**Synbiotics in Aquaculture**

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**Introduction**

Aquaculture has grown tremendously over the past decades and has become an economically important industry. It is the fastest-growing food producing sector globally with the greatest potential to meet the increasing demand for aquatic food. The intensive culture of aquatic organisms to fulfill the demands of a ever-increasing human population has come with a number of difficulties. Aquaculture can have a negative impact on the environment by spreading illnesses, destroying wetlands and mangroves, reducing the richness of wild fish populations by allowing non-native fish to escape, and polluting surface and groundwater through effluent discharge.

The modernization of aquaculture, which includes extensive stocking, feeding and manuring for high fish production has resulted in a number of issues including drop in water quality, decline in fish health and resulting in occurrence of a range of diseases, which is the significant issue that makes aquaculture systems non-viable leading to economic losses. Several workers have identified a large number of disease causing organisms including bacteria, viruses and parasites which have been studied in greater detail. As a result, fish health management has become one of the most important factors in achieving safe and well-grown fish from aquaculture. In recent years, nearly 20% of the production has been lost due to diseases resulting in economic losses. It was common practice for several decades to use chemotherapeutic/antimicrobial agents to prevent and control infectious diseases which had limited success in prevention or cure of aquatic diseases. However, routine use of these agents leads to several adverse consequences such as environmental degradations, food security problems which also led to evolution of resistant strains of bacteria. To address these issues, immunostimulants such as probiotics, prebiotics and their synergistics have been proposed as an alternative method for disease outbreak prevention and control.

**Immunostimulants**

Immunostimulant are one that elevates the non-specific defense mechanisms by increasing phagocytosis, leucocytic activity, macrophage and neutrophil migration or specific immune response. In addition, they also reduce the immune suppressive effects of stress. Immunostimulants have been derived from diverse natural sources and a large number were later synthesized chemically with the natural products as structural models. They show varying degrees of effectiveness in preventing disease caused by microbial pathogens. Different compounds like select proteins, lipids, carbohydrate based cell wall extracts and synthetic compounds have been used as immunostimulants in farm reared fish and shellfish. Depending on origin, the immunostimulants are classified as bacteria and bacterial products, complex carbohydrates, immunoenhancing drugs, nutritional factors, animal extracts, cytokines and lectin and plant extracts. The most common ones include glycans, yeasts, glucans from yeasts, abalone extracts, rough mutants, levamisole, Ferund's adjuvant and other naturally or synthetically produced substances.

**Probiotics**

The term probiotic was originated from the Greek words “pro” and “bios” which mean “for life” and are often called as promoter of life that help in a natural way to improve the overall health status of the host organism and is currently used to name bacteria associated with beneficial effects on human and animals. According to WHO/FAO (2002), the probiotics are defined as live microorganism which when administered in adequate amount confers a health benefit on the host. Probiotic bacteria were first studied by Elie Metchnikoff, a Nobel laureate of 1908 in the field of medicine. The term probiotic was first mentioned by Kollath (1953), defined as organic and inorganic supplements required to restore the health of malnourished patients. Interest in the probiotic concept also reached the field of animal husbandry in the 1960s. Dietary supplements became available for farm animals with the inclusion of LAB as health promoting agents. The application of probiotics in aquaculture has been related to several beneficial effects, namely modulation of the intestinal microbiota and immune system, as well as enhances survival, development and nutrition and disease resistance. However, further research is required to establish their exact modes of action.

The probiotic may play a considerable role as immunostimulants and antimicrobial agents. Merrifield *et al*. (2010) proposed a more inclusive and broader definition for probiotic intended for use with aquatic animals: ‘any microbial cell provided via diet or rearing water that benefit the host fish, fish farmer or fish consumer which is achieved in part at least by improving the microbial balance of the fish’. The direct benefits to the host were considered as immunomodulation, improved disease resistance, reduce stress response and improved GI morphology and benefit to the fish farmer or fish consumer as improved fish appetite, growth performance, feed utilization, improvements in carcass and flesh quality and reduce malformation. Typically, the lactic acid bacteria (LAB) have been widely used and researched for human and animal purposes, and these LAB are also known to be present in the intestine of the healthy fish. The increasing interest in the possible use of probiotics in aquaculture and thus, the research into the use of probiotics for aquatic animals is necessary owing to the demand for environment-friendly sustainable aquaculture. The probiotic improve the water quality and the immune responses of a host animal by balancing microbial load. Some of the beneficial effects of probiotics can manifest as enhanced feed utilization of cultured aquatic animals through the supplementation of digestive enzyme, improved feed efficiency and higher growth, the prevention of intestinal disorder and the pre-digestion of anti-nutritional factor present in the mixed feed. Most probiotic colonize in the host and affect the digestive processes through increased numbers and production of microbial enzymes improving the intestinal microbial balance and consequently the digestibility and absorption of feed and feed utilization.

Probiotics are marketed in two forms a) Dry forms: the dry probiotics that come in packets can be given with feed or applied to water and have to be brewed at a farm site before application. Each kit of dry probiotics contains a packet of dry powder and a packet of enzyme catalyst. Brewing has to be done in clean disinfected water after emptying the packets and blending thoroughly. Usually, it is brewed at 27-32°C for 16 to 18 hours with continuous aeration. The finished products must be used within 72 hours. Maximum aeration is required in semi-intensive culture ponds. If aeration is less, the application of probiotics has to be spread for two consecutive days, applying 50% of the dose each time.

b) Liquid forms: The hatcheries generally use liquid forms which are live and ready to act. These liquid forms are directly added to hatchery tanks or blended with farm feed. The liquid forms can be applied any time of the day in indoor hatchery tanks, while it should be applied either in the morning or in the evening in outdoor tanks. Liquid forms give positive results in lesser time when compared to the dry and spore form bacteria, though they are lower in density. There are no reports of any harmful effect of probiotics but it is found that the biological oxygen demand level may be temporarily increased on its application; therefore it is advisable to provide subsurface aeration to expedite the establishment of probiotic organisms. A minimum dissolved oxygen level of 3 mg/l is recommended during probiotic treatment.

**Prebiotics**

Prebiotic, unlike probiotic, is not an organism and has less influence in the natural environment. Based on the definition of Gibson and Roberfroid (1995), prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon and thus improves host health. Some of the non-digestible carbohydrates are considered as prebiotics, they include resistant inulin and oligofructose, trans-galactooligosaccharides (TOS), lactulose, isomaltooligosaccharides (IMO), lactosucrose, xylooligosaccharides (XOS), soybean oligosaccharides and glucooligosaccharides. Bongers and Van Den Heuvel (2003), demonstrated that increasing effect of prebiotics on mineral absorption, the osmotic effect of 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 can alter the microbial community in the gastrointestinal tract to enhance nonspecific immune responses. Mannan oligosaccharide (MOS) extracted from yeast outer cell wall (*Saccharomyces spp.*) maintain gut health by adsorption of pathogenic bacteria, containing type-I fimbriae or by agglutinating different bacterial strains. Lower the pH value of the colon resulting from the production of SCFA resulted in inhibiting the growth of certain harmful pathogen by enhancing the growth of the bifidobacteria and other lactic acid species. Soleimani *et al*. (2012) reported that upraised growth performance (final weight, SGR and FCR) was observed in fish fed with 2% and 3% FOS. So that FOS can be considered as a beneficial dietary supplement for improving the immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach fry. The fish blood profile and growth performance could be affected by probiotic (Gro-Biotic A, 1-3%). Prebiotic supplementation and lipid concentration significantly affected several aspects of fish performance and body composition, some of the differences were numerically small. Most of the information regarding the role of prebiotics in the gut physiology includes the study of the microbiota. These microbial communities in the gut varied with the prebiotic used (nature, concentration, duration, etc.) and the fish species. In general, the dietary intake of prebiotics provokes the microbiota with a higher number of “good” bacteria (namely *Lactobacillus* and *Bifidobacterium* species) and lower of “bad” bacteria (potential pathogenic bacteria such as *Aeromonas* sp. or *Vibrio* spp).

**Synbiotics**

Synbiotic refers to nutritional supplements combining a mixture of probiotics and prebiotics in a form of synergism. The concept of synbiotics was proposed to characterise some colonic foods with interesting nutritional properties that make these compound candidates for classification as health-enhancing functional ingredients’. A mixture of prebiotics and probiotics can beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the GI tract by selectively stimulating the growth and by activating the metabolism of one or a limited number of health-promoting bacteria and thus improving the host welfare. An effective pairing would allow alteration of the gut environment by a prebiotic that would select for preferential growth conditions of known beneficial probiotics. The benefits of this approach are that fish farmers are able to control and provide favourable conditions in the colon as well as ensure that a beneficial probiotic is present in sufficient numbers. Dietary in the combination of *Jerusalem artichoke* and *L. plantarum* showed significantly enhanced SGR, FCR, serum lysozyme activity, phagocytic activity, respiratory burst activity when compared with control and individual applications. Dietary *Jerusalem artichoke* and *L. plantarum* significantly stimulated growth, immunity and disease resistance of *P. bocourti* was reported by many authors. The juvenile angelfish fed with *Artemia* enriched with synbiotic (*Pediococcus acidilactici* and fructooligosaccharide) improved growth performance, mucosal immune response, stress resistance as well as modulation of intestinal microbiota toward potentially beneficial bacteria such as Lactic acid bacteria and the diet supplementation with synbiotic (*Enterococcus faecium* and FOS) significantly increased blood factors at all treatments and the synbiotic fed groups showed significantly higher survival rate after challenges with *Saprolegnia parasitica*. Synbiotic supplementation in the host affects beneficially by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and activating the metabolism of a limited number of health-promoting bacteria studied in rainbow trout (*Oncorhynchus mykiss*). Partida-Arangure *et al.* (2013) found that insulin and bacteria improved immunity in the cultured shrimp, *Litopenaeus vannamei.* Synbiotics in combination with plant products or yeast have recently been utilized in aquaculture with promising results on gut microbiota, gut morphology and mucosal immune responses. Synbiotics promote immunity by allowing helpful bacteria to colonise the mucus lining and preventing harmful bacteria from colonising by competing for substrates and adhesion sites. Synbiotics have been shown to improve growth characteristics by promoting fat decomposition.

Synbiotics are now becoming an important aspect of aquaculture procedures in order to achieve high yield. Despite the promising potential benefits of these feed supplements as demonstrated in the current literature, the use of synbiotics in fish farms has been poorly investigated to date and the above account clearly summarizes a limited amount of work on the effect of synbiotics on carps as well as the influence of synbiotics on histo-pathological changes including the gut and other associated body organs.

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