# **Effects of Probiotic Bacteria on Fish Performance**

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Received date: April 12, 2017; Accepted date: May 28, 2017; Published date: June 4, 2017

Citation: Allameh SK, Noaman V, Nahavandi R. Effects of Probiotic Bacteria on Fish Performance. Adv Tech Clin Microbiol. 2017, 1:2.

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# Abstract

According to many reports, the beneficial effects of probiotic bacteria on fish have well been documented. Probiotics as an alternative strategy have been suggested to be used as replacement for antimicrobial drugs and growth promoters. In addition, some researchers believe that probiotics have advantages for improving the health of fish in aquaculture and increasing fish performance. Probiotic bacteria can be used in feed and play a beneficial role in the ecosystem of the fish gastrointestinal tract and finally enhance the growth performance and other rearing parameters such as feed conversion ratio, protein efficiency ratio, digestibility and body composition.

Keywords: Probiotic bacteria; Fish; Performance

## Introduction

Vila et al. [1] demonstrated that Elie Metchnikoff was the first scientist who established the basis of the use of lactic acid bacteria (LAB) in human in 1908, which is now called probiotic. Therefore, it has been over 100 years that the benefits of probiotic microorganisms have been recognized [1]. The last definition of probiotic was presented by Gram and Ringo as "live microbial cultures added to feed or environment (water) to increase viability (survival) of the host" [2]. The application of probiotic bacteria as promoter in animal feeds dates back to the 1970. Probiotics were used in the feed to enhance the growth and health of the host by increasing its resistance to disease [1,3,4]. The beneficial effects of probiotic bacteria have well been documented in human, pig, ruminant and poultry nutrition [1,4,5], although the application of such probiotics for aquatic animals is fairly a new concept [4]. The interest in probiotics and changes in intestinal microorganisms developed mainly for supporting and maintaining the host health. The studies are stimulated by a need to find alternatives to antibiotics and medicines that have very severe side effects [6]. In general, antibiotics have been used to inhibit and control the pathogenic bacteria in aquaculture. However, because of their negative effects (bacterial resistance, remain in animal products and environment), several alternative strategies such as probiotic bacteria have been suggested [7,8]. Furthermore, chemotherapy can disturb the homeostasis of gut physiology and cause fish to be vulnerable and sensitive to infections [9]. Probiotic bacteria which competes with bacterial pathogens for nutrients and attachment sites, adhere to the gut and inhibit the growth of pathogens is valuable and valid alternative for prophylactic use of antibiotics and biocides [10]. In total, it is necessary for aquaculture to be supported by effective alternatives to protect fish farming. Some alternatives such as using probiotics have been proposed to improve fish performance [11,12]. Several experiments recommended probiotic bacteria for increasing fish production and improving the health of fish by controlling pathogen bacteria [13-15].

Lactic acid bacteria as a main group of probiotic are used in animal nutrition to improve growth, survivability, feed efficiency, and also prevention intestinal disorders and neutralize of antinutritional factors present in the feedstuffs [16-18]. They are also applied to increase microbial monitoring, growth and feed efficiency [18,19]. In this review, you will find the effects of probiotic bacteria on rearing parameters, immune system response and inhibitor activities against pathogenic bacteria in fish.

#### Growth

The effects of probiotics on the growth performance and other beneficial activities in fish have well been documented [16,20-22]. In general, the common probiotics that are used for aquatic animals comprised of Lactobacillus, Lactococcus, Leuconostoc, Carnobacterium, Shewanella, Bacillus, Aeromonas, Vibrio, Enterobacter, Pseudomonas, Bifidobacteria, Clostridium and Saccharomyces [22].

The effects of different microbial probiotics in the diet of Rohu fingerling (*Labeo rohita*) on growth, nutrient digestibility, digestive enzymes and intestinal microbiota were studied [23]. They reported that the fish fed a combination of three probiotics (*Bacillus subtilis, Lactococcus lactis* and *Saccharomyces cerevisiae*) showed a higher growth, protein efficiency ratio, nutrient digestibility and lower feed conversion ratio compared to the other groups. Lara-Flores et al. reported that all the probiotic-containing diets resulted in growth higher than that of the control diets for tilapia (Oreochromis niloticus L.) [24]. They described that the addition of probiotics mitigated the effects of the stress factors and resulted in better fish performance, with better growth results in the diets containing the yeast. Similar results were observed when yeast was isolated from the intestines of wild rainbow trout. This probiotic showed a significant enhancement in growth of the cultured trout when it was introduced into the digestive tract of domestic rainbow trout [25]. In contrast, the bacterial mixture used with the optimum protein in diets caused no significant growth increases when compared to the control and yeast treatments. These results may be described by the greater adaptive capacity of yeasts in aquatic environments than bacteria [25]. Furthermore, better growth response was observed with probiotic fortified diets, but the best growth was obtained with a bacterium, instead of yeast. Kennedy et al. showed that the addition of a gram-positive probiotic bacterium increased the survival, size uniformity and the growth rate of marine fish larvae [26]. Hidalgo et al. tested two kinds of probiotics on the growth performance of juvenile dentex (Dentex dentex) but received no significant increase in comparison with the control treatment [27].

### Feed conversion ratio (FCR)

The addition of probiotics could improve feed utilization even under stress conditions [24]. The best FCR values were observed when probiotic-containing diets were fed to Nile tilapia (*Oreochromis niloticus* L.). The use of Spirulina as a probiotic in Nile tilapia diet improved feed conversion ratio compared to the control [12]. In addition, Nile tilapia treated with commercial probiotic showed significantly higher feed conversion efficiency compared to the control [28]. Furthermore, improved production was recorded for shrimp with significant lower feed conversion ratio than control using probiotic [29]. Also, Mohapatra et al. reported a lower FCR for Rohu fingerling (*Labeo rohita*) when they fed the diets containing probiotics [23]. On the contrary, Hidalgo et al. did not observe any significant influence for FCR when the probiotics were used for juvenile dentex (*Dentex dentex*) [27].

### Protein efficiency ratio (PER)

Some reports indicated that probiotics fortified diets could significantly improve the protein efficiency ratio (PER) or apparent nitrogen utilization (ANU) [24]. This results in optimizing protein use, which is the most expensive feed nutrient for growth. In addition, the application of probiotics causes a higher feed nutrient efficiency in stress conditions [24,30]. Abdel-Tawwab and Ahmad [12] and EL-Haroun et al. [28] showed a significant effect of probiotics on the protein efficiency ratios compared to the control in Nile tilapia (*Oreochromis niloticus* L.).

### Digestibility

In general, the application of probiotics in diets results in more nutrient digestibility for feedstuffs [23]. This suggests that the addition of probiotics will improve the diet and protein digestibility, which may in turn explain the better performance [24]. The positive effects of probiotics in fish diets on feed nutrients digestion were reported attributable to the digestive enzyme activity of bacteria [31]. Askarian et al. [32] evaluated the effect of chitin on the adherent aerobic intestinal microbiota of Atlantic salmon (Salmo salar L.) and further tested for protease, amylase, cellulose, phytase and chitinase activities particularly for LAB. They reported that the LABs have the ability to produce digestive enzymes such as amylase, lipase and protease. The positive effects on nutrient digestibility in Rohu fish (Labeo rohita) were observed when the diets supplemented with different microbial probiotics [23]. Similar effects have been reported for terrestrial animals (such as poultry) in which digestibility is shown to increase considerably with the use of probiotics in diet [33,34]. Tovar-Ramirez et al. recorded an increase in the digestive enzyme activities of amylase, trypsin and lipase in sea bass (Dicentrachus labrax) using live yeast [35]. Wang and Xu investigated the effect of Bacillus sp. probiotics on protease, amylase and lipase specific activities in the common carp and a significant increase in digestive enzyme activities in the all probiotics treatment groups were observed [36]. Also, a significant effect of the probiotic treatment on amylase and trypsin activities in the shrimp was reported by Castex et al. [29]. Suzer et al. [18] demonstrated that probiotics affect the digestive process by enhancing the population of beneficial microorganisms and then microbial enzyme activity, consequently improving the digestibility and absorption of feed and feed utilization. They also illustrated that the high growth performance can enhance specific activities of digestive enzymes as well.

#### **Body composition**

The administration of probiotics in the diet resulted in an improvement in the protein and lipid content, but no significant effect on the moisture and ash content of Nile tilapia (Oreochromis niloticus L.). EL-Haroun et al. suggested that no statistical differences were observed in carcass moisture, ash and protein content among the different treatments when commercial probiotic were used in Nile tilapia [28]. They observed differences in the carcass lipid and gross energy content, with the highest value recorded in fish fed a control diet. Lara-Flores et al. reported that diets containing probiotics could not significantly affect the body composition of Nile tilapia [24]. In addition, the effects of administration of Bacillus subtilis in the diet on body composition showed that this probiotic improved the fat content of the carcass, but no significant differences were observed for moisture, ash and protein content. Therefore, it seems that probiotics have no significant influence on the body composition of fish and do not affect strongly tissue synthesis [37].

#### Immune system response

The role of dietary nutrients or feed additives on the functions of the immune system in fish has been studied since 1980s. Some of these additives have been investigated for their potential to protect fish from stressors or diseases [38]. Among the numerous beneficial effects that are usually attributed to probiotics, the modulation of immune system is one of the most important subjects [22]. There has been a growing interest in recent years to control disease problems through alternative methods. One of them is the use of antagonistic bacterial such as LAB to control populations of potential pathogens through competitive exclusion or enhancement of immunity. In aquaculture, this goal may be achieved by the use of probiotics in a number of ways such as enrichment of larval food, inclusion in the diet or addition to the water [16,39]. The probiotics can stimulate specific and non-specific immune systems in fish. Monospecies or multispecies probiotics can promote phagocytic and lysozyme activities and also expression of various cytokines in fish. In addition, probiotic bacteria can stimulate the gut immune system of fish and increase immunoglobulin cells and acidophilic granulocytes. Different factors such as source, type, dose and duration of supplementation of probiotics can significantly influence the immunomodulatory activity of probiotics [22]. Many immunostimulants have been investigated on fish and shellfish. There are some reports that autochthonous microbiota may stimulate the immune response of aquatic animals to enteric pathogens. Some of them originated from microbial cell walls such as muramyl dipeptide, glucans and lipopolysaccharides. Hence, probiotic LAB consider as an immunostimulant in aquatic animals [13].

The use of LAB as a probiotic not only can stimulate the immune system but also improve water quality and nutrition, as a means to increase larval survival and aquaculture output [40]. Rengpipat et al. declared that Bacillus S11 provided disease protection by activating both the cellular and humoral immune defences, as well as presumably providing competitive exclusion in the shrimp's gut [41]. The addition of yeast-glucan, yeast zymosan and dead bacterial cells have also stimulated immune responses in shrimp (*Penaeus monodon*). Panigrahi et al. reported that the immune response was induced by different forms of the probiotic *Lactobacillus rhamnosus* (JCM 1136) in the rainbow trout (*Oncorhynchus mykiss*) [40].

It is thought that the use of probiotics and immunostimulants in fish feeding can be an important supplement to vaccines to prevent infectious disease [42]. Probiotics can enhance the natural immune function by dietary administration. They may adhere transiently and colonize the gastrointestinal tract and increase the antibody level [43]. In animal rearing, the application of probiotics has presented positive effects in both disease resistance and animal growth [42]. Therefore, the effects of probiotics on the immune system responses and bacterial population in aquatic organisms and the environment are well evidenced [13,18].

### Pathogenicity and challenge abilities

Around 40% of the world's aquatic products are obtained from aquaculture. The importance and higher demand of aquaculture products causes an increase in seafood production. However, production stocks will be lost through disease problem [44].

There are three major routes of infection in fish namely through the skin, gills and the GI tract. The GI tract is a muscular tube that exhibits a regional variation in structure and function and also the main site for pathogenic bacteria entry [45]. Two

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major groups of pathogenic bacteria in fish include *Vibrio* spp. and *Aeromonas* spp., the gram-negative species which are commonly implicated in mortality. These bacteria cause severe diseases such as vibriosis and furunculosis and particularly *A*. *hydrophila* causes small surface lesions, sloughing of scales, local haemorrhage and septicaemia [44,45]. Since commercial-scale aquaculture systems are developing, disease can be a significant limiting factor. These diseases are common worldwide and cause considerable economic losses during intensive aquaculture [46].

It is well known that probiotics may decrease the incidence of disease or reduce the danger of disease outbreaks. Probiotics can also produce inhibitory substances against pathogens, competition for essential nutrients and adhesion sites [16]. In addition, they supply essential nutrients and enzymes resulting in enhanced nutrition in the host. Furthermore, the modulation of interactions with the environment and the development of beneficial immune responses are exerted by probiotics [16,46].

Moreover, some reports have noted that the gut microorganisms are important for fish health by inhibiting the establishment of pathogenic bacteria in the alimentary tract. These results should be attended because the digestive tract is one of the main infection routes for pathogenic bacteria [47].

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