Potential Application of Prebiotics in Aquaculture

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Abstract

Various sectors of the aquaculture industry would benefit if cultured organisms were conferred with enhanced growth performance, feed efficiency and disease resistance. As such, the cost of medication and production costs could be reduced and consumer perceptions would be improved. It has been documented in a number of terrestrial animals and humans that microbiota of the gastrointestinal tract plays important roles in affecting the nutrition and health of the host. Dietary application of probiotics, which are live microbial organisms, may be restricted due to regulatory approvals and technical constraints such as heat inactivation during feed manufacturing. Thus, prebiotics, which are non-digestible feed ingredients that benefit the host by stimulating growth and activity of health-promoting bacteria, recently has attracted attention. Probiotics may promote growth of bacteria such as *Lactobacillus* and *Bifidobacter* spp. in the intestine and limit potentially pathogenic bacteria such as *Salmonella*. Such compounds have been reported to favorably affect various terrestrial species; however, such information in currently limited for aquatic organisms although some positive effects of prebiotic supplements on fish and crustaceans recently have been published. This paper will review recent studies in which the effects of various prebiotics have been evaluated for potential application in the aquacultural production of fish and shrimp.

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Introduction

A persistent goal in various types of aquaculture is to maximize the efficiency of production to optimize profitability. Intensification of production is one means of increasing production efficiency but also may lead to greater disease susceptibility of the cultured organism due to deterioration of water quality and elevation of other stressful conditions. Bacterial infections often affect cultured organisms whose immune system may be compromised by stressful conditions. One of the most common means of treating such infections is to administer antibiotics. However, as a traditional strategy for aquatic disease management, antibiotics have been extensively criticized for potential development of antibiotic-resistant bacteria and destruction of environmental microbial flora, as well as having relatively high cost and marginal effects in some cases. Certain antibiotics also have been shown to suppress the immune system, potentially making aquacultured organisms more susceptible to viral or parasitic infections. Increasing concerns of antibiotic use have resulted in a ban on subtherapeutic antibiotic usage in Europe and stringent regulations on application of antibiotics in the United States and other countries. These policy alterations may impact aquaculture and have therefore prompted interest in developing alternative strategies for disease control. Prepared diets not only provide essential nutrients to support growth and development of the cultured organism, but also may be one of the most promising means to influence the cultured organism's health and resistance to stress and disease-causing agents (Gatlin 2002). Thus, in recent years there has been heightened research in developing dietary supplementation strategies in which various health-promoting compounds have been evaluated. These compounds can be broadly classified as immunonutrients and immunostimulants with the difference between the two related to their mechanisms of action. Immunonutrients confer their benefits to animals by serving as a substrate or energy source for the immune system, while immunostimulants upregulate immunity by conferring signals to the animal's neuro-immuno-endocrine system or various cell signaling pathways. One group of immunostimulants that has shown numerous beneficial effects in terrestrial animals as well as in some aquatic animals is referred to as prebiotics which are defined as non-digestible food ingredients that beneficially affect the host by stimulating growth and/or activity of a limited number of beneficial bacteria in the gastrointestinal (GI) tract (Gibson and Roberfroid 1995). Pertinent information about this group of immunostimulants will be the subject of this article.

Results and Discussion

It is well established that various inactivated natural microbes or microbial products such as lipopolysaccharides and β-glucans can stimulate the cell-mediated immune system of various animals. Some of these products can be delivered orally without complete degradation in the digestive system and thus may be used as potential immunostimulants for aquaculture. For example, β-glucans have received extensive recognition as an immunostimulant (reviewed by Sakai 1999; Sealey and Gatlin 2001), although optimal administration protocols especially administration length still needs to be refined. The influences of numerous other microbial immunostimulants including peptidoglycan, liposaccharides and sulfated polysaccharides on aquatic animal health have been reported but more supporting research is warranted.

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Dietary supplements of live microbial organisms are classifies as probiotics (Fuller 1989), and have been observed to beneficially affect the intestinal microbial balance of the host organism and confer various beneficial effects included immunostimulation and enhanced disease resistance. These compounds have received heightened attention in aquaculture over the past several years (Gatesoupe 1999; Gatlin 2002; Irianto and Austin 2002). The currently recognized probiotics that may influence fish immunity, disease resistance and other performance indices include: *Bacillus subtillis*, *Bacillus licheniformis*, *Bacillus circulans*, *Lactobacillus rhamnosus*, *Lactobacillus delbrüeckii*, *Carnobacterium maltaromaticum*, *Carnobacterium divergens, Carnobacterium inhibens, Enterococcus faecium, Saccharomyces cerevisiae* and *Candida sake*. Probiotic research related to aquaculture has been directed towards application of the microbes to the aquatic medium as well as to the diet as noted in the reviews by Gatesoupe (1999), Irianto and Austin (2002) and Burr, Gatlin & Ricke (2005). The function of probiotics in treating the aquatic environment is to reduce the presence of potentially pathogenic bacteria by competitive exclusion. The current restrictions on application of probiotics in aquaculture include cost as well as insufficient evaluation of biological consequences and potential influences on natural microbial biodiversity. In addition, there are a variety of potential constraints to dietary application of probiotics in aquaculture including susceptibility of these live organisms to inactivation by the heat of extrusion processing and uncertain regulatory approval procedures in some countries.

The various potential constraints to probiotic application as well as some of the positive influences of these live microbes such as increased growth and disease resistance of various aquatic species have resulted in heightened interest in evaluating prebiotics. Some of the more common prebiotics established to date include fructooligosaccharide (FOS), transgalactooligosaccharide (TOS) and inulin (Vulevic Rastall, & Gibson 2004). Such health-promoting bacteria including *Lactobacillus* and *Bifidobacter* spp. have been shown to be stimulated by various prebiotics and consequently limit potentially pathogenic bacteria such as *Salmonella*, *Listeria* and *Escherichia coli* in various terrestrial animals (Manning and Gibson 2004). It has become readily evident that the microbiota affected by prebiotics plays integral roles in numerous processes including growth, digestion, immunity and disease resistance of the host organism as demonstrated in poultry (Patterson and Burkholder 2003), other terrestrial livestock and companion animals (Flickinger, van Loo & Fahey 2003), as well as in humans (Gibson and Roberfroid 1995). However, at this time, the application of prebiotics in aquaculture has been rather limited but holds considerable potential. In addition, the effective application of prebiotics to aquatic organisms will require their microbial community to be better characterized and understood.

The GI tract of invertebrates and vertebrates provide habitat for a diverse ecosystem of microorganisms. These microorganisms play an important role in the health and nutrition of the host. The vertebrate GI tract is predominantly an anaerobic environment, and the GI tract microbial community of fishes, especially the anaerobic microbial community, is poorly studied and understood. Most studies characterizing the fishes' intestinal microbial community have been aerobic studies (reviewed in Cahill 1990; Ringø 1993; Spanggaard, Huber, Neilsen, Neilsen, Appel & Gram 2000; and Huber, Spanggaard, Appel, Rossen, Nielsen & Gram 2004), which identify the dominant facultative anaerobic bacteria, but do not cultivate the strictly anaerobic

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bacteria. Using aerobic methods to culture bacteria have led some investigators to conclude that anaerobic bacteria play a minor role in the GI tract microbial community of fishes. In the first *in vitro* prebiotic trial ever conducted with fish (Burr, Hume, Ricke & Gatlin 2006), a FOS concentration of 0.375% was determined to significantly alter the microbial population of red drum *Sciaenops ocellatus* GI tract inoculum based on denaturing gradient gel electrophoresis (DGGE) analysis (Hume, Kubena, Edrington, Donskey, Moore, Ricke & Nisbet 2003; Ricke, Park, Moore, Birkhold, Kubena & Nisbet 2004).

The current research on prebiotics with fish is even more limited than with probiotics, although several preliminary studies recently have been conducted. The main advantage of prebiotics over probiotics is that they are natural feed ingredients and thus regulatory control over dietary supplementation should be limited. One commercial prebiotic, GroBiotic-A® (consisting of a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products), has been shown to enhance resistance of hybrid striped bass (Li and Gatlin 2004, 2005), rainbow trout (Sealey W.M., personal communication) and golden shiner (Lochmann, R.T., personal communication) to a variety of bacterial pathogens. Dietary supplementation of GroBiotic®-A also improved survival of Pacific white shrimp *Litopenaeus vannamei* cultured in low-salinity (2 ppt) water. In addition, a freshwater 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. Another recent investigation with *Litopenaeus vannamei* also showed that dietary FOS enhanced hemocyte respiratory burst, which is one measure of non-specific immunity; however, a live disease challenge was not conducted. Another recent study showed that dietary supplementation of 2% inulin significantly changed GI microflora in turbot *Psetta maxima* larvae by increasing *Bacillus* species to 14% and decreasing *Vibrio* species (Mahious, Gatesoupe, Hervi, Metailler & Ollevier 2006). In addition, the turbot larvae fed 2% oligofructose had significantly higher growth rate than fish fed 2% inulin, 2% lactosucrose or 2% cellulose.

Inclusion of prebiotics in the diet has been reported to increase the uptake of glucose (Breves, Sztkuti &Schröder 2001) and bioavailability of trace elements (Bongers and van den Heuvel 2003). The increased availability of trace elements was attributed to decreasing the pH of the intestinal tract due to the increased concentrations of VFAs (Bongers and van den Heuvel 2003). There also may be an 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 (Bongers and van den Heuvel 2003). A recent study in our laboratory examined the effects of four prebiotics on digestibility of a soybean meal-based diet by red drum. The four prebiotics were GroBiotic®-A, mannanoligosaccharide (MOS), galactooligosaccharide (GOS), and FOS, each added to a basal diet at 1% by weight. The diets were formulated so that 50% of the protein was provided by soybean meal and the other 50% was from menhaden fish meal. Fish were fed the diets for three weeks and then manually stripped to obtain fecal material. The dried fecal material was analyzed for protein, lipid, organic matter and chromium in order to compute coefficients of digestibility. The GroBiotic-A supplemented diet showed the most significant ($P \leq 0.05$) increase in protein and organic matter digestibility compared to the basal diet although the other prebiotics also tended to increase protein and organic matter digestibility as well.

Prebiotics also may alter the fermentation products of the GI tract as demonstrated by Smiricky-Tjardes, Grieshop, Flickinger, Bauer & Fahey (2003) who reported that TOS increased the concentrations of the volatile fatty acids (VFAs) propionate and butyrate in the small intestines of swine. However, in that study nutrient digestibility was reported to be lower even though the intestinal microbiota had shifted to a more beneficial community for the host (Smiricky-Tjardes et al. 2003). Recent in vitro studies in our laboratory with both red drum and hybrid striped bass have established changes in VFA production with the addition of GroBiotic®-A and FOS.

One other class of compounds are synbiotics, which were defined by Gibson and Roberfroid (1995) as "a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare". Synbiotics represent a very new concept for aquaculture. To the best of our knowledge, evaluation of these products has not been conducted to date in aquatic species. Much more in-depth research to more fully characterize the effects of prebiotics and possibly synbiotics on microbial ecology of the GI tract should be pursued for development of efficient management strategies to manipulate GI tract microflora of aquatic animals and enhance their production in aquaculture.

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