



Emerging roles of probiotics, prebiotics, and synbiotics in fish gut health and oxidative stress regulation

Sadia Islam, Most. Sinthia Rahman, Riad Ahmed, Md. Nurul Haider¹, Mahmudul Hasan², Mohammad Shafiqul Alam and Md. Shahanoor Alam*

Department of Genetics and Fish Breeding, Gazipur Agricultural University, Gazipur 1706, Bangladesh; ¹Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh; ²Department of Fisheries, Jamalpur Science and Technology University, Jamalpur 2012, Bangladesh

*Corresponding e-mail: shahanoor@gau.edu.bd

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Abstract

The intensification of aquaculture has increased the prevalence of stress-related disorders and gastrointestinal imbalances in cultured fish, often leading to impaired growth, reduced disease resistance, and elevated oxidative stress. In this context, functional feed additives such as probiotics, prebiotics, and synbiotics have emerged as promising alternatives to antibiotics for enhancing fish health and production efficiency. This review synthesizes current knowledge on the emerging roles of these biologically active compounds in modulating fish gut health and oxidative stress regulation. Probiotics contribute to maintaining a balanced intestinal microbiota, improving digestive enzyme activity, enhancing nutrient assimilation, and stimulating innate immune responses. Prebiotics selectively promote the growth of beneficial microbial communities by serving as fermentable substrates, thereby strengthening gut integrity and metabolic functions. Synbiotics exhibit enhanced efficacy in optimizing gut microbial balance and improving physiological resilience. Furthermore, these functional additives play a critical role in mitigating oxidative stress by enhancing antioxidant enzyme activities and reducing reactive oxygen species accumulation, ultimately improving fish health and survival under intensive farming conditions. Overall, the integration of probiotics, prebiotics, and synbiotics into aquafeeds represents a sustainable strategy to promote gut health, strengthen immune competence, and regulate oxidative stress in aquaculture systems.

Keywords: Digestive health, growth performance, disease resistance, gut microbiota, water quality

Introduction

In contemporary aquaculture, optimizing fish production maintaining health and welfare is a major challenge (Dawood and Koshio, 2016; Mukherjee *et al.* 2019). Achieving this objective requires a clear understanding of the complex interactions among digestive enzyme activity, oxidative stress, and antioxidant defense systems in fish (Hoseinifar *et al.* 2021). These interconnected physiological processes play a critical role in regulating nutrient utilization, maintaining cellular homeostasis, and ultimately enhancing fish growth performance and resilience.

The digestive system of fish is a highly coordinated network of enzymatic processes that governs the breakdown, absorption, and assimilation of nutrients required for growth, development, and physiological functions (Mata-Sotres *et al.* 2016, Assan *et al.* 2022).

In parallel with digestion, the balance between oxidative stress and antioxidant defense mechanisms is essential for maintaining cellular integrity and metabolic homeostasis (Hoseinifar *et al.* 2021). Recent research has highlighted the regulatory role of dietary functional additives, particularly probiotics, prebiotics, and synbiotics, in modulating these physiological processes (Wang *et al.* 2021, Assan *et al.* 2022). Probiotics are beneficial live microorganisms that improve host health, whereas prebiotics are non-digestible dietary components that selectively stimulate the growth and activity of advantageous gut microbiota. Synbiotics further enhance these effects (Van Doan *et al.* 2016). Collectively, these functional feed additives have gained considerable attention due to their potential to improve digestive enzyme activity, enhance nutrient utilization, and strengthen antioxidant defense systems, thereby contributing to improved growth performance and increased resilience against oxidative stress in fish (Wang

et al. 2021). Although numerous studies have investigated the effects of probiotics, prebiotics, and synbiotics on either digestive enzyme activity or oxidative stress in fish (Table 1), these aspects are often examined independently, resulting in a fragmented understanding of their combined roles (Wang *et al.* 2019, Wang *et al.* 2021, Assan *et al.* 2022). Consequently, a clear gap remains in integrating these interconnected physiological processes within a comprehensive framework.

This review aims to address this limitation by systematically synthesizing existing research, with a particular focus on the interactions between digestive function and oxidative stress regulation. By consolidating current evidence, it seeks to identify key mechanisms, highlight existing knowledge gaps, and suggest directions for future research. Ultimately, this approach provides a more holistic perspective on how probiotics, prebiotics, and synbiotics collectively influence digestive enzyme activity and oxidative stress responses in fish (Wang *et al.* 2019, Wang *et al.* 2021, Assan *et al.* 2022).

Importance of digestive health in fish

Digestive enzymes are a group of specialized biomolecules present in the gastrointestinal tract of fish that catalyze the breakdown of complex macromolecules into simpler, absorbable forms, thereby supporting nutrient assimilation, energy metabolism, and overall physiological performance (Xiong *et al.* 2019, Assan *et al.* 2022). A comprehensive understanding of fish digestive physiology is therefore fundamental for improving aquaculture productivity, conserving natural fish populations, and promoting sustainable fisheries management (Xiong *et al.* 2019).

Research has demonstrated that the assessment of digestive enzyme activity provides valuable insights into feeding strategies and ecological adaptations of fish in natural environments. For instance, larval fish exhibit rapid growth rates largely due to efficient digestion, which is driven by high feeding intensity and elevated enzymatic activity (Mata-Sotres *et al.* 2016). Consequently, digestive efficiency is closely linked to growth performance, developmental processes, and overall health status in fish. Different classes of digestive enzymes, including amylolytic, proteolytic, lipolytic, and cellulolytic enzymes, are responsible for the hydrolysis of

carbohydrates, proteins, lipids, and cellulose, respectively. These enzymatic processes are critical for optimizing nutrient utilization and play a pivotal role in supporting the growth and development of aquaculture species (Kavitha *et al.* 2018).

The role of oxidative stress in fish

High levels of reactive oxygen species (ROS) within cells, associated with oxidative stress, can exert detrimental effects on lipids, proteins, and DNA (Hoseinifar *et al.* 2021). Oxidative stress develops when the production of reactive oxygen species (ROS) exceeds the capacity of the fish's antioxidant defense system to neutralize them. This imbalance between ROS generation and antioxidant defense, known as oxidative stress, may result in DNA hydroxylation, protein denaturation, lipid peroxidation, apoptosis, and ultimately cell damage (Hoseinifar *et al.* 2021). Hydroxyl radicals and hydrogen peroxides are among the principal oxygen radicals contributing to ROS. To off-set the adverse effects of naturally generated ROS, living organisms have developed an antioxidant defense system consisting of enzymatic antioxidants (e.g., superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase) and non-enzymatic antioxidants (e.g., glutathione, thioredoxin, vitamin C, and vitamin E) (Hoseinifar *et al.* 2021). ROS, such as superoxide radicals and hydrogen peroxide, are capable of damaging lipids, proteins, and DNA within fish cells (Schieber and Chande 2014, Hoseinifar *et al.* 2021). Fish naturally produce ROS as byproducts during normal metabolic activity. Factors such as pollution, temperature variation, and poor water quality can increase ROS production (Schieber and Chande 2014, Halliwell and Gutteridge 2015). Infections and diseases can likewise induce ROS production as part of the fish's immune response. ROS are capable of damaging cell membranes, proteins, and DNA, which leads to impaired cellular function. Oxidative stress may also suppress growth rates in fish (Li *et al.* 2016).

Significance of antioxidant defense in fish

Fish have evolved complex antioxidant defense mechanisms to counteract oxidative stress and maintain cellular homeostasis (Halliwell and Gutteridge 2015,

Li *et al.* 2016, Hoseinifar *et al.* 2021). These defense mechanisms consist of both enzymatic and non-enzymatic antioxidants. It functions by converting superoxide radicals into hydrogen peroxide, followed by the conversion of hydrogen peroxide into water and oxygen (Yang *et al.*, 2018; Gao *et al.*, 2022). It neutralizes hydrogen peroxide and lipid peroxides. Non-enzymatic antioxidants comprise vitamins C and E, which serve as scavengers of free radicals. Glutathione functions as an essential cellular antioxidant. Carotenoids have antioxidant properties and also play a role in fish coloration (Xie *et al.* 2019). In the aquaculture setting, where oxidative stress is particularly important, especially in intensive and stressful modern culture systems, researchers are exploring whether beneficial microbes can enhance the antioxidant defense of cultured organisms. Previous studies in aquaculture have reported promising antioxidative effects of beneficial additives, including probiotics, prebiotics, and synbiotics, in a range of fish species (Hoseinifar *et al.* 2021).

Effects of probiotic, prebiotics and synbiotics on digestive enzymes in fish

Probiotic supplementation has been widely reported to stimulate digestive enzyme activity, leading to enhanced nutrient utilization and growth performance in various aquatic species, including the white shrimp *Litopenaeus vannamei*, Indian white shrimp *Fenneropenaeus indicus*, abalone *Haliotis asinina*, and humpback grouper *Cromileptes altivelis* (Marlida *et al.* 2014). In addition, studies focusing on digestive physiology have significantly advanced the understanding of how nutrients are digested, absorbed, and metabolized in fish (Table 1) (Nie *et al.* 2017). These findings highlight the important role of gut health and microbial interactions in improving digestive efficiency and overall fish performance.

Probiotics are commonly linked with the promotion of gut health through modulation of the composition and function of the gut microbiota (Sumon *et al.* 2018). They may influence digestive enzymes through a variety

Table 1. Probiotic use in regulating digestive enzymes in cultured fish through dietary administration

Probiotics	Sources	Recommended dosages	Duration	Stimulated enzyme	Fish species	Reference
<i>Lactobacillus casei</i>	Commercial	5×10^7 cfu g ⁻¹	75 day	A, L, P, T ↑	<i>Cyprinus carpio</i>	Mohammadian <i>et al.</i> 2019
<i>L. acidophilus</i>	<i>Ctenopharyngodon idella</i>	1×10^6 cfu g ⁻¹	60 days	P, A, C ↑	<i>Ctenopharyngodon idella</i>	(Wang 2011)
<i>Bacillus subtilis</i> Ch9	<i>C. idella</i>	3×10^9 cfu kg ⁻¹	56 days	P, L, A ↑	<i>C. idella</i>	(Wu <i>et al.</i> 2012)
<i>L. plantarum</i> NIOFSD018	<i>Oreochromis niloticus</i>	1×10^7 cfu g ⁻¹	60 days	P, L, A ↑	<i>Oreochromis niloticus</i>	(Essa <i>et al.</i> 2010)
<i>Saccharomyces cerevisiae</i> NIOFSD019	<i>O. niloticus</i>	1×10^4 cfu g ⁻¹	60 days	P, L, A ↑	<i>O. niloticus</i>	(Essa <i>et al.</i> 2010)
<i>B. licheniformis</i>	Commercial	1×10^6 cfu g ⁻¹	8 weeks	P, L, A ↑	<i>Lates calcarifer</i>	(Adorian <i>et al.</i> 2018)
<i>Lactobacillus sp.</i>	<i>Cyprinus carpio</i>	2×10^7 cfu g ⁻¹	60 days	P, L, A ↑	<i>C. carpio</i>	(Yanbo <i>et al.</i> 2006)
<i>B. subtilis</i>	Commercial	1×10^6 cfu g ⁻¹	8 weeks	P, L, A ↑	<i>L. calcarifer</i>	(Adorian <i>et al.</i> 2018)
<i>B. subtilis</i>	<i>C. carpio</i>	5 g kg ⁻¹	28 days	P, L, A, C ↑	<i>Penaeus vannamei</i>	(Wang 2007)
<i>Rhodopseudomonas</i>	<i>C. carpio</i>	1×10^6 cfu g ⁻¹	60 days	P, A, C ↑	<i>C. idella</i>	(Wang 2011)
<i>B. amyloliquefaciens</i>	<i>Clarias gariepinus</i>	1×10^{10} cfu kg ⁻¹	60 days	P, L, A ↑	<i>C. gariepinus</i>	(Reda <i>et al.</i> 2018)
<i>B. cereus</i>	<i>C. gariepinus</i>	1×10^{10} cfu kg ⁻¹	60 days	P, L, A ↑	<i>C. gariepinus</i>	(Reda <i>et al.</i> 2018)
<i>B. subtilis</i>	<i>C. gariepinus</i>	1×10^{10} cfu kg ⁻¹	60 days	P, L, A ↑	<i>C. gariepinus</i>	(Reda <i>et al.</i> 2018)
<i>Clostridium butyricum</i>	<i>Macrobrachium rosenbergii</i>	2×10^9 cfu g ⁻¹	60 days	P, A ↑	<i>M. rosenbergii</i>	(Sumon <i>et al.</i> 2018)

A, Amylase; C, Cellulase; T, Trypsin; P, Protease; L, Lipase; '↑'- Up Arrow indicates "increase in assessment to the control".

of mechanisms (Assan *et al.* 2022). Probiotic strains such as *Lactobacillus* and *Bifidobacterium* can enhance lactase activity. (Mardani *et al.* 2018). Some probiotics are also able to produce proteases, enzymes involved in protein breakdown (Naidu 2011). These proteases may work alongside the host's digestive enzymes to aid in the breakdown of dietary proteins into smaller peptides and amino acids (Mirghaed *et al.* 2018). Probiotics may further affect amylase activity, which is responsible for degrading carbohydrates into simpler sugars (Mardani *et al.* 2018). By modulating carbohydrate metabolism, probiotics may influence nutrient availability for both the host and the gut microbiota. In addition, some probiotic strains, particularly certain *Lactobacillus* and *Bifidobacterium*, may exhibit bile salt hydrolase activity (Liu *et al.* 2017). This enzyme is involved in bile salt metabolism and may affect the digestion and absorption of dietary fats.

Prebiotics are not enzymes themselves, yet they can indirectly influence digestive enzymes through their effects on the gut microbiota. SCFAs (Short-Chain Fatty Acids) may regulate the activity of various digestive enzymes, including amylases and proteases (Akrami *et al.* 2015). The fermentation of prebiotics by gut bacteria can alter gut pH (Dawood and Koshio, 2016). These pH shifts can influence digestive enzyme activity by creating an environment that may be either more or less favorable for their function (Feckaninova *et al.* 2017).

The interaction between probiotics and prebiotics can strongly influence digestive enzymes. Prebiotics can act as an energy source for probiotics, thereby supporting their growth and survival in the gut (Dawood and Koshio 2016) enhancing the overall effect of probiotics on digestive enzyme activities. Synbiotics function together to selectively stimulate the growth of beneficial bacteria (Dawood and Koshio 2016). This selective stimulation can affect the overall balance of the gut microbiota, potentially influencing the production and activity of different digestive enzymes. Probiotics, prebiotics, and synbiotics can exert a multifaceted influence on digestive enzymes (Dawood and Koshio 2016).

Impacts of probiotics, prebiotics, and synbiotics on oxidative stress in fish

Research has shown that probiotics can positively affect the antioxidant defense system in fish (Hoseinifar *et al.* 2021). These microorganisms can stimulate the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Figure 1) (Hoseinifar *et al.* 2021, Wang *et al.* 2019). As a result, probiotics contribute to the removal of reactive oxygen species (ROS) and lessen oxidative stress in fish tissues (Hoseinifar *et al.* 2021). Additionally, probiotics may help preserve the balance of the gut microbiota, thereby promoting a healthy gut environment. A well-balanced gut microbiota is essential for nutrient absorption and can indirectly influence the antioxidant status of fish. Prebiotics indirectly affect oxidative stress by supporting the growth of probiotics (Abdel-Latif *et al.* 2023). By creating a favorable environment for beneficial bacteria in the gut, prebiotics contribute to maintaining a healthy gut microbiota. This, in turn, helps prevent oxidative stress by ensuring optimal nutrient absorption and gut function. The combination of probiotics and prebiotics in synbiotics often produces stronger effects on oxidative stress than individual administration (Figure 1) (Mohammadi *et al.* 2021, Wang *et al.* 2019). Synbiotics can support the survival and activity of probiotics in the gut, creating a more resilient and diverse microbial community. This improved gut health can enhance antioxidant defense mechanisms and lower the risk of oxidative stress in fish (Figure 3) (Hoseinifar *et al.* 2021, Abdel-Latif *et al.* 2023).

Interactions of probiotics, prebiotics, and synbiotics with antioxidant defenses in fish

Probiotics have been widely demonstrated to enhance antioxidant defense systems in fish through multiple complementary mechanisms (Wang *et al.* 2019). One of the primary actions of probiotics is their ability to scavenge free radicals and neutralize reactive oxygen species (ROS), thereby reducing oxidative stress and minimizing cellular damage (Salem *et al.* 2018). In addition, probiotics can stimulate the activity of antioxidant enzymes and modulate the immune system, which is closely associated

with oxidative stress regulation, ultimately improving the overall physiological resilience of fish.

Prebiotics, although not directly involved in antioxidant activity, contribute indirectly by promoting the growth and activity of beneficial gut microbiota (Guerreiro *et al.* 2016). This enhanced microbial activity can lead to the production of bioactive compounds with antioxidant properties and exert anti-inflammatory effects, both of which play important roles in reducing oxidative stress (Zhang *et al.* 2014). Since inflammation is closely linked with the generation of free radicals, the anti-inflammatory effects of prebiotics further support the maintenance of antioxidant balance in fish.

Synbiotics, defined as a synergistic combination of probiotics and prebiotics, provide enhanced benefits by improving the survival, colonization, and activity of probiotic microorganisms in the gastrointestinal tract (Abdel-Latif *et al.* 2023). The presence of prebiotics as a substrate ensures sustained probiotic functionality, resulting in prolonged and more effective antioxidant

responses (Zhang *et al.* 2013). Consequently, synbiotics offer a more comprehensive strategy for strengthening antioxidant defenses through both direct mechanisms, such as ROS scavenging, and indirect pathways involving immune modulation and gut microbiota regulation (Ashouri *et al.* 2020).

Overall, probiotics, prebiotics, and synbiotics play pivotal roles in enhancing antioxidant capacity and stress tolerance in fish. Their application in aquaculture holds significant promise for improving fish health, reducing oxidative damage, and promoting sustainable production. However, their effectiveness may vary depending on species-specific responses, environmental conditions, and the types and combinations of microbial strains used.

Advantages of probiotics, prebiotics, and synbiotics in fish farming

Probiotics are well known for producing beneficial enzymes that enhance digestion and protect the gastrointestinal tract (GIT) (Sumon *et al.* 2018). Their application helps maintain a balanced intestinal microbiota, leading to

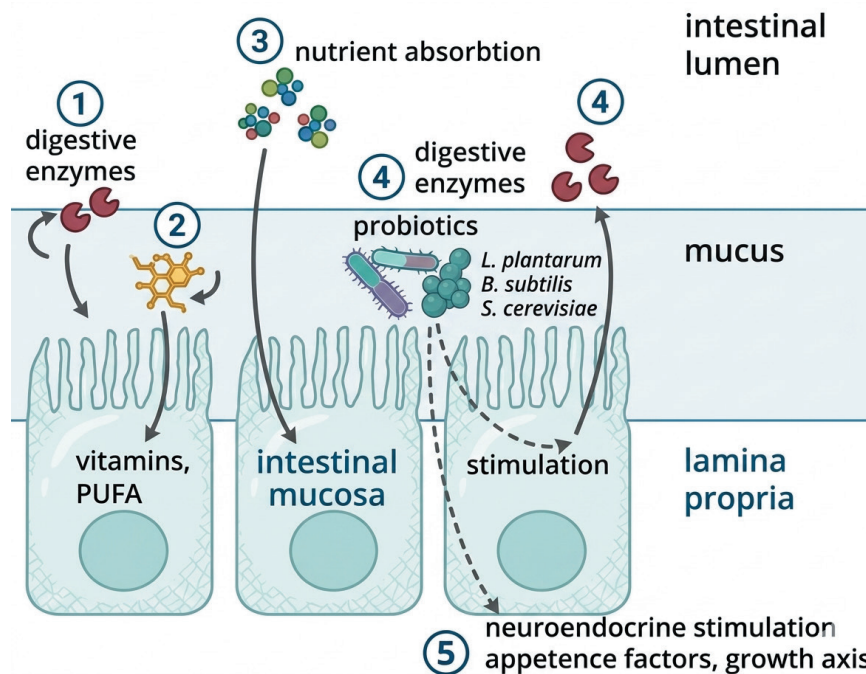


Figure 1. Probiotics in the GIT enhance nutrition and growth by producing digestive enzymes, improving micronutrient absorption, stimulating enzyme secretion, and modulating neuroendocrine functions that promote appetite and growth.

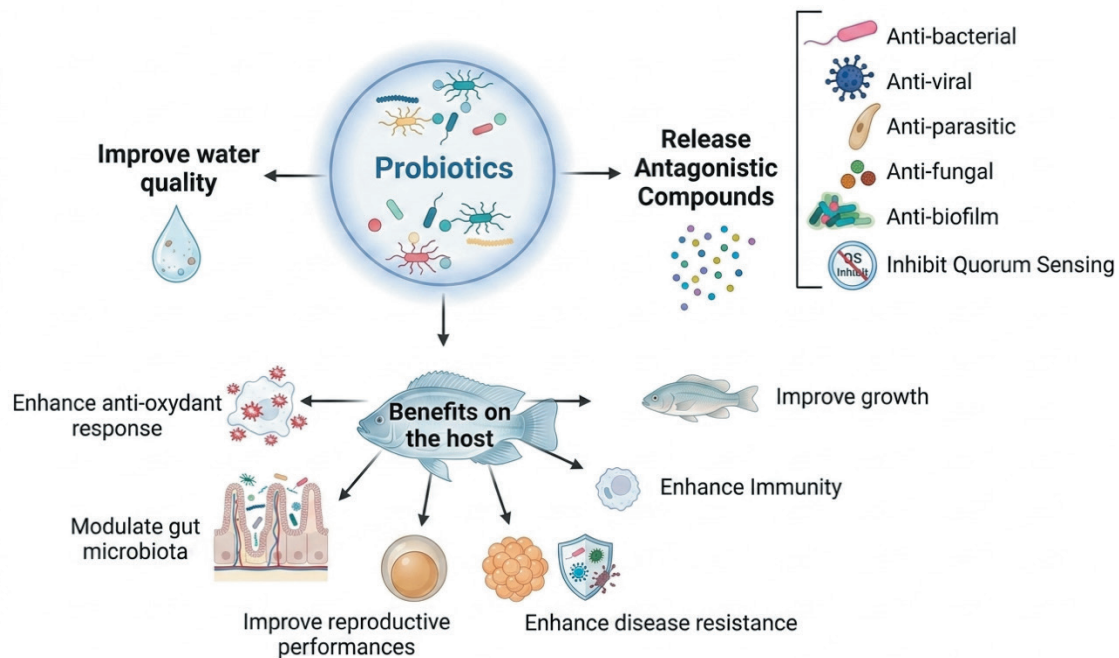


Figure 2. Functional roles of probiotics in aquaculture.

improved digestive enzyme activity, better nutrient absorption, and reduced pathogenic infections (Chen *et al.* 2019).

A key mechanism by which probiotics enhance fish performance is through the production of additional digestive enzymes such as amylase, protease, and lipase, which complement host enzymes and improve feed utilization and growth. For example, probiotic supplementation (e.g. PrimaLac®) has been shown to increase amylase activity and enhance growth performance in fish species like *Rutilus frisii kutum* (Mirghaed *et al.* 2018).

Probiotics have been shown to reduce the adverse effects of stress in fish by maintaining a stable gut environment. A balanced microbial community in the fish gut can also support the breakdown of organic matter, thereby helping to preserve good water quality in aquaculture systems. Indeed, the use of probiotics in fish farming represents a promising strategy for improving the health and productivity of farmed fish (Amenyogbe, 2023). This approach involves manipulating the gut microbiota to establish a favorable environment that supports fish

well-being and reduces disease risk. Probiotics are able to produce substances such as organic acids, bacteriocins, and hydrogen peroxide, which inhibit the growth of harmful bacteria (Liu *et al.* 2021). They can also improve nutrient absorption by increasing the surface area of the intestinal mucosa (Rohani *et al.* 2021, Mounir *et al.* 2022).

Prebiotics are non-digestible dietary components that selectively stimulate the growth and activity of beneficial microorganisms in the gastrointestinal tract of fish, and they are increasingly considered as effective alternatives to antibiotics in aquaculture (Assan *et al.*, 2022). Common prebiotics used in fish diets include oligosaccharides such as fructo-oligosaccharides (FOS), inulin, and mannan oligosaccharides (MOS), as well as fiber-rich plant materials like cellulose and hemicellulose (Akhter *et al.* 2015). These compounds are resistant to digestion in the upper gastrointestinal tract and reach the intestine, where they serve as substrates for beneficial bacteria, promoting their proliferation and activity. This process enhances microbial diversity and helps establish a stable and balanced gut microbiota (Andrews *et al.* 2009). As a result, prebiotics contribute to improved nutrient utilization,

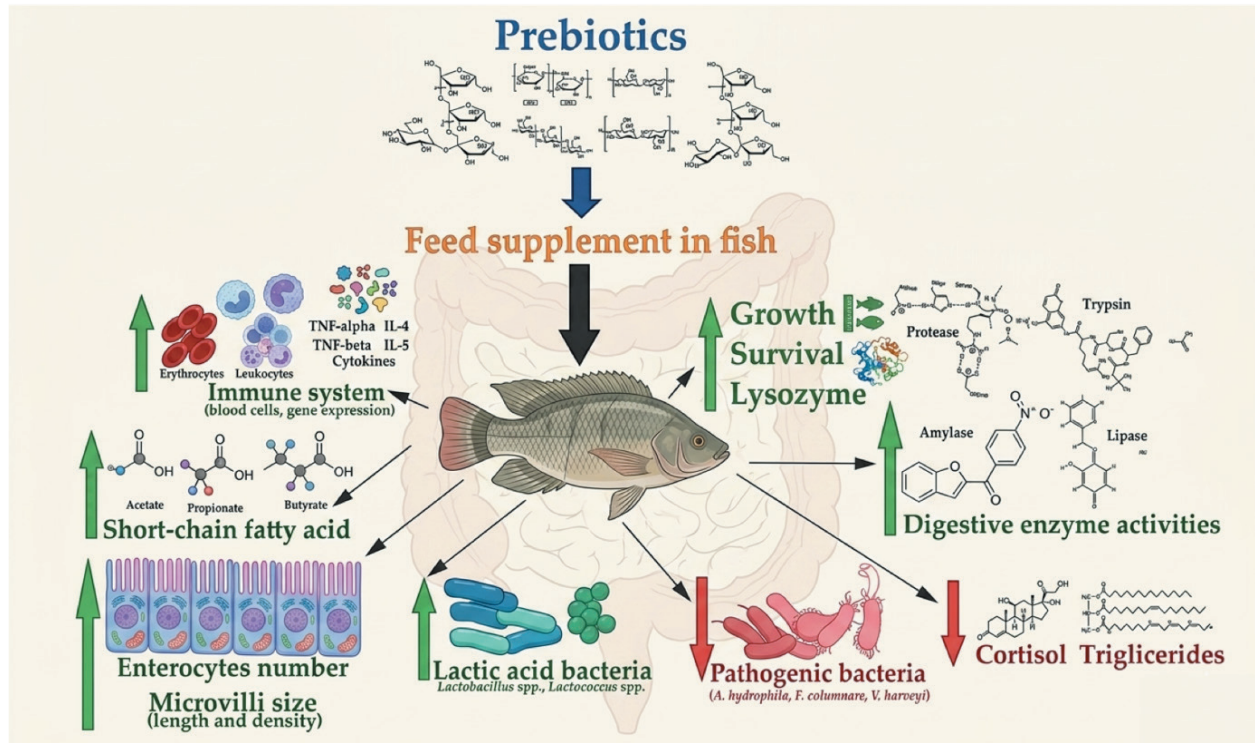


Figure 3. Functional roles of prebiotics in aquaculture. Green arrows and letters indicate additive effects. Red arrows and letters indicate inhibitory effects. Black letters indicate the description of the action mechanisms.

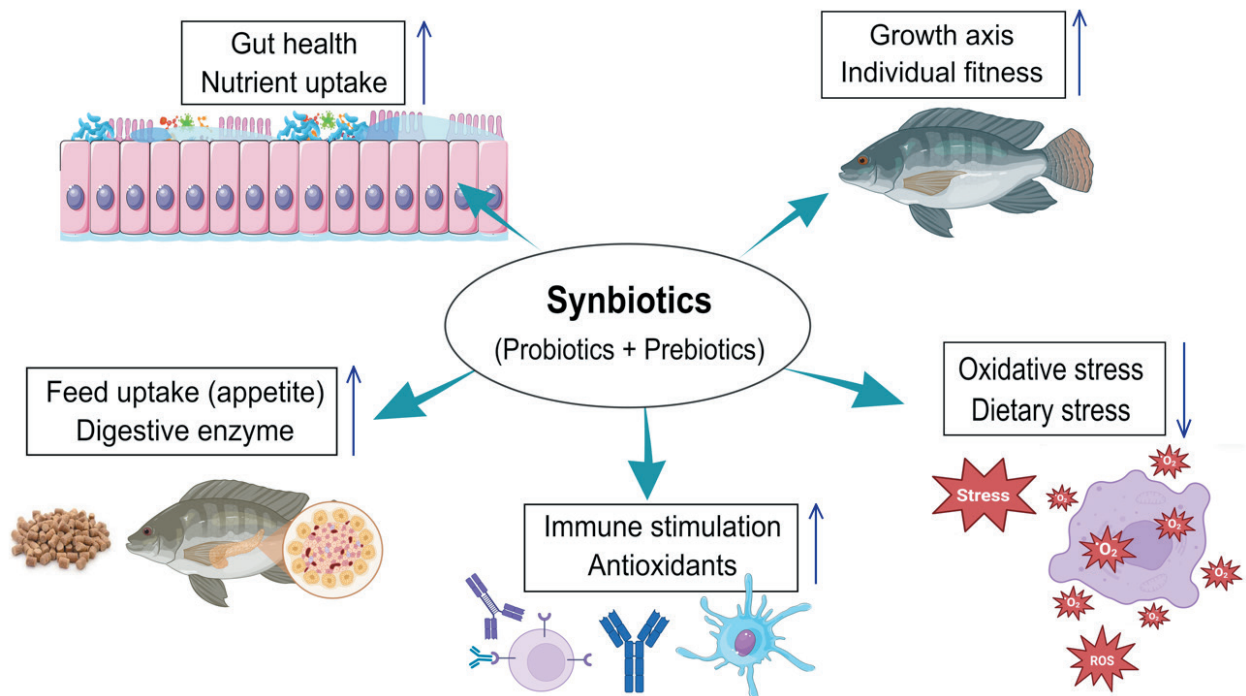


Figure 4. Combined modes of action of synbiotics in fish. Through synergistic interactions between probiotics and prebiotics, synbiotics enhance growth, gut health, nutrient absorption, stress resistance, and immune function.

better gut health, and increased disease resistance in fish (Mata-Sotres *et al.* 2016).

Synbiotics are defined as combinations of probiotics and prebiotics that create a synergistic effect to enhance health-promoting benefits in the host organism (Mohapatra *et al.* 2013).

In fish farming, synbiotics are applied to maximize the colonization and activity of probiotics in the gut by supplying a favorable nutritional environment through prebiotics. This combination offers several advantages, including improved survival of probiotics during storage and transportation, greater adherence of probiotics to the gut lining, and prolonged activity of beneficial microorganisms in the fish gastrointestinal tract.

Conclusions

Probiotics, prebiotics, and synbiotics are promising functional feed additives that enhance gut health, immune response, and oxidative stress regulation in aquaculture species. Probiotics (e.g., *Lactobacillus*, *Bacillus*) improve microbial balance and nutrient utilization, while prebiotics like FOS and inulin stimulate beneficial microbiota and intestinal integrity. Their combined use as synbiotics provides synergistic benefits, including better growth, disease resistance, and stress tolerance. These compounds also strengthen antioxidant defenses and reduce ROS-induced damage. However, responses vary among species and conditions, and data on long-term effects and optimal dosages remain limited. Future research should focus on species-specific and field-based studies. Overall, their integration into aquafeeds offers a sustainable alternative to antibiotics in aquaculture.

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Conflicts of Interest

The authors declare no conflict of interest.

Author's Contributions

SI, MSR, and MSA designed and wrote the review; RA, MNH and MH reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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