

## Enhancing Aquaculture through Prebiotics, Probiotics, and Synbiotics in Nile Tilapia Farming

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### ABSTRACT

Sustainable aquaculture relies significantly on optimizing fish growth, health, and feed utilization. In recent years, synbiotics, a combination of prebiotics and probiotics, have emerged as promising feed additives to enhance aquaculture productivity. This review analyzes the current knowledge on dietary synbiotic supplementation in tilapia farming, focusing on the types of prebiotics, probiotics, and their combinations (synbiotics) used in aquafeeds. It explores their mechanisms of action and evaluates their individual and synergistic effects on tilapia growth, digestive efficiency, immune responses, and disease resistance. Furthermore, the potential benefits of incorporating synbiotics into tilapia diets are discussed in relation to overall fish health and well-being. This analysis aims to provide valuable insights for researchers, farmers, and industry professionals, contributing to the development of sustainable and efficient tilapia farming practices through dietary prebiotic, probiotic, and synbiotic supplementation.

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

### INTRODUCTION

Aquaculture has experienced significant growth in recent years, driven by the rising demand for fishery products and the depletion of wild fish stocks. As of 2022, aquaculture production accounted for more than 50% of global fishery products (FAO, 2024a). Among various cultured fish species, Nile tilapia (*Oreochromis niloticus*) ranked second only to carps, with a global production exceeding 5 million tonnes in 2022 (FAO, 2024b). Despite its rapid expansion, tilapia culture faces several challenges, particularly devastating disease outbreaks resulting from factors such as high stocking densities and global warming (Debnath *et al.*, 2023). The widespread use of various drugs and chemicals has compounded these issues, leading to problems such as antibiotic-resistant pathogens, chemical residues in fish products and environmental contamination (Pepi and Focardi, 2021).

To address these concerns, probiotics, prebiotics, and synbiotics offer a transformative approach in tilapia farming, serving as sustainable alternatives to traditional antibiotic and chemical treatments. These supplements have been shown to enhance growth rates, feed efficiency, immunity, disease resistance, and survival under environmental stress, making them viable, safe, and eco-friendly options for sustainable aquaculture. Their inclusion in fish diets or rearing water has consistently demonstrated improvements in fish health and overall aquaculture performance (Mugwanya *et al.*, 2022).

This review aims to provide a comprehensive and critical evaluation of the existing literature on the effects of dietary prebiotics, probiotics, and synbiotics on the growth performance and physiological parameters of Nile tilapia. It systematically examines the types of prebiotics and probiotics used in aquafeeds, their mechanisms

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of action, and their individual and combined impacts on tilapia growth, digestive efficiency, immune responses, and disease resistance. By critically examining the current state 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 aims to provide valuable insights 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

Prebiotics are non-digestible carbohydrates, often categorized by their molecular size or the number of monosaccharide units. These compounds stimulate the growth and activity of gut microorganisms, particularly beneficial bacteria, thereby promoting the health and development of aquatic organisms (Mountzouris, 2022). Prebiotics are characterized by their resistance to acidic conditions in the stomach, their ability to undergo fermentation by gut microbiota, and their role in facilitating the proliferation of beneficial microbes, thus enhancing the overall well-being of the host (Davani-Davari *et al.*, 2019). Tran *et al.* (2022) reported that prebiotics serve as energy sources for gut microbes, contributing to improved health outcomes in the host. Generally, prebiotics are complex carbohydrates with long chains that act as energy sources for beneficial microorganisms, ultimately improving the overall health of the host. The primary sources of prebiotics are predominantly of botanical origin (Mohammadi *et al.*, 2020). Edible mushrooms have also been identified as significant sources of prebiotics (Balakrishnan *et al.*, 2021), while animal dairy products contribute to a smaller extent. Prebiotics, essential for maintaining a healthy gut microbiome, are abundantly found in a diverse range of natural food sources, including vegetables, fruits, beans, seaweed, microalgae, and animal milk (Ahmadifar *et al.*, 2019; Elumalai *et al.*, 2020).

In Nile tilapia farming, prebiotics have demonstrated several beneficial effects. They enhance growth by fostering the proliferation of beneficial gut bacteria, which improves nutrient absorption and utilization (Ramos *et al.*, 2017). Additionally, prebiotics have also 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; Dias *et al.*, 2020). Incorporating prebiotics into aquaculture practices minimizes reliance on antibiotics by promoting fish health and reducing the likelihood of disease outbreaks, thereby supporting more sustainable and responsible aquaculture practice (Song *et al.*, 2014; Abdul-Kari *et al.*, 2021).

### *Prebiotics mode of action in tilapia*

#### *Nurturing beneficial gut microbes through selective fermentation*

Prebiotics play a vital role in tilapia farming by providing a nutrient-rich environment that fosters the growth of beneficial gut bacteria, which in turn, significantly impact the health and growth of aquatic organisms (Wee *et al.*, 2022). A key mechanisms of their effectiveness is their function as a nutrient source for specific bacteria in the gastrointestinal tract (GIT). For instance, prebiotics such as inulin undergo selective fermentation by microbes like *Bifidobacterium*, which secretes enzymes such as beta-fructosidases to break down inulin's glycosidic bonds. This fermentation process alters the gut microbial composition, favoring the proliferation of beneficial microbes over pathogenic ones (Merrifield *et al.*, 2010). Studies on tilapia have shown that dietary supplementation with specific prebiotics, such as glucan, increased populations of beneficial *Firmicutes* and *Proteobacteria* in the gut, thereby enhancing growth and overall health (Souza *et al.*, 2020).

#### *Stimulation of digestive enzyme production via microbial proliferation*

The proliferation of beneficial gut microbes, facilitated by prebiotic supplementation, leads to increased secretion of digestive enzymes such as

amylase, protease, and lipase, thereby enhancing the fish's nutrient digestion and absorption capabilities. Recent research by Aryati *et al.* (2021) demonstrated that incorporating honey, a prebiotic, into Nile tilapia diets increased the population of gut microbes responsible for producing these enzymes. This enhanced enzyme activity is positively correlated with improved growth performance in tilapia. Similarly, Abd El-latif *et al.* (2015) highlighted the role of prebiotics in boosting digestive enzyme activity and production in tilapia, resulting in better nutrient utilization, increased body weight and improved feed efficiency. These findings are further supported by Hoseinifar *et al.* (2015), emphasizing the critical role of prebiotics in optimizing digestive function and overall fish productivity.

#### *Increasing disease resistance and the innate immune system*

Prebiotics have a crucial impact on the innate immune system of tilapia in aquaculture, significantly enhancing its resilience, overall health, and the maintenance of gut microbial equilibrium. Immune system activation and enhanced protection against infections are closely associated with the regulation of beneficial gut bacteria, which is influenced by prebiotics. Akhter *et al.* (2015) demonstrated that prebiotics enhance the innate immune response in fish and shellfish, providing increased resistance to infections. Additionally, prebiotics exhibit diverse biological activities, including the secretion of extracellular enzymes by gut bacteria and the production of bioactive metabolites. These compounds, as reported by Huynh *et al.* (2017), indirectly influence tilapia growth and health, offering a holistic approach to optimizing growth, reproduction, immunity, and disease resistance.

#### *Application of prebiotics in Nile tilapia farming*

The prebiotics commonly used in aquaculture include  $\beta$ -glucan, inulin, arabinoxylan oligosaccharide (AXOS), mannan oligosaccharide (MOS), galacto-oligosaccharide (GOS), fructo-oligosaccharides (FOS), and various other oligosaccharides. Extensive research has highlighted the advantages of prebiotics,

which include improved growth performance (Li *et al.*, 2019), enhanced feed efficiency (Shoaei *et al.*, 2015), strengthened immune systems (Li *et al.*, 2021), and improve disease resistance (Li *et al.*, 2018; Abdel-Latif *et al.*, 2022; Yilmaz *et al.*, 2022). Consequently, incorporating prebiotics into aquaculture practices is expected to significantly enhance production efficiency. However, it is essential to recognize that the response of host gut microbiota to prebiotics is selective (Gibson *et al.*, 2017). Prebiotics such as fructooligosaccharides (FOS) and galactooligosaccharides (GOS) have been observed to preferentially stimulate the growth of specific gut microbiota, such as *Lactobacillus* and *Bifidobacterium* species, through selective fermentation. This fermentation enhances enzymatic activity, which, in turn, promotes gut health (Gibson *et al.*, 2017). Prebiotics also modulate the gut microbiota by stimulating the proliferation of beneficial bacteria while inhibiting pathogenic strains (Kaewarsar *et al.*, 2023). The fermentation of FOS and GOS by probiotic bacteria results in the production of short-chain fatty acids (SCFA), which confer various health benefits and contribute to gut metabolic activity (Bamigbade *et al.*, 2022). The selective interactions between specific prebiotics and gut microbiota highlight the importance of understanding these dynamics to promote gut health and overall well-being in aquaculture species. Supplementing aquaculture diets with prebiotics can further diversify the gut microbiota in aquatic organisms, ultimately leading to substantial improvements in their overall health.

The publications on the application of prebiotics in Nile tilapia aquaculture has been steadily increasing since 2010 (Figure 1).

Table 1 summarizes the applications of prebiotics in Nile tilapia farming, detailing their dosages, durations, and the observed responses. These findings highlight the significant positive effects of prebiotics on the health and performance of Nile tilapia, including enhanced growth, improved immune function, and increased 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. A summary of probiotic applications in Nile tilapia farming.

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

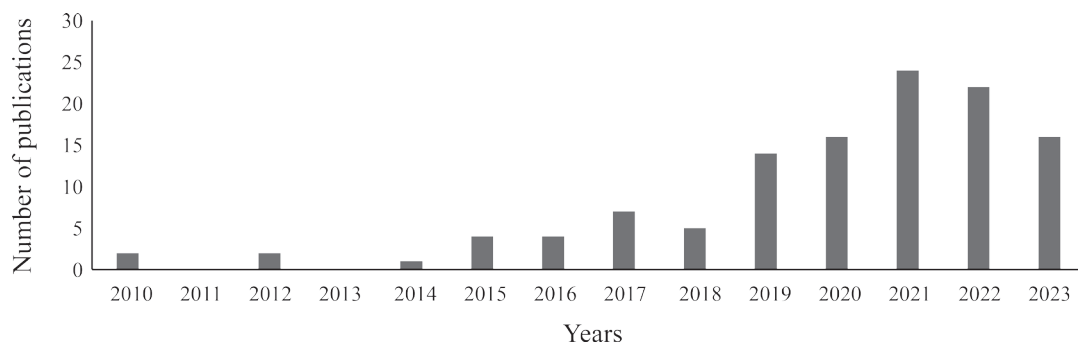


Figure 1. Number of publications on application of prebiotics in Nile tilapia appeared in Scopus indexed journals during 2010–2023.

Sources: Scopus database (www.scopus.com)

## PROBIOTICS

Probiotics are defined as live microorganisms introduced into the gastro-intestinal tract (GIT) through food or water to promote good health by enhancing the internal microbial balance (Gatesoupe, 1999; Azad *et al.*, 2019). Their utilization in aquaculture has been recognized as an ecologically sustainable method for preventing disease outbreaks, enhancing growth, and improving digestion (Wang *et al.*, 2008; Guo *et al.*, 2016; Adel *et al.*, 2017).

In Nile tilapia farming, probiotics have demonstrated several positive effects, including improved immune responses, enhanced growth performance, increased resistance to ammonia, better digestive health, and reduced stress levels (Islam and Rohani, 2021). Probiotics strengthen the immune system of Nile tilapia, enabling them to resist diseases and infections more effectively, which results in higher survival rates and overall health improvements (Shija *et al.*, 2023). Additionally, they promote the proliferation of beneficial gut microbes, which supports overall health and development of the fish (Zabidi *et al.*, 2021). Probiotics have also been shown to increase Nile tilapia's resistance to ammonia, helping them tolerate the stressors of aquaculture environments more effectively (Cavalcante *et al.*, 2020). Additionally, they enhance digestive health by boosting the activity of digestive enzymes, which is crucial for efficient nutrient breakdown and assimilation (Xia *et al.*, 2020). Furthermore, probiotics can help reduce stress levels in Nile tilapia, which is particularly important in aquaculture settings where elevated stress levels can negatively impact growth and increase vulnerability to diseases (Munni *et al.*, 2023).

### *Probiotics mode of action in tilapia*

The application of probiotics in tilapia aquaculture aims to stimulate growth and enhance the fish's ability to resist diseases (Shija *et al.*, 2023). While the precise mechanisms by which probiotics exert their effects are not fully understood, research indicates that their influence varies depending on the host and the type of probiotic used (Hai, 2015).

Several potential mechanisms of action have been identified, as describes in the following sections.

### *Competition for nutrients*

In general, bacteria require iron as a vital minerals for their growth, however, its availability in animal tissues and fluids is limited. Siderophores, iron-binding molecules produced by bacteria, facilitate iron acquisition, which is essential for bacterial proliferation (Mugwanya *et al.*, 2022). Gram *et al.* (1999) highlighted a correlation between siderophore synthesis and the pathogenicity of certain microorganisms. Beneficial probiotic bacteria can produce siderophores and thrive in low-iron environments, aiding nutrient absorption and improving feed efficiency in the host. Iron sequestration play a crucial role in host defense, as it restricts pathogens' access to this essential nutrient, thereby inhibiting their growth. Host organisms further employ iron-binding proteins, such as calprotectin and lipocalin-2, to limit iron availability to pathogens (Spiga *et al.*, 2023). These mechanisms collectively enhance feed utilization and overall performance in aquatic organisms (Steinfeld *et al.*, 2015).

Additionally, probiotics have been shown to influence the activity of digestive enzymes, including carbohydrase's, lipases, and proteases (Eshaghzadeh *et al.*, 2015), which contribute to efficient nutrient breakdown. Taoka *et al.* (2007) reported that the administration of probiotics such as *Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum*, and *S. cerevisiae* increased enzyme activity tilapia. Similarly, Tan *et al.* (2019) observed enhanced digestive enzyme activity in Nile tilapia when supplemented with *Rumeliibacillus stabekisii* at concentrations of  $10^6$  and  $10^7$  CFU·g<sup>-1</sup>.

### *Effect of probiotics on intestinal health*

Probiotics can establish themselves within the intestinal tract of animals, which is a complex ecosystem comprising non-pathogenic, pathogenic, and commensal bacteria (Nayak, 2010). In Nile tilapia, the development and function of the intestines

are closely linked to the presence of beneficial endogenous intestinal microbiota. These microbiotas play a vital role in regulating mucosal development and tolerance, contributing significantly to the overall health of the fish (Akhter *et al.*, 2015). By reducing the presence of pathogenic organisms in the gastrointestinal tract, probiotics promote a healthy intestinal environment. This allows for the development of a robust intestinal epithelial layer, characterized by minimal mucosal damage and enhanced nutrient absorption. Such improvements in gut health support optimal growth and development, ensuring the overall well-being of tilapia (Merrifield *et al.*, 2010).

#### *Production of inhibitory substances*

Probiotics are known to produce a wide range of inhibitory compounds that exhibit antimicrobial and antiviral properties. These compounds, produced by beneficial gut bacteria in Nile tilapia, include siderophores, bacteriocins, hydrogen peroxide, lysozymes, proteases, volatile fatty acids (such as lactic, propionic, acetic, and butyric acid), organic acids, and extracellular enzymes. These substances collectively play a crucial role in suppressing viral replication (Chauhan and Singh, 2019). In addition, these compounds lower the pH of the GIT, creating an environment that inhibits the growth of opportunistic pathogens (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* (Villamil *et al.*, 2014). This ability to combat pathogenic bacteria explains the enhanced disease resistance observed in Nile tilapia 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, probiotics contribute to improved growth rates, enhanced nutrient digestion, and strengthened

host's immunity. Moreover, probiotics promote the balance of beneficial gut bacteria, which is essential for maintaining overall health. This balanced gut microbiota not only supports fish health but also reduces the need for antibiotics, fostering a healthier aquatic ecosystem (Ringø *et al.*, 2010; El-Saadony *et al.*, 2021). Probiotics are recognized as a sustainable and environmentally friendly alternative to antibiotics, offering a safe 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 eco-friendly agents (Anokyewaa *et al.*, 2021). These probiotics function within the host's intestine to enhance digestion and growth by either secreting digestive enzymes or stimulating the host's enzyme production (Nunes, 2018). Additionally, they inhibit pathogen adhesion, maintain the balance of gut microbiota, and strengthen the intestinal mucosal barrier. Probiotics also modulate immune responses and antioxidant levels, ultimately enhancing the host's disease resistance (Amoah *et al.*, 2019).

Since 2008, the application of probiotics in Nile tilapia aquaculture has grown steadily, reflecting increasing recognition of their benefits for fish health and farm productivity (Hai, 2015). Among the extensively studied probiotics, *Bacillus subtilis* has shown substantial improvements in growth performance, feed conversion efficiency, and profitability. Research highlights its ability to optimize reproductive performance, increase resilience against pathogens like *Streptococcus agalactiae*, and improve economic outcomes for fish farmers. These benefits have solidified probiotics as a valuable tool for sustainable and productive tilapia farming (Figure 2).

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>	$1 \times 10^8$ and $10^9$ CFU·kg <sup>-1</sup>	4 weeks	Weight gain ↑, SR ↑, FCR ↓, Disease resistance against <i>S. agalactiae</i> ↑, superoxide anion levels ↑.	(Srisapoom and Areechon, 2017)
<i>Bacillus</i> spp. (ANSCI9, BFAR9, RM3, and RM10)	$10^8$ CFU·kg <sup>-1</sup> of feed	30 days	Average body weight ↑, absolute growth ↑, specific growth rate ↑, relative growth rate ↑, FCR ↓, disease resistance ↑.	(Samson and Quiazon, 2020)
<i>Bacillus</i> spp. (RM10 and BFAR9)	$10^8$ CFU·kg <sup>-1</sup> 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, and <i>Bacillus licheniformis</i> DF001)	$1 \times 10^6$ CFU·kg <sup>-1</sup>	120 days	Immune parameters ↑, <i>IL-1β</i> and <i>TNF-α</i> gene expressions ↑,	(Van Doan <i>et al.</i> , 2023)
<i>Saccharomyces cerevisiae</i> + <i>Lactobacillus Acidophilus</i>	$10^8$ CFU·mL <sup>-1</sup>	12 weeks	Final weight ↑, body length ↑, specific growth rate ↑, weight gain ↑, feed intake ↑, FCR ↓, protein efficiency ratio ↑, RBCs ↑, Hb ↑, mortality rate ↓.	(Hassanien <i>et al.</i> , 2017)
<i>Saccharomyces cerevisiae</i> + <i>Bacillus</i> spp. ( <i>B. subtilis</i> , <i>B. megaterium</i> , and <i>B. licheniformis</i> )	$5.6 \times 10^8$ CFU·g <sup>-1</sup> and $1 \times 10^6$ CFU·g <sup>-1</sup>	90 days	Final body weight ↑, weight gain ↑, average daily growth gain ↑, aspartate aminotransferase (AST) ↓, alanine aminotransferase (ALT) levels ↓.	(Sutthi <i>et al.</i> , 2018)

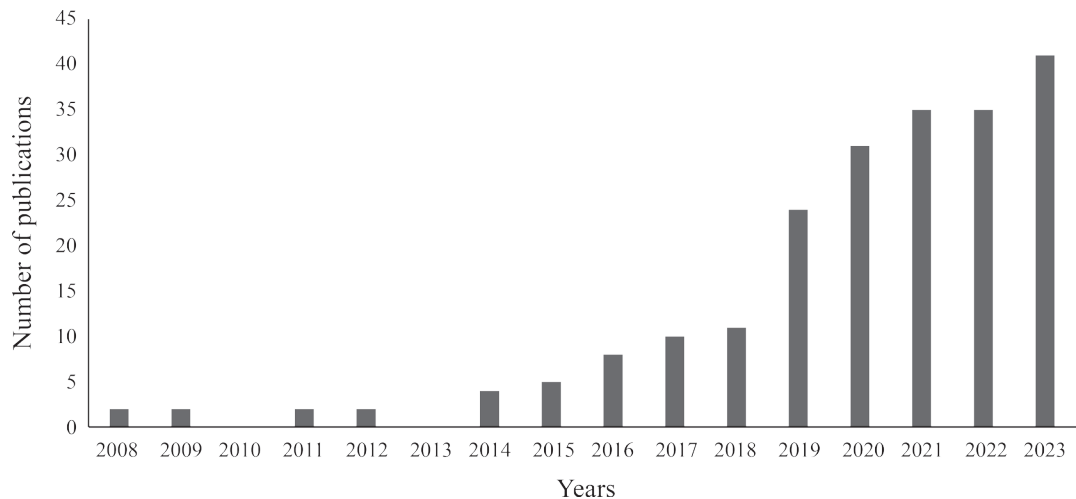


Figure 2. Number of publications on application of probiotics in Nile tilapia appeared in Scopus indexed journals during 2010–2023.

Sources: Scopus database (www.scopus.com)

## SYNBIOTICS

Synbiotics are defined as “a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host” (Swanson *et al.*, 2020). As a combination of probiotics and prebiotics, synbiotics have demonstrated their potential to significantly enhance aquaculture productivity. They promote rapid growth in fish, strengthen immune systems, and improve digestion and nutrient absorption. Additionally, synbiotics effectively manage diseases and maintain water quality, making them a valuable tool in sustainable aquaculture development (Nguyen *et al.*, 2019; Waagbø and Remø, 2020).

The benefits of synbiotics stem from their ability to optimize the gut environment by enhancing the implantation of live microbial dietary supplements in the digestive tract. This process selectively stimulates the growth and metabolism of health-promoting microorganisms, contributing to the overall welfare of the organism (Cerezuela *et al.*, 2011; Das *et al.*, 2017). Studies have consistently shown that synbiotics synergistically improve growth, immunity, and disease resistance

in aquatic organisms (Dawood *et al.*, 2018; 2020).

The term "synbiotic" specifically refers to a combination of probiotic bacteria and a prebiotic growth substrate, such as inulin. The synergistic interaction between these components results in distinct improvements in growth, immunity, disease prevention, and other parameters that are not achievable by the individual components alone. When introduced as feed additives to Nile tilapia, synbiotics serve as substrates for probiotics, supporting the secretion of supplementary nutrients and enhancing fish growth. In addition to promoting growth, synbiotics modulate the gut microbiota, fostering rebiosis to maintain gut equilibrium and improve gut function. They stimulate mucosal immunity, promote cell growth and differentiation, and upregulate genes coding for digestive enzymes such as amylase, protease, and lipase. These effects collectively improve digestibility, nutrient utilization, and absorption. Furthermore, synbiotics contribute to better water quality by promoting the proliferation of beneficial bacteria, suppressing pathogenic bacteria, and enhancing nutrient cycling. These improvements lead to increased disease resistance and support sustainable aquaculture practices (Figure 3).

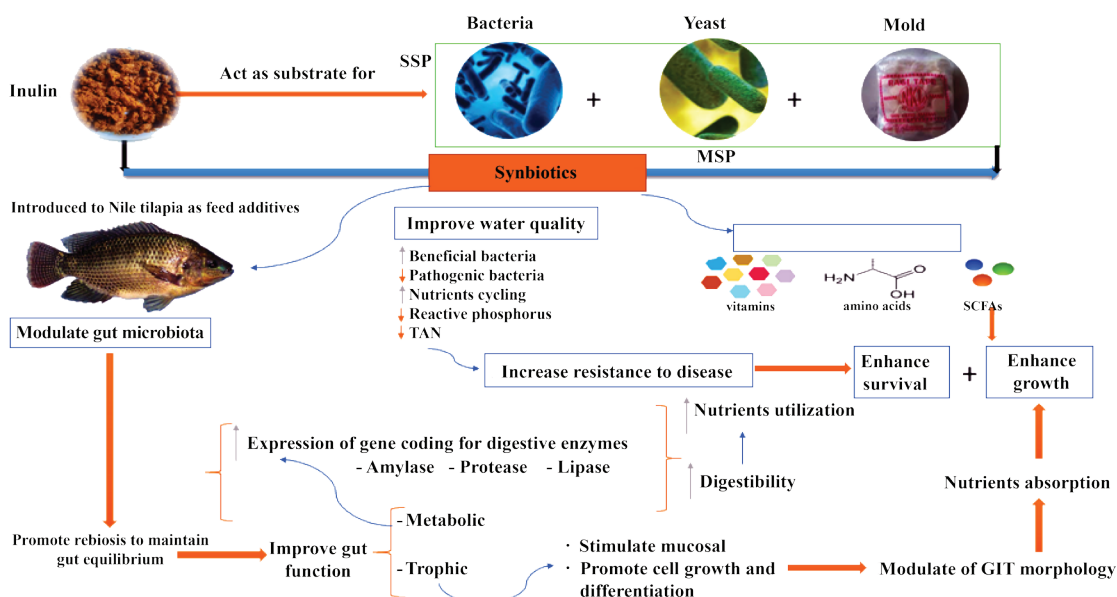


Figure 3. Mechanisms and immunomodulatory impact of synbiotics on Nile tilapia health.

Note: SSP = Single strain probiotics; MSP = Multi strain probiotics



### *Selection of prebiotics and probiotics for effective synbiotic formulations in aquaculture*

Research has consistently demonstrated that both individual applications of prebiotics and probiotics, as well as their combined use in synbiotics, positively impact the health of aquatic animals. These dietary supplements have been shown to enhance growth, immune function, and disease resistance in aquatic organisms (Yeh *et al.*, 2014). Notably, the synergistic effects observed when prebiotics and probiotics are combined as synbiotics highlight their superior efficacy compared to their individual use, underscoring the significant benefits of their interaction (Zhang *et al.*, 2015).

The beneficial effects of synbiotics largely stem from the synergy between prebiotics and probiotics, with prebiotics playing a key role in enhancing the survival and implantation of probiotics. Developing effective synbiotic formulations requires evaluating the ability of prebiotics to selectively stimulate specific probiotic strains *in vitro*. This involves ensuring that probiotic bacteria can utilize prebiotics as carbon sources, enable substantial growth and high cell yields during fermentation. Nevertheless, this approach is constrained by limited knowledge of interactions between selective prebiotics and the native microbiota (Kolida and Gibson, 2011). The suitability of specific prebiotic carbohydrates for stimulating the growth of particular probiotic strains remains unclear, making it a critical area of focus in the development of synbiotics for aquaculture.

Probiotics can metabolize certain prebiotic oligosaccharides, leading to their selective proliferation within the intestinal tract. This metabolism generates primary and secondary metabolites that confer health benefits to the host's health. To create effective synbiotic formulations, it is essential to assess and optimize combinations of probiotics and prebiotics based on their prebiotic activity, which measures the substrate's effectiveness in supporting probiotic growth (Mazzola *et al.*, 2015). The complementary effect in synbiotics involves selecting a probiotic based on their specific benefits to the host and then with prebiotics that selectively enhance the growth and activity of the chosen probiotics. While prebiotics do not directly interact with probiotics, they support their proliferation and functionality within the target environment, amplifying the overall benefits of the synbiotics (Figure 4).

### *Application of synbiotics in tilapia farming*

The application of synbiotics in tilapia farming has gained substantial attention due to its practicality and numerous benefits for the aquaculture industry (Sewaka *et al.*, 2018). Extensive research has investigated the sources and impacts of synbiotics on various critical aspects, including growth rates, feed efficiency, digestive enzyme activity, intestinal morphology, blood parameters, immune response, disease resistance, and survival rates (Ismail *et al.*, 2019; Van Doan *et al.*, 2020).

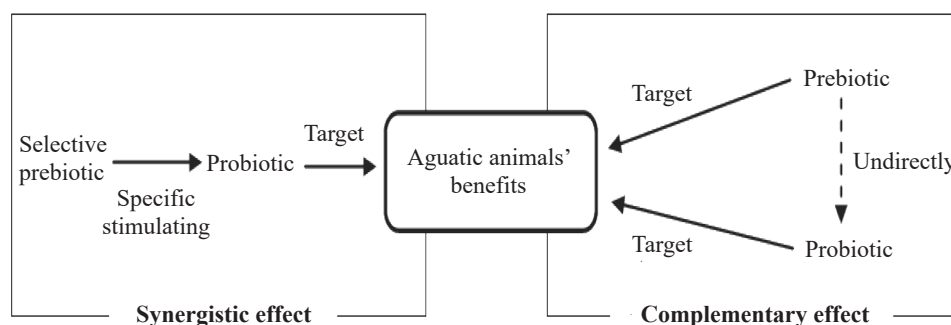


Figure 4. A flow chart representing concept in developing synbiotics in aquaculture.

Source: Huynh *et al.*, 2017

Synbiotics are recognized as potent growth and immunity enhancers in both freshwater and marine fish, thereby expanding their potential benefits to a broad spectrum of aquatic species (Rohani *et al.*, 2022). Incorporating synbiotics into tilapia diets fosters the proliferation of beneficial intestinal bacteria, resulting in heightened enzymatic activity. This increased enzymatic function improves feed digestibility, subsequently enhancing growth performance in the host species (Ahmadnia *et al.*, 2012). Moreover, exogenous enzymes from synbiotics complement endogenous digestive enzymes, prolonging digestion and facilitating the hydrolysis of complex substances. This process ensures the steady supply of essential nutrients necessary for optimal growth (Dehaghani *et al.*, 2015).

Furthermore, synbiotics stimulate the secretion of digestive enzymes, enhancing the breakdown of complex molecules into simpler, absorbable forms. This improved nutrient degradation enhances digestion efficiency and feed utilization (Cerezuela *et al.*, 2011; Rohani *et al.*, 2022). Numerous studies have consistently demonstrated positive effects on Nile tilapia, linking their use to improved growth rates, enhanced innate immunity, and increased resistance to bacterial infections. These findings highlight the potential of synbiotics to promote overall health and resilience of Nile tilapia (Hassaan *et al.*, 2015; Sirbu *et al.*, 2022). Notably, synbiotics have proven effective in mitigating the negative effects of low-temperature

conditions on Nile tilapia, including reduced growth performance, blood health, and immune response, making them a valuable tool for enhancing the resilience of this economically significant fish species (Gewaily *et al.*, 2021). Since 2010, research on the application of synbiotics in Nile tilapia farming has gained increasing attention, as evidenced by the growing number of publications in Scopus-indexed journals, as illustrated in Figure 5.

Research has consistently demonstrated that dietary supplementation with probiotics, prebiotics, and beta-glucans significantly enhanced the growth, immune response, oxidative status, and overall health of Nile tilapia and other fish species. These supplements have been shown to improve growth performance, increase body protein content, elevate digestive enzyme activity, and strengthen immune and oxidative responses. Furthermore, studies indicate that these dietary additives contribute to maintaining intestinal microbial balance, enhancing intestinal morphology, and providing protection against bacterial infections. Such findings highlight their potential in promoting the overall well-being of fish (Table 3). Collectively, the evidence underscores the efficacy of synbiotics, probiotics, and prebiotics as sustainable and efficient strategies for improving fish health and production (Sewaka *et al.*, 2018; Dawood *et al.*, 2020; Van Doan *et al.*, 2020; El-Nobi *et al.*, 2021; Mohammadi *et al.*, 2021; Fath El-Bab *et al.*, 2022; Hersi *et al.*, 2023).

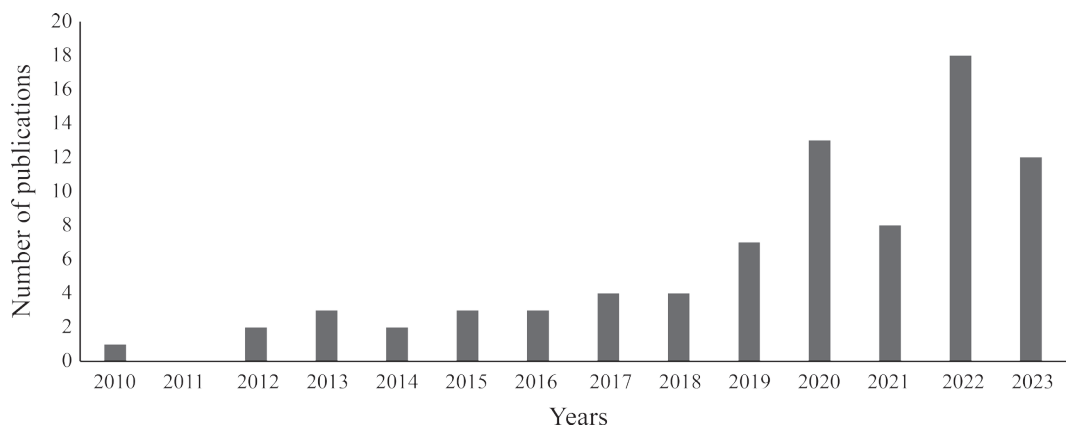


Figure 5. Number of publications on application of synbiotics in Nile tilapia appeared in Scopus indexed journals during 2010–2023.

Source: Scopus database ([www.scopus.com](http://www.scopus.com))

Table 3. Summary of synbiotic applications in Nile tilapia farming.

Synbiotics	Dosage	Duration	Response	Reference
<i>Aspergillus oryzae</i> (ASP) and $\beta$ -glucan (BG)	1 ASP + 1 BG g·kg <sup>-1</sup>	60 days	FBW ↑, WG ↑, SGR↑, FER ↑, PER ↑, activity of antioxidative enzymes (SOD and CAT) ↑, oxidative enzyme (MDA)↓, NBT↑, IgM↑, lysozyme↑, bactericidal ↑, phagocytosis ↑.	Dawood <i>et al.</i> , 2020
$\beta$ -glucan and <i>Bacillus coagulans</i>	0.1 g BG + 1 g <i>B. coagulans</i> ·kg <sup>-1</sup> ; 0.1 g BG + 2 g <i>B. coagulans</i> ·kg <sup>-1</sup> .	14 weeks	Growth performance ↑, feed efficiency parameters ↑, body composition ↑, antioxidant activity ↑, anterior intestine villus ↑, <i>HSP70</i> and <i>IL-1<math>\beta</math></i> gene expression ↓, <i>IL-8</i> and <i>GH</i> gene expression ↑.	Fath El-Bab <i>et al.</i> , 2022
Jerusalem artichoke (JA) and <i>Lactobacillus rhamnosus</i>	10 g·kg <sup>-1</sup> JA+ 10 <sup>8</sup> CFU g <sup>-1</sup> <i>L. rhamnosus</i>	30 days	SGR ↑, ADG↑, FCR ↓, glucose ↑, total protein ↑, total cholesterol levels ↑, lysozyme activity ↑.	Sewaka <i>et al.</i> , 2018
<i>Saccharomyces cerevisiae</i> , $\beta$ -glucan, and MOS	1×1.011 CFU·L <sup>-1</sup> , 25 g·L <sup>-1</sup> , 35 g·L <sup>-1</sup>	8 weeks	Total protein↑, globulin↑, albumin↑, nitric oxide ↑, lysozyme activity ↑, serum glucose ↑, cholesterol ↑, triglycerides ↑, catalase ↑, superoxide dismutase ↑, glutathione peroxidase ↑, cumulative mortality rate ↓.	El-Nobi <i>et al.</i> , 2021
MOS+ <i>Lactobacillus Plantarum</i> , <i>Saccharomyces boulardii</i> , and <i>Lactobacillus acidophilus</i>	2 g·kg <sup>-1</sup> +1.5 × 10 <sup>9</sup> cob g <sup>-1</sup> +5 × 10 <sup>9</sup> cob g <sup>-1</sup> +5 × 10 <sup>9</sup> cob g <sup>-1</sup>	85 days	Gut microbiota ↑, body composition ↑, tissue histomorphology ↑.	Hersi <i>et al.</i> , 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 <i>et al.</i> , 2021
CDXOS + <i>Lactobacillus plantarum</i> CR1T5 (LP)	10 g kg <sup>-1</sup> CDXOS + 10 <sup>8</sup> CFU·g <sup>-1</sup> <i>L. plantarum</i> CR1T5	12 weeks	FBW ↑, WG ↑, SGR ↑, FCR ↓, lysozyme and peroxidase activities ↑, resistance against <i>Streptococcus agalactiae</i>	Van Doan <i>et al.</i> , 2020
Mushroom+ <i>Lactobacillus plantarum</i>	10 g·kg <sup>-1</sup> Mushroom +10 <sup>8</sup> CFU·g <sup>-1</sup> <i>Lactobacillus plantarum</i>	8 weeks	FW ↑, SGR ↑, WG ↑, FCR ↓, skin mucus lysozyme ↑, peroxidase activities ↑	Van Doan <i>et al.</i> , 2017
<i>Lactobacillus acidophilus</i> + fructooligosaccharides + mannan oligosaccharides	84×10 <sup>7</sup> CFU·g <sup>-1</sup>	84 days	FBW ↑, FCR ↑, PER ↑, body chemical composition ↑, hematological parameters ↑, insulin-like growth factor ↑, expression of <i>GH</i> and <i>GHR1</i>	Hassaan <i>et al.</i> , 2015
Betaplus® + Technomos®	1% BW BetaPlus® probiotics (1×10 <sup>12</sup> CFU·kg <sup>-1</sup> feed) and TechnoMos® prebiotics (936,000 mg·kg <sup>-1</sup> )	50 days	Growth performance ↑, health profiles ↑, resistance to infection with <i>Aeromonas hydrophila</i> and <i>Pseudomonas fluorescens</i> ↑, survival rate ↑	Sirbu <i>et al.</i> , 2022

**Note:** FBW = Final body weight; WG = weight gain; SGR = specific growth rate; FER = Final energy retention; PER = Protein efficiency ratio; CDXOS = corn-cob-derived xylooligosaccharides; FCR = feed conversion ratio

## CONCLUSIONS

The integration of synbiotics, a combination of prebiotics and probiotics, offers a promising strategy for enhancing growth performance, immune response, and disease resistance in Nile tilapia aquaculture. Prebiotics serve as a substrate that selectively nurtures beneficial gut bacteria, indirectly enhancing digestive enzyme production and bolstering innate immunity, and improving disease resistance. Probiotics, in turn, promote a favorable balance between beneficial and pathogenic microorganisms within the fish's intestinal and skin mucus microbiota. The effectiveness synbiotics lies in the synergistic interaction between prebiotics and probiotics, providing superior benefits compared to their individual use.

Synbiotics present a sustainable and efficient approach to tilapia farming, offering multiple benefits such as improved growth performance, enhanced immune function, reduced dependency on antibiotics, better gut health, and increased resilience to disease. To maximize these advantages, future research should prioritize the development of cost-effective and sustainable synbiotic formulations tailored for incorporation into tilapia diets. This effort will require a careful selection of prebiotics and probiotics based on their proven efficacy, specificity, and safety to ensure optimal outcomes in aquaculture.

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