



# The Use of Synbiotics (Prebiotic and Probiotic) in Aquaculture Development

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## Abstract

Synbiotic is a dietary supplement that combine probiotic and prebiotic, which beneficially affects the host animal by improving its intestinal balance, health and growth. Probiotics are live microbial feed supplement, while prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating growth and activity of the intestinal bacteria. Researches have shown that gastrointestinal bacteria play important role in affecting the nutrient and health of the host organism. Several methods of altering the intestinal microflora to achieve positive effects such as better resistance to pathogen, enhancing growth and stimulation of the host immune system have been studied in fish and shrimps. Synbiotics is been used as a replacement for antibiotics used to enhance growth and feed efficiency in aquatic organism and is associated with incalculable risks for human health. Studies on the use of prebiotic singly or in combination with probiotic (synbiotic) in farm animals and aquaculture have shown to improve the energy expenditure derive from other sources of food, increase the incorporation of protein for growth, increase the immunity and disease resistance of the host organism. Probiotic approaches have been extensively used but viability after ingestion is yet to be ascertain. Prebiotic are chosen to stimulate some intestinal flora (*Bifidobacteria spp* and *Lactobacilli*) but may reduce the population of some beneficial bacteria (*Aeromonas spp* and *Carnobacterium spp*) in the colon. The concept of synbiotic is quite new and is introduced to solve the problems encounter by single application of probiotic and prebiotic. despite the beneficial effect on the use of probiotic and prebiotic on aquaculture species research on the benefits on the use of symbiotic is still inadequate. This paper therefore gives reviews of researches on the effects, mode of action of synbiotics and their potential for further application in aquaculture production.

**Keywords:** Aquaculture; Synbiotic; Probiotic; Prebiotic; Fish growth.

## 1. Introduction

Aquaculture is one of the fastest growing food producing sectors in the world estimated at the rate of 18% per year in production and 17.8% per year of the aquaculture business since 1997-2008 [1]. The global aquaculture industry currently accounts for over 45% of all sea food consumed and has become an important economic activity in many countries [2]. Intensification of production is one means of increasing production efficiency but may also lead to increased susceptibility of the cultured organism due to deterioration of water quality and elevation of stress. However, the rapid development of aquaculture is accompanied by problems such as widespread epizootics, feed efficiency and growth performance [3]. Disease outbreak, poor growth and mortality are some of the contributing factor to the poor growth of aquaculture in Africa. Poor growth and drug resistance of pathogens in aquacultured organisms are two of the most important concerns in the aquaculture industry whose aims are to increase the growth or survival performance, feed efficiency and resistance of aquatic organism [4]. Traditionally, antibiotics have been supplemented in aquafeeds for prevention and/or treatment of bacterial disease of aquatic animals. It has been reported that antibiotics may enhance growth and feed efficiency by killing intestinal microflora and thus increasing amino acid utilization by the host in some animal species [5]. However, the use of antibiotics may pose threats such as development of bacterial strains that are more resistant to antibiotic treatment, destruction of environmental microbial flora, immuno-suppression, and the occurrence of antibiotic residues in cultured organisms for human consumers [1]. Besides the use of antibiotics for fish and shrimps is expensive and in many farms unavailable, therefore dietary supplements including probiotic, prebiotics, and / or synbiotics and immuno stimulants are the major fields that have received heightened attention. Functional additive, as synbiotics is a new concept on aquaculture where the additions of microorganisms on diets show a positive effect in growth caused by the best use of carbohydrates, protein, and energy hence, diminishing mortality by disease, antagonism to pathogen and better microbial intestinal balance [6, 7]. Synbiotic is compounded from the combination of non digestible food ingredients (prebiotic) and live organism (probiotic). Synbiotics refer to nutritional supplements combining probiotics and prebiotics to form a symbiotic relationship [8]. This new concept for aquaculture needs further evaluation and research to more fully characterize the effects in aquatic organisms. Probiotics are dietary supplements containing potentially beneficial live bacteria, yeast or algae that affect the host organism by improving its intestinal balance

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[9]. Prebiotics are non digestible food ingredient that beneficially affects the host organism by selectively stimulating the growth and activity of bacteria in the colon [8]. Literature have established that many intestinal microbial species may have beneficial influences on the growth performance of fish [10], and dietary composition is capable of influencing the intestinal microflora of fishes [11, 12]. Dietary supplements are substances which added in trace amounts provide a mechanism by which such dietary deficiencies can be addressed which benefits not only the nutrition and thus the growth rate of the animal concerned, but also its health and welfare in modern day fish farming. Some of the most utilized growth-promoting feed additives include hormones, antibiotics, and some salts [13, 14].

The main purpose of using synbiotic is to improve the health of their host and increase growth rate. Application of synbiotics in aquaculture has shown positive result. Many beneficial effects such as growth performance, immune stimulation, gastrointestinal microbial balance, biological control agents and water quality management has been reported in aquaculture systems where synbiotics the have been used [15]. Competition with pathogens, adhesion, and antagonism to pathogen, ability to provide enzymes, metabolites, digestion and absorption are some properties of synbiotics [16].

## 2. Synbiotics in Aquaculture

Combination of prebiotics and probiotics are known as synbiotic. Their usage is two of the several approaches that have potential to reduce enteric disease in aquaculture and subsequent contamination of fish product. Probiotic are dietary supplements containing potentially beneficial live bacteria, yeast or algae. Examples of probiotics include gram-positive bacteria such as *Bacillus sp*, *Carnobacterium inhibens* K1 and *Lactobacillus sp* as well as gram-negative bacteria such as *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Vibrio fluvialis*. Other probiotics are bacteriophages and yeast (*Saccharomyces cerevisiae*, *Phaffia Rhodozoma*, *Debaryomyces hansenii*) as well as microalgae (*Tetraselmis suecica*). Probiotics are usually selected to control specific pathogens through competitive exclusion or direct mild stimulation of fish immune system. Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the intestinal tract. The health benefit is established by directly affecting the intestinal microbial balance of the host organism hence, stimulating the fish immune system and also controlling specific pathogen through competitive exclusion [17]. Prebiotics are non- digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activating metabolism of a number of health promoting bacteria in the intestinal tract thus improving the host intestinal balance. The main advantage of prebiotics over probiotics is that they are natural feed ingredients and thus regulatory control over dietary supplementation should be limited. There are several food mainly carbohydrates used as prebiotic nowadays, but for a food to be classified as a probiotic, must have some characteristics. Fooks, *et al.* [18] and Gibson, *et al.* [19] stated that any foodstuff that reaches the colon such as carbohydrates, protein, peptides and certain lipids undigested are prebiotics. Other example of prebiotic includes Inulin, Oligofructose, Manno Oligossacharides (MOS), Transgalactooligosacharides (TOS), glucooligosacharide (GOS), Isomalto Oligosaccharides (IMO), lactosucrose, xyloligosaccharides (XOS), Soyabean Oligosaccharides and Lactulose. Gibson, *et al.* [19] noted that prebiotic have the following characteristics namely 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. Prebiotic in the diet are selectively fermented by probiotics bacteria *Bifidobacteria*, *Lactobacillus* and *Bacteriodes* to produce short chain compounds such as propionate, lactose and some lipid which have been reported to increase the uptake of glucose [20] and bioavailability of trace elements [21]. Some commercial synbiotics used in aquaculture are Biogen, Pronifer, Biomin, Mycofix, Bacterins and Grobiotic. The application of synbiotics in aquaculture have shown positive results such as increase fermentation and production of short chain fatty acid (SCFA), reducing the pH of the colon thereby inhibiting the growth of certain pathogenic bacterial while stimulating the growth of *bifidobacteria* and *Lactobacillus* [21, 22]. The SCFA (propionate) produced 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 [23, 24]. Improving general health and growth performance in aquaculture by stimulating the growth of specific microbes in the intestinal tract or directly stimulating the immune system basically is a good and rational strategy. The prospective of simultaneously improving performance and the general health of the animals by addition of specific feed supplements is appealing, not only to farmers but also feed producers in times of increasing market competition. Nevertheless, the basic requirements of a diet to supply adequate quantities of essential and conditionally essential nutrients for the various organisms must not be neglected. The stimulation of growth of intestinal bacteria by synbiotics involves the supply of sufficient amounts of other nutrients to enable the multiplication of these microbes. If the supply of basic nutrients cannot be guaranteed by the diet or the animal itself, the beneficial effects of synbiotic (probiotic and prebiotics) will be reduced. Arguably with aquaculture systems, application of potential synbiotic (prebiotic and probiotic) materials to the water column will no doubt have an impact on the culture. Some include the improvement of water quality in culture systems since this removes environmental stressors like ammonia and nitrite and lessen the prevalence of pathogens like *Vibrio* or viral infections. There fore synbiotics can be added to tanks and ponds in which animals live, as biological control or for their capacity of modified the bacterial composition of aquatic animal's intestine, water and sediment, or used with feed as health supplement. The probiotics and prebiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of

nutrition of host species through the production of supplemental digestive enzymes [25-27] and Carnevali, *et al.* [27]. Thus, the use of probiotics and prebiotics is attracting aquaculture much attention in recent times [10, 28-31].

### 3. Effects of Synbiotics on Fish and Shrimps

The potential for reducing stress and enhancing immunity and disease resistance by nutritional factors feed additives (such as synbiotic, immunostimulants and bacterins) has been demonstrated in warm-blooded animals. However, very little work in this area has been conducted in aquaculture. Thus, the effects of dietary nutrients and their interactions, anti-nutritional factors, additives, feed and feeding strategy need to be assessed to develop economically viable feeds and feeding practices to optimize growth, improve stress resistance, immune response and disease resistance, and improve product quality of aquaculture species. Therefore, this seminar shall discuss the following effects of synbiotics in aquaculture on

1. Growth performance
2. Immune enhancement
3. Digestion and absorption
4. Disease management
5. Water quality control

### 4. Growth Performance

Functional feeds extend beyond the satisfying basic nutritional requirements of the cultured organism but to improving growth, feed utilization and furthermore the general health and stress resistance of the animals. 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. They have been several reports on the use of prebiotics and probiotics singly or combined (synbiotics) to enhance growth of fish and shrimps. Supplementation of Beluga's (*Huso huso*) diet with 1-3% inulin (prebiotic) showed positive relationship between some performance indices including weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), energy retention (ER), feed efficiency (FE) and protein retention (PR). The growth performance parameters of Nile tilapia (*Oreochromis niloticus*) fingerlings fed with diets supplemented with either feed additives of (Biogen<sup>®</sup>) or (Pronifer<sup>®</sup>) for a period (90 days) showed that the group of fish fed the supplemented diets grew better than the group fed the control diet. Khatlab, *et al.* [32] and Mohamed, *et al.* [33] reported that Nile tilapia (*O. niloticus*) fingerlings fed on diets supplemented with Biogen and Yeast exhibited greater growth than those fed with the control diet respectively. They also reported that the diet containing 30% protein supplemented with Biogen (synbiotic) at level of 0.1% produced the best growth performance and feed efficiency. The authors concluded that Biogen<sup>®</sup> is an appropriate growth-stimulating additive in tilapia cultivation. Enhanced growth performance was generally observed in hybrid striped bass fed diets supplemented with GroBiotic or brewers yeast compared to fish fed the basal diet throughout the feeding trial with significantly weight gain observed after 12 weeks of feeding [34]. Increased weight gain and feed efficiency were generally observed in hybrid striped bass fed diets supplemented with partially autolyzed brewers yeast and GroBioticR-A (synbiotic) for 16 weeks. Supplementation of the GroBiotic significantly enhanced survival of hybrid striped bass during the *in situ* mycobacterial challenge, suggesting a potential use of this prebiotic in aquaculture. *Bacillus subtilis* have been shown to produce digestive enzymes such as amylase, protease and lipase which enrich the concentration of intestinal digestive enzymes [35]. The bacteria have also been shown to improve digestive activity via synthesis of vitamins and cofactors or via enzymatic improvement [15]. Gullian, *et al.* [36] demonstrated a significant growth increase in shrimp inoculated with *Bacillus* sp whilst found that enhanced growth was generally obtained in shrimp fed diets with *B. subtilis* inclusion. Adding spices and medicinal herbs such as garlic, onion, marjoram, caraway, basil, anise, fennel, licorice, black seeds and fenugreek to fish diets resulted in improvement of protein digestibility and energy retention [37]; and enhancement of growth and feed conversion [38, 39]. The better digestibility obtained with the addition of probiotics improved diet and protein digestibility, which may in turn explain the better growth and feed efficiency noticed with the supplemented diets [33]. El-Dakar, *et al.* [31] reported that the final body weight, specific growth rate (SGR), protein ratio (PR), energy ratio (ER) and food conversion ratio (FCR) of rabbit fish offered feeds with commercial Biogen (synbiotic) had better growth rate than those without Biogen. Refstie, *et al.* [40] found that Atlantic salmon fed with a fish meal based diet supplemented with 75 g kg<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup> inulin itself had no effect on growth [41]. Growth, feed efficiency and survival were improved in two experiments with rainbow trout that were fed a diet containing 2 g kg<sup>-1</sup> mannanoligosaccharide compared with those fed the basal diet [42, 43]. The body composition and the body protein concentration in rainbow trout and hybrid tilapia, have been reported to increase as the level of MOS was increased in the diet from 1.5 to 4.5 g kg<sup>-1</sup> [44, 45].

### 5. Digestion and Absorption

A mixture of probiotics and prebiotics (synbiotics) beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (GIT), and thus improving digestion and absorption of the host. Useful gut microbiota growth facilitates the fermentation process in all kinds of animals including man. This fermentation is of nutritional significance in all the animals. The bacterial breakdown produces

various organic acids namely acetic, butyric acid, propionic acid and other substances which cause reduction in pH which in turns reduces the activity of enzymes in the small intestine. They are also useful for the production of vitamins such as Vitamins A and K [13]. Symbiotic have effects on the main physiological functions of the gastrointestinal tract which affect digestion, absorption and propulsion [46]. Major functions of the gut microbiota include metabolic activities that result in salvage of energy and absorbable nutrients, trophic effects on the intestinal epithelium and protection of the host against invasion by harmful microbes [30]. 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. [47], explained that the GIT of invertebrates and vertebrates provide habitat for a diverse ecosystem of microorganisms. Ahmed, *et al.* [48] reported an increase of crypt cells proliferation in the small intestines with the use of Biogen. Gastric bacterial population may also play an important role with regards to immunostimulation and development of gut associated lymphoid tissue [49]. Adding linoleic acid to the diet altered the intestinal microbial community by inhibiting the growth of *Lactobacillus* spp and enhanced the growth of *Aeromonas* pp, *pseudomonas* spp and *Vibrio* spp [11]. Lactosucrose has been shown to increase the thickness of the intestinal tunica muscularis of Red sea bream [34]. When linoleic acid was supplemented to the diet of Arctic char (*Salvelinus alpinus*) the total viable count of microbiota in the GIT increased by an order of magnitude (10 fold) as compared with fish fed a diet without linoleic acid [11, 12]. Dietary MOS improved intestinal morphology in both the anterior and posterior intestinal regions. Mannan oligosaccharide supplementation increased absorptive surface area by promoting longer mucosal folding. Furthermore, increased absorptive surface area was confirmed at the electron microscopy level, where micrographs showed that MOS was able to increase both microvilli density and length. MOS supplementation significantly increased microvilli length in cobia (*Rachycentron canadum*) larvae [50]. It was observed that a diet supplemented with 15% inulin caused harmful effects on the enterocytes of Arctic char while 2% inulin significantly changed GIT microflora in turbot *Psetta maxima* larvae by increasing bacillus species to 14% and decreasing vibrio spp [41]. According to Ringø, *et al.* [51] when 15% inulin was replaced with dextrin the bacterial population in the hindgut of Arctic char was reduced from  $4.8 \times 10^5$  to  $3.6 \times 10^4$ . Supplementation of the diet of pacific white shrimp (*Litopenaeus vannamei*) with Fructooligosaccharide (FOS) at concentrations ranging from 0.025 – 0.80% by weight changed the microbial community in the gastrointestinal track [52]. Gatlin and Burr [53] reported that red drum (*Sciaenops ocellatus*) fed with Soya bean and fish meals based diets had no effect on gastrointestinal track microbial community but when supplemented with Grobiotic they was a significant effect of microbial community.

## 6. Immune Enhancement

Prebiotic and probiotic applied singly or combined (synbiotics) produces substances (immunostimulant) that stimulate the immune systems, thus enhancing the host protection against infection. Immunostimulant are chemical compounds that activate the immune systems of animal and render them more resistant to infections by viruses, bacteria, fungi and parasites. They are many reports that bacterial compound act as an immunostimulant in fish and shrimps (Sakai, 1999). Isolauri, *et al.* [54] have reported the synbiotics stimulate the immunity of organism in two ways. The flora from synbiotic migrates throughout the gut wall and multiplies to a limited extent and when they die they release antigens which when absorbed stimulate the immune system. Fish larvae, shrimps and other invertebrates have immune systems that are less well developed than adult fish and are dependent primarily on non-specific immune responses for their resistance to infections [55]. According to ingestion of bacterial and subsequent endocytosis in cod and herring larvae stimulated the development of a strong immune system. Song, *et al.* [56] observed an increase in acid phosphatase activity in *Miichthys miiuy* fed with *C. butyricum* as an indication of increase immune system. [7] show that administration of a mixture of *Bacillus* spp and *Vibrio* spp promotes the resistance of juvenile of *L. vannamei* against *V. harveyi*. *Vibrio* cells, lipopolysaccharides peptidoglycan, laminaria, yeast and glucans have experimentally tested in small scale culture and proven to be important feed ingredients in the control of disease through production of immunostimulant [46]. Mussatto and Mancilha [22] stated that the *bifidobacteria* in the caeco colon produces immunostimulants which inhibits the growth of putrefactive and pathogenic bacteria by fermenting some oligosaccharides. The use of Grobiotic has been reported to significantly increase the immunity of stripe bass hence improving survival rates [34]. The non-specific immune system was positively affected when the diet of Rainbow trout and common carp were supplemented with MOS [42, 57]. Torrecillas, *et al.* [58] reported dietary incorporation of MOS at 0.4% activated sea bass immune system and increased its resistance to bacterial infection. Fructo-oligosaccharides (FOS) supplementation at the concentration from 0.025 – 0.8% by weight enhances hemocyte respiratory burst (measure of non-specific immunity) of Pacific white shrimp cultured in a recirculation system. Synbiotic modify the gastrointestinal tract microflora to enhance non specific immuno- responses [59]. El-Boushy and El-Ashram [60] showed that the  $\beta$ -glucans was able to enhance the non-specific immunity of the African catfish (*Clarias gariepinus*) efficiently more than *Saccharomyces cerevisiae*. Yeast  $\beta$ -glucans seem to modulate the specific immune response by increasing the serum antibodies secreted by plasma cells against *Edwardsiella ictaluri* in catfish [61] and against *Yersinia ruckeri* in rainbow trout, when given in combination with vitamin C [62].

## 7. Biological Control Agents

The use of different chemotherapeutic is to avoid bacterial infection in fish. Using several antibiotics to treat bacterial infection however have increased drug resistant bacteria in fish. Yasuda and Taga [63] suggested that synbiotics were found to be useful not only as food but also as biological controllers of fish disease and activators of nutrient regeneration. Review the concept of probiotics and prebiotics as a viable therapeutic modality in the

treatment of gastrointestinal disease. The synbiotic when use for the hope of growth stimulation affects the gut microflora which results in the reduction of the resistance to infection caused by certain bacteria. The probiotics supplementation help and repairs the deficiencies in the gut flora and a balanced in intestinal micro biota enhances resistance and reduction to infection [9, 55]. Probiotics mostly combined with prebiotics use as biological control agents in aquaculture belong to the lactic acids bacteria (*Lactobacillus*, *Carnobacterium*), vibrio spp, *Bacillus* spp and *Pseudomonas*. Dietary supplementation of 2% Grobiotic and brewer yeast (synbiotic) in the diet of hybrid bass *Morone chrysops* showed a significant protection against Mycobacterial infection [34]. Supplementation of probiotic in fish diet was also observed to produce resistance against *streptococcus iniae*, *Aeromonas hydrophilia* and *mycobacterium marinum*, which are prevalent pathogenic mycobacteria in wild and cultured hybrid striped bass [64, 65]. Mycobacteriosis is generally chronic and causes severe infection and high cumulative mortality in closed recirculating system [66]. Mannan oligosaccharide supplementation is able to bind to certain Gram-negative bacteria preventing intestinal colonization; thus, resulting in a removal mechanism of bacteria from the gut [67]. A large reduction of the number of these organisms provides protection against diseases. Torrecillas, et al. [58] observed that dietary MOS can provide protection against *V. alginolyticus* infection in European sea bass.

Addition of lactic acid bacteria to a diet in a rearing medium containing turbot larva, significantly reduced larval mortality which were challenged with a pathogenic vibrio spp [15]. Several strains of *Pseudomonas fluorescens* were able to exclude pathogenic *Aeromonas salmonica* strain from Atlantic salmon presmolt with stress inducible furunculosis infection and limit the mortality of rainbow trout infected with *Vibrio anguillarum* [7, 68]. Ahmed, et al. [48] observed that the two doses of super biobuds (synbiotic) enhance the growth rate, improve the survival and increase resistance of Nile tilapia to the challenge infection.

**Table-1.** Overview of literature on the synbiotics use as biological control agents in aquaculture

Type of synbiotics	Effect	Suggested mode of action	Reference(s)
<i>Lactobacillus plantarum</i> Plus diet	Increase of survival of halibut larvae 2 weeks after hatching	Immun-stimulation	Lara-Flores, et al. [69]
<i>Bacillus</i> strain IP5832 plus diet	Increase of weight of turbot larvae, decrease of mortality when challenged with an <i>Vibrionaceae</i> member	Antagonism	Gatesoupe [70]
<i>Carnobacterium divergens</i> Plus diet	Decrease of mortality of Atlantic cod fry challenged with a pathogenic <i>V. anguillarum</i> after the infection;	Antagonism	Gildberg and Mikkelsen [71]
Biogen and Pronifer	Increase in body weight, feed efficiency and utilization of <i>O. niloticus</i>		Mohamed, et al. [33]
GroBiotic- A	<i>Morone chrysops</i> growth Performance and decreased severity of the mycobacterial infection.	antagonism	Li and Gatlin [34]

## 8. Water Quality Control

Accumulation of organic matter and appearance of black, anaerobic mud in pond bottom sediments is a concern to shrimp growers. This accumulation increase oxygen demand and acidic conditions in the bottom soils which may adversely affect the growth of cultured organism [72]. There fore any method that reduces the accumulation of organic matter in pond sediments should enhance the quality of the pond ecosystem. Deterioration of soil and water quality in aquaculture systems is often associated with decomposition of organic matter over time. Research have proven that the use of synbiotics in aquaculture have enhance the rate of degradation of organic matter, increase level of dissolved oxygen, eliminate undesirable waste products (nitrite ammonia, carbon dioxide and sulfide), reduce the proportion of blue green algae and increase aquatics production [72]. The application of prebiotic and probiotic have proven to be effective in shrimp's hatcheries by out competing pathogenic bacteria for nutrient and other resources, thus reducing the risk of disease and improving larval growth r; [73].

## 9. Properties and Mode of Action of A Synbiotic (Prebiotic and Probiotic)

The major purpose of using symbiotic is to maintain a favourable relationship between beneficial and pathogenic microorganisms that constitutes the flora of intestine and skin of aquatic animals. Therefore a symbiotic is expected to have a few specific properties in order to certify a beneficial effect. These properties include

- a) Competitive exclusion
- b) Antagonism to pathogens
- c) Adhesion and
- d) Immunity stimulation

## 10. Competitive Exclusion Adhesion

One possible mechanism for preventing colonization by pathogens is competition for adhesion sites on the gut and other tissues. The ability to adhere to enteric mucus and wall surfaces is necessary for bacteria to establish in fish intestines [46]. Bacterial adhesion to tissue surface is important during the initial stages of pathogenic infection [74]. *Lactobacilli*, *Bifidobacteria* and *Cornobacteria* constitute the major microflora colonizing the gastrointestinal tract of many vertebrates including fishes [75]. These bacteria adhere firmly to the mucosal epithelium thereby prevented from being swept away by peristalsis along the intestinal wall [76]. Competition for adhesion receptors with pathogens might be first synbiotic effects [77]. The attachment ability have been tested in vitro and in vivo and the results suggest that the pathogen was displaced by the potential symbiotic based on its ability to attach to mucus, where the growth of the pathogen in the digestive tract was suppressed by the synbiotic applied [78]. This effect prevents the pathogenic bacterial colonization along the intestinal wall and therefore, prevents disease development [76]. Numerous studies have shown that synbiotics inhabits pathogens and the use of antibiotics disturbs the intestinal microbiota increases susceptibility to infection but addition of symbiotic increases resistance to infection [79]. Proposed mechanism of pathogens inhibition by the intestinal microbiota includes competition for nutrient, production of toxic compound (low pH and bacterocins), competition for binding sites on the intestinal epithelium and stimulation of the immune system [9].

Competitive exclusion as it applies to the gastrointestinal tract is a phenomenon whereby an established microflora prevents or reduces the colonization of a competing bacterial challenge for the same location in the intestine. The aim of synbiotics designed under competitive exclusion is to obtain a stable, agree and controlled microbiota on culture based on competition for attachment sites on the mucosa, competition for nutrients, and production of inhibitory substance by the microflora which prevents replication and/or destroys the pathogenic bacteria and with this reduce its colonization [78]. Lactic acid bacteria are known to produce compounds such as bacteriocins that inhibit the growth of other microorganisms [56]. Virtually all microorganisms require iron for growth. Successful bacterial pathogens are able to compete successfully for iron in the highly iron-stressed environment of the tissues and body fluids of the host. The omission of iron from the diet of early-weaned sea bass (*Dicentrarchus labrax*) larvae had no detrimental effect on the survival or the growth rate of the fish but significantly limited the bacterial load of the larvae and increased the diversity of the microbiota [78]. The requirement for iron is high for many pathogens, including *V. anguillarum*. In a challenge test with this bacterium, salmon mortality increased linearly with dietary iron content [78]. Smith and Davey [80] showed that the fluorescent pseudomonad F19/3 is capable of inhibiting the growth of *Aeromonas salmonicida* in culture media and that this inhibition is due to competition for free iron. Strain F19/3 was also capable of excluding *A. salmonicida* from Atlantic salmon (*Salmo salar*) presmolts with stress-inducible infections. Competitive exclusion resulting from preemptive colonization has been shown for the cecal walls of chickens, which kept their effect after the ceca were washed four times in buffered saline [67]. Adhesion capacity and growth on or in intestinal or external mucus has been demonstrated in vitro for fish pathogens like *V. anguillarum* and *A. hydrophila* [74] and for candidate probiotics such as *Carnobacterium* strain K1 [81] and several isolates inhibitory to *V. anguillarum* [46]. In one of these studies, the aim was to measure the *in vitro* capacity of the strains to adhere to and grow in turbot intestinal mucus in order to investigate their potential to colonize farmed turbot as a means of protecting the host from infection by *V. anguillarum* [46]. The intestinal isolates generally adhered much better to a film of turbot intestinal mucus, skin mucus, and bovine serum albumin than did *V. anguillarum*, indicating that they could compete effectively with the pathogen for adhesion sites on the mucosal intestinal surface.

## 11. Antagonism

Microbial communities can disperse the effect caused by the invasion or addition of certain extrinsic pathogenic organisms. Bacterial antagonism is a common phenomenon in nature and plays a major role in the equilibrium between beneficial and potentially pathogenic microorganisms [82]. In addition, microorganism can be sources of a variety of bioactive natural products of basic research and commercial interest that have inhibitory effect on microbial growth [83]. Bacteria of the genus *Vibrio* spp are part of the autochthonous flora of marine organism and caused mortalities in shrimp larvae and juveniles [73]. *Bacillus* spp, *Lactobacillus* pp and yeast had been used as probiotic or combine with prebiotic for their antagonism effect to *Vibrio* spp [36, 84]. in aquaculture synbiotics are more extensively used for prevent and/or combat bacteria disease for example the vertebral column compression syndrome caused by *Flavobacterium psychrophilum* where the use of lactic acid bacteria [85] or yeast [86] caused a decrease of this bacteria. *Vibrio* sp. and *Aeromonas* sp. have become the most pathogenic microorganisms in fish aquaculture. pseudomonad's have receive special attention as disease-protecting microorganisms and have been used as plant biocontrol [87]. In recent years, there has been great interest in the use of lactic acid bacteria as

disinfection treatment Gatesoupe [68] and for control the populations of the native microflora such as *Aeromonas* and *Vibrio* species [88]. *In vitro* antagonism test is based on the nature phenomenon of antimicrobial metabolites production of some bacteria strains and is a frequent way for screening probiotics and or prebiotics. For example, antagonism of *B. subtilis* (strain BT23) against *V. harveyi* did confer protection to *Penaeus monodon* [26].

## 12. Summary

There is a lot of attention these days on the use of microbial supplements to improve aquaculture practices. Arguably with aquaculture systems, application of potential prebiotic and probiotic materials to the water column will no doubt have an impact on the culture and this is where the definitions of prebiotic and probiotic become less clear. Some include the improvement of water quality in culture systems since this removes the stressors like ammonia and nitrite and lessen the prevalence of pathogens like *Vibrio* or viral infections. Plus microbial additions to the water column will no doubt make their way into the gut of the culture species. The antibiotics used for the hope of growth stimulation affect the gut microflora, which, increase digestion and absorption.

The direct use results in the reduction of the resistance to infection caused by certain bacteria. There is increasing evidence to suggest that synbiotic act by stimulating the host's immune systems, increase gastrointestinal micro flora, improve resistance to infection of probiotic on water (from fresh to seawater of farms and laboratories) is a special point of environment research consideration. Those products (probiotics) are commonly foreign or exogenous strain, and represent a possible risk of microorganism pollution, especially with the use of strain with genetic modification, specific adhesions or colonization niche, antibiotic production, synergistic action. However, a number of synbiotic products have been thoroughly researched, and agree evidenced their efficacy a possible use on aquaculture. Beneficial bacterial preparations that are species-specific probiotics have become more widely available to the aquaculture community. These preparation show specific benefic effect as disease prevention and offer a natural element to obtain a stable healthy gut environment and immune system. The establishing of strong disease prevention program, which includes probiotic and good management practices can be hope to raise aquatic organisms production.

Despite the works of different farmers and researcher, the intake of prebiotic and probiotic is primarily dependent on the types of ingredients used in diet formulation and will therefore vary widely among species and diets. Considerations in supplementing synbiotics in fish diets have been arisen to some extent. The types of synbiotics to supplement specific animal characteristics (species, age, stage of production) and type of diet are important considerations.

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