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The role of prebiotics, probiotics, and synbiotics supplementation in Nile tilapia farming: A comprehensive review

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Abstract

Aquaculture's sustainability heavily relies on optimizing fish growth, health, and feed utilization. In recent years, synbiotics, a combination of prebiotics and probiotics, have gained significant attention as potential feed additives to enhance aquaculture productivity. This review provides a comprehensive analysis of the current state of knowledge regarding dietary synbiotics supplementation in tilapia farming. This review explores the various types of prebiotics, probiotics, and their combination (synbiotics) used in aquafeeds, their mechanisms of action, and their individual and combined effects on tilapia growth, digestive efficiency, immune responses, and disease resistance. Additionally, this review discusses the implications of incorporating synbiotics in tilapia diets for overall fish health and well-being. This review aims to provide valuable insights for researchers, farmers, and industry stakeholders, contributing to the development of sustainable and efficient tilapia farming practices through dietary prebiotics, probiotics, and synbiotics supplementation.

Keywords: Aquaculture sustainability, Prebiotics, Probiotics, Synbiotics, Tilapia farming

Introduction

Aquaculture has experienced significant growth in recent years due to the rising demand for fishery products and the depletion of wild fish stocks. With the global population increasing rapidly, the need for affordable protein sources has become crucial, making aquaculture a vital

solution (El-Saadony et al., 2021). Governments and development agencies are increasingly focusing on aquaculture as a promising activity to meet global food demands. It outpaces other food production methods in growth rate, making it essential for addressing food shortages and malnutrition (FAO, 2020). Successful aquaculture involves maintaining water quality, ensuring proper nutrition, promoting breeding, and preventing diseases and predators. However, the industry faces challenges such as the high cost of fish feed ingredients and frequent disease outbreaks, which hinder the full potential of aquaculture's intensification and commercialization (Tabassum et al., 2021).

Among various cultured species, Nile tilapia (*Oreochromis niloticus*) stands out as one of the most prominent choices for aquaculturists due to its fast growth rate, high fecundity, high adaptability to different environmental conditions, and palatability to consumers (Hasan et al., 2019). As the demand for sustainable aquaculture rises, there is a growing urgency to enhance the overall performance and health of farmed fish. *O. niloticus* is a significant species cultivated in tropical, subtropical, and temperate regions (El-Sayed, 2020), providing approximately 4.5 million metric tons yearly, serving as an affordable protein source for global food security (Senapin et al., 2018). To enhance the efficiency of tilapia aquaculture, significant research efforts are required to optimize feeding costs and improve feed utilization. This will ultimately lead to better growth performance, characterized by increased weight gain, growth rates, and survival rates (García-Ulloa et al., 2013).

The sustainability of aquaculture depends on various factors, including site selection, seed quality, disease outbreak, water quality, and cost-effective feed production. Recent research indicates that the majority of the costs associated with aquaculture operations are primarily attributed to the production of feeds, accounting for a significant proportion of the total production cost, ranging from 66% to 84%, making it essential to reduce feed expenses to ensure profitability (Jahangiri and Esteban, 2018). To address this concern, researchers have increasingly focused on developing feed additives, particularly prebiotics and probiotics, to enhance feed efficiency in aquaculture. Incorporating these additives into aquafeed has shown promising results in improving food value, digestive enzymes, growth, and immune responses in fish (Huynh et al., 2017). Probiotics, prebiotics, and synbiotics have been identified as promising alternatives to traditional methods in tilapia farming. These supplements have been found to improve growth rates, feed efficiency, immunity, disease resistance, and overall survival rates of tilapia in the face of pathogens and environmental stressors, making them a viable, safe, and eco-friendly option for sustainable aquaculture practices. Overall, their

inclusion in fish diets or rearing water has been found to improve the well-being of fish in aquaculture (Mugwanya et al., 2022).

This review aims to comprehensively evaluate the existing body of literature on the effect of dietary prebiotics, probiotics, and synbiotics supplemented diets on the growth performance and various physiological parameters of Nile tilapia (*Oreochromis niloticus*). It will delve into the types of prebiotics and probiotics used in aquafeeds, their mechanisms of action, and their individual and combined effects on tilapia growth, digestive efficiency, immune responses, and disease resistance. By critically examining the existing body of knowledge, this review seeks to contribute to the advancement of sustainable and efficient tilapia farming practices through the integration of synbiotics in aquafeeds. Ultimately, the findings of this review may prove valuable for researchers, farmers, and industry stakeholders, promoting the growth and success of the tilapia aquaculture sector while addressing the global challenges of food security and environmental sustainability.

Prebiotics

A prebiotic refers to a substance, such as a substrate, long chain sugar, nutrient, or fiber, that functions as nourishment for the advantageous bacteria residing within the digestive tract of a host (Mountzouris, 2022). Moreover, a prebiotic can be characterized as a substance that exhibits resistance to the acidic conditions prevailing in the stomach, possesses the ability to undergo fermentation by the gut microbiota, and facilitates the proliferation of gut microbiota, hence enhancing the overall well-being of the host (Davani-Davari et al., 2019). Tran et al. (2022), have indicated that prebiotics serve as energy sources for gut microbes, hence enhancing health outcomes in the host. In general, prebiotics can be described as complex carbohydrates with long chains that serve as a source of energy for beneficial microorganisms with the aim of improving the overall health of an organism. The primary origins of prebiotics are predominantly sourced from botanical sources (Mohammadi et al., 2020). Additionally, edible mushrooms have been identified as a source of prebiotics (Balakrishnan et al., 2021), whereas animal dairy products contribute to a smaller proportion of prebiotic sources. Prebiotics, essential for maintaining a healthy gut microbiome, are abundantly found in a diverse range of natural food sources. These compounds, which include vegetables, fruits, beans, seaweed, microalgae, and animal milk, provide a rich and accessible source of prebiotics that can be easily incorporated into a balanced diet (Ahmadifar et al., 2019; Elumalai et al., 2020).

Prebiotics in Nile tilapia farming have been found to have several beneficial effects. They enhance growth by fostering the proliferation of beneficial gut bacteria, which improves nutrient absorption and utilization, ultimately resulting in better growth performance (Ramos *et al.*, 2017). Prebiotics have been shown to boost the immune system of Nile tilapia, thereby increasing their resistance to diseases and infections (Selim and Reda, 2015; Li *et al.*, 2019). The incorporation of prebiotics in fish farming can minimize the need for antibiotics by promoting fish health and reducing the likelihood of disease outbreaks, thereby contributing to a more sustainable and responsible aquaculture practice (Song *et al.*, 2014; Abdul-Kari *et al.*, 2021). Prebiotics play a crucial role in enhancing the health of Nile tilapia by fostering the growth of beneficial gut bacteria. This leads to a balanced gut microbiome, which in turn contributes to improved overall health and productivity in fish farming. Additionally, incorporating prebiotics into Nile tilapia diets can be economically beneficial due to enhanced growth performance, reduced feed consumption, and increased resistance to pathogens (Dias *et al.*, 2019) (Figure 1).

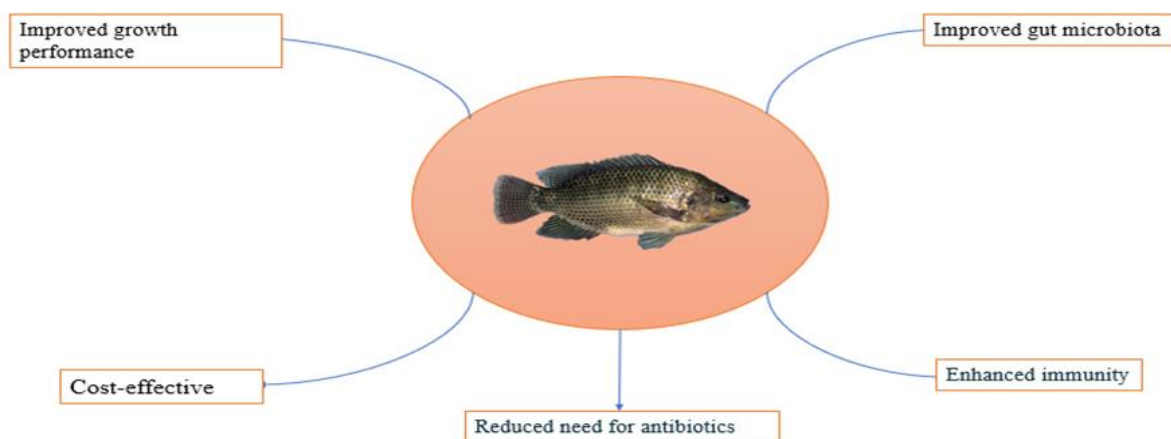


Figure 1. Positive effects of prebiotic on Nile tilapia Farming

Prebiotics mode of action in tilapia

Nurturing beneficial gut microbes through selective fermentation

Prebiotics are essential in tilapia farming, as they provide a nutrient-rich environment for beneficial gut bacteria, which in turn, significantly impact the health and growth of the aquatic organisms (Wee *et al.*, 2022). One of the key mechanisms underlying their effectiveness is their role as a nutrient source for specific bacteria in the gastrointestinal tract (GIT). Notably, prebiotics like inulin undergo selective fermentation by microbes such as *Bifidobacterium*, leading to the secretion of enzymes like beta-fructosidases that degrade

inulin's glycosidic bond. This fermentation process alters the gut microbial composition, favoring the proliferation of beneficial microbes over pathogens (Merrifield et al., 2010). Studies on tilapia have shown that supplementing diets with specific prebiotics, such as glucan, leads to increased populations of beneficial firmicutes and proteobacteria in the gut, which subsequently contribute to improved growth and health (Souza et al., 2020).

Stimulation of digestive enzyme production via microbial proliferation

Prebiotics also exert their impact on tilapia farming by indirectly stimulating the production of essential digestive enzymes. The proliferation of beneficial gut microbes, promoted by prebiotics, results in increased secretion of these enzymes, thereby enhancing the fish's ability to digest and absorb nutrients. Recent research by Aryati et al. (2021) demonstrated that incorporating honey, a prebiotic, into *O. niloticus* diets led to a higher population of gut microbes that externally produce crucial digestive enzymes like amylase, protease, and lipase. This positive correlation between prebiotic supplementation and enzyme activity translates into improved fish growth performance. This phenomenon is consistent with findings by Abd El-latif et al. (2015), who highlighted the role of prebiotics in enhancing digestive enzyme activity and production in tilapia. Enhanced enzyme activity not only supports nutrient utilization but also positively correlates with overall body weight and feed efficiency (Hoseinifar et al., 2015).

Increasing disease resistance and the innate immune system

Prebiotics have a crucial impact on the innate immune system of tilapia in aquaculture, significantly boosting its resilience and overall health, while also promoting the maintenance of gut microbial equilibrium. The immune system activation and protection against possible infections have been associated with the presence of beneficial gut bacteria, which is regulated by prebiotics. The research conducted by Akhter et al. (2015) revealed that prebiotics can enhance the innate immune response in fish and shellfish, hence providing them with increased resistance against infections. Moreover, the diverse range of activities exhibited by prebiotics encompasses the secretion of extracellular enzymes produced by bacteria and the production of bioactive metabolites. These compounds, as shown by Huynh et al. (2017), can indirectly influence tilapia growth and health, contributing to a comprehensive strategy for optimizing growth, reproduction, immunity, and disease resistance. ficiency (Hoseinifar et al., 2015).

Application of prebiotics in Nile tilapia farming

The utilization of prebiotics in tilapia aquaculture holds significant promise, as it offers a multitude of potential advantages such as enhanced growth performance, enhance immune response, diminished reliance on antibiotics, improved gut health, and heightened resistance against diseases. Prebiotics commonly utilized in aquaculture encompass a range of substances such as β -glucan, inulin, arabinoxylan oligosaccharide (AXOS), mannan oligosaccharide (MOS), galacto-oligosaccharide (GOS), fructo-oligosaccharides (FOS), and oligosaccharides. Prebiotics have been extensively studied and are known to offer numerous advantages in aquaculture. Research indicates that they can significantly boost growth performance (Li et al., 2019), enhance feed efficiency (Shoaei et al., 2015), strengthen the immune system (Li et al., 2021), and improve disease resistance (Abdel-Latif et al., 2022; Li et al., 2018; Yilmaz et al., 2022). As a result, the incorporation of prebiotics into aquaculture methodologies is expected to augment the output of species. Nevertheless, it is crucial to acknowledge that the reaction of the host gut microbiota to prebiotics exhibits selectivity (Gibson et al. 2017). Prebiotics like fructooligosaccharides (FOS) and galactooligosaccharides (GOS) have been observed to selectively promote the growth of specific gut microbiota, such as *Lactobacillus* and *Bifidobacterium* species, due to their ability to be fermented by these bacteria, enhancing their enzymatic activity (Gibson et al., 2017). These prebiotics play a crucial role in modulating the gut microbiota by stimulating the proliferation of beneficial bacteria while inhibiting the growth of pathogenic strains (Kaewarsar et al., 2023). The fermentation of FOS and GOS by probiotic bacteria leads to the production of short-chain fatty acids (SCFA), which have various health benefits and contribute to the overall metabolic activity of the gut (Bamigbade et al., 2023). Therefore, the preference of certain gut microbiota for FOS and GOS may be attributed to the specific enzymatic processes involved in fermenting these prebiotics, highlighting the importance of understanding the interactions between prebiotics and gut bacteria for promoting gut health and overall well-being. However, the incorporation of supplementary prebiotics into aquaculture diet has the potential to broaden the range of gut microbiota in aquatic organisms, hence promoting improvements in their overall health.

The application of prebiotics in Nile tilapia has been ongoing since 2010 and has been increasing every year. This is achieved by fostering the proliferation of beneficial gut bacteria, thereby strengthening the fish's immune system and increasing their resistance to pathogens (Figure 2).

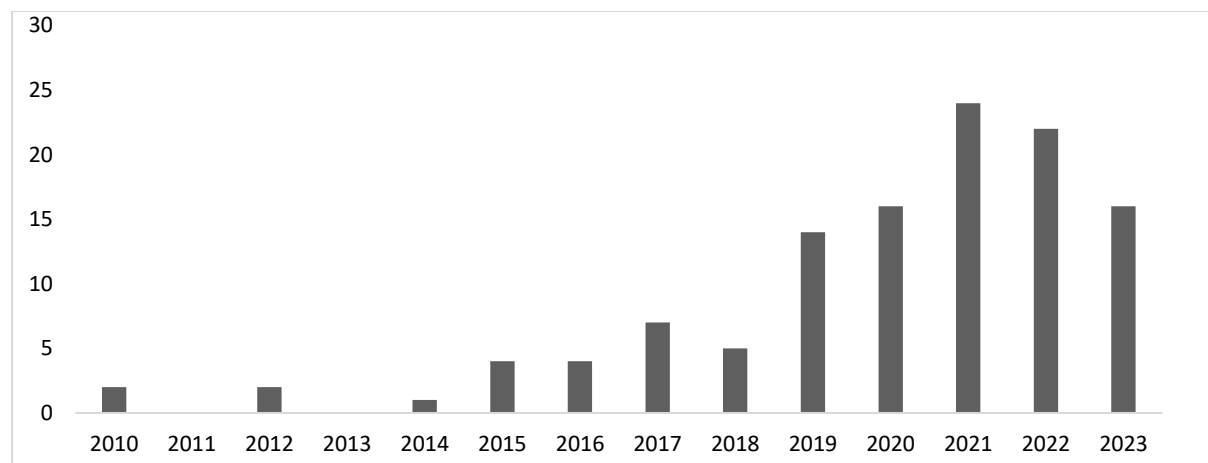


Figure 2. Application of prebiotics in Nile tilapia farming 2010 - 2023

Table 1 provides a summary of prebiotic applications in Nile tilapia farming, their dosages, durations, and the responses observed in Nile tilapia. These findings suggest that prebiotics can have significant positive effects on the health and performance of Nile tilapia, including improved growth, immune function, and resistance to pathogens. However, the optimal dosage and duration of prebiotic use may vary depending on the specific prebiotic and the fish species.

Table 1. Summary of prebiotic applications in Nile tilapia farming

Prebiotics	Dosage	Duration	Response	Reference
Inulin	5g kg ⁻¹	1 and 2 months	Hematocrit ↑, NBT (Superoxide activity) ↑, lysozyme activity ↑, Protection from <i>A. hydrophila</i> challenge ↑.	Ibrahim et al., 2010
Inulin	15–20 g.kg ⁻¹	60 days	intestine and glycogen deposition in liver ↑, immune parameters ↑, Haematological parameters ↑, Biochemical parameters ↑	Ali et al., 2017
Inulin	5 g g.kg ⁻¹	8 weeks	growth performance ↑, blood chemicals parameter↑, lysozyme activity ↑, ACH50 activity ↑	Tiengtam et al., 2015
Inulin	2.5 g.kg ⁻¹	2 weeks	Growth performance ↑, Immuno- haematological indices ↑, liver and spleen structure ↑, parasitic infection ↓	Yones et al., 2019
β-glucans (BG01 & BG02)	0.1 g.kg ⁻¹ BG01 & 0.1 g kg ⁻¹ BG02	30 days	Growth performance ↑, survival rate after bacterial infection ↑	Pilarski et al., 2017
MOS	0.5 %	12 weeks	Weight gain ↑, FCR ↓, lysozyme activity ↑, Mortality rates against <i>A. hydrophila</i> ↓	Kishawy et al., 2020
MOS	4 g g.kg ⁻¹	16 weeks	Growth performance ↑, serum albumin ↑, total protein ↑, RBCs ↑, and Hb levels in lead-contaminated diets ↑	Ayyat et al., 2020
FOS	2% & 3 %	8 weeks	Final weight ↑, weight gain ↑, SGR ↑, FCR ↓, intestinal enzymes activities ↑, histological feature of intestinal villi ↑	Abd El-latif et al., 2015
FOS	2 & 4 g.kg ⁻¹	8 weeks	Weight gain ↑, FCR ↓, digestive enzyme activity ↑, protein and lipid retention ↑.	Poolsawat et al., 2020
MOS	6 & 6 g.kg ⁻¹	21 days	ADG ↑, FCR ↓, disease resistance ↑	Samrongpan et al., 2008
β-glucan + Mos	1.5 & 3 g.kg ⁻¹	60 days	Growth ↑, feed utilization ↑, FCR ↓, Villus height ↑, goblet cells ↑, intraepithelial lymphocytes ↑, body composition ↑, serum total protein ↑, albumin ↑, globulin ↑	Selim and Reda, 2015
β-glucan	0.1% of diet	30–45 days	Growth ↑, immune performance ↑.	Koch et al., 2021

Probiotics

Probiotics refer to live microorganisms that are introduced into the gastrointestinal tract (GIT) through food or water with the aim of improving the internal microbial balance and thus supporting overall well-being (Gatesoupe, 1999; Azad et al., 2019). The utilization of probiotics in aquaculture has been recognized as an ecologically sustainable method for prevention of disease outbreaks, enhanced growth, and improved digestion (Guo et al., 2016; Adel et al., 2017).

Probiotics have been demonstrated to offer several positive effects on Nile tilapia farming. These benefits encompass improved immunological responses, enhanced growth, increased resistance to ammonia, better digestive health, and reduced stress levels. Probiotics can enhance the immunological responses of Nile tilapia, leading to a stronger immune system that helps the fish better resist diseases and infections, resulting in improved survival rates and overall health (Shija et al., 2023). Furthermore, probiotics can improve the growth rate of Nile tilapia by promoting the growth of beneficial gut microbes, which, in turn, support the overall health and development of the fish (Zabidi et al., 2021). They also aid in increasing the fish's resistance to ammonia, making them more resilient to the stressors of aquaculture environments (Cavalcante et al., 2020). Additionally, probiotics can enhance the digestive health of Nile tilapia by boosting the activity of digestive enzymes, which is crucial for the efficient breakdown of feed and the overall health of the fish (Xia et al., 2019). The use of probiotics also can help reduce stress levels in Nile tilapia, which is particularly important in aquaculture settings where high stress levels can lead to decreased growth rates and increased susceptibility to diseases (Munni et al., 2023). The Positive effects of probiotic on Nile tilapia Farming presented in Figure 3.

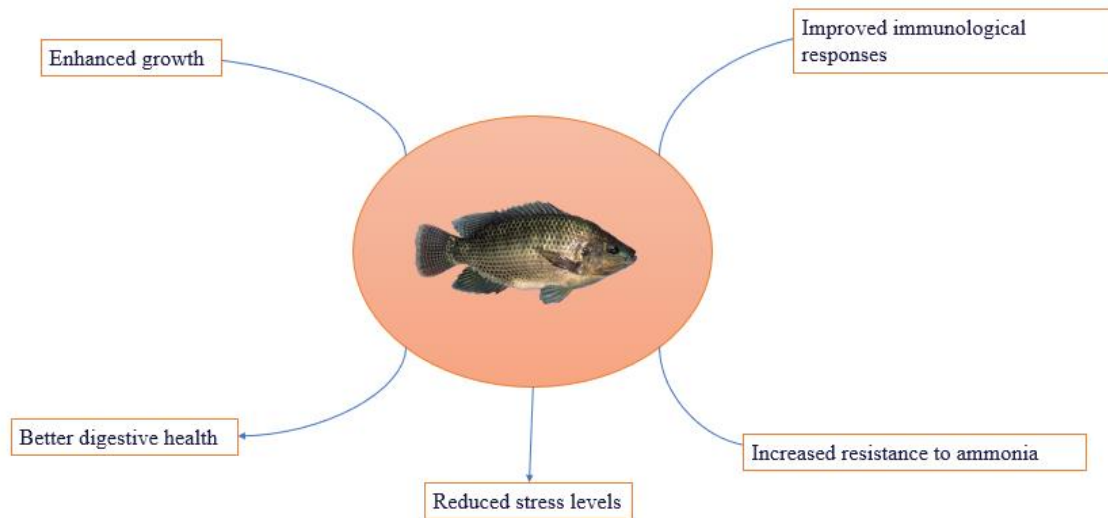


Figure 3. Positive effects of probiotic on Nile tilapia Farming

Probiotics mode of action in tilapia

The use of probiotics in tilapia aquaculture is employed with the intention of stimulating growth and bolstering the tilapia's ability to withstand diseases (Shija *et al.*, 2023). While the specific mechanism through which probiotics affect fish is still not fully understood, studies have demonstrated that probiotics can produce varying effects that are specific to the host and the type of probiotic used (Hai, 2015). Several potential mechanisms of action of probiotics in tilapia aquaculture can be identified, including:

Competition for nutrients

In general, bacteria have a requirement for iron, which is recognized as one of the vital minerals necessary for their growth. However, the growth of bacteria is hindered due to the restricted availability of iron in animal tissues and fluids. Siderophores that bind to iron make it easier for bacteria to get the iron they need to grow (Mugwany *et al.*, 2022). Gram *et al.* (1999), earlier posited a correlation between the synthesis of siderophores and the pathogenicity of specific microorganisms. Bacteria that possess the ability to produce siderophores exhibit the capacity to sequester iron in an environment characterized by low iron levels. This sequestration process is a challenge for pathogenic bacteria, as it hinders their ability to absorb iron and thus inhibits their growth. Furthermore, it has been observed that some probiotic microorganisms can produce digestive enzymes, which can effectively diminish the presence of anti-nutrient compounds in feed and enhance the absorption of nutrients. Consequently, this leads to an improvement in feed utilization and the overall performance of aquatic organisms (Steinfeld *et al.*, 2015). Carbohydrases, lipases, and

proteases are enzyme types that have been identified in previous research (Eshaghzadeh *et al.*, 2015). Taoka *et al.* (2007) reported that the administration of probiotics (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum*, and *S. cerevisiae*) directly or by dietary inclusion resulted in enhanced activity of digestive enzymes in tilapia. In their study, Tan *et al.* (2019) reported an observed augmentation in digestive enzyme activity in *O. niloticus* when subjected to nutritional supplementation of 10^6 and 10^7 CFU g^{-1} of *Rumeliibacillus stabekisii*.

Effect of probiotics on intestinal morphology

According to Nayak (2010), it is believed that probiotics have the ability to establish themselves and exert their advantageous effects within the intestinal tract of animals, which is recognized as a multifaceted ecosystem comprising non-pathogenic, pathogenic, and commensal bacteria. The development and function of the intestines in Nile tilapia are intricately linked to the presence of beneficial endogenous intestinal microbiota. These microbiotas play a vital role in regulating mucosal development and tolerance, highlighting their essential contribution to the overall health and well-being of the fish (Akter *et al.*, 2015). Probiotics have been shown to maintain a healthy intestinal environment in fish by significantly reducing the presence of harmful pathogenic microorganisms within the gastrointestinal tract. This reduction in pathogenic microorganisms allows for the development of a robust intestinal epithelial layer, characterized by lower mucosal damage and enhanced nutrient absorption. By promoting a healthy gut environment, probiotics can support the overall health and well-being of fish, ensuring optimal growth and development (Merrifield *et al.*, 2010).

Production of Inhibitory Substances

Probiotics are known to produce a variety of inhibitory compounds that exhibit antimicrobial and antiviral properties. The compounds produced by beneficial gut bacteria in Nile tilapia include a diverse array of substances that collectively contribute to the fish's overall health. These substances include siderophores, bacteriocins, hydrogen peroxide, lysozymes, proteases, volatile fatty acids (such as lactic, propionic, acetic, and butyric acid), organic acids, and extracellular enzymes. The production of these compounds is significant, particularly in the context of aquatic animals, where they are thought to play a crucial role in suppressing viral replication (Chauhan and Singh, 2019). Furthermore, inhibitory compounds lower the pH of the GIT, inhibiting the growth of opportunistic infections (Tinh *et al.*, 2008). Extracellular compounds produced by *L. acidophilus* were found to have excellent antibacterial activity against *A. hydrophila* and *Streptococcus agalactiae* in vitro, according to Villamil *et al.* (2014).

This could explain why *O. niloticus* developed better resistance to fish infections after consuming probiotic *L. acidophilus*.

Application of probiotics in tilapia farming

Probiotics have garnered substantial interest in the aquaculture industry due to their multifaceted advantages. As dietary additives, they offer a high level of security and contribute to improved growth rates in fish. Additionally, probiotics enhance nutrient digestion, boost the host's immunity, and modulate the balance of beneficial gut bacteria. This balanced gut microbiota, in turn, enhances the overall health of the fish. Furthermore, the application of probiotics in aquaculture has been demonstrated to have a profound impact on the environment by significantly reducing the reliance on antibiotics and fostering a healthier aquatic ecosystem (Ringø, 2010; El-Saadony et al., 2021). Probiotics are emerging as a highly promising and environmentally friendly alternative to antibiotics, offering a safe and sustainable solution for aquaculture (Assefa and Abunna, 2018). The primary probiotics used in this context include *Bacillus*, *Lactobacillus*, *Enterococcus*, and *Saccharomyces*, which have been extensively studied and classified as effective and non-polluting agents (Anokyewaa et al., 2021). Probiotics function in the host's intestine to enhance growth and digestion by either secreting digestive enzymes themselves or stimulating the production of such enzymes (Nunes, 2018); Additionally, they inhibit the adhesion and growth of pathogens, maintain the balance of beneficial gut bacteria, and strengthen the mucosal barrier. These actions also modulate the immune response and antioxidant levels, ultimately enhancing the host's resistance to disease (Amoah et al., 2019).

The application of probiotics in Nile tilapia has been ongoing since 2008, and the use of probiotics has been increasing every year (Figure 4). There are numerous publications related to the use of probiotics in Nile tilapia, which optimize reproductive performance and result in economic advantages. *Bacillus subtilis* is one of the probiotic strains that has been used in Nile tilapia, and it has been shown to improve growth performance and be profitable when applied by fish farmers. Studies have delved into the effects of probiotic supplementation on the growth, gut health, and disease resistance of juvenile Nile tilapia, revealing significant and promising results. Specifically, studies have shown improvements in weight gain, feed conversion ratio, and resistance to pathogens such as *Streptococcus agalactiae*, indicating the potential benefits of probiotics in enhancing the health and well-being of Nile tilapia. The use of probiotics in Nile tilapia is expected to continue to increase, as they provide benefits in terms

of growth, health, and disease resistance, leading to improved survival and overall performance in aquaculture.

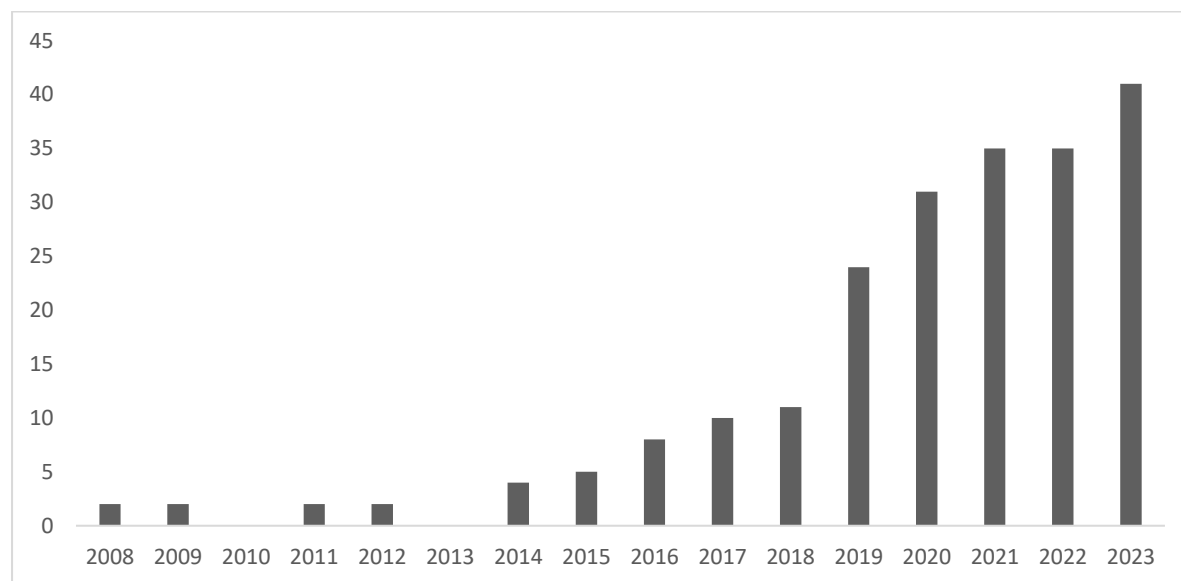


Figure 4. Application of Probiotics in Nile tilapia Farming 2008 – 2023

Table 2 provides summary about the application of probiotics in Nile tilapia farming, their dosage, duration, and response of various probiotics used in tilapia farming. These findings suggest that probiotics can have a beneficial impact on the growth and health of Nile tilapia.

Table 2. Summary of probiotics applications in Nile tilapia farming

Probiotics	Dosage	Duration	Response	Reference
<i>Bacillus pumilus</i>	1×10^8 and 10^9 CFU/kg	4 weeks	Weight gain ↑, SR ↑, FCR ↓, Disease resistance against <i>S. agalactiae</i> ↑, superoxide anion levels ↑.	(Srisapomee and Areechon, 2017)
<i>Bacillus</i> spp. (ANSCI9, BFAR9, RM3, and RM10)	10^8 CFU g ⁻¹ of feed.	30 days	Average body weight ↑, absolute growth ↑, specific growth rate ↑, relative growth rate ↑, FCR ↓, disease resistance ↑.	(Samson et al., 2020)
<i>Bacillus</i> spp. (RM10 and BFAR9)	10^8 CFU g ⁻¹ of feed.	56 days	average body weight ↑, specific growth rate ↑, FCR ↓, condition factor (k) ↑,	(Samson, 2022)
<i>Bacillus</i> spp. Mixture (<i>Bacillus subtilis</i> TISTR001, <i>Bacillus megaterium</i> TISTR067,	$(1 \times 10^6$ CFU/g)	120 days	Immune parameters ↑, <i>IL-1β</i> and <i>TNF-α</i> gene expressions ↑,	(Doan et al., 2023)

and <i>Bacillus licheniformis</i> DF001)				
<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus</i> <i>Acidophilus</i>	10 ⁸ cfu/ml	12 weeks	Final weight ↑, body length ↑, specific growth rate ↑, weight gain ↑, feed intake ↑, FCR ↓, protein efficiency ratio ↑, RBCs ↑, Hb ↑, mortality rate ↓.	(Hassanien et al., 2017)
<i>Saccharomyces cerevisiae</i> + <i>Bacillus</i> spp. (<i>B. subtilis</i> , <i>B. megaterium</i> , and <i>B. licheniformis</i>)	5.6 × 10 ⁸ cfu g ⁻¹ and 1 × 10 ⁶ cfu g ⁻¹	90 days	Final body weight ↑, weight gain ↑, average daily growth gain↑, aspartate aminotransferase (AST) ↓, alanine aminotransferase (ALT) levels ↓.	(Sutthi et al., 2018)

Synbiotics

Synbiotics, a dietary supplement, combines the benefits of both probiotics and prebiotics. By doing so, it positively impacts the host animal by promoting a balanced gut microbiome, enhancing overall health, and supporting growth (Okey et al., 2018). Synbiotics, a harmonious blend of probiotics and prebiotics, have been found to significantly enhance aquaculture productivity by promoting rapid growth in fish, strengthening their immune systems, and improving digestion and absorption of nutrients. Additionally, synbiotics have been shown to effectively manage diseases and maintain water quality, making them a valuable tool in the sustainable development of aquaculture (Nguyen et al., 2019; Waagbø and Remø 2020).

Synbiotics, a combination of probiotics and prebiotics, have a profound impact on organisms by not only enhancing their overall well-being but also by facilitating the implantation of live microbial dietary supplements in the digestive tract, thereby optimizing the gut environment and supporting the fish's health. This is achieved through the selective stimulation of the growth and metabolism of a limited number of health-promoting microorganisms, ultimately contributing to the overall welfare of the organism (Cerezuela et al., 2011; Das et al., 2017). The utilization of synbiotics in aquaculture demonstrates a remarkable synergistic effect on enhancing growth performance, immune response, and disease

and probiotics in aquaculture, most studies have consistently demonstrated synergistic effects when these components are combined, indicating that the interaction between them produces a more significant and beneficial impact on aquatic animal health compared to their individual use (Zhang et al., 2015). The effects of synbiotics are now considered crucial due to their synergistic interactions, whereas the primary function of prebiotics is to enhance the survival and implantation of probiotics. Developing an effective synbiotic requires evaluating the *in vitro* ability of prebiotics to selectively stimulate specific probiotics. This process involves ensuring that probiotic bacteria can utilize prebiotics as a carbon source, thereby achieving substantial growth rates and cell yields during fermentation. Nevertheless, this approach is constrained by the limited understanding of the interactions between selective prebiotics and the native microbiota (Kolida and Gibson, 2011). The suitability of specific prebiotic carbohydrates for the selective growth of specific probiotic strains is unclear, which is a crucial consideration in the development of synbiotics for aquaculture.

Probiotics can metabolize certain prebiotic oligosaccharides, enabling their selective proliferation within the intestinal tract. This metabolism results in the production of primary and secondary metabolites that confer health benefits to the host. To create potent synbiotic formulations, it is essential to assess and fine-tune the combinations of probiotics and prebiotics based on their prebiotic activity, which indicates the substrate's effectiveness in supporting probiotic growth, as reported by Mazzola et al. (2015). The complementary effect in synbiotics involves selecting a probiotic based on its specific effects on the host, and then choosing a prebiotic that selectively enhances the growth and activity of the probiotic. The prebiotic does not directly interact with the probiotic but rather supports its growth and activity within its target range, enhancing the overall benefits of the synbiotic (Figure 6).

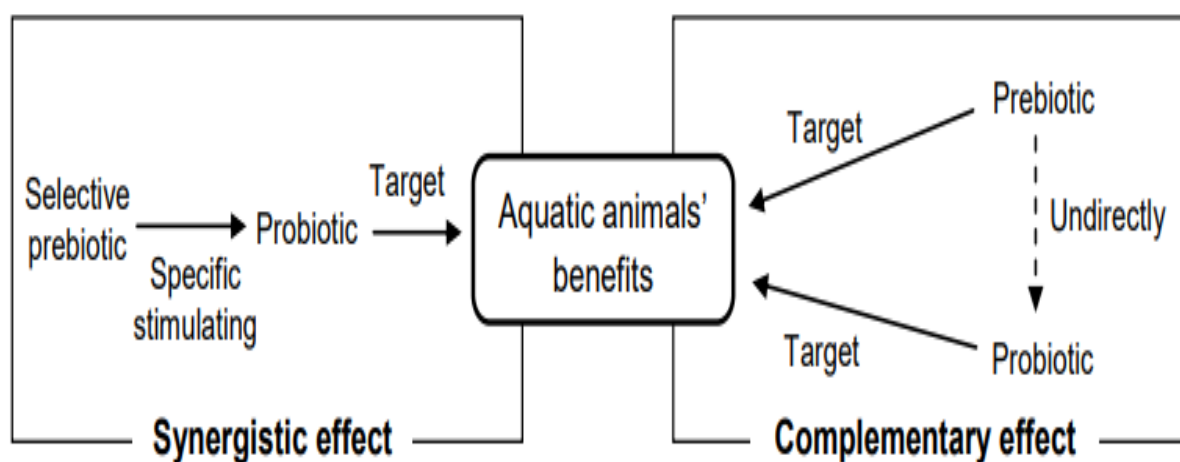


Figure 6. Synbiotic approaches in aquaculture

Source: (Huynh et al., 2017).

Application of synbiotics in tilapia farming

The use of synbiotics in tilapia farming has gained significant attention in recent years due to its practicality and numerous benefits for the aquaculture industry (Sewaka et al., 2018). Research has extensively investigated the sources and effects of synbiotics on various aspects of tilapia farming, including growth rates, feed efficiency, digestive enzyme function, intestinal morphology, blood parameters, immune response, disease resistance, and survival rates. (Ismail et al., 2019; Van Doan et al., 2019).

Synbiotics have been recognized as crucial growth and immunity-enhancing factors in both freshwater and marine fish, thereby expanding their potential benefits to a broad spectrum of aquatic species (Rohani et al., 2022). By introducing synbiotics into the diet, the favorable bacteria in the intestinal tract can thrive, enhancing overall intestinal enzyme activity. This heightened enzymatic activity increases feed digestibility, ultimately leading to improved growth performance in the host species (Ahmadnia et al., 2012). Moreover, exogenous enzymes complement the action of endogenous enzymes, extending the digestion process and enabling more complete hydrolysis of complex substances. The addition of supplemental synbiotics also triggers an increase in the secretion of digestive enzymes, thereby facilitating the breakdown of complex molecules into simpler ones. This process ensures the availability of essential nutrients crucial for growth (Dehaghani et al., 2015). Enhanced enzyme activities bolster the host fish species' capacity to efficiently degrade nutrients, resulting in improved digestion rates and feed utilization (Cerezuela and Meseguer, 2011; Rohani et al., 2022). Extensive studies have focused on the impact of synbiotics on Nile tilapia growth performance, consistently demonstrating positive outcomes. The application of synbiotics has been linked to enhanced growth rates, improved innate immunity, and increased resistance to bacterial infections, highlighting their potential in improving the overall health and well-being of Nile tilapia (Hassaan et al., 2015; Sirbu et al., 2022). Notably, synbiotics have been shown to mitigate the adverse effects on the growth performance, blood health, and immune response of Nile tilapia reared under low-temperature conditions, making them a valuable tool for enhancing the resilience of this economically significant fish species (Gewaily et al., 2021). The application of synbiotics in Nile tilapia farming has been a significant area of research since 2010. The use of probiotics and prebiotics as feed additives has been explored to improve growth performance, physiological conditions, and disease resistance in Nile tilapia. This

approach involves combining probiotics with prebiotics to enhance the beneficial effects on the fish, leading to better overall health and well-being (Figure 7).

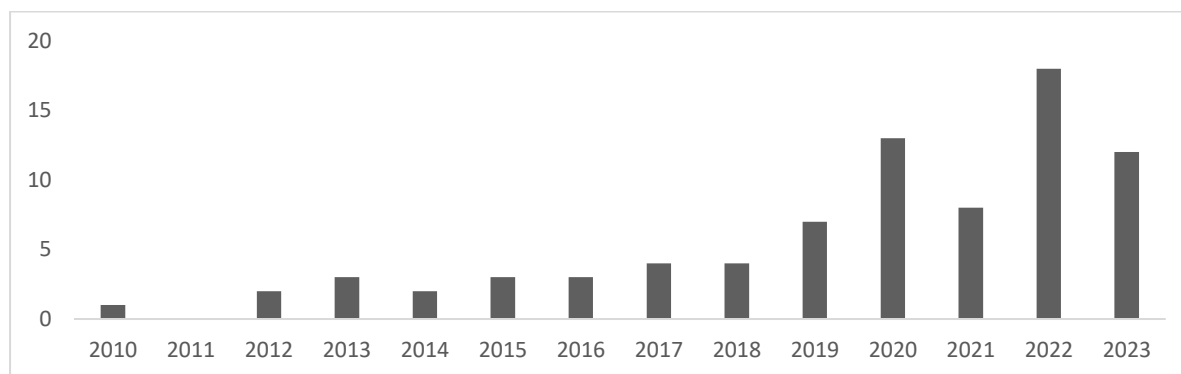


Figure 7. Application of synbiotics in Nile tilapia farming 2010 – 2023

Research has consistently demonstrated that dietary supplementation with probiotics, prebiotics, and beta-glucans can have substantial positive effects on the growth, immune response, oxidative status, and overall health of Nile tilapia and other fish species. These supplements have been found to enhance growth performance, improve body protein content, increase digestive enzyme activity, and boost immune and oxidative responses. Furthermore, studies suggest that these dietary additives may also influence the balance of intestinal microbiota, improve intestinal morphology, and protect against bacterial infections, highlighting their potential in promoting the overall well-being of fish (Table 3). Overall, the evidence supports the potential of using synbiotics, probiotics, and prebiotics as efficient and sustainable approaches for enhancing fish health and production (Sewaka et al., 2018; Dawood et al., 2019; Van Doan et al., 2019; El-Nobi et al., 2021; Mohammadi et al., 2021; Fath El-Bab et al., 2022; Hersi et al., 2023).

Table 3. Summary of synbiotic applications in Nile tilapia farming

Synbiotics	Dosage	Duration	Response	Reference
<i>Aspergillus oryzae</i> (ASP) and β -glucan (BG)	1 ASP + 1 BG g.kg ⁻¹	60 days	FBW ↑, WG ↑, SGR↑, FER ↑, PER ↑, activity of antioxidative enzymes (SOD and CAT) ↑, oxidative enzyme (MDA)↓, NBT↑, IgM↑, lysozyme↑, bactericidal ↑, phagocytosis ↑.	Dawood et al., 2019)
β -glucan and <i>Bacillus coagulans</i>	0.1 g BG + 1 g <i>B. coagulans</i> kg ⁻¹ ; 0.1 g BG + 2 g <i>B. coagulans</i> kg ⁻¹ .	14 weeks	Growth performance ↑, feed efficiency parameters ↑, body Composition ↑, antioxidant activity ↑, anterior intestine villus ↑, <i>HSP70</i> and <i>IL-1β</i> gene expression ↓, <i>IL-8</i> and <i>GH</i> gene Expression ↑.	Fath El-Bab et al., 2022

Jerusalem artichoke (JA) and <i>Lactobacillus rhamnosus</i>	10 g kg ⁻¹ JA+10 ⁸ CFU g ⁻¹ L. <i>rhamnosus</i>	30 days	SGR ↑, ADG↑, FCR ↓, glucose ↑, total protein ↑, total cholesterol levels ↑, lysozyme activity ↑.	Sewaka et al., 2018
<i>Saccharomyces cerevisiae</i> , β-glucan, and MOS	1 × 10 ¹¹ cfu/L, 25 g/L, 35 g/L	8 weeks	Total protein↑, globulin↑, albumin↑, nitric oxide ↑, lysozyme activity ↑, serum glucose ↑, cholesterol ↑, triglycerides ↑, catalase ↑, superoxide dismutase ↑, glutathione peroxidase ↑, cumulative mortality rate ↓.	El-Nobi et al., 2021
MOS+ <i>Lactobacillus Plantarum</i> , <i>Saccharomyces boulardii</i> , and <i>Lactobacillus acidophilus</i>	2g kg ⁻¹ + 1.5 × 10 ⁹ cob g ⁻¹ + 5 × 10 ⁹ cob g ⁻¹ + 5 × 10 ⁹ cob g ⁻¹	85 days	Gut microbiota ↑, body composition ↑, tissue histomorphology ↑.	Hersi et al., 2023
PHDP + <i>Pediococcus acidilactici</i> (PA)	PHDP (0.1%) + PA (0.2%)	56 days	SGR ↑, digestive enzymes activity ↑, immune response ↑, resistance against <i>Aeromonas hydrophila</i> ↑.	Mohammadi et al., 2021
CDXOS + <i>Lactobacillus plantarum</i> CR1T5 (LP)	10 g kg ⁻¹ CDXOS +10 ⁸ CFU g ⁻¹ L. <i>plantarum</i> CR1T5	12 weeks	FBW ↑, WG ↑, SGR ↑, FCR ↓, lysozyme and peroxidase activities ↑, resistance against <i>Streptococcus agalactiae</i>	Van Doan et al., 2019
Mushroom+ <i>Lactobacillus plantarum</i>	10 g kg ⁻¹ Mushroom + 10 ⁸ CFU g ⁻¹ <i>Lactobacillus plantarum</i>	8 weeks	FW ↑, SGR ↑, WG ↑, FCR ↓, skin mucus lysozyme ↑, peroxidase activities ↑	Van Doan et al., 2017
<i>Lactobacillus acidophilus</i> + fructooligosaccharides + mannan oligosaccharides	84×10 ⁷ CFU g ⁻¹	84 days	FBW ↑, FCR ↑, PER ↑, body chemical composition ↑, hematological parameters ↑, insulin-like growth factor ↑, expression of <i>GH</i> and <i>GHR1</i>	Hassaan et al., 2015
Betaplus® + Technomos®	BetaPlus® probiotics and TechnoMos® prebiotics in a ratio of 1:1%	50 days	Growth performance ↑, health profiles ↑, resistance to infection with <i>Aeromonas hydrophila</i> and <i>Pseudomonas fluorescens</i> ↑, survival rate ↑	Sîrbu et al., 2022

Note: FBW: Final body weight, WG: weight gain, SGR: specific growth rate, FER: Final energy retention, PER: Protein efficiency ratio, CDXOS: corncob-derived xylooligosaccharides, FCR: feed conversion ratio

CONCLUSIONS

The integration of synbiotics, a combination of prebiotics and probiotics, stands as a transformative approach to boosting growth performance, immune response, and disease

resistance in Nile tilapia aquaculture. Prebiotics act as a nurturing substrate for beneficial gut bacteria, indirectly enhancing digestive enzyme production and bolstering disease resistance and innate immunity. Probiotics, on the other hand, help maintain a beneficial balance between friendly and pathogenic microorganisms in the fish's intestinal or skin mucus flora. The careful selection of prebiotics and probiotics is crucial for developing potent synbiotic formulations.

Synbiotics hold immense promise for tilapia farming, offering numerous benefits such as improved growth performance, stronger immune responses, reduced dependency on antibiotics, better gut health, and increased disease resistance. To fully harness these advantages, future research should focus on developing cost-effective and sustainable synbiotic formulations that can be seamlessly incorporated into tilapia diets. It's essential to select prebiotics and probiotics based on their proven efficacy, specificity, and safety.

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