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A REVIEW ON THE ROLE OF BACILLUS SPP. AS PROBIOTICS IN TILAPIA CULTURE

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ABSTRACT

Probiotics, such as Bacillus spp., have been found to have beneficial effects in animal and human nutrition when used in the right amount. When fed to aquatic animals, Bacillus spp. acts as non-pathogenic probiotics and are widely known for their numerous benefits in aquaculture. These benefits include enhancing the immune system, improving growth performance, controlling diseases, and optimizing reproductive and digestive health in tilapia. Bacillus spp. is particularly suitable as probiotics because they can produce antimicrobial agents and have the sporulation capacity to survive in harsh environments. This review explores the significant role of Bacillus probiotics in cultivating Nile tilapia (*Oreochromis niloticus*). The article delves into the mechanisms by which Bacillus probiotics exert these beneficial effects, including enhanced phagocytosis, stress reduction, and disease control. Additionally, the review addresses critical factors influencing the efficacy of Bacillus probiotics, such as strain selection, dosage, environmental conditions, supplementation duration, feed composition, and water quality. Despite the promising benefits, challenges still need to be addressed in the consistent application and optimization of these probiotics in aquaculture. It highlights future research directions, emphasizing the need for a deeper understanding of the interactions between Bacillus probiotics and their host organisms to improve their practical application in tilapia culture.

Keywords: Probiotics, Tilapia, Antimicrobial agent

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1. INTRODUCTION

Aquaculture is the fastest food production sector of the world, accounting for 52% of fish farmed for human consumption and 46% of all cattle produced. In 2018, the world produced 179 million tons (MT) of fish, generating USD 401 billion in revenue (FAO 2020). Asia is the leading continent in fish production (34%), followed by America (14%), Africa (7%), Europe (10%), and Oceania (1%) (Mugwanya et al. 2021). Over the past 20 years, Africa and Asia have shown significant growth in overall fish production compared to other continents (FAO 2020). Tilapia, a warm water fish and a member of the cichlid family is the world's second most popular fish raised for food. It is mainly produced, consumed, and exported from China (Ansah et al. 2014; Naylor et al. 2021). Tilapia is highly sought after due to its high protein and nutrient content (Athulya et al. 2024), and is rich in vitamins, manganese, omega-3 fatty acids, iron, zinc, phosphorus, salt, and potassium (Penarubia et al. 2023). Its quick growth rate and adaptability to different environments make it a popular choice for aquaculture in developing nations, particularly in low-income countries like Bangladesh (Obiero et al. 2014; Pratiwi and Pratiwy 2022; Hasan et al. 2023). However, the intensive aquaculture of Nile tilapia faces challenges such as poor water quality, overcrowding, and low oxygen levels, which can weaken the fish's immune system. These conditions are linked to environmental stress and can lead to disease outbreaks, resulting in higher fish mortality rates and reduced production (Elsabagh et al. 2018; El-Son et al. 2020; Elbahnaswy et al. 2021; Kord et al. 2022). The chemotherapeutic agents and antibiotics to manage disease outbreaks has weakened aquatic organisms' natural immunity and increased their susceptibility to various aquatic pathogens (Romero et al. 2012; Guzm´ an-Villanueva et al. 2014). The excessive use of antibiotics since the 1950s has led to water contamination and poses a health risk to humans (Almansour et al. 2023). Therefore, finding safe antibiotic alternatives is crucial for the long-term viability and well-being of aquaculture (Chen et al. 2024). Scientists are currently exploring strategies to enhance fish immune systems to control and prevent disease outbreaks (Cerezuela et al. 2012).

Probiotics are beneficial live bacteria that support immune function, growth, and the balance of gut microorganisms in animals (Omar et al. 2024). The term "probiotics" was first coined by Parker in 1974. Probiotics are organisms and materials that promote the balance of gut microbiota (Parker 1974). They can be considered a nutrient-dense dietary source and a biologically beneficial environmental control agent (Ahmad et al. 2017). In

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aquaculture, the use of probiotics as feed additives has shown significant effects on immune response, growth, disease resistance, and other favorable benefits for the cultured host (Puvanasundram et al. 2021). Probiotics serve as a viable alternative to reduce the need for antibiotics in treating illnesses while also enhancing immune response, health, growth, feed utilization, and water bioremediation in an environmentally responsible and sustainable manner (Fig. 1). As a result, probiotics are beneficial to aquaculture, especially in organic production systems (Hei and Fotedar 2010). Bacillus species have been identified as an increasing trend (Wang et al. 2019). Among the most popular probiotic bacteria are *Bacillus subtilis* and *B. licheniformis*. Different researchers have shown that these types of bacteria can improve fish growth performance, immunity and intestinal absorption surface. Therefore, more investigation is required to determine the effects of these bacteria on fish nutrition (Aly et al. 2008; Gobi et al. 2018; Veiga et al. 2020). Consequently, this study designed to estimate the impacts of Bacillus probiotic on hemato-immunology, growth and intestinal health of Nile tilapia (*O. niloticus*) as a feed additive (Ferrarezi et al. 2024). It has been discovered that Bacillus probiotics aid in the breakdown of proteins, carbs, and fats as well as the improvement of vitamin and mineral absorption from meals, ensuring that the body is getting the nutrients it needs for optimum health (Olmos 2017; Kuebutornye et al. 2020; Haque et al. 2021).

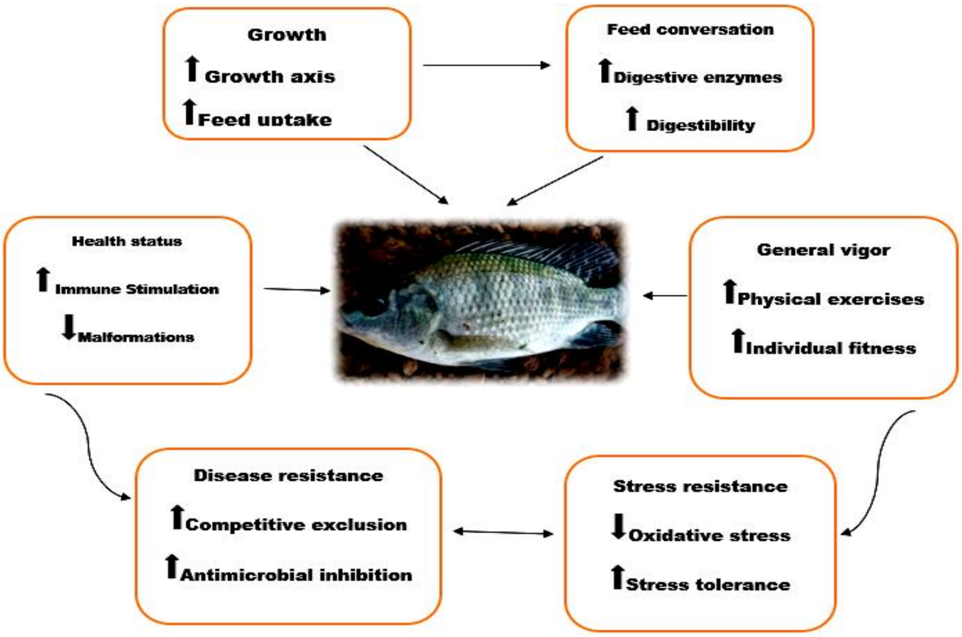

Fig. 1: General impact of Bacillus probiotic on Nile tilapia.

2. BENEFITS OF BACILLUS PROBIOTICS IN TILAPIA CULTURE

Bacillus probiotics stand out among the many types of microbial options due to their ability to survive tough environmental conditions via sporulation. They are also noted for being non-pathogenic and non-toxic to fish, in addition to their capability to synthesize antimicrobial products, rendering them superior candidates to other probiotics. This study aimed to assess the positive impacts of administering Bacillus spp. probiotics to tilapia fish, as indicated by increased lipase, protease, and amylase activity in the fish's gastrointestinal tract post-colonization (Abarike et al. 2018).

Additionally, Bacillus spp. can be added to the aquatic ecosystem to manage and outcompete pathogenic bacteria. Various species of probiotics from the Bacillus genus are currently employed for this purpose, with *B. subtilis* being extensively researched and widely utilized in tilapia aquaculture (Davis et al. 2017). While its probiotic application has long been integral to diverse aquaculture practices, its potential for enhancing water quality, contribution to bio flocs/bioremediation, and its role as a carrier for expressing and delivering heterologous antigens are noteworthy. Table 1 presents the effect of different doses of probiotics on fish health and immunity.

This microorganism utilizes a range of diverse and intersecting mechanisms, including synergistic, antagonistic, competitive exclusion, and immune-stimulating effects. Nile tilapia (*O. niloticus*), categorized as the world's third most farmed fish species (4.41 million tons in 2020) (FAO 2020), is significant for global food security and is highly suitable for freshwater aquaculture. This research investigated the potential beneficial impacts of administering the commercial probiotic Bio plus 2BC® [comprising *B. licheniformis* and *B. subtilis* of 1.6×10^{10} CFU/g] on Nile tilapia. The study focused on various parameters, including nutrient digestibility, growth performance, gut microbiology, hematology (hematocrit index, leukocyte, erythrocyte, and thrombocytes), and innate immunology (respiratory burst, lysozyme, and phagocyte activities) (Al-Fattah and Abdelqader 2014). Fish

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having diets of probiotics (0.04 and 0.08%) showed greater weight gain compared to the control category, as well as more thrombocytes in the bloodstream. The probiotics positively influenced the gut microbiota, demonstrated by higher diversity and viability indexes and increased genetic variability (Aly et al. 2008). Incorporating *B. subtilis* and *B. licheniformis* (0.04 and 0.08%) into the diet enhances growth and alters the intestinal microbiota, dropping the presence of potentially pathogenic species. Establishing a population of useful microorganisms may improve overall host health.

Table 1: The effects of different *Bacillus* probiotics on fish

Probiotics based on	Fish	Dose	Bacteria	Effect	References
Bacillus subtilis	Indian Major Carp	1.5×10^7CFU/g	*Aeromonas hydrophila*	Max. survival	Kumar et al. (2006)
	Channel catfish and striped catfish	8×10^7 CFU/g	*Edwardsiella ictaluri*	Reduced mortality	Ran et al. (2012)
	Red hybrid tilapia	0.3%	*Streptococcus agalactiae*	Reduced mortality	Ng et al. (2014)
	Grouper	10^4, 10^6, and 10^8 CFU/g	*Streptococcus sp.*	Enhanced growth and immunity	Liu et al. (2012)
	Olive founder	0.5%	*Streptococcus iniae*	Max. survival ratio	Cha et al. (2012)
	Catfish (*Clarias gariepinus*)	15%	*Aeromonas hydrophila*	High growth, water quality	Aini et al. (2024)
	Rainbow trout	10^7 cells per gram	*Aeromonas sp.*	Max. immunity	Newaj-Fyzul et al. (2007)
Bacillus pumilus	Tilapia	10^6 and 10^{12} g^{-1} diet fed	*Aeromonas Hydrophila*	Improved immunity and disease resistance	Aly et al. (2008)
	Tilapia	PRO 67%	*Streptococcus Agalactiae*	Improved immunity and disease resistance	Guimarães et al. (2022)
-Bacillus subtilis and Bacillus licheniformis	Nile Tilapia	0.04% and 0.08%	*γ-Proteobacteria*	Better growth performance and modifies the intestinal microbiota, reducing pathogenic species.	Tachibana et al. (2021)
	Trout		*Y ersinia ruckeri*	Increased survival rate	Raida et al. (2003)
Bacillus circulans	*Catla catla*	-	*Aeromonas hydrophila*	Improved immunity and survival	Bandyopadhyay and Mohapatra (2009)
Bacillus licheniformis	Tilapia	0.02%, 0.04%, 0.06%, 0.08% and 0.1%	*Streptococcus iniae*	Improved growth, disease resistance	Han et al. (2015)

Studies have shown that dietary inclusion of Bacillus probiotics stimulates disease resistance and faster growth in farmed fish species. By leveraging immunological data, including lysozyme activity, phagocytosis, respiratory burst, complement activity, antioxidants, and immunity-associated gene expression, researchers aim to grow active approaches for utilizing Bacillus probiotics in farming Nile tilapia. The antibiotics has long been prevalent in commercial aquaculture. Although effective in controlling infectious diseases, this practice has led to several significant issues (Akanmu 2018; Hoseinifar et al. 2018).

Recently, unselective pesticides and antibiotics have caused the appearance of antibiotic-resilient bacteria, with residues of these substances detectable in aquatic yields. Using these products to manage disease occurrences is no longer promoted because of their adverse environmental impacts including creating mutagenic microbes and damaged fish species, such as Nile tilapia (Zorriehzahra et al. 2016; Okocha et al. 2018).

Bacillus probiotics enhance the absorption of minerals and vitamins from food, aiding in the breakdown of carbohydrates, proteins and fats to confirm that the body receives essential nourishment for prime health (Olmos 2017; Kuebutornye et al. 2020). Nile tilapia, a widely farmed freshwater fish species, is known for its resilience, rapid growth, and good nutritional value, making it an appealing option for farmers. Its effective farming structures also make it an additional justifiable choice for the ecosystem (Pilling et al. 2020; Richardson et al. 2021; Zhang 2021). There are following useful effects of probiotics (bacillus spp.) on Nile tilapia culture are described below.

2.1. Improved Immune System

Nile tilapia have been observed to derive advantages from probiotics (such as *B. licheniformis, B. safensis, B. subtilis,*), offering various health benefits (Telli et al. 2014; Han et al. 2015; Wu et al. 2021). These probiotics can promote the well-being and growth of Nile tilapia by fostering the proliferation of useful gut bacteria, improving feed conversion efficiency, and enhancing resistance to diseases (Kuebutornye et al. 2019b). Although Nile tilapia have been noted to take advantage of Bacillus probiotics, the specific mode of action and the optimal dose may differ among individual fish (Saputra et al. 2016; Srisapoome and Areechon 2017). Tilapia is well-known for its

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robust immune system, composed of three main factors (Saurabh and Sahoo 2008). The First factor is the physical barrier, primarily the skin which formed by various cellular layers that provide protection against many infections. The Second factor comprises humoral components, including various proteins and essential molecules that combat against different pathogens. Third factor involves cellular components, which attack quickly on all foreign particles upon recognizing them that can enter in fish body (Liu et al. 2017) (Fig. 2). Various kinds of immune cells can react with probiotics like *B. subtilis*. These cells include macrophages, granulocytes, monocytes, natural killer cells, neutrophils, and lymphocytes (Ashraf and Shah 2014). These interactions can enhance Nile tilapia's innate immune responses to pathogens. This enhancement occurs through signals from cell surface pattern recognition receptors (Roayaei et al. 2015). In fact, Opiyo et al. (2019) observed that administering *B. subtilis* as a probiotic led to an increase in white blood cells in Nile tilapia. Additionally, (Srisapoome and Areechon 2017) found that tilapia fed diets containing *B. pumilus* exhibited higher peripheral blood leukocyte counts. Furthermore, when tilapia was fed diets supplemented with *B. subtilis* and *B. licheniformis*, there was an increase in acidophilic granulocytes and intraepithelial lymphocyte proliferation (Tachibana et al. 2021).

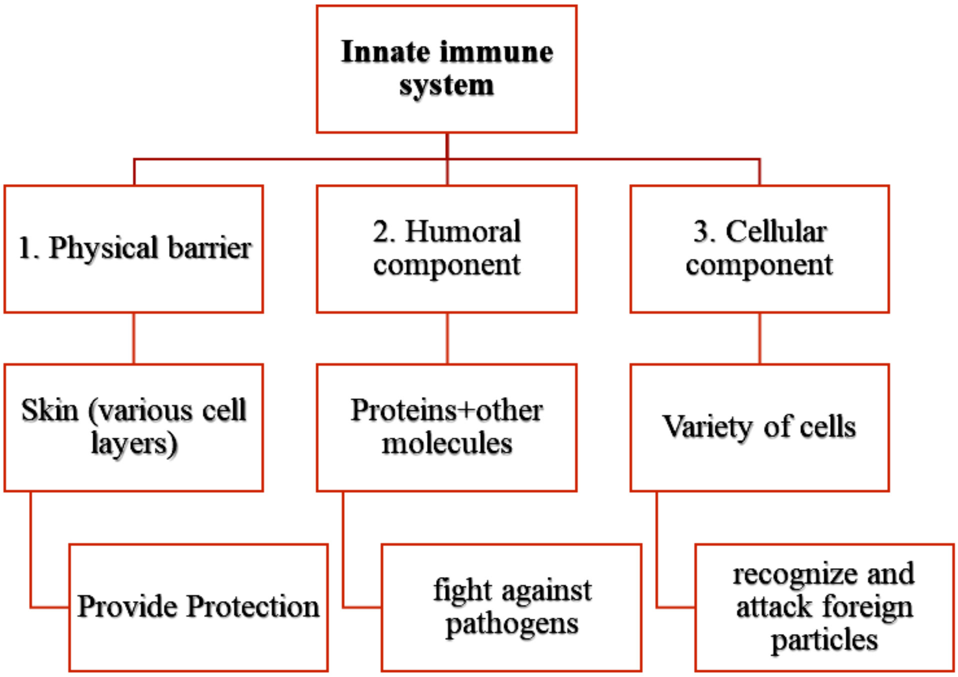

Fig. 2: Key factors involved in the innate immune system of tilapia (Source??).

In the gut mucosa, probiotics interact with immune cells. PRRs (Pathogen pattern recognition receptors) are proteins that help to identify pathogens by detecting their molecular patterns. These receptors recognize pathogen-associated (PAMPs) and damage-associated molecular patterns (DAMPs), such as lipoic acid, lipopolysaccharides and peptidoglycan. Additionally, PRRs can identify microbe-associated molecular patterns (MAMPs), which are present in both pathogenic and non-pathogenic microorganisms. MAMPs enhance the transcription process of pro-inflammatory chemokines and cytokines, which recruit innate immune cells when *probiotics* such as interact with the immune system (Fig. 3) (Kuebutornye et al. 2020). In both fish and higher vertebrates, serum immunoglobulins are integral to the humoral immune system, significantly contributing to the organism's ability to resist diseases. These antibodies are produced by B lymphocytes, which attach to antigens they encounter, thereby preventing these harmful antigens from infiltrating the body. Various Bacillus probiotics, including *B. amyloliquefaciens* and different strains like Lactobacillus sp., *B. subtilis* TPS4, *B. amyloliquefaciens* TPS1 and *B. velezensis* TPS3N play a role in this process. Whether administered individually or in grouping, have been demonstrated to elevate immunoglobulin and leukocytes (Ridha and Azad 2012; Kuebutornye et al. 2020).

2.2. Increased Phagocytosis

The innate immunity is the primary protective mechanism against infections (Cruvinel et al. 2010). Diets containing *B. coagulans* increased phagocytosis in the farming of Nile tilapia (Ghalwash et al. 2022). *B. amyloliquefaciens* and *B. subtilis* have both explained the stimulation of lysozyme in tilapia (Liu et al. 2017). Saurabh and Sahoo (2008) proposed that the high level of lysozymes in Nile tilapia could be recognized as increased phagocytes that secrete more lysozyme or lysozyme-manufacturing cells. Tilapia showed immune resistance to *A. hydrophila* after the addition of *E. faecium* to their diet (Tachibana et al. 2021).

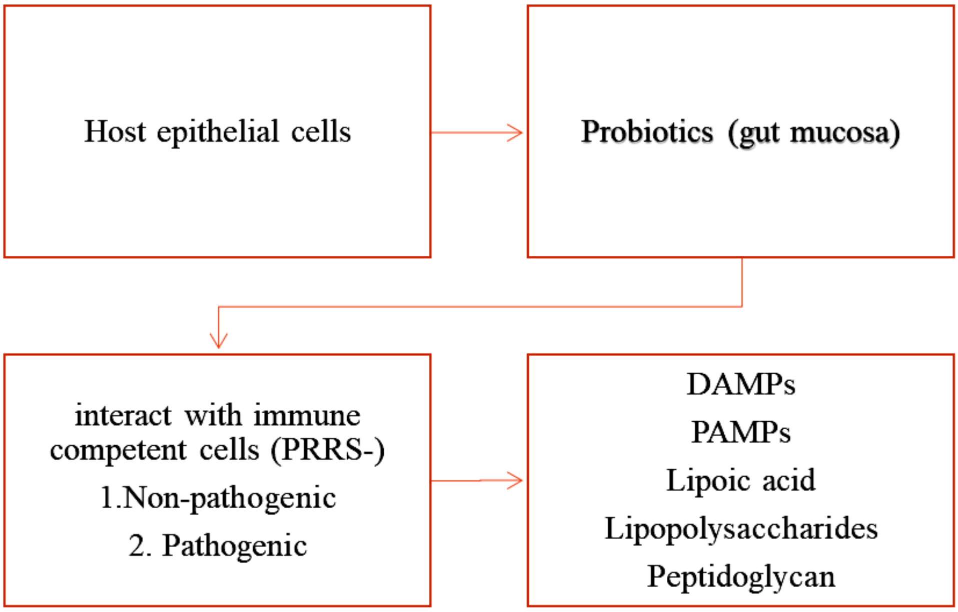

Fig. 3: The effects of probiotics (*Bacillus*) on immunological responses of fish. In fish immune system, host and microbe react and host easily identifies MAMPs (microbe-associated molecular patterns) in nonpathogenic and pathogenic microorganisms, immune signaling substances produced when MAMPs and PRRs react with host cells (Kuebutornye et al. 2020).

2.3. Regulation of Humoral Immune Activity

Lysozyme is an enzyme with multiple functions, found across various animals, plants, and microorganisms. Notably, lysozyme also targets chitin, a crucial component of fungal cell walls (Saurabh, et al. 2008). When Nile tilapia face low oxygen levels, *B. pumilus* as a probiotic has been shown to significantly enhance both cellular and humoral immune responses, including lysozymal activity (Liu et al. 2017). Additionally, *B. subtilis* modulates lysozyme activity, helping to mitigate the adverse properties of stress of salinity on Nile tilapia (Mahboob 2013).

2.4. Improved Growth Performance

Host-associated Bacillus strains like B. subtilis TPS4, *B. amyloliquefaciens* TPS17 and *B. velezensis* TPS3N have shown significant benefits for Nile tilapia. They enhance intestinal structure by increasing villus height, villus width, and muscle thickness. These strains also boost lipase activity of intestine and support a healthy balance of intestinal microbes (Kuebutornye et al. 2020). Bacillus species promote beneficial intestinal flora in fish, improving growth and immune function (Ma et al. 2022).

In fisheries, the use of probiotics as dietary additives has confirmed favorable results in improving fish growth and immunity rate (Atef et al. 2024). Probiotics offer advantages such as better growth, feed efficiency, enhanced immunity, and the promotion of beneficial gut bacteria in aquatic animals. These microorganisms produce enzymes that aid in better digestion and nutrient absorption by fish (Assan et al. 2022).

Previous studies have shown that probiotics containing *B. subtilis* or other genera, including commercial mixtures with Bacillus, Bifidobacterium, Enterococcus, Lactobacillus, and Pediococcus, contribute to the growth and health of Nile tilapia in recirculating aquaculture systems. Bacillus species are commonly found in fermented soybean products and are used in animal feeds (Phinyo et al. 2024).

In Nile tilapia diets, *Saccharomyces cerevisiae* yeast and fungi are used to ferment soybean meal, improving digestive enzymes in the gut, feed utilization, immune response, and resistance against aquatic pathogens (Phinyo et al. 2024). In aquaculture, probiotics enhance growth and health by colonizing larval fish, stimulating health through competitive exclusion, and producing antimicrobial products against harmful bacteria. Farmers utilize intensive and super-intensive aquaculture systems to increase production economically. Herbal additives and extracts have also been shown to enhance growth performance, antioxidant properties, and immune responses in aquatic animals (Ahmadifar et al. 2020)

2.5. Control of Diseases

Bacillus species are essential in boosting fish disease resistance and improving water quality (James et al. 2021). Their ability to form spores makes Bacillus spp. particularly resilient to harsh gastric conditions when used as dietary supplements (Cao et al. 2020). Nile tilapia, economically significant in developing countries, has recently seen a rise in aquaculture production (Kuebutornye et al. 2020; FAO 2020).

This increase, however, has led to heightened stress and greater susceptibility to bacterial contaminations in fish, resulting in the increased use of antibiotics (Phinyo et al. 2024). The administration of antibiotics not only lessens useful bacteria but also promotes antibiotic-resistant pathogens. Therefore, alternative methods to strengthen fish immunity and reduce antibiotic reliance are urgently needed. Bacillus spp., found in Thua nao and involved in soybean meal (SBM) fermentation, has been known as a probiotic for aquatic faunas (Kuebutornye et al. 2019a). Including Bacillus as a dietary supplement in fish feed enhance immunity, antioxidant enzymes, and increase disease-resistance against pathogenic bacteria (Gobi et al. 2018).

2.6. Enhanced Reproductive Performance

Nile tilapia (*O. niloticus*) is a highly valued species known for its economic significance, resilience, rapid growth, and adaptability to diverse rearing conditions. Previous research has shown that probiotic supplementation can enhance growth and survival rates, strengthen innate immunity (Galagarza et al. 2018), and improve disease resistance (Kuebutornye et al. 2020) in Nile tilapia. However, studies on the effects of probiotics on reproductive regulation in this species are scarce, with no existing research on their impact on reproductive hormone expression. Therefore, this study seeks to investigate the effects of probiotics on GnRH (gnrh1, gnrh2, gnrh3), kisspeptin (kiss2) and its receptor (kiss2r), and its receptor (GnRH), GTHs subunits (FSHB, LHB) and growth hormone (GH), in addition to assessing the gonad histology and gonadosomatic index (GSI) in Nile tilapia. Female tilapia undergo multiple spawning events annually under favorable photothermal conditions (26-28°C, 14L: 10D), with the environmental cues influencing sex differentiation usually achieved within 2-3 months (Biswas et al. 2005). Oreochromis species are considered maternal mouthbrooders, where eggs are incubated in the mouth (Lapeyre et al. 2009). However, regulating reproduction timing can be challenging in tilapia due to early maturation, asynchronous oocyte development, prolific breeding, and low fecundity. Furthermore, artificial fertilization in tilapia poses difficulties, hindering the actual selective breeding in captivity (Lapeyre et al. 2009). Intriguingly, despite the significant commercial attention in Nile tilapia, there is limited understanding of the presence and features of biological rhythms affecting reproductive factors in this species.

2.7. Stress Reduction

The study aimed to evaluate the impact of probiotics on growth, blood chemistry, hematology, and immune gene expression in Nile tilapia subjected to transport stress. Transporting fish, a common practice in aquaculture, often causes mechanical stress and water quality issues (Sutthi and Van 2020). Fish respond to this stress by changing their levels of circulating catecholamines and corticosteroids, including cortisol, a key stress hormone.

Tilapia, a popular farmed fish (Sutthi and Van 2020), is raised in over 100 countries because of its rapid growth, adaptability, and high market demand. However, increased tilapia farming has led to water quality problems and greater vulnerability to infectious diseases, especially bacterial infections. These issues cause high mortality rates and economic losses in the industry. Pathogens like Aeromonas spp. and Streptococcus spp. significantly contribute to these financial setbacks in tilapia farming (Sutthi and Van 2020). Additionally, limited space can increase the risk of injuries and disease spread (Telli et al. 2014). Therefore, incorporating oregano essential oil (OEO) into tilapia diets might improve their ability to handle stress from intensive farming practices. Additionally, fluctuations in temperature significantly impact feeding behaviors, often leading to reduced feeding activity and decreased feeding demand, resulting in stress and subsequent physiological changes, including compromised immunity and increased susceptibility to infections.

2.8. Improved the Digestive Health

Probiotics are increasingly being recognized as a valuable addition to animal feed, thanks to their ability to support intestinal microbiota and overall health. Extensive research has shown their benefits in enhancing the use of plant-based proteins in fish diets (Hassaan et al. 2021, 2014). Studies suggest that probiotics can beneficially alter the microbial communities in the host or farming environment (Wang et al. 2019). For instance, supplementing diets with probiotics like Bacillus and Lactobacillus can significantly improve digestion by increasing intestinal length and boosting growth performance (Hossain et al. 2022).

B. amyloliquefaciens, a rod-shaped, Gram-positive bacterium that thrives in anaerobic conditions, has been studied for its impact on Nile tilapia. Research indicates that diets enriched with *B. amyloliquefaciens* can significantly enhance the height of intestinal villi, leading to better nutrient absorption (Silva et al. 2017). Additionally, Nile tilapia fed with *B. amyloliquefaciens* have shown considerable weight gain (Reda 2015). Notably, *B. amyloliquefaciens* produces various metabolites during its growth, warranting further investigation into its composition and potential applications in aquaculture. This experiment aimed to explore whether *B. amyloliquefaciens* has a positive effect on Nile tilapia, given the crucial role of intestinal microbiota in digestion, nutrient metabolism, and immune function.

3. MECHANISMS OF ACTION

Probiotics containing *B. subtilis* have the ability to prevent illness in the intestines, create an environment that is hostile to pathogens, compete with them for essential nutrients, limit epithelial adhesion sites, and modify immune responses both physiologically and molecularly (Thirabunyanon and Thongwittaya 2012) (Fig. 4). The genus contains probiotics that can be added to water, taken as food supplements, and as bio-encapsulated Bacillus particles (Kumar et al. 2016). It is well-recognized that *B. amyloliquefaciens* produces organic acids and short-chain fatty acids, which can enhance digestibility and help break down food into proteins, carbs, and fats (Maas et al. 2021; Cai et al. 2020). Bacillus probiotics (*B. subtilis* and *B. licheniformis*) increase feed digestibility by increasing the synthesis of digestive enzymes such lipase, amylase, and protease (Yang et al. 2020). After feeding Nile tilapia two host-associated Bacillus species (*B. licheniformis* and *B. amyloliquefaciens*), significant lipase activity was seen in the intestines of *B. subtilis* (Kuebutornye et al. 2020).

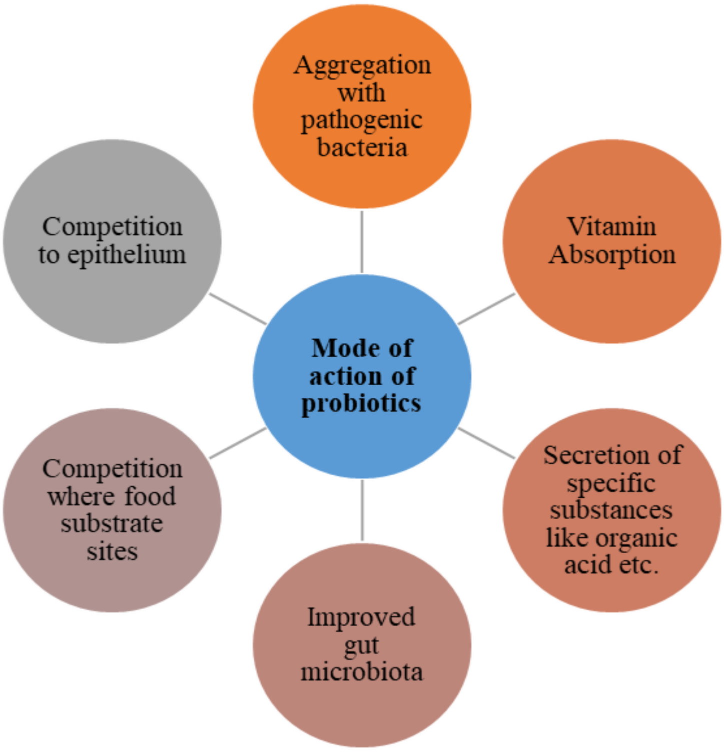

Fig. 4: Diagram illustrating the mechanism of action of Bacillus probiotics: Bacillus probiotics guard the intestines against illnesses by competing with pathogens for essential nutrients, creating an unwelcoming environment for them, and limiting epithelial attachment sites (Thirabunyanon and Thongwittaya 2012).

It has been demonstrated that Bacillus probiotics, including Bacillus spp. KUAQ1 and KUAQ2, *B. subtilis* C-3102, *B. subtilis*, and *B. velezensis* LF01, compete with pathogens for attachment sites on the gastrointestinal epithelium of Nile tilapia (He et al. 2013; Eladawy, 2019; Sookchaiyaporn et al. 2020). *B. clausii* produces a number of proteases, which are inhibitory compounds that can help prevent pathogens from adhering to mucosal epithelium. Nile tilapia is protected from illnesses by a variety of Bacillus species, including as *B. cereus*, *B. subtilis*, and *B. amyloliquefaciens*, which can compete with pathogens for adhesion sites (Tabassum et al. 2021). For example, the use of *B. pumilus*, *B. firmus*, *B. subtilis*, and *B. cereus* as probiotics inhibited the growth of Vibrio sp. (*V. harveyi* and *V. parahaemolyticus*, V. *alginolyticus*) and Mycobacterium species in Nile tilapia (Das et al. 2010).

Bacillus probiotic bacteria, such as Bacillus cereus and *B. subtilis*, can reduce aluminum-induced oxidative stress and tissue damage by preventing intestinal absorption and hepatic buildup of aluminum (Arun et al. 2021). *B. subtilis*, one of the most widely used probiotics in aquaculture, supports several defense mechanisms, including immunological stimulation, competitive exclusion, antagonistic mechanisms, and synergistic mechanisms (Nayak 2021). By including *B. subtilis* and *B. cereus* in the diet of tilapia, intestinal cell and microvillus density was increased, increasing disease resistance through alterations in the intestinal microbiota (Xia et al. 2020).

According to Kuebutornye et al. (2020), the pace at which harmful microbes colonize fish intestines may be reduced by increasing the density of microvilli. The absorptive surface area of the gut has been found to be influenced by the density of microvilli, which are tiny, finger-like protrusions that line the surface (Al Masri et al. 2015). Better absorption of nutrients and other substances is made possible by an increased absorptive surface area caused by a larger density of microvilli. The rate at which pathogenic microorganisms cling and colonize also reduces because the increased absorptive surface area creates fewer sites for them to attach to and colonize (Shim et al. 2017). It has been discovered that feeding Bacillus probiotics to fish increases their resistance to a variety of illnesses by stimulating and modifying their immune system (Vallejos-Vidal et al. 2016).

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4. FACTORS AFFECTING BACILLUS PROBIOTIC EFFICACY

Administering live bacteria orally to aquaculture species has shown promising benefits, as documented in numerous studies. However, many probiotic bacteria struggle to survive the intense conditions of feed processing and the high temperatures within the host animal's gastrointestinal tract (GIT). The public commonly recognizes lactobacillus spp. as probiotics. Research indicates that direct feeding of probiotics to fish or shrimp yields measurable and reproducible benefits, particularly improved health and resistance to infectious diseases. Probiotics are generally considered feed supplements that work in the digestive tract by inhibiting potentially harmful organisms through competitive exclusion. In the context of aquatic species, research sometimes challenges the idea that probiotics bind to and colonize GIT. Instead, there is substantial evidence suggesting that probiotics act as non-specific immune stimulants, enhancing protective immunity by influencing the innate component of the immune system (Nya 2022).

4.1. Selection of Probiotic Strains

In this study, two potential probiotics, Bacillus sp. KUAQ1 and Bacillus sp. KUAQ2, were isolated from the intestines of Nile tilapia (*O. niloticus* Linn.) and evaluated for their biological functions. The bacteria species were identified through traditional microbiological and biochemical assays, as well as 16S ribosomal RNA polymerase chain reaction analysis. Both Bacillus strains demonstrated resilience, surviving 6 hours at a pH range of 2 to 9 and 2 hours in bile salts.

The probiotics were tested for specific protease activity and their ability to inhibit the growth of *Aeromonas hydrophila* and *Streptococcus agalactiae*. Over an 8-week feeding trial, the probiotics showed no significant effect ($P>0.05$) on the average weight, average daily growth, specific growth rate, or feed conversion ratio of tilapia fry. Additionally, the probiotics did not affect the survival rate of tilapia when challenged with *S. agalactiae*. However, the juvenile fish treated with probiotics exhibited significantly higher levels of several immune parameters ($P<0.05$) compared to the control group. These parameters included lysozyme, phagocytic activity, and respiratory burst activity. There were no significant differences ($P>0.05$) in superoxide anion levels or alternative complement activity. The probiotics also did not significantly improve the fish's ability to handle stress in brackish water at 25ppt NaCl ($P>0.05$). Overall, these two Bacillus probiotics contribute to enhancing the immune system and managing disease in farm-raised tilapia (Selim and Reda 2015).

Since most probiotics utilized in aquaculture come from non-fish sources, they are unable to give aquatic animals the desired effects. Three Bacillus species were identified in this study using their biochemical, morphological, and evolutionary relationships after being extracted from the digestive system of the freshwater fish, *O. niloticus*. Based on their capacity to withstand high temperatures, bile salt concentrations, adhesion (hydrophobicity and auto-aggregation), low pH, hemolytic activity, and antimicrobial activity (including biosafety assay), their probiotic potential was assessed. Three Bacillus strains were designated as TPS3N, TPS17, and TPS4, respectively. These strains were identified as *B. subtilis* TPS4 (MK130899), *B. velezensis* TPS3N (MK130897), and *B. amyloliquefaciens* TPS17 (MK130898). TPS4 was γ-hemolytic, whereas TPS3N and TPS17 were α-hemolytic. The three isolates exhibited increased viability at higher temperatures (80, 90, and 100°C). They also demonstrated resistance to low pH (1), high cell surface hydrophobicity, bile salt concentration (0.5%), and auto-aggregation. As a result of their compatibility, the three isolates can be employed in consortia. Gentamicin, ampicillin, cephalexin, ceftriaxone, amikacin, kanamycin, chloramphenicol, penicillin, erythromycin, cefoperazone, doxycycline, tetracycline, ciprofloxacin, furazolidone (not including TPS17) and clindamycin (not including TPS4) were all effective against these strains. According to the antimicrobial assessment, TPS4 was only effective against Streptococcus agalactiae, while TPS3N and TPS17 demonstrated strong antimicrobial activity against all three fish pathogens (*A. hydrophila*, *S. agalactiae*, and *V. harveyi*) (Kuebutornye et al. 2020).

4.2. Dosage and Delivery

Probiotics have demonstrated significant promise in the treatment and prevention of numerous illnesses, including neurological disorders, cancer, cardiovascular disease, and inflammatory diseases. Probiotics are useful against pathogens that are resistant to numerous medications and assist in maintaining a balanced gut macrobiotic ecology. Probiotic *B. pumilus* AQAHBS01 extracted from Nile tilapia farms were investigated in both farm and laboratory settings. Fish immune responses were increased in the lab when fed feed with viable *B. pumilus* at levels of 1×10^7-10^9 colony forming units (CFU)/kg.

This was evidenced by the fish's increased phagocytic activity and elevated superoxide anion levels, which contributed to improved disease resistance against *Streptococcus agalactiae*. The fish, weighing approximately 50 grams, showed significant results when *B. pumilus* AQAHBS01 was administered at concentrations of 1 × 10^8 and 10^9 CFU/kg diet. These specific concentrations proved effective in enhancing disease resistance in Nile tilapia cultured in cages during the crucial period from early to mid-April when temperatures rose to 33°C. On the other hand, the control group and fish that received *B. pumilus* AQAHBS01 at 1×10^7 CFU/kg experienced rapid mortality due to streptococcosis (Elsabagh et al. 2018).

Additionally, the river's dissolved oxygen concentrations dropped to critical values of 1.0-1.5mg/L, which gave fish long-lasting anorectic effects. The cultivated fish may have been gradually killed by this effect until the experiment's conclusion. This data clearly shows that *B. pumilus* can be used as a probiotic to treat streptococcus's resistance in both field and laboratory culture settings. Variations in water quality, however, continue to be a major barrier to the application of probiotics in on-farm cage culture practices because they typically have detrimental effects on fish health. Farmers must carefully consider that fish are becoming more delicate and vulnerable to issues from infectious and non-infectious diseases as a result of this decline in health. Despite the increased interest in Bacillus probiotics, little is known about their exact mechanism of action, ideal dosage, and administration methods. A common freshwater fish cultivated all over the world is the Nile tilapia. Farmers find it appealing due to its hardiness, quick growth rate, and high nutritional value.

4.3. Environmental Conditions

The widespread use of intensive farming techniques has resulted in a quadrupling of tilapia production over the last ten years. However, there have been ecological effects of growing production as well, such as the rise of various pathogens and disease states. The indiscriminate use of chemicals and antibiotics, which pollutes the environment and breeds drug-resistant pathogens, contributes to the spread of diseases. As a result, the use of chemicals and antibiotics to prevent disease in fish culture has been strictly limited. Finding safe and efficient ways to manage and stop infectious disease outbreaks in fish has become more important in recent times. Since probiotic bacteria have been shown to have excellent effects on both fish health and growth and environmental safety, they have emerged as a focal point of current fish culture research. Numerous studies have documented increased growth in fish culture subsequent to the administration of probiotics. Furthermore, it has been observed that serum and mucosal surfaces exhibit elevated levels of lysozyme, superoxide dismutase (SOD), peroxidase, catalase (CAT), anti-protease, protease, and immunoglobulin M (Igm). These molecules are crucial defense mechanisms against various infectious pathogens in a variety of cultured fish. Furthermore, it has been observed that the use of probiotics in fish culture alters the expression of a number of significant genes, such as heat shock protein 70, which is linked to immune and stress responses (Wang and Lu 2016).

Bacillus species are highly regarded as probiotic bacteria due to their positive impact on growth, immune response, and disease resistance, making them excellent candidates for use as feed additives. For instance, incorporating *B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, and Saccharomyces cerevisiae into the diets of various cultured fish has shown promising results in enhancing growth, serum and mucosal immunity and disease resistance. The spore-forming ability of Bacillus species is particularly advantageous, allowing them to withstand the heat of feed palletization and survive the fish's digestive process, ultimately colonizing the intestines where they can produce essential digestive enzymes such as amylase, protease, and lipase.

Despite the growing body of research on Bacillus species, most studies have focused on single-species or single-strain probiotics. There is a scarcity of research on the use of multispecies or multi-strain probiotics, such as Bacillus spp., in fish diets to boost immune response and resistance to pathogens. Additionally, there is limited knowledge about the molecular mechanisms through which probiotics influence gene regulation and differential expression, as well as their impact on mucosal immunity. Mucosal immunity, which involves the production of immune substances like lysozyme, immunoglobulin, and proteolytic enzymes by mucosal tissues such as the skin, plays a crucial role in defending against infectious agents. To better understand the role of probiotics in disease prevention and control, further research is needed to explore their effects on gene expression and mucosal immunity (Nayak 2021).

4.4. Duration of Supplementation

Because of their probiotic qualities, Bacillus species are frequently used in animal production. Feed supplementation with particular strains of Bacillus can improve digestibility, immune modulation, gut microbiota, and growth performance in various animal species. Animals are fed bacilli as spores because they can withstand the rigorous feed processing and extended storage. Though it is generally acknowledged that probiotics must be at a metabolically active level to carry out specific probiotic functions like the emission of antimicrobial enzymes and compounds, the production of short-chain fatty acids, and the struggle for vital nutrients, the spores are metabolically quiescent. These processes are supposed to kick in in the host gastrointestinal tract (GIT) shortly after spore digestion in order to support host metabolism and microbiota. Given that vegetative cells offer numerous health benefits while bacterial spores are metabolically dormant, it is particularly relevant to talk about the life cycle of Bacillus in animal GIT. This review attempts to summarize the key traits of both vegetative cells and spores as well as to address the most recent findings regarding the life cycle of useful Bacillus in diverse intestinal systems (Soltani et al. 2019).

Beneficial microbial cells known as probiotics are frequently used as immune modulators. Probiotic supplements to fish diets may influence particular immune system and gut functions and offer disease prevention. Bacillus sp., Lactobacillus sp., and Saccharomyces sp. are the most often utilized probiotics in aquaculture. Fish are given Bacillus species, which are aerobic, nonpathogenic gram-positive bacteria, through the water or by mouth to improve their physical state and the populations of GI microbes. Adding *B. amyloliquefaciens* to fish diets has positive effects. There is still a dearth of knowledge regarding the application of *B. amyloliquefaciens*, despite numerous studies focusing on the effects of probiotics both in vitro and in vivo on the immune systems of various fish species. Growth, intestinal villous heights, goblet cell counts, intraepithelial lymphocyte (IEL) counts, and GI bacterial populations are all improved by *B. amyloliquefaciens*. There is still little knowledge regarding how *B. amyloliquefaciens* affects tilapia's immune system and ability to withstand disease. Thus, the purpose of this study was to examine how two levels of *B. amyloliquefaciens* dietary supplementation affected the immune parameters of Nile tilapia as determined by serum lysozyme, nitric oxide (NO), phagocytic, and bactericidal activity as well as the expressions of interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) in kidney tissue in the head. Furthermore, the effectiveness of *B. amyloliquefaciens* as a novel substitute technique for managing *Y. ruckeri* and *C. perfringens* type D was assessed (Selim and Reda 2015).

4.5. Feed Composition

Given that 50% of production costs in an intensive aquaculture system are related to nutrition, attention must be paid to this aspect. Improving management and nutritional quality in contemporary aquaculture can reduce these expenses by up to 20%, enabling larger savings. High density rates result in high feed consumption and declining water quality, which raises issues with pollution of the environment, fish health (disease outbreaks, for example), and low feed efficiency rates (disease outbreaks, for example). In this regard, probiotic administration in aquaculture is regarded as a workable substitute that has minimal negative effects on the environment for enhancing animal health and growth. The primary effects of probiotic feeding include immune system stimulation, improved growth and performance, modulation of the gut microbiota, and bioremediation. Probiotics' ability to inhibit pathogen growth, produce various substances (like bacteriocin, organic acids, and volatile compounds), compete for nutrients and adhesion sites, and enhance the innate immune response (like by boosting lysozyme and burst respiratory activities and favoring interactions with leucocytes, phagocytes, and natural killer cells) is highlighted by several authors. The Bacillus species can directly or indirectly improve the host's ability to utilize nutrients by modulating gut microbiota and promoting intestinal physiology through the secretion of exogenous enzymes. Fish fed certain species of Bacillus orally may experience faster growth rates. Furthermore, altering the intestinal microbiota's composition to include a larger percentage of commensal bacterial communities may support the integrity of the host intestine and aid in innate and adaptive functions. Through their interactions with the gut-associated lymphoid tissue, commensal bacteria support the host immune response (GALT) (Tachibana et al. 2021).

4.6. Water Quality

Numerous studies have shown that probiotics can reduce production costs and enhance the growth performance of farmed tilapia. Probiotics are safe substitutes for antibiotics that have a number of advantageous effects on the aquaculture sector through a variety of mechanisms, including improvements in water quality, immune system and stress responses in fish, competitive inhibition of pathogenic bacteria through the production of inhibitory compounds, and enhanced activity of digestive enzymes that increase the host's availability of nutrients.

According to earlier research, Bacillus isolates show promise as probiotic candidates for fish. Probiotics based on Bacillus enhanced tilapia growth and health, the activity of digestive enzymes, and the microbiota and morphology of the intestinal tract. These advantageous outcomes for *B. subtilis* were shown. The positive effects of *B. amyloliquefaciens* in tilapia raised in cages and *B. pumilus* in Nile tilapia raised in captivity and in the wild were also shown. The effects of digestive enzymes, probiotics based on Bacillus, and a probiotic blend of Bacillus with other viable bacteria in tilapia fingerlings were assessed. Moreover, several reports have indicated that probiotics, including Bacillus, improve fish habitats by lowering the growth of pathogenic bacteria and harmful phytoplankton and by bioremediation of organic wastes in rearing water. On the other hand, not much is known about the effects of commercial probiotics made of a combination of Bacillus strains on tilapia raised in Egypt's environmental conditions. Thus, the purpose of this work was to examine the effects of a probiotic mixture of Bacillus strains (*B. subtilis, B. licheniformis*, and *B. pumilus*) on the intestinal morphometry, growth performance, water quality and hemobiochemical parameters in Nile tilapia (Elsabagh et al. 2018).

5. CHALLENGES AND FUTURE DIRECTIONS

High stocking densities and rising feed costs pose significant challenges to the aquaculture sector, leading to increased infections and diseases that affect fish production. In response, the use of prescription drugs and antibiotics has risen, sparking debates among scientists regarding their effectiveness. To combat diseases and promote growth, fish farming utilizes various beneficial feed additives, such as prebiotics, synbiotics, and probiotics. These not only enhance the immune response of aquatic organisms but also serve as alternatives to antimicrobial agents (Soltani et al. 2019). The Food and Agriculture Organization (FAO) defines probiotics as live microbial supplements that benefit the host's health, while prebiotics are indigestible compounds that support beneficial gut microorganisms. Both have shown promise in improving the health and productivity of fish. Research continues to explore innovative dietary supplements, including probiotics, prebiotics, and other beneficial compounds (Boyd et al. 2020).

The contamination of nitrogenous compounds like ammonia, nitrate, and nitrite poses significant challenges for aquaculture, leading to mass mortality at high concentrations and risks even at low levels. Lactobacillus species help eliminate pathogens and remove nitrogen from contaminated shrimp farms. Gram-positive bacteria, such as Bacillus species, are more efficient at converting organic matter into microbial biomass. They are associated with improved water quality, reduced pathogens, and enhanced survival and growth rates in young *Penaeus monodon* (Kuebutornye et al. 2019a). Probiotics, prebiotics, and synbiotics play a crucial role in enhancing the health and digestive efficiency of aquaculture species, contributing to sustainability in the industry. Aquaculturists should receive clear and transparent results from investigations. Incorporating these dietary additives is currently the best strategy for sustainable aquaculture. Future research should focus on species complexities, culture techniques, and the types of additives used, as well as challenges within aquafeed manufacturing. Additionally, methods to enhance Nile tilapia's immune systems with probiotics and maintain Bacillus probiotics during treatment warrant further investigation.

6. CONCLUSION

This study explores probiotics in tilapia culture. Probiotics can be sourced from intestines, gonads, rearing water, sediments, or commercial products and are mainly used as feed additives. Their effectiveness depends on proper dosage and treatment duration. While they can enhance immune responses and improve growth and survival rates, further research is needed to fully understand their effects compared to other fish species. Current evidence does not definitively confirm the positive impacts of probiotics and prebiotics on animal health, raising concerns over survival during application. Although commercially available, these products require investment but can increase production efficiency and decrease disease incidence. Synbiotics, which combine prebiotics and probiotics, may improve gastrointestinal health, but research on their use in aquaculture remains limited. Preparation methods, species, and environmental challenges influence limitations in aquaculture. Laboratory findings often do not apply to field settings, emphasizing the need for extensive field testing. Probiotics and prebiotics can effectively replace antibiotics, enhancing health, survival rates, and producing safe organic products. More studies are needed to determine how probiotics and prebiotics promote growth in aquaculture species. Advanced techniques, such as immunohistochemistry and genomics, are essential for understanding these mechanisms. Increased digestive enzyme activity has been noted in groups fed probiotics, highlighting the importance of optimizing dietary levels to prevent adverse effects while reaping their benefits in aquaculture.

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