formed during fermentation of food/feed in the large bowel. Toxic compounds formed at that site include ammonia (a liver toxin), amines (liver toxins), nitrosoamines (carcinogens), phenols and cresols (cancer promoters), indole and skatole (carcinogens), estrogens (suspected carcinogens/breast cancer promoters), secondary bile acids (carcinogens /active colon cancer promoters) and aglycones (mutagenic substances) (Flickinger et al., 2003). In case of beneficial bacteria, Merri¢eld et al. (2009) by study of a couple of articles, suggested that the beneficial bacteria plays a role as a defensive barrier against pathogenic species in addition to contributing towards digestive function via the production of a range of vitamins and enzymes (Rimmer and Wiebe, 1987; Moriarty, 1990; Sugita et al., 1997; Sugita et al., 1998; Ramirez and Dixon, 2003). Gastric bacterial populations may also play an important role with regard to immunostimulation and development of gut-associated lymphoid tissues (Picchietti et al., 2007). Furthermore, several researches have demonstrated the influence of mucosal bacterial populations on the integrity of the epithelial surface (Ringø et al., 2003; Ringø et al. 2007). It is demonstrated that the lactic acid bacteria (e.g., *Bifidobacterium, Lactobacillus*) have the ability to tolerate the acidic and bile environment of the intestinal tract. Lactic acid bacteria (LAB) also functions to convert lactose into lactic acid, thereby reducing the pH in the GIT and naturally preventing the colonization by many bacteria (Mombelli and Gismondo, 2000; Klewicki and Klewicka, 2004).

In aquaculture, few reports are available on the influence of prebiotics on growth and intestinal microflora in fish. In the earliest of studies with fish, certain nutrients such as linoleic acid, linolenic acid and soluble carbohydrate were investigated mainly by Ringo and his colleagues their effects on the aerobic/facultative anaerobic intestinal microbiota of Arctic char *Salvelinus afpinus*. When linoleic acid was supplemented to the diet of Artic char, the total viable counts increased by an order of magnitude (10 fold) as compared with fish fed a diet without linoleic acid (Ringø, 1993; Ringø et al., 1998; Ringø and Olsen, 1999). Adding linoleic acid to the diet altered the intestinal microbial community by inhibiting the growth of *Lactobacillus* sp. and enhancing the growth of *Aeromonas* sp., *Pseudomonas* sp. and *Vibrio* sp. Polyunsaturated fatty acids of the n-3 and n-6 series also were shown to alter the microbial population of Arctic char, with the lactic acid bacteria *Carnobacterium* spp. being the dominant facultative anaerobe cultivated (Ringø et al., 1998). Lactosucrose has been shown to increase the thickness of intestinal tunica muscularis of red sea bream, while this dietary supplement was used as substrate by the intestinal microflora (Kihara et al., 1995). However, lactosucrose was poorly used by trout (Kihara and Sakata, 2001a) and carp microbiota (Kihara and Sakata, 2001b). Olsen et al. (2001), have observed a damaging effect of inulin on enterocytes of Arctic charr, when the amount of the prebiotic in the diet was 15% of the diet.

In another investigation using dextrin instead, researchers reported that substituting dextrin with 15% inulin reduced the bacterial population from 4.8×10^5 to 3.56×10^4 level in the hindgut of Arctic charr, however the composition of bacteria colonizing the hindgut of Arctic charr fed inulin were dominanted by Gram-positive bacteria of the genera *Staphylococcus*, *Streptococcus*, *Carnobacterium* and *Bacillus* (Ringø et al., 2006).

Supplementation of Beluga's (Huso huso) diet with 1, 2 and 3% inulin showed that all bacteria levels increased during the first 4 weeks and started to decrease in inulin fed fish during the next 4 weeks and there were no significant differences between all treatments, but the intestinal lactic acid bacteria (LAB) increased in the 1% inulin group (Akrami et al., in press).

Olsen et al. (2001) observed that a diet supplemented with 15% inulin caused harmful effects on enterocytes to Arctic charr, *Salvelinus alpinus*. Dietary supplementation of 2% inulin significantly changed GI microflora in turbot

Psseta maxima larvae by increasing *Bacillus* species to 14% and decreasing *Vibrio* species (Mahious et al., 2006).

Supplementation of Pacific white shrimp's (*Litopenaeus vannamei*) diet with short- chain Fructooligosaccharides at concentrations from 0.025, 0.0500, 0.075, 0.100, 0.200, 0.400 and 0.800% by weight, changed the microbial community in the GI track. Although most of the bacterial species contributing to the GI microbial in this study were uncultured species but two of the denaturing gradient gel electrophoresis (DGGE) bands intensified by the addition of short chain FOS were found to be *Alkalibacillus* spp. and *Micrococcus* spp. or an unidentified seawater bacterium. Both *Alkalibacillus* spp. and *Micrococcus* spp. are gram-positive aerobic microbes that can tolerate saline environments (Li et al., 2007). In an 8-week trial, Gatlin and Burr (2009) found that red drum fed fish meal-based and soybean meal/fish meal-based diets supplemented with either GroBiotic®-A or inulin at 1% of dry weight had no effect on gastrointestinal track microbial community.

In summary, prebiotics have been reported to have numerous beneficial effects in fish such as increased disease resistance and improved nutrient availability. The reasons for the different results are not clear yet. It may be due to the different basal diet, inclusion level, type of monosaccharide, adaptation period, chemical structure (degree of polymerization, linear or branched, type of linkages between monometric sugars), origin of prebiotic, animal characteristics (species, age, and stage of production), duration of use and hygienic conditions of the experiment. If beneficial effects of prebiotics are manifested in fishes, then prebiotics have much potential to increase the efficiency and sustainability of aquacultural production. Therefore, comprehensive research to more fully characterize the intestinal microbiota of prominent fish species and their responses to prebiotics is warranted.

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