**Review Article****Review of some prebiotics and probiotics supplementation effects on farmed fishes: with special reference to Mannan oligosaccharides**

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Aquaculture has been increasingly contributing to animal protein production during the last few decades. Tilapia is known as one of the highly valuable fish cultured in wide geographical areas in several countries as a source of animal protein including Egypt. Tilapia has the merit of tolerance to a wide range of environmental conditions and ability to utilize food from the lowest trophic levels. The intensification of aquaculture production is usually challenged by infections including bacterial and parasitic burdens, which obligates the use of chemicals and antibiotics to control disease outbreaks. The injudicious use of antibiotics inevitably led to expansion of resistance, mutant pathogenic strains and detrimental effects to fish and consumer health. Therefore, it is imperative to find alternative ecofriendly sources as prebiotics and probiotics which can improve fish health, performance, and immunity without any side effects to the fish themselves or the consumer health. Prebiotics are known as a group of non-digestible food ingredients which encourage the growth of advantageous microorganisms in the gastro-intestinal tract. Like probiotics and synbiotics, inclusion of prebiotics as feed supplements into diets of farmed fishes is usually accompanied by immunomodulation and increased resistance of fish against serious bacterial agents as *Aeromonas hydrophila* infection. The current review article focuses on the potential effects of probiotics and MOS-containing prebiotics on health status, immune response and survivability of farmed fishes specially referring to Nile tilapia.

**1. Farming of Nile tilapia**

The Nile tilapia, *Oreochromis niloticus*, is generally spread in Africa and worldwide. In the 1950s, it was introduced in Lake Victoria, one of the Nile River resources, together with *Tilapia rendalii*, *Oreochromis leucostictus* and *Tilapia zillii* (Elsaied *et al.*, 2019).

Tilapia is among the most cultivated fish across the globe during the past three decades (Rezk *et al.*, 2009). Nevertheless, the contribution of fish and tilapia to the overall animal protein production in Africa is still limited (FAO, 2006). Increasing the productivity of tilapia, may represent an enhancing tool for increasing animal protein and raising the presently very low protein consumption in Africa. Interest in commercial production of tilapia has been associated with development of several breeding programs with the aim of improving its productivity (Rutten *et al.*, 2005; Ponzoni *et al.*, 2005; Maluwa and Gjerde, 2006).

Murphy *et al.* (2020) reviewed the trend of national aquaculture production from the year 2000 through 2016 and found that it has been increased from  $3.4 \times 10^5$  mt to  $13.71 \times 10^5$  mt. Tilapia in particular increased from nearly

$1.7 \times 10^5$  mt in 2000 to more than  $9 \times 10^5$  mt in 2016. The authors added that tilapia farming in Egypt contributes to 47.7% of fish supply in Egypt through 940.309 mt tilapia aquaculture production. Genetically improved Nile tilapia has been introduced in Egypt recently and has resulted in an increased performance, where the improved tilapia strains have recorded 28% superior harvest rate (Ibrahim *et al.*, 2013, Dickson *et al.*, 2016; and Marjanovic *et al.*, 2016).

Comparative studies revealed that fish consumption, particularly tilapia, has become a strategic food sector, where it has been cited to play an increasingly important role than other meat sources in the daily diets, particularly those of low-income consumers as reported by Wally (2016). When compared to other African countries, Egypt comes as the top aquaculture tilapia producer, the third global tilapia producer after China and Indonesia as estimated by Ali *et al.* (2020). This indicates the importance of tilapia farming as a chief source of animal protein for Egyptian community. Notably, aquaculture hosts about 580,000 job opportunities in this sector (El-Sayed *et al.*, 2015). Hence, unexpected mortalities are believed to cause great economic losses to fish farming

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practices. It should be noted that tilapia farms in Egypt were distributed mainly in small-holder farms 10 feddan or less which constituted about 50% of tilapia farms (Eltholth *et al.*, 2015).

## 2. Feeding habit of Nile tilapia

The feeding preferences of Nile tilapia are herbivorous, while it tends to be omnivorous animal. The cost of tilapia farming is low as it feeds on a short feed chain in a fishpond which also reduces feeding pressure on prey species. Also, tilapia feeding habits ensures the limitation of biomagnification of toxins in food chain (Barlow, 2000). Planktons, aquatic invertebrates, decaying organic matter are the main feeding materials of tilapia and they have the ability to catch plankton in gills mucous (Fryer and Iles, 1972). Tilapia has long intestine which possesses the ability to digest plant materials and their feeding habits are classified as continuous feeding activity so that they should receive their daily diet on three to four occasions (Lim, 1989).

High levels of production are expected from culturing tilapia as they can effectively use both artificial and natural feed under high stocking densities conditions. The main feature of *Oreochromis niloticus* is the sensitivity to low temperatures with optimum growth rates achieved between 28 and 32 °C. Meanwhile, a 30% reduction than optimal growth rate has been observed with temperature fall to 20-22 °C as documented by Teichert-coddington *et al.* (1997).

## 3. Probiotics, prebiotics, and synbiotics

Probiotics were scientifically defined as live microbes, which if supplied in ample amounts will benefit the host [reviewed in Hill *et al.* (2014)]. Thus, probiotics can be distinguished from commensal micro-organisms. While the term "Prebiotic" was described earlier as unite structural living organisms (Hutkins *et al.*, 2016) with beneficial effects on gut health. However, the term was confusing, and we needed to differentiate between different types of chemical-prebiotics and food-prebiotics. In their review article, Khangwal and Shukla (2019) defined prebiotics as non-digestible carbohydrates which can be utilized as a priming source of energy which can be used by gut microflora. The authors added that it is possible to find prebiotics in natural sources or it can be synthesized through enzymatic digestion. Prebiotics are also documented in another definition where they are known as food ingredients that improves gastrointestinal tract health status (Bindels *et al.*, 2015).

Prebiotics can be fermented by the beneficial intestinal microbes, additionally, they resist the gastric acidity, and enhance the growth of beneficial micro-organisms which support health of the host (Guerriero *et al.*, 2017). Prebiotics have been well-known for their beneficial effects on health of gut microflora and have been cited to help the recovery process of gut micro-organisms after gut dysbiosis in disease conditions. The health benefits conferred by prebiotics to the gut are thought to be due to their effects on increasing the number of gut microflora on the expense of harmful organisms (Joshi *et al.*, 2018). Regarding synbiotics, they are food supplements produced by synergistic combination between prebiotics and probiotics. Synbiotics confer stronger beneficial effects to the host, when compared to prebiotics or probiotics (Khangwal and Shukla, 2019).

### 3.1. Effect of probiotics on fish health status

Studies explain the ability of dietary supplements to enhance fish immunity and improve protection against diseases (Conte, 2004; Caipang and Lazado, 2015). Hematology is a favorable technique that may reveal abnormalities caused by pathogenic or environmental conditions. The leucocytes and erythrocytes counts may be affected by underlying conditions as contaminants, diseases and immunostimulants (Grant 2015, Carraschi *et al.*, 2017). The conservation of fish health through dietary improvement is essential for successful farming and fish production, as dietary incorporations improve the nutritional status of fish, and directly affects the immune and physiological responses and resistance of the host (Kiron, 2012).

In-water application is the only procedure that can be applied for fish all ages. Feed supplements administration, however, has constraints during larval development. Moreover, injection, that is not also applicable for larval stages, causes great stress. On contrary, direct probiotics supplementation in water can be applied from day-one after hatching. The mixture of probiotic administration through water and supplemented feed is clearly recommended as the proper method to apply probiotics in larval stages. Remus® commercial probiotic applied in water to cod larvae up-regulated growth-related proteins but down-regulated stress-related proteins (Park *et al.*, 2012). Based on results, most of the microbial content in cod larvae intestine was comprised of *L. plantarum* (Strom and Ringo, 1993). To summarize, administration of probiotics in water has been considered the most helpful application technique of probiotics in cod larval stages (Lauzon *et al.*, 2014).

### 3.2. Effect of probiotics on water quality

Aquaculture re-circulating systems must include regular control of water quality parameter. Adequate management of carbon: nitrogen proportion precedes a buildup of nitrogenous wastes in aquaculture practices. It typically expands the microbial population, and the great variety of pathogens fosters the equilibrium of the system by taking the nitrogen combinations which produce in situ microbial protein (Lopez-Elias *et al.*, 2015), development of nutrition (Moreno-Arias *et al.*, 2018), diminution of food conversion ratio and feed expenses besides promoting the health of the cultured organisms.

Naturally, the management of toxic combinations (nitrogenous substances) is hypothetically carried out by denitrifying bacteria; this is a starring role which probiotics could perform. Lately, various types of probiotics have demonstrated efficiency in removal of ammonia. Consequently, these eco-friendly probiotics can participate to enhance water quality. For instance, *Bacillus subtilis* has been commonly dispensed as an applicable probiotic to regulate the water quality. The application of *B. subtilis* as a water supplement in the raising water of olive flounder led markedly to diminish ammonia levels and mortality (Grant, 2015).

*Bacillus* spp. have been competent to transform organic ingredients to carbon dioxide more effectively if matched with gram-negative bacteria transforming a bigger amount of organic substances to bacterial slum. Probiotic usage in water to enhance water parameters, and their dispensation to cleanse aquaculture wastewater is beneficial in places with limited water resources, as the water can be recycled for aquacultural events after passing through filtration and

purification. The *Bacillus licheniformis* probiotic from large yellow croaker not only substantially reduced ammonia levels, but also the protein and starch from indigestible feed in wastewater (Zhang *et al.*, 2011).

A study on probiotics application for disinfecting wastewater in ponds revealed reduction in nitrogenous levels, along with diminishing propensity in chlorophyll a levels. Probiotics can improve the water microbial variety and enhance its composition (Mohapatra *et al.*, 2013). Additionally, better water quality after in-water probiotics application may result from the improvement of parameters, like dissolved oxygen, temperature, and pH. (Aguirre-Guzman *et al.*, 2012). Nevertheless, applying various probiotic species (e.g., *Pseudomonas*, *Nitrobacter*, *Cellulomonas* and *Enterobacter*) was not effective for channel catfish to enhance water quality; therefore, knowledge of the mechanisms of action of probiotics in cleaning water is still in its early stages (Ibrahim, 2015).

### 3.3. Effect of probiotics on fish survivability

Skin mucosal epithelia are considered the first line of defense. A compilation of innate and adaptive immune cells describe the immune response owed to mucosal epithelia of fish, controlled by skin, gill and gut related lymphoid tissues (Mohapatra *et al.*, 2013).

Probiotic applications (Figure 1) through insertion in water can promote the non-specific immune response, as the mucus layer constitutes the primary barrier against the aquatic environment (Cordero *et al.*, 2015). A substantially higher Atlantic halibut (*Hippoglossus hippoglossus* L. larval survival was procured by probiotic *L. plantarum* in-water supplementation (Ottasen and Olfasen, 2000).

To guarantee a viable aquaculture, guarding of egg clusters against infections is a crucial requirement. Presently, there is no commercial preparation focused on this area, particularly in the brief case of marine species. The usage of probiotics is extremely promising in efforts to reduce infections and reduce the larval mortalities. Also, helps to prevent egg pathogenic infestations. Therefore, probiotic application in water might improve the status of substrate spawners' aquaculture (Wesseling *et al.*, 2015).

### 3.4. Probiotics administration in aquaculture

Massive aquacultural improvements are achieved by application of probiotics as replacements to chemical therapeutics, so this accomplishment deserves more studies to less explored issues. One of these matters is the effectiveness of various administration techniques. The direct supplement of probiotics into water in aquaculture systems as a possible method has been less studied related to the other application techniques, although some studies reveal high effectiveness of probiotic administration through water. Additionally, as this technique is more operative in marine environments for the reason that higher probiotic is up taken by treated fish, more research concerning in-water probiotics applications in various marine fish species must be taken out. Additional studies on environmental safety of these additives are required (Chinabut and Puttinaowarat, 2005). The minimum examined issues in the area of probiotics in-water administration involve the relationship between the quantity and quality of probiotics required to control ammonia in fish farms (Gross *et al.*, 2004; Skjermo *et al.*, 2015). The use of various probiotics have been recognized very helpful also in shrimp production (Hostins *et al.*, 2017).

Studies on this application route would include various molecular biotechnology devices. DNA microarrays are generally used for the evaluation of immune responses in fish, but the information delivering transcriptomic effects accelerated by probiotics in-water application is very restricted; therefore, additional studies are required for better understanding of probiotics in-water application (Murray *et al.*, 2010; Reyes-Lpez *et al.*, 2015).

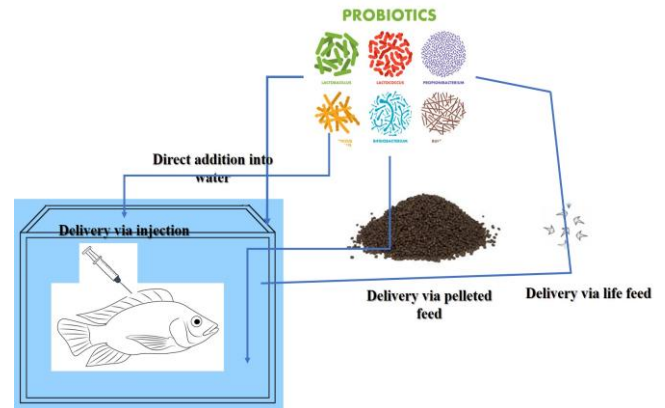


Fig.1. Various probiotics application methods in aquaculture.

## 4. Probiotics

### 4.1. Chemistry of probiotics with special reference to Mannan oligosaccharides-probiotics

Mannans are widely known in several plants species in the form of storage or structural components. Mannans typically consist of repeating mannose medieties which are linked with  $\beta$ -1, 4-glycosidic bonds (Soni & Kango, 2013). Galacto-mannan is purified from the various plants endosperm as coconut and gum, meanwhile glucomannan can be obtained from konjac and orchid species. One of the popular uses of heteromannans in food industry is their routine use as gelling, thickening or stabilizing agents (Nopvichai *et al.*, 2019).

MOS are carbohydrates of short chain comprising of 3–10 mannose. Presently, they are produced by mannans cell wall hydrolysis which originates from *Saccharomyces cerevisiae* yeast or plant galactomannans. The structural variety in-between  $\alpha$ - and  $\beta$ -MOS depends on the parent mannan polymer glycosidic link.  $\alpha$ -MOS are drawn from hydrolysis of yeast cell wall  $\alpha$ -mannan, meanwhile,  $\beta$ -MOS are acquired from  $\beta$ -glycosidic bonds plant mannans. Commercial products, as Active MOS<sup>®</sup>, FerMos<sup>®</sup> and Bio-Mos<sup>®</sup> consist of  $\alpha$ -MOS that is obtained from yeast and used as probiotics in the poultry and recently in aquaculture (Prajapati *et al.*, 2018).

MOS are produced by the hydrolysis of different natural mannans that ends in the release of  $\beta$ -MOS of differing degree of polymerization. Recent advancements indicate that MOS is an essential supplement and could be a considerable intervention as an antioxidant agent (Jana and Kango, 2020).

### 4.2. Probiotics as feed additives in aquaculture

Mannan-oligosaccharide (MOS)-containing probiotic has been widely examined for its potential role in improving fish responses and immunity. It has been administered to Zebra fish Juveniles at a rate of 0.4% MOS/Kg basal diet and succeeded to modulate fish behavior to starvation from

anxiety to near normal behavior (Forsatkar *et al.*, 2017). A two percentage of the yeast-based prebiotic, Grow Biotic added to the basal diet offered to *Tototaba macdonaldi* has been associated with significant reduction in lysozyme activity, compared to control group, despite different bacterial clustering patterns in response to diet supplementation (Gonzalez-Felix *et al.*, 2018).

Prebiotics are dietary supplements which represent a distinct approach for avoidance of diseases in various animals including aquatic animals. Prebiotics can be described as indigestible food ingredients which alter the structure and/or function of microbiota via selectively stimulating the proliferation and/or activity of beneficial bacteria, subsequently improving the overall aquaculture production (Burr *et al.*, 2010).

The positive effects include improving growth and feed utilization, plus enhancing the immune response in fish (Zhou *et al.*, 2010), and improved survival after challenges against pathogens (Li and Gatlin, 2005) or parasites (Buentello *et al.*, 2010).

A recent study in Rainbow trout (*Oncorhynchus mykiss*), to investigate if selenium-enriched prebiotic effect on fish, showed no significantly higher weight gain, hepatosomatic index, and viscerosomatic index than the fish fed control (Gonzalez-Felix *et al.*, 2018).

Rossi *et al.* (2021) studied the effects of prebiotic and glycine application on growth performance, nutrient composition of fish body and intestinal histopathology of largemouth bass (*Micropterus salmoides*). They concluded that glycine incorporation may improve growth of Largemouth bass after complete replacement of fish meal with glycine.

Most MOS are derived from yeast cell wall (*S. cerevisiae*) that stimulates growth and fortifies the fish immune system as cited by Rungrassamee *et al.* (2014). In a similar context, MOS supplementation had the ability to modulate immune-stimulatory effects by interaction with a specific receptor in the innate immune system of fish (Iqbal *et al.*, 2018). Li *et al.* (2018) observed that MOS and/or inulin enhanced immune-related genes transcription like TLR and STAT in shrimp. Supplemented diet improved protection against *Vibrio alginolyticus* and whispo virus.

Recently, Serradell *et al.* (2020) studied effects of supplementation of the prebiotic MOS to the diet of European sea bass (*Dicentrarchus labrax*) at a rate of 0.5% for nine weeks which was followed by stress induction through confinement of combined confinement and challenge with intestinal infection by *Vibrio anguillarum*. Serum cortisol was significantly decreased in fish exposed to confinement challenge. In addition, the prebiotic increased fish lysozyme levels after infection (Al-Sherji *et al.*, 2013).

## CONCLUSION

Supplementation of farmed fishes' diets with probiotics and prebiotics, and the synchronization between both could be a promising solution for improving the overall performance of farmed fishes, increasing survivability, and expectedly enhancing digestibility and intestinal microflora. This will in turn results in maximizing productivity and profitability. Further studies are needed to illustrate the mechanism of action of synergetic effect of both probiotics and prebiotics in aquaculture.

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