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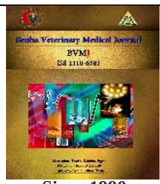

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Review Article

Effects of prebiotics supplementation on Nile tilapia, emphasizing phospholipids aquafeed - A review.

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ABSTRACT

Aquaculture has been a growing provider of animal protein industry in recent decades. Tilapia is a very valued fish cultivated across a vast geographical region in numerous countries, including Egypt. Tilapia has the advantage of being tolerant of a wide range of environmental circumstances and utilizing food from the lowest trophic levels. In recent decades, aquaculture has become a greater contribution to animal protein supply. Tilapia is a highly valued fish grown in a wide range of nations, including Egypt, as a source of animal protein. Tilapia could be tolerant to various environmental conditions. Some natural bio-friendly feed additives such as probiotics, prebiotics, and synbiotics are become a new approach in aquaculture industry to maintain aquaculture species health and a good environment at the same time produce chemical and antibiotic-free aquaculture product they become popular dietary supplements with the potential to increase not only growth performance, but also immunological competence and general well-being of fish and crustaceans. The prebiotics preparations are known to be important feed additives to be used in the aquaculture industry to sustain both aquatic species and environmental health. This makes them popular incorporations to enhance not only the growth indicators but also the immunological competence and general well-being of fish and crustaceans. The current review paper focuses on the possible effects of phospholipids (P.L) containing prebiotics on the health, immunological response, growth performance, and survival of *oreochromis niloticus*.

1. *Oreochromis niloticus* culture

The Nile tilapia *Oreochromis niloticus* is typically found throughout the African countries and worldwide (Elsaied *et al.*, 2019). It is a member of cichlids and comes in the second place of the world produced freshwater fishes (F.A.O., 2012, 2014). However, the input of aquaculture and tilapia to the total African production of animal protein, it is still inadequate (F.A.O., 2018, 2020). Boosting tilapia production may offer an improving strategy for adopting aquaculture to increase animal protein and raise Africa's currently very low protein intake. Tilapia is among the most extensively cultivated fish worldwide. Farmed tilapia accounts for more than 77% of global tilapia output (F.A.O., 2009).

Murphy *et al.* (2020) has reported an increase in the national aquaculture production, that review was prepared in the period from 2000 to 2016 and detected that increase starting 3.5×10^5 Mt toward 13.7×10^5 Mt. Tilapia particularly improved from almost 1.6×10^5 Mt in 2000 to 10 Mt 10^5 Mt in 2016.The Egyptian tilapia industry provides 47.9% of the total fish supply, which weighs around 941.308 Mt tilapia productions. Recently, the genetically improved *O. niloticus* was presented in Egypt, resulting in higher performance; with better-quality strains recording a 27.9 percent greater harvest level (Ibrahim *et al*., 2013; Marjanovic *et al*., 2016 and Dickson *et al*., 2016).

According to estimates, Egypt ranks first and places in superior levels in aquaculture tilapia production compared to other African nations (Ali *et al.*, 2020). Remarkably, the aquaculture industry secures around 585,000 jobs for workers in this area (El-Sayed *et al*., 2021). As a result, unanticipated deaths are thought to generate significant financial losses in aquaculture processes. It should be emphasized that the Egyptian Tilapia farms were mostly presented as small-scale farms of 10 feddan or less, accounting for approximately half of all *O. niloticus* farms (Eltholth *et al*., 2015).

2. *Nile tilapia feeding behavior*

Nile tilapia's dietary habits are herbivorous, even though it is an omnivore species. The farming of Tilapia is inexpensive because it nurses on a short chain of the feed in a fish farm, reducing the pressure on the species for preying. Furthermore, tilapia feeding patterns avoid the accumulation of contaminants at higher food chain levels (Barlow, 2000). Tilapia feed mostly on decomposing organic debris, and they can capture plankton in their gill mucus (Fryer and Iles, 1972). Tilapia has a lengthy gut that can digest planktons, and its feeding habits are classed as unceasing feeding

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action, which means they should have their daily meal on almost from 3 to 4 times. The important characteristic of *Oreochromis niloticus* is its sensitivity to cold weather, with an optimal level for development at 28 to 32 degrees Celsius. In the interim, a decrease of 31% in growth performance was reported with low temperature of 20 to 22^{o}C (Lim, 1989).

3. Prebiotics

Prebiotics could be defined as any material, nutrient, substrate or fiber that assists as nutriment to the useful microbes in a host digestive tract (Mountzouris, 2022).

Prebiotics should be able to withstand stomach acidity. Thus, a prebiotic could also be described as a material that resist acidic gastric conditions, might be fermented by gut microorganisms and effectively stimulate the gut microbiota growth to advance the gut well-being and host status (Davani-Davari *et al*., 2019). They should be fermentable by the helpful gut microorganisms. Furthermore, prebiotics should encourage the beneficial microorganism's growth that benefits the host's health (Guerriero *et al.,* 2017). Prebiotics are famous for their favorable properties on gut micro-flora health and were reported to aid in the healing route of gut microorganisms following gut symbiosis in illness circumstances (Akram *et al.,* 2019). Prebiotics are hypothesized to provide health advantages to the gut by boosting the amount of gut micro-flora at the expense of dangerous microorganisms (Khangwal and Shukla, 2019).

One method to overcome antibiotic overuse in aquatic health management is to employ prebiotics as feed additives (Song *et al*., 2014; Zulhisyam *et al.,* 2020; Kari *et al*., 2021, 2022). These incorporations are being widely described to produce encouraging results in terms of growth enhancement (Ramos *et al.,* 2017), improving immunological reactions (Selim and Reda, 2015), resistance to various diseases (Li *et al.,* 2018), and relieving abiotic stressors in aquatic animals (Hoseinifar *et al*., 2014).

3.1. Prebiotics in aquaculture

Prebiotics, often known as helpful compounds, offer food or energy to beneficial microbes. Prebiotics were used to improve fish health by favoring gut microbiota growth (Rohani *et al.,* 2021). Arabinoxylan oligosaccharide (AXOS), β-glucan, inulin, galacto-oligosaccharide (GOS), oligosaccharides, fructo-oligo saccharides (FOS), and mannan-oligosaccharide (MOS) were shown to improve aquaculture species' growth indicators (Li *et al.,* 2018), feed consumption efficacy (Shoaei *et al.,* 2015), immune response (Li *et al.,* 2005, Li *et al.,* 2021), and disease resistance (Figure, 1) (Yilmaz *et al.,* 2022). As a result, no doubt using prebiotics can aid to boost aquatic animals' output. Nevertheless, the fish gut biota is prebiotic-selective. Not all prebiotics are universal in their ability to encourage the expansion of the gut flora. Prebiotics like FOS (fructo-oligosaccharides) and GOS (galacto-oligosaccharides), for example, have been shown to enhance gut bacteria like *Bifido bacterium* and *Lactobacillus* sp. (Gibson *et al.,* 2017). This is due to the enzymes that *Bifido bacterium* and *Lactobacillus* sp. both have that ferment oligosaccharides. Regardless of the facts, more prebiotics added to fish feed can encourage more gut flora species of aquatic animals, improving their health (Teitelbaum and Walker, 2002).

A Prebiotic can promote host gut flora growth (Dawood and Koshio, 2016). This might help in the development of aquatic creatures. Prebiotics can also improve feed efficacy (Figure 2). As a result, it can reduce feed conversion ratio (FCR) and boost aquatic animal growth performance. According to a literature review, numerous different forms of prebiotic mixes efficiently support the growth of several aquatic species (Ganguly *et al.,* 2013). According to the literature review, several prebiotic mixtures successfully stimulate the growth of many fish and aquaculture species.

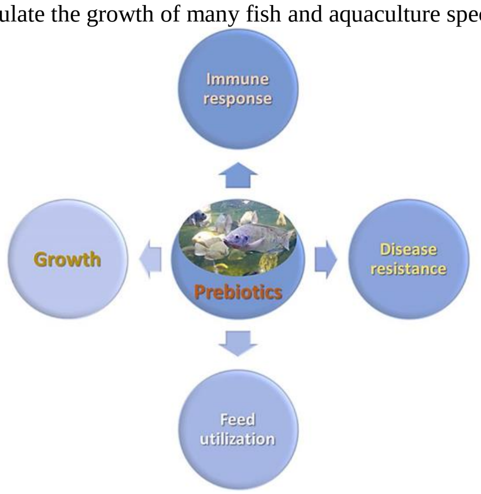

Figure 1 Prebiotics usage in aquaculture

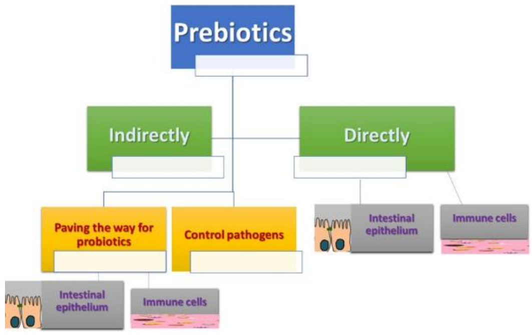

Figure 2 Prebiotics mode of action in aquaculture

3.1.1. Prebiotic supplementations effects on aquatic animals' growth performance

Gut microbiota could efficiently promote the growth of fish (Dawood and Koshio, 2016). This will assist in boosting the growth of fish and other aquatic animals. In addition, Prebiotics preparations can likewise upsurge feed usage and efficiency. Thus, it can raise the growth rate and minor the feed conversion rate (FCR) of aquatic animal (Ganguly *et al.,* 2013). It was reported that various mixtures of prebiotic and supplementations successfully promote the growth and weight gain of several aquatic species (Lu e*t al.,* 2019). For instance, red drum (*Sciaenops ocellatus*) supplemented with GOS + FOS + Galacto-gluco-mannans (GGM) + MOS mixtures (Zhou *et al.,* 2010), post-larvae of *Litopenaeus vannamei* supplemented with GOS + inulin + FOS (Oktaviana and Yuhana, 2014), Chinese mitten crab (*Eriocheir sinensis*) incorporated with MOS + inulin + β-glucan (Lu *et al.,* 2019) and much more for example presented in Table 1. β-glucan prebiotic, was reported to improve the growth of many aquatic animals when used separately or in combination with several additional prebiotics. β-glucan, when combined with MOS will represent a perfect mixture that successfully can boost the Nile tilapia (*O. niloticus*) growth performance (Selim and Reda, 2015), and was reported to exert the same actions in other species for example, shabout (*Tor grypus*) (Mohammadian *et al.,* 2021), Chinese mitten crab (*Eriocheir sinensis*) (Lu *et al*., 2019), snake head (*Channa striata*) (Munir *et al.,* 2016), and Caspian trout (*Salmotrutta caspius*) (Jami *et al*., 2019).

Table 1 Effect of prebiotic supplementations on aquatic animals' growth performance.

Supplement	Fish spp.	Concentration g/kg diet	Reference
Glucans	Nile tilapia *O. niloticus*	0.1 for two weeks	(Lu et *al.*, 2019)
Mannan oligosaccharide and β-glucan		0.4-2	(Abdel gayed et *al.*, 2021)
Mannan oligosaccharide, β-glucan, and dextrose		0.5–1.6	(Ismail *et al.*, 2019)
lecithin Lysomax®		0.45	(El-Sayed *et al.*, 2021)
β-glucan, MOS	Caspian trout	4	(Jami et *al.*, 2019)

Calcium format prebiotics when used separately or as a mixture with others at the rate of 0.5 and 1.5 percent can enhance the growth and feed conversion of fishes. Moreover, ACIDLAC® organic acids mixture when used as a replacement will have growth enhancement and other advantageous effects (Benedetto, 2003). Mairoka *et al.* (2004) similarly conveyed that organic acids compounds might successfully substitute antibiotic growth promoters (AGPs) and other chemicals for improved overall performance.

Almost synchronization occurs between probiotics and prebiotics, where probiotics augments the theory of prebiotics, through selectively stimulating the growth of gut beneficiary bacteria plus activating the metabolism of those bacteria that will improve the intestinal stability. However, the data about the prebiotics applications in aquaculture is still inadequate. It is reported that prebiotic Fermacto® addition (1, 2 and 3 g/kg) in aqua feeds displayed considerably higher body weight and better food conversion ratio in common carp fry. Rearing conditions of carp fry were improved after feeding Fermacto prebiotic at an optimal level of 3 g/ kg (Mazurkiewicz *et al.*, 2008).

3.1.2. Effect of dietary phospholipid (PL) supplementation in aquafeeds

In larval and juvenile stages of several species of fish and crustaceans, dietary phospholipid (PL) supplementation in purified diets has been shown to positively affect survival, growth, resistance to stress testing, and abnormalities. Due to the difficulties of bio-encapsulating PL in live prey, it is difficult to determine the precise PL needs in larvae. It is also challenging to compare needs estimated with artificial diets due to the wide variation in purity and content of PL sources and experimental settings (such as diet formulation and degree of co/pre-feeding with real food). Larval stages need more nutritional PL than juveniles because they are so vulnerable to a dietary PL shortage. Red sea bream larvae and goldfish larvae both had substantial reductions in survival. As evidenced by the survival of young fish, such as rock bream *Oplegnathus fasciatus* and sea bass, juvenile fish appear to be less impacted by the dietary PL supply than larvae (Kanazawa, 1993).

There is substantial debate about whether high dietary phospholipid levels are harmful to fish and crustaceans. Increasing dietary PL levels over the necessary level did not affect survival or growth in several trials (Kanazawa, 1993). However, when more than 3% soybean lecithin was included in the diet, larval *P. japonicus* survival was reduced (Coutteau *et al.,* 1996).

It has been proposed that lecithin is needed as a surfactant for effective lipid emulsification and digestion in early crustacean and fish larval stages (Koven *et al.,* 1998). Dietary PL is more important in larval fish and crustaceans than in juveniles. Because of their capacity to transport lipids and retain fatty acids, PL fractions such as Phosphatidyl choline (PC) and PI are more effective in larvae. Furthermore, PLs increase food features regarding water stability via their gum characteristics and chemo-attractive capabilities. Because of its increased digestibility, it outperforms neutral lipids in terms of essential fatty acid and energy content. The favorable effects of PL lead to increased development, survival, and quality in fish and crustacean larvae. Sea bass larvae can use PL more effectively than neutral lipids, resulting in increased growth, survival, and skeletal development Cahu *et al*., 2003;Olsen *et al*., 2014). The key bottleneck is the reduced survival of freshwater catfish (*Clarius batrachus* and *Heteropneustus fossilis*) larvae, which might be addressed by PL supplementation in the diet (Olsen *et al*., 2014). As a result, further studies on these species are required.

3.1.3. Effects of prebiotic mixtures containing soybean lecithin on the growth performance of aquatic animals

The fish performance and innate immune response can be significantly enhanced by using the lecithin-containing bio-emulsifier Lysomax® for farmed Nile tilapia to perform at their optimum and maintain good health, around 0.45 g/kg of feed would be sufficient (El-Sayed *et al*., 2021).

The aquafeed industry has been obliged to lower the quantities of dietary fish oil in feed formulations due to the constant rise in fish oil costs and their unsustainable supply. An extensive study has been done on partially substituting plant oil with fish oil (Santigosa *et al.,* 2011).

Freshwater fish respond differently to additional PLs depending on the species, stage of growth, and environmental factors. In larval common carp (*Cyprinus carpio*), for instance, dietary PL was crucial for growth, survival, feed utilization, and optimal health, most likely because immature fish cannot synthesis PL (Fontagne *et al.*, 2000). However, because they can produce PL from nutritional precursors, bigger fish may not benefit from more PL. Supplemental soy lecithin, on the other hand, had no impact on fingerling and juvenile channel catfish survival, growth rates, or feed efficiency (Sink and Lochmann, 2014).

3.1.4. Prebiotic supplementations effect on aquatic animals' immune response and disease resistance

Prebiotic could be an intricate carbohydrate that might offer a source for energy for gut macrobiotic abundance, therefore it could improve the immune response of aquatic animals and fishes (Mohammadi *et al*., 2022). Prebiotics can motivate immune reactions in fishes. The immune saccharides might be an additional selection to be administered in aquaculture as an alternative to the usage of antibiotics for enhancing the health management of aquatic animals (Hoseinifar *et al.*, 2020). There are two expending methods for the prebiotics to improve the immune reactions in aquatic animals, whichever through demotion of gut flora growth or innate immune system activation (Song *et al*., 2014). Nevertheless, the microbiota of animal gut is selective to an exact prebiotic. Thus, the prebiotic mixtures application will provide superior effects on the immune response of aquatic animals (Elumalai *et al.,* 2022) as revealed in Table 2.

Table 2 Effect of prebiotic supplementation on the aquatic animals' immune response.

Supplement	Species	Dose g/kg diet	Reference
Chito-oligosaccharides and Shrimp shell chitin (hydrolyzed)	Tilapia hybrid (*O.aureus×O.niloticus*)	1	(Qin *et al.*, 2014)
GOS + FOS + GGM+ MOS	Red drum	10	(Zhou *et al.*, 2010)
MOS and β-glucan	Caspian trout	4	(Jami *et al.*, 2019)
	Gilthead sea bream	1	(Guzmán-Villanueva *et al.*, 2014)
	Nile tilapia	0.5-2	(Abdel gayed *et al.*, 2021)

There are very few studies on immune modulation by dietary PL. Phosphatidyl serine has the immune modulatory role in white shrimp (*Penaeus vannamei*) when supplemented in diet that can enhance total hemocyte count and phenoloxidase values of hemocyte and plasma. Besides these, it can also modulate antibacterial and bacteriolytic activities in blood plasma, and PL can modulate anti-stress capacity in shellfish. Inclusion of PL in diet improves the gill health of grass carp, indicating enhanced immunity and antioxidant status and it is also reported that supplementation of PL in diet can improve gut health and immunity through modulation of lysozyme, up-regulating expression level of interleukin 10, down-regulated tumor necrosis factor á (TNF-á), superoxide dismutase, glutathione peroxidase and glutathione reductase (Cui *et al.,* 2018).

Similar to this, immune responses (phagocytic activity, respiratory burst activity lysozyme activity, and phenoloxidase values) in *O. niloticus* fed lecithin-containing diets, particularly at 0.4 g/kg, were greatly improved compared to the control diet-fed fishes. These findings unequivocally show that Lysomax enhances innate immunity in this species. This may be explained by the fact that dietary PLs and their unsaturated fatty acids can make cell membranes more permeable and fluid, which strengthens fish immune (Balfry and Higgs, 2001).

The increased phospholipid composition and fatty acid outline of Lysomax may also be responsible for improving fish immunity as a result of supplementation. Phospholipids are the precursor of the substrate fatty acids needed for producing eicosanoids (EPA, 20:5n-3) (Balfry and Higgs, 2001). Most of the research highlighted in this review focused on the favorable benefits of prebiotic combinations as feed additives, which can boost aquaculture output by encouraging growth, strengthening the immune system, raising disease resistance, and lowering stress in aquatic animals. Research has been undertaken to assess the potential of prebiotics or prebiotic combinations to lower stress in aquatic animals. However, when introducing prebiotics to aquaculture species, correct dose and prebiotic type considerations must be made. A high dose of prebiotics can be harmful to aquatic animals, whilst other types of prebiotics can only boost growth but not the immune response or resistance to diseases of aquatic animals. There is still a gap between practical applications and scientific findings of prebiotics in aquatic animals.

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