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THE EFFECT OF LACTIPLANTIBACILLUS PLANTARUM BE7 AND LACTICASEIBACILLUS PARACASEI BUM6 AS FEED SUPPLEMENT ON GROWTH PERFORMANCE AND PATHOGEN RESISTANCE IN OREOCHROMIS SPP.

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MSc

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CONFIRMATION BY PANEL EXAMINERS

I certify that a Panel of Examiners has met on 22nd December 2025 to conduct the final examination of Muhammad Harith Haqeem Bin Zunaide on his Master of Science thesis entitled “The Effect of Lactiplantibacillus plantarum Be7 and Lacticaseibacillus paracasei Bum6 As Feed Supplement on Growth Performance and Pathogen Resistance in Oreochromis spp.” in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiner recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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ABSTRACT

Probiotic inclusion as a dietary supplement has demonstrated numerous benefits for the aquaculture industry, particularly in improving productivity and profitability. As the use of antibiotics can promote the emergence of resistant strains and fish vaccines are often costly to implement, probiotics present a more practical and sustainable alternative for disease mitigation in cultured fish. However, the effectiveness of probiotic supplementation is strain-specific and varies according to tolerance to gastrointestinal stress conditions and aquaculture environments, limiting the efficacy of many commercially available products. This study aimed to evaluate the probiotic potential of two locally isolated probiotic strains from Malaysian fermented foods, *Lactiplantibacillus plantarum* BE7 (belacan) and *Lacticaseibacillus paracasei* BUM6 (budu) and their effects on the growth performance, survival, and disease resistance of red hybrid tilapia (*Oreochromis* spp.) against fish pathogens, *Streptococcus agalactiae* and *Aeromonas hydrophila*. In vitro screening was conducted to assess tolerance to gastrointestinal, environmental stress conditions and antagonistic activity against *S. agalactiae* and *A. hydrophila*. This was followed by a 14-week feeding trial to evaluate growth performance, feed utilization, survival, and disease resistance to pathogens. Both probiotic strains demonstrated high tolerance (>60%) to bile, pH, and salinity stress and exhibited strong inhibitory activity against the tested pathogens. After 14 weeks of feeding trial, fish fed with *L. plantarum* BE7 showed significantly higher survival rate, specific growth rate (SGR), absolute growth rate (AGR), and improved feed conversion ratio (FCR) compared to other groups ($P < 0.05$). Following pathogen challenge, *L. plantarum* BE7-fed fish exhibited significantly improved survival and reduced disease severity. These findings suggest that *L. plantarum* BE7 has potential as functional probiotic for dietary application to enhance growth performance and disease resistance of tilapia culture in Malaysia.

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LIST OF SYMBOLS

Symbols

CFU/mL	Colony forming unit per milliliter
cm	Centimeters
cm/day	Centimeters per day
C	Degree Celcius
g	Grams
g/day	Grams per day
hrs	Hours
mg/L	Milligrams per liter
ppm	Parts per million

LIST OF ABBREVIATIONS

Abbreviations

AGR	Absolute Growth Rate
FCR	Feed Conversion Ratio
LAB	Lactic Acid Bacteria
SGR	Specific Growth Rate

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Aquaculture is a sector that provide food supply to the world’s global population. With an expanding global population comes a rising need for aquaculture goods from fish farming activities. Aquaculture produced the most fish products in Southeast Asia in 2021, accounting for about 54.2% of total fisheries production. Marine capture fisheries came in second with 39.2% and inland capture fisheries with 6.6% (Department of Fisheries, 2021). In Malaysia, in 2018, the cultivation of the red hybrid tilapia (*Oreochromis* spp.), black tilapia (*Oreochromis mossambicus*), and Nile tilapia (*Oreochromis niloticus*) had contributed to 30.7% of the total freshwater aquaculture production in Malaysia (Mohamad *et al.,* 2021). In between January and July 2024, Malaysia's fisheries industry had generated 1.3 million metric tonnes, guaranteeing the nation's food security with red hybrid tilapia is the most significant freshwater fish species that have been cultivated by aquaculture sector, making up to 94% of the total tilapia produced worldwide (Mohamad et al., 2021).

The demand for fish products as a major daily source of protein, as opposed to other animal proteins such as poultry or beef, has increased due to the health benefits associated with eating fish and other aquatic species (Luthada-Raswiswi et al., 2021). Fish are rich with nutrients such as high-quality protein, omega-3 fatty acids, vitamins, and minerals, which promote cardiovascular health, cognitive function, and general well-being (FAO, 2022). This rising demand puts tremendous pressure on the aquaculture business to increase productivity and provide a sustainable supply to the world's growing population. Despite the fact that red hybrid tilapia is well-known for its rapid growth and more resistance towards diseases compared to other farmed fish species, the industry confronts a number of problems. Disease outbreaks are among the serious impediment in farming operations, reducing production and profitability (Azmai, 2015; Sanches-Fernandes et al., 2022). Furthermore, the stress factors coming from high stocking densities, fluctuating water quality, and environmental stressors also aggravate farmed fish's susceptibility to viral illnesses.

A severe disease outbreak in rearing culture might result in significant economic losses for the worldwide aquaculture production. Streptococcosis remains a common bacterial illness affecting tilapia culture, predominantly caused by *Streptococcus agalactiae*, resulting in considerable fish mortality and global economic losses (Yong et al., 2022). Furthermore, *Aeromonas hydrophila*, a prominent aquatic pathogen, causes motile Aeromonas septicaemia (MAS), a disease that kills many freshwater fish species (Semwal et al., 2023). In January 2020, a farm in Selangor reported a 70% death rate of *Oreochromis* sp. as a result of bacterial co- infection by *A. hydrophila*, and *S. agalactiae* (Basri et al., 2020). Streptococcosis and motile Aeromonas septicaemia are the common fish diseases that can cause outbreaks in rearing cultures, either through single infections or co-infections (Omar et al., 2023). Lethargy, exophthalmia, and irregular swimming behaviour in infected farmed tilapia are strong markers of disease outbreaks (Shahjahan et al., 2023). Motile Aeromonas septicaemia and streptococcosis have a significant economic impact on Malaysian aquaculture, wreaking havoc on both ornamental and food fish businesses by increasing death rates, disrupting market supply, and raising production costs (Lulijwa et al., 2023).

In aquaculture, antibiotics have traditionally been used to control disease outbreaks. However, the extensive and frequently uncontrolled use of antibiotics has resulted in the emergence of antimicrobial resistance (AMR) in aquatic settings, posing serious threats to both ecosystem health and human public health (Salam et al., 2023). While vaccination is a promising technique for managing viral illnesses in farmed fish, its actual implementation confronts significant challenges. These include high operating expenses that are sometimes prohibitively expensive for small-scale farmers, technological complexity in vaccine production, and a lengthy and strict regulatory clearance procedure that hinders market access (Kim et al., 2022). Given these obstacles, probiotics have emerged as a viable option for improving fish health and disease resistance. The use of probiotics in aquaculture is widely viewed as a viable and environmentally friendly way to fulfil expanding worldwide demand for fish while reducing dependency on antibiotics and overcoming the constraints associated with immunisation techniques.

The application of probiotics has been increasingly recognized as a promising alternative and complementary approach to conventional antibiotic treatments and vaccination strategies in aquaculture. Probiotic supplementation improves the health and growth of aquatic species by enhancing gut integrity, stimulating immunological responses, and maintaining microbial balance within aquatic environments (Chizhayeva et al., 2022). Moreover, probiotics contribute to improved feed utilization, stress tolerance, and disease resistance among cultured species, leading to higher productivity and survival rates (Newaj-Fyzul and Austin, 2021). Beyond their biological benefits, probiotics offer significant ecological advantages, promoting sustainable aquaculture practices due to their environmentally friendly and non-toxic nature (Hai, 2015). In the previous study done by Ilyanie et al., 2023, *Lactiplantibacillus plantarum* BE7 and *Lacticaseibacillus paracasei* BUM6 were identified and confirmed as probiotic strains that were locally isolated from traditional Malaysian fermented foods which were *belacan* and *budu*, respectively.

As locally isolated strains, *L. plantarum* BE7 and *L. paracasei* BUM6 may exhibit enhanced tolerance to gastrointestinal conditions and local aquaculture environments compared to commonly used commercial probiotics dominated by mixed or *Bacillus*-based formulations. However, their probiotic potential has not yet been comprehensively validated through in vivo feeding trials and pathogen challenge studies in tilapia. Therefore, this study aimed to evaluate the survival properties of *L. plantarum* BE7 and *L. paracasei* BUM6 under harsh environmental conditions encountered during dietary administration, such as pH, bile concentration, and salinity, as well as to assess their effects on the growth performance and survival of red hybrid tilapia during a 14-week feeding trial and their protective efficacy against common fish pathogens that cause disease outbreak among tilapia culture which are *Streptococcus agalactiae* and *Aeromonas hydrophila*. The findings of this study are expected to contribute to the development of strain-specific, locally adapted probiotic applications to support sustainable tilapia aquaculture in Malaysia.

1.2 Problem Statement

Streptococcosis and Aeromonas septicaemia are among the most prevalent bacterial diseases affecting tilapia aquaculture worldwide, resulting in significant economic losses and reduced production efficiency. Current disease management strategies rely heavily on antibiotics and vaccines; however, the effectiveness of antibiotics is increasingly compromised due to antimicrobial resistance, while vaccine application remains costly, labour-intensive, and variable in efficacy depending on administration methods (Ridzuan et al., 2022; Salam et al., 2023). Consequently, probiotics have emerged as a promising alternative for sustainable disease control in aquaculture. Nevertheless, the efficacy of probiotic supplementation is highly strain-specific and depends on the ability of individual strains to tolerate gastrointestinal stress, remain viable under aquaculture conditions, and exert functional benefits when administered via feed (Ringø et al., 2020). Although numerous probiotic candidates have been identified through in vitro screening, there is still a lack of in vivo evidence, particularly long-term feeding trials and pathogen challenge studies, to validate their effects on growth performance and disease resistance in tilapia. Furthermore, the probiotic potential of locally isolated strains, such as *Lactiplantibacillus plantarum* BE7 and *Lacticaseibacillus paracasei* BUM6, has not been comprehensively evaluated in red hybrid tilapia, highlighting a critical knowledge gap that this study aims to address

1.3 Research Objectives

The objectives of this study were:

i. To evaluate the growth and survival properties of the probiotics *L. plantarum* BE7 and *L. paracasei* BUM6 in relation to various environmental parameters (pH, bile, salinity).

ii. To determine the effect of probiotic supplemented feed, *L. plantarum* BE7 and *L. paracasei* BUM6 on the growth performance and survivability of red hybrid tilapia.

iii. To analyze the protection ability of *L. plantarum* BE7 and *L. paracasei* BUM6 against aquatic pathogens, *Streptococcus agalactiae* and *Aeromonas hydrophila*.

1.4 Research Questions

The research questions of this study were:

i. How do *L. plantarum* BE7 and *L. paracasei* BUM6 respond to different environmental parameters (pH, bile concentration, and salinity) in terms of growth and survival?

ii. To what extent does probiotic supplementation with *L. plantarum* BE7 and *L. paracasei* BUM6 improve the growth performance and survivability of red hybrid tilapia compared to a control diet?

iii. How does supplementation with *L. plantarum* BE7 and *L. paracasei* BUM6 enhance the resistance of red hybrid tilapia against infections by *S. agalactiae* and *A. hydrophila*?

1.5 Hypotheses

The hypotheses according to each research objectives for this study were:

Objective i – Viability of probiotic strains against stress

H : There is no significant difference in the growth and survival of *L. plantarum* BE7 and *L. paracasei* BUM6 under varying pH, bile concentration, and salinity levels.

Ha: *L. plantarum* BE7 and *L. paracasei* BUM6 show significantly different growth and survival rates under varying pH, bile concentration, and salinity levels.

Objective ii – Growth and survival of tilapia

H : Supplementation with *L. plantarum* BE7 or *L. paracasei* BUM6 does not significantly affect the growth performance and survivability of red hybrid tilapia compared to the control group.

Ha: Supplementation with *L. plantarum* BE7 or *L. paracasei* BUM6 significantly improves the growth performance and survivability of red hybrid tilapia compared to the control group.

Objective iii – Pathogen resistance

H : Supplementation with *L. plantarum* BE7 or *L. paracasei* BUM6 does not provide significant protection to red hybrid tilapia against *S. agalactiae* or *A. hydrophila* infections.

Ha: Supplementation with *L. plantarum* BE7 or *L. paracasei* BUM6 significantly enhances protection of red hybrid tilapia against *S. agalactiae* or *A. hydrophila* infections.

1.6 Significance of Study

This study is significant as it contributes to strengthening scientific knowledge regarding the benefits of probiotics as supplemental feed for improving efficiency and productivity in aquaculture. Probiotic supplementation has been reported to enhance growth performance, feed efficiency, immune responses, and disease resistance in farmed fish, however, the effectiveness of these benefits is highly strain-dependent and requires systematic validation. The present study evaluates locally isolated probiotic strains from traditional fermented sources, namely *L. plantarum* BE7 (belacan) and *L. paracasei* BUM6 (budu), which may exhibit superior tolerance to gastrointestinal stress and aquaculture environmental conditions. Through controlled feeding trials and pathogen challenge experiments, this study provides empirical evidence on growth performance, survival, and disease resistance in red hybrid tilapia. The findings offer potential benefits to the Malaysian aquaculture industry by identifying locally sourced and cost-effective probiotic candidates that may serve as alternatives or complements to commercial probiotics, which are predominantly based on *Bacillus* spp. By validating the functional efficacy of these local isolates through in vitro assays and in vivo evaluations, this research supports the development of strain-specific, sustainable probiotic applications for tilapia aquaculture in Malaysia.

1.7 Limitations

This study has several limitations that should be acknowledged when interpreting the findings. First, the histopathological examination was conducted using a limited number of samples, with only one representative fish selected from each experimental group. As a result, the histological observations were primarily descriptive in nature and did not allow for robust statistical analysis, thereby limiting the statistical power and generalizability of the histopathological findings. In addition, although the probiotic-coated feed was prepared according to standardized procedures, this study did not include specific assessments to verify probiotic viability or stability on the feed during delivery following the coating process. Variations in probiotic survival during handling and feeding may have influenced the effective dose ingested by the fish. Despite these limitations, the study provides valuable preliminary evidence on the probiotic potential of locally isolated strains and highlights important considerations for future research. Further studies incorporating larger sample sizes for histopathological analysis and quantitative evaluation of probiotic viability and feed stability are recommended to strengthen the reliability of the findings.

1.8 Chapter Summarizations

This chapter discusses about the aquaculture plays a vital role in Malaysia’s food security, with red hybrid tilapia being a key cultured species. However, bacterial diseases caused by *S. agalactiae* and *A. hydrophila* significantly reduce production and profitability. Conventional control measures like antibiotics and vaccines face challenges such as resistance and high costs. Probiotics provide a sustainable alternative, improving fish health, growth, and disease resistance. However, only suitable strain might offer an effective outcome since the probiotic is strain-specific and has vary tolerance toward gastrointestinal stress condition and aquaculture environments. This study evaluates two local strains, *L. plantarum* BE7 and *L. paracasei* BUM6, for their stress tolerance, growth benefits, and protective effects, aiming to enhance sustainable tilapia farming practices. The following chapter reviews relevant literature on tilapia aquaculture, major bacterial pathogens, current disease management strategies, and the role of strain-specific probiotics in enhancing fish health and disease resistance.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Aquaculture

There are three major subsectors in charge of fish production for the global consumers: marine capture fisheries, inland capture fisheries, and aquaculture. In 2021, aquaculture sector had accounted for the greatest part of total production volume offish in Southeast Asia's countries as shown in Figure 2.1 (Southeast Asian Fisheries Development Center, 2021). Aquaculture firms have expanded fast over the world, establishing themselves as significant contributors to the global food production economy ensuring the nation's food security. Aquaculture is known as the practice of cultivating aquatic organisms such as fish, shellfish, and aquatic plants in controlled conditions from their juvenile stage until they are ready to be commercialized. It comprises breeding, raising, and harvesting aquatic creatures in ponds, tanks, cages, and other waterways. Unlike fishing, aquaculture does not exploit fish's natural environment, which may lead to problems such as overfishing, pollution, and habitat deterioration in the future. Aquaculture greatly satisfy the rising demand for seafood products while reducing the impact on wild fish populations. Hence, it become the most sustainable practice to supply fish product to the global growing population (Cooke *et al*, 2023).

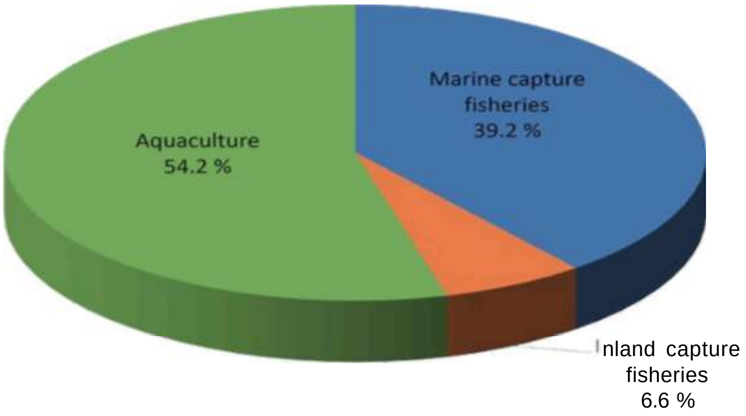


Figure 2.1 Proportion (%) of Production of Fishery Subsectors of Southeast Asia in 2021 by Quantity (Southeast Asian Fisheries Development Center, 2021)

Recently, people tend to eat fish as their daily protein consumption rather than meat and poultry due to health benefits reason. These shifting demands for different protein selections for fish have changed the percentage of world fish consumption over other animal proteins. The United Nations Food and Agriculture Organization (FAO) reports that aquaculture's contribution of global fish output climbed from 25.7% in 2000 to 46% in 2018 (FAO, 2020). Between 1961 and 2017, fish consumption climbed by an average of 3.1% per year, outperforming increases in consumption of all other animal protein meals (meat, dairy, milk, etc.) and the world population growth rate of 1.6% (FAO, 2020). In 2017, fish consumption accounted for 7% of all proteins consumed and 17% of the total animal protein intake of the world's population (FAO, 2020). Department of Fisheries (DOF) stated that Malaysia has established aquaculture facilities for at least 49 different marine and freshwater fish species to strengthen this sector’s ability to maintain production. The most popular marine fish species cultured among the facilities include Asian seabass, snapper, and grouper. For the farmed freshwater fish species like catfish, tilapia, and riverine catfish were commonly cultured, with a total production of 121,553.75 metric tonnes by 2020 (DOF, 2020).

As of 2024, Malaysia's aquaculture sector accounts for around 30% of overall fisheries production, with the government aiming for a 40% rise by 2030 to fulfil increasing domestic demand and boost food security (Obi et al. 2025). In 2023, the sector generated 492,680 metric tonnes of aquaculture goods worth MYR 3.9 billion, employing around 148,000 fishermen and aquaculture farmers. In order to achieve the 2030 targets, Malaysia is investing in sophisticated technology like biofloc systems to improve sustainability and efficiency. Biofloc system functions by adding a carbon source to the water, which promotes the growth of heterotrophic bacteria and other microbes thus enhancing water quality (Raza et al. 2024). Because it supports the objectives of sustainable aquaculture, increased yields, and food security while lessening environmental pressure, biofloc technology is being advocated more and more in Malaysia and other Southeast Asian nations. The initiative is consistent with wider regional goals promoting sustainable aquaculture, which contributes to economic development and environmental protection (Reuters, 2025). Within this expanding aquaculture landscape, red hybrid tilapia represents one of the most economically important freshwater species in Malaysia, making it a suitable model for evaluating strategies to enhance productivity and sustainability.

2.2 Red Hybrid Tilapia

Oreochromis spp., commonly referred to as red hybrid tilapia, represents one of the most extensively cultured freshwater fish species worldwide. Native to Sub-Saharan Africa and the Middle East, tilapia species have been widely disseminated due to their robust aquaculture potential (Mohamad et al., 2021). The Mozambique tilapia (*Oreochromis mossambicus*) was the first species introduced to Southeast Asia, particularly the Philippines, in 1950 (Dullah, 2020). Subsequent developments in the early 1970s, following the introduction of Nile tilapia (*Oreochromis niloticus*), led to the rapid expansion of tilapia farming in ponds and cages. By the 1980s, the introduction of a hybrid variety, the red hybrid tilapia, a cross between *O. mossambicus* and *O. niloticus*, further advanced the global prominence of tilapia aquaculture. Red hybrid tilapia is particularly valued for several special characteristics, including rapid growth rates, high feed conversion efficiency, tolerance to a wide range of environmental conditions, resistance to common pathogens, and attractive red pigmentation, which significantly enhances their market appeal (Mohamad et al., 2021).

Red hybrid tilapia, black tilapia, and Nile tilapia are all commercially significant aquaculture species, each with unique characteristics that determine farming suitability. Nile tilapia, the most often farmed, is prized for its rapid development, adaptability to a variety of conditions, and mild flavour (Campos et al., 2025). Red hybrid tilapia, which is often a mix between Nile and other tilapia species, is popular because of its appealing look and customer preference, particularly in markets where red is associated with premium quality, despite being slightly less resilient than its parent species (Olsson et al., 2023). Black tilapia, which refers to pure or hybrid strains with darker pigmentation, has high disease resistance and efficient feed conversion, making it ideal for intensive culture systems (Kassim et al., 2024). While Nile tilapia remains the benchmark for growth and global production, red hybrids and black strains offer targeted advantages depending on market demands and farming conditions. Figure 2.2 shows the differences in the general body shape and structure among Nile tilapia, Black tilapia, and Red hybrid tilapia.

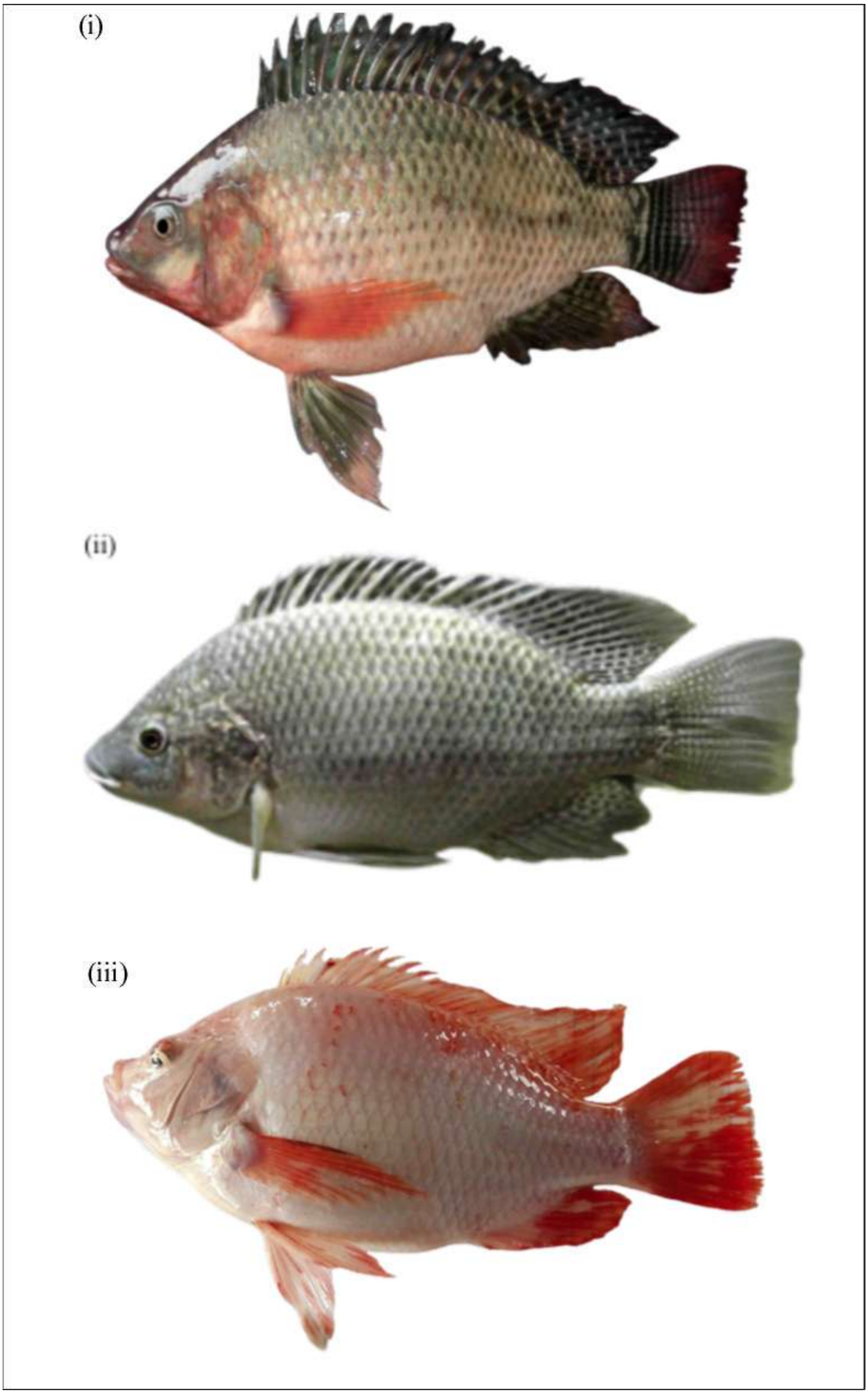


Figure 2.2 (i) General Body Shape and Structure of Nile tilapia
(ii) Black Tilapia and (iii) Red Hybrid Tilapia (Al-Faisal and Mutlak, 2022)

In Malaysia, red hybrid tilapia constitutes a major component of the cultured fish by aquaculture industry, with production reaching 33,437 tonnes, positioning it as the second-highest farmed species after the African catfish *{Glorias gariepinus)* (Mohamad et al., 2021). However, in 2019, red hybrid tilapia account for approximately 30% of the freshwater and brackish water fish species production which higher than the African catfish as shown in Figure 2.1 (Department of Fisheries Malaysia, 2020). Their physiological resilience, such as tolerance to low dissolved oxygen, variable salinity, and fluctuating temperatures, makes them ideal candidates for diverse aquaculture environments (Tan et al., 2024). Moreover, their strong resistance to disease reduces the dependence on chemical treatments, thereby supporting more sustainable farming practices. Beyond their biological advantages, red hybrid tilapia offered considerable commercial flexibility that they can be marketed as whole fish, processed into fillets, or used in the development of value- added products such as fish nuggets and burgers (Department of Fisheries Malaysia, 2020). This versatility not only enhances profitability but also allows farmers to cater to different consumer preferences, thereby improving the overall economic viability and sustainability of tilapia farming operations (Dullah, 2020).

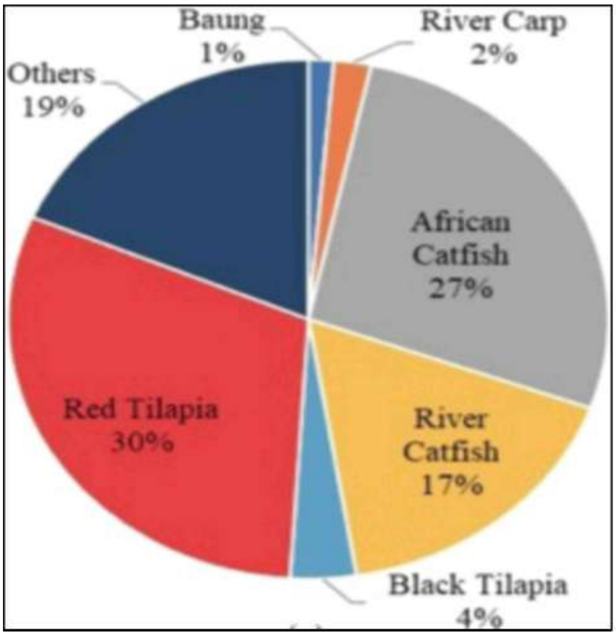


Figure 2.3 Aquaculture Production by Quantity of Popular Freshwater Water and Brackish Water Species in Malaysia in 2019 (Tan Et AL, 2024)

In addition to their adaptability and commercial versatility, red hybrid tilapia continues to gain attention for their aesthetic and premium market appeal, especially in export-oriented aquaculture. Their distinct red pigmentation as shown in Figure 2.3 (iii) resembles that of higher- value marine species like red snapper, making them particularly attractive in both domestic and international markets (UPM, 2023). This visual appeal contributes to higher consumer demand and market prices compared to conventional tilapia strains. Furthermore, ongoing selective breeding programs, such as Malaysia's Putra Red Premium initiative, have focused on improving key traits including flesh quality, coloration consistency, and growth performance, enhancing the overall value chain for this species (Abdullah et al., 2021). As a result, red hybrid tilapia emerged as a strategic species for sustainable aquaculture development in Malaysia and the broader Southeast Asian region, supporting national food security goals and boosting rural livelihoods through high-value fish production compare to other tilapia fish species.

Red hybrid tilapia was selected as the model species for this study due to its substantial economic importance and biological suitability for aquaculture research. In Malaysian aquaculture, this species is among the most widely cultivated freshwater fish due to its rapid growth rate, high adaptability, and strong tolerance to handling and environmental stress compared to other cultured species (FAO, 2023). In addition, the global demand for red hybrid tilapia continues to increase, reflecting its significant commercial value and contribution to food security (Ashouri et al., 2023). These characteristics make red hybrid tilapia an ideal model organism for controlled feeding trials and nutritional intervention studies. Furthermore, red hybrid tilapia is highly susceptible to bacterial pathogens that cause frequent disease outbreaks in intensive aquaculture systems, particularly *Streptococcus agalactiae* and *Aeromonas hydrophila*. This susceptibility provides a strong justification for selecting this species to evaluate the effectiveness of dietary probiotic supplementation in enhancing disease resistance under controlled laboratory conditions. In this study, probiotic feeding was initiated at an early developmental stage following a seven-day acclimatization period after hatchery stocking. A feeding duration of 14 weeks was selected, as this period corresponds to the typical grow-out phase required for red hybrid tilapia to reach marketable size under tropical culture conditions, allowing sufficient time to evaluate probiotic effects on growth performance, physiological development, and resilience to pathogenic challenges (Nyinondi et al., 2022)

2.3 *Streptococcus agalactiae* and *Aeromonas hydrophila* Outbreaks in Aquaculture

Streptococcus agalactiae and *A. hydrophila* are two of the common bacterial pathogens causing large illness outbreaks in aquaculture, notably among tilapia species. *S. agalactiae* is a Gram- positive bacterium that causes streptococcosis in fish, a systemic bacterial infection that causes severe clinical symptoms such as meningoencephalitis, septicaemia, and internal organ destruction (Delannoy et al., 2021). The infection cause by the pathogens may spread horizontally through water and vertically through broodstock, making it extremely infectious in aquaculture settings. *A. hydrophila*, on the other hand, is a Gram- negative bacteria that causes motile Aeromonas septicaemia (MAS), which is characterised by hemorrhagic septicaemia, skin ulcers, fin rot, and high death rates, especially in stressed or immunocompromised fish (Raji et al., 2023).

Bacteria and viruses are the major causes of Malaysia's outbreaks of infectious diseases in the aquaculture business. The most prevalent bacterial infections affecting farmed fish are *Streptococcus agalactiae*, *Streptococcus iniae*, *Aeromonas hydrophila*, and *Vibrio alginolyticus*. Streptococcosis is the most concerning fish disease among fish farmers in aquaculture due to the aggressive sequence of outbreaks impacting tilapia culture that began in 1997. The first reported incidence of streptococcosis in Malaysia occurred in the late 1990s at Pahang River, Pahang, resulted in 60% mortality of cultivated red hybrid tilapia (Zamri-Saad et al., 2014). In 2000, a series of illness outbreaks were reported in Kenyir Lake, Terengganu, and Pergau Lake, Kelantan, killing nearly 50% of farmed tilapia (Najiah et al., 2012). Repeated occurrences were seen in the Aquaculture Industrial Zone at Kenyir Lake and Temerloh, Pahang, resulting in huge death of cage-cultured red hybrid tilapia (Laith et al., 2017). Global tilapia output is mostly impacted by the Gram-positive bacteria *S. agalactiae* (Maulu *et al.*, 2021).

In recent years, *A. hydrophila* has emerged as a significant threat to tilapia aquaculture in Malaysia, leading to substantial economic losses among fish farmers. One notable outbreak occurred in 2020, where multiple farms across Selangor and Perak reported sudden mass mortalities among adult red hybrid tilapia (Basri et al., 2020). Key virulence factors of *A. hydrophila* include aerolysin and haemolysin, which contribute to its ability to cause disease (Yadav et al., 2022). Infected fish may experience metabolic disruptions, cardiac dysfunction, and tissue damage (Ahmed et al., 2023). Environmental factors such as temperature, pH, and ammonia levels can accelerate bacterial growth and increase its pathogenic potential (Singh et al., 2023). Furthermore, *A. hydrophila* is known for its resistance to multiple antibiotics, including oxacillin and amoxicillin, complicating treatment efforts (Nair et al., 2021). The disease caused by *A. hydrophila*, known as motile Aeromonas septicaemia (MAS), can affect several organs in the fish. External symptoms of infection may include exophthalmia (popeye), abdominal distension (swelling), and pale gills (Anjur et al. 2021). Scaled fish often exhibit oedema (fluid retention) in the scale pockets, resulting in a roughened or bristling appearance, a condition referred to as lepidorthosis (Khan et al., 2022).

In January 2020, a significant epidemic occurred in a tilapia farm in Selangor, Malaysia, killing 70% of adult red hybrid tilapia. Tilapia Lake Virus (TiLV), *A. hydrophila*, and *S. agalactiae* were detected using bacterial isolation, polymerase chain reaction (PCR), and sequencing study (Mohamad et al., 2022). This instance demonstrated the disastrous effects of co-infections in aquaculture systems, emphasising the necessity for strict disease management techniques. Streptococcosis continues to be one of the most common bacterial infections in tilapia aquaculture worldwide. In Taiwan, clinical research revealed that bacterial infections dominated tilapia illness cases, with streptococcosis accounting for roughly 53.7% of all diagnoses and a documented infection rate of 29.5% (Chang et al., 2021). Infected tilapia generally displays prominent clinical symptoms such as aberrant swimming patterns (e.g., spiralling or lethargy), skin ulcerations, haemorrhages, and exophthalmia (protruding eyes), all of which are signs of severe systemic infections as shown in Figure 2.4 (i) (Dong et al., 2020; Raji et al., 2023).

Infections with *S. agalactiae* and *A. hydrophila* can cause significant histopathological damage in red hybrid tilapia, leading to severe impacts on fish health and survival. *S. agalactiae* infection is often associated with meningitis, characterized by inflammation, neuronal degeneration, and perivascular cuffing in the brain, which correlate with neurological symptoms such as erratic swimming and lethargy (Abdallah et al., 2024). Liver tissues typically display multifocal necrosis, congestion, and mononuclear cell infiltration (Dong et al., 2020). Meanwhile, *A. hydrophila* infection leads to pronounced hemorrhages, hepatocellular degeneration, renal tubular necrosis, and splenic lymphoid depletion, indicating systemic infection and septicemia as shown in Figure 2.4 (ii) (Mohamad et al., 2022). In co- infection scenarios, tilapia experience more exacerbated lesions with compounded tissue destruction, elevated mortality rates, and accelerated disease progression (Raji et al., 2023). Table 2.1 shows the comparison between the clinical signs and symptoms of naturally *S. agalactiae* and *A. hydrophila* infection.

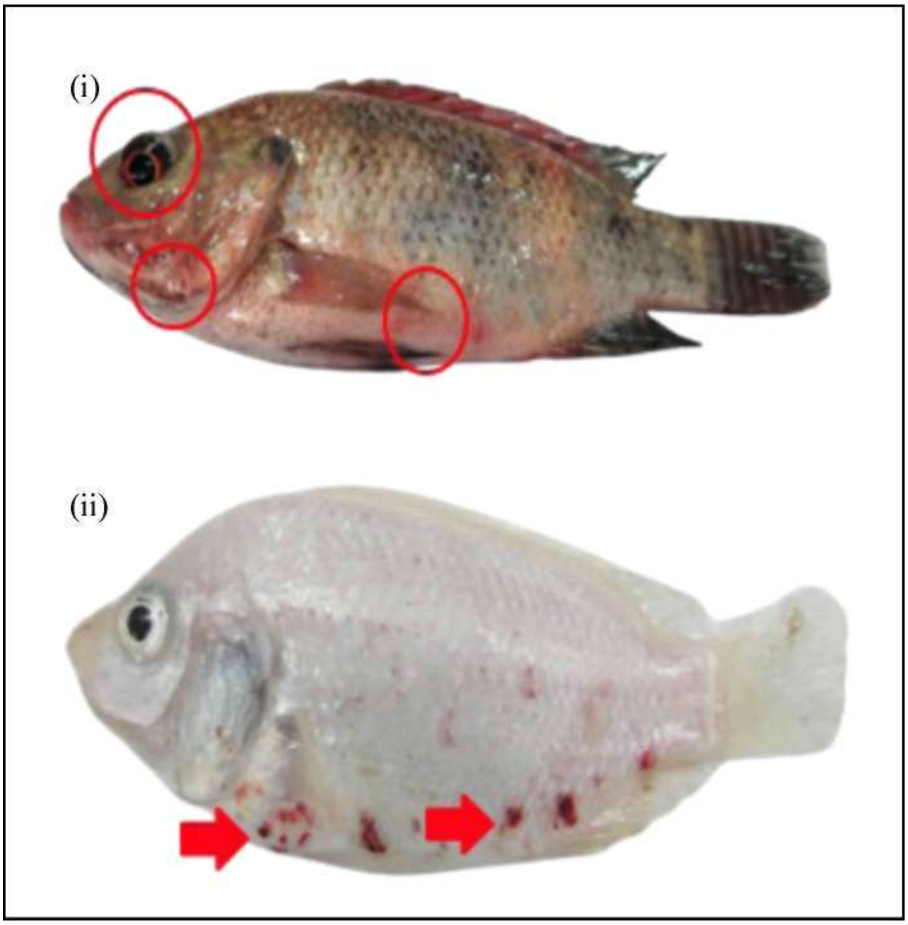
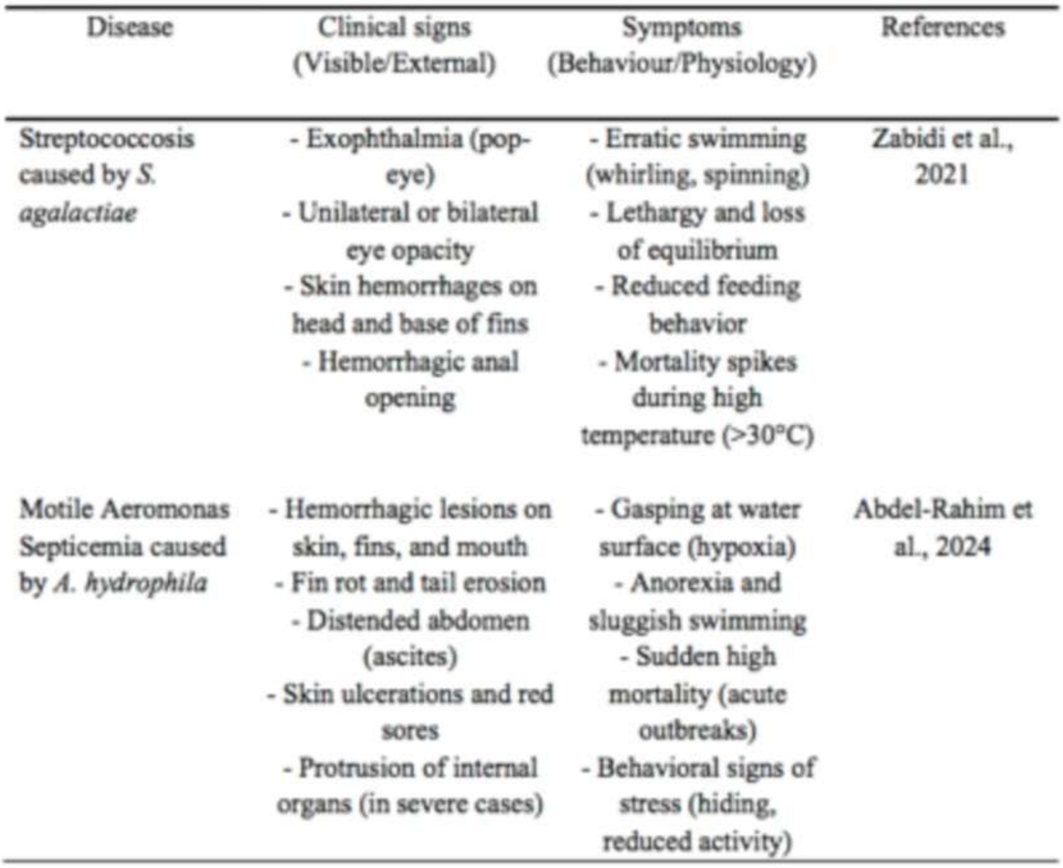


Figure 2.4 (i) Nile Tilapia Exhibit Septicaemia (red circle) Following *S. agalactiae* Infection (Dong et al., 2020), (ii) Red Hybrid Tilapia Showing Severe Haemorrhages (Red Arrow) on The Abdomen following Infection with A *hydrophila* (Mohamad et al., 2022).

Table 2.1
Clinical Signs and Symptoms of Fish Infected with Streptococcosis and Motile Aeromonas Septicemia



Disease	Clinical signs (Visible/External)	Symptoms (Behaviour/Physiology)	References
Streptococcosis caused by *S. agalactiae*	- Exophthalmia (pop-eye) - Unilateral or bilateral eye opacity - Skin hemorrhages on head and base of fins - Hemorrhagic anal opening	- Erratic swimming (whirling, spinning) - Lethargy and loss of equilibrium - Reduced feeding behavior - Mortality spikes during high temperature (>30°C)	Zabidi et al., 2021
Motile Aeromonas Septicemia caused by *A. hydrophila*	- Hemorrhagic lesions on skin, fins, and mouth - Fin rot and tail erosion - Distended abdomen (ascites) - Skin ulcerations and red sores - Protrusion of internal organs (in severe cases)	- Gasping at water surface (hypoxia) - Anorexia and sluggish swimming - Sudden high mortality (acute outbreaks) - Behavioral signs of stress (hiding, reduced activity)	Abdel-Rahim et al., 2024

Given the high prevalence and severe impacts of *S. agalactiae* and *A. hydrophila* on tilapia health and aquaculture productivity, effective disease management strategies are critically required. In current aquaculture practices, the primary approach for controlling bacterial infections relies heavily on the use of antibiotics, which are commonly administered through medicated feed or immersion treatments to reduce mortality and disease spread. Antibiotics such as oxytetracycline, florfenicol, enrofloxacin, and amoxicillin are frequently used to treat streptococcosis and motile Aeromonas septicaemia in cultured fish. However, the extensive and often uncontrolled use of antibiotics has raised growing concerns regarding antimicrobial resistance, environmental contamination, and food safety risks. Therefore, understanding the role and limitations of antibiotics in aquaculture is essential before exploring alternative disease mitigation strategies.

2.4 Antibiotics Used in Aquaculture

The introduction of antibiotics into aquaculture began in the mid-20th century as a revolutionary method to combat infectious diseases and enhance fish survival rates, especially as the industry shifted toward intensive farming practices (Okeke et al., 2022). It is commonly used prophylactically to prevent bacterial infections in fish populations, particularly in high- density farming environments. In addition to direct treatment, antibiotics are often employed to manage bacterial populations in the water, especially in recirculating aquaculture systems (RAS), where maintaining water quality is critical to prevent disease transmission (Kraemer et al., 2019). Common aquaculture antibiotics such as amoxicillin and sulphadiazine- trimethoprim, are critical in managing bacterial infections in fish populations. Amoxicillin, a β-lactam antibiotic, inhibits bacterial cell wall formation, resulting in cell lysis and death. It is commonly used to treat Gram-positive pathogens including *S. agalactiae* and *S. iniae* infections in tilapia aquaculture (Okeke et al., 2022). Meanwhile, sulphadiazine- trimethoprim is a synergistic combination of two antibiotics that inhibit folic acid production in bacteria, making it an effective treatment for systemic bacterial infections and septicaemia caused by species such as *A. hydrophila* (Lulijwa et al., 2020).

Resistant strains of *Vibrio*, *Aeromonas*, and *Pseudomonas* have been isolated from aquaculture environments, complicating disease treatment and threatening food security. In Malaysia, multiple studies have reported the presence of multidrug-resistant bacteria in farmed fish, shrimp, and aquaculture water systems (Tan et al., 2021). For example, resistant *Vibrio* spp. have been frequently detected in Malaysian shrimp farms, showing resistance to commonly used antibiotics such as tetracycline, oxytetracycline, and erythromycin (Mohamad et al., 2019). Most Southeast Asian nations lack sufficient laws, regulatory oversight, and monitoring mechanisms for the use of antibiotics in aquaculture (Chuah et al., 2016). Although antibiotics serve an important role in controlling infectious diseases and maintaining fish health, their overuse or misuse presents significant risks.

The most concerning of these risks is the development of antimicrobial resistance (AMR), which occurs when pathogens adapt to the effects of antibiotics, rendering them less effective or ineffective in future treatments (Okeke et al. 2022). Furthermore, the AMR from aquaculture systems can spread to humans via contaminated food, water, or direct exposure, posing serious public health risks

(Chowdhury et al., 2018). Consequently, while antibiotics are crucial for disease management in aquaculture, their use must be carefully regulated to minimize negative impacts on both fish health and the environment. The survival of antibiotics in aquatic environments further exacerbates the situation, as these compounds continue to affect microbial communities and may lead to the emergence of multi-resistant pathogens (Larsson and Flach, 2022).

Additionally, the accumulation of antibiotic residues in aquatic ecosystems disrupts the natural microbial balance, potentially harming biodiversity and the overall health of marine life (Chowdhury et al., 2018). These residues can have far-reaching consequences, affecting wild aquatic organisms and potentially entering the food chain, which ultimately poses risks to human health through seafood consumption or contaminated water supplies. Although Malaysia has banned the use of nitrofurans and chloramphenicol in aquaculture production, these antibiotic residues continue to be detected in Malaysian seafood. The US Food and Drug Administration (FDA) reported 44 instances of such residues between 2009 and 2018, highlighting ongoing concerns regarding antibiotic contamination (Food and Drug Administration, 2018). In conclusion, antibiotic treatment in aquaculture is not a reliable or safe method for disease prevention, as the rise of antibiotic-resistant bacteria in aquaculture ecosystems presents a significant threat to public health. These resistant pathogens can contaminate seafood or expose people to polluted water, underscoring the urgent need for better regulatory practices and alternative disease management strategies (Pepi and Focardi, 2021).

Therefore, while antibiotics remain widely used in aquaculture, their limitations have driven increasing interest in alternative disease control strategies, including vaccination and probiotic supplementation. Probiotics work by enhancing gut flora, boosting immune responses, and competitively excluding pathogens, unlike antibiotics that kill both harmful and beneficial bacteria, resulting in microbial imbalance and resistance (Mohammed et al., 2022). Plus, the probiotics boost feed utilisation, growth, and disease resistance without increasing antibiotic resistance or leaving hazardous residues in the environment (Newaj-Fyzul and Austin, 2015). Furthermore, probiotics give long-term advantages by modifying the host immunity and increasing water quality through microbial balance, which antibiotics are not capable of doing (Mohammed et al., 2025). Thus, the use of probiotics is consistent with sustainable aquaculture practices and worldwide initiatives to prevent antibiotic resistance in food production systems.

Lastly, probiotics provide long-term benefits by modulating immunity and improving water quality through the maintenance of a balanced microbial ecosystem which effects that antibiotics cannot replicate. As such, probiotics align with sustainable aquaculture practices and global initiatives aimed at reducing the reliance on antibiotics in food production systems, helping to mitigate the growing threat of antibiotic resistance (Saba et al., 2024).

In response to the growing concerns associated with antibiotic use, including antimicrobial resistance and environmental contamination, vaccination has emerged as an alternative disease prevention strategy in aquaculture. Unlike antibiotics, which are typically applied after disease onset, vaccines function by stimulating the host immune system to provide long-term protection against specific pathogens. Fish vaccination has been increasingly adopted for the control of major bacterial diseases such as streptococcosis and aeromoniasis, particularly in intensive aquaculture systems. Therefore, the application of fish vaccines represents an important biological approach for disease management and warrants further discussion in the context of sustainable aquaculture practices.

2.5 Fish Vaccine Use in Aquaculture

Vaccine use in aquaculture has become more important for disease prevention and control in farmed fish, providing a more environmentally friendly alternative to antibiotics. Over the last few decades, the prevalence of new and re-emerging infectious illnesses has increased across a wide variety of aquaculture species, resulting in large global economic losses (Muktar et al. 2016). Fish vaccines have been produced and commercialised over the world to battle key diseases such as Vibrio spp., Aeromonas spp., Streptococcus spp., and viruses including Infectious Salmon Anaemia Virus (ISAV) and Viral Nervous Necrosis (VNN) (Somvanshi et al., 2022). Norway, Chile, and Canada are among the top countries in vaccine research and implementation, notably in salmon farming, where immunisation programs have significantly decreased antibiotic usage while improving fish health (Brudeseth et al., 2013).

Despite notable advancements in aquatic vaccine development worldwide, Malaysia continues to face significant challenges in this area. Although substantial investments have been made by local researchers in aquatic vaccine research, no locally developed fish vaccines have yet reached commercialization (Ridzuan et al. 2022). Currently, Malaysia has approved only four aquatic vaccines for commercial use such as AquaVac® Strep SI, AquaVac® IridoV, and VibriFishvax®, AquaVac® Strep SA1 targeting streptococcosis, iridovirus infections, and vibriosis, respectively (Department of Veterinary Services Malaysia, 2025). The reliance on imported vaccines not only raises production costs but may also result in suboptimal protection against region-specific pathogen strains. Consequently, the demand for affordable, locally tailored vaccines remains high to improve disease management and strengthen the resilience of Malaysia’s aquaculture industry (Barnes et al., 2022). Recent global trends emphasize the urgent need for the development of polyvalent and oral vaccines to make immunization more practical and cost-effective for mass aquaculture operations (Zhao et al., 2023).

Vaccination is widely recognized as one of the most effective method for controlling infectious diseases in aquaculture, primarily by promoting herd immunity within farmed fish populations (Ma et al., 2019). However, the route of vaccine administration plays a crucial role in determining its overall success. Injectable vaccines, though commonly employed, can induce considerable physical and psychological stress in fish, sometimes resulting in mortality and diminishing the intended immunological response (Brudeseth et al., 2013). Conversely, oral vaccines offer a less stressful alternative but face major limitations, as the harsh acidic conditions of the gastrointestinal tract often degrade vaccine antigens before they can elicit a sufficient immune response (Somvanshi et al., 2022). In this context, current vaccine research in Malaysia primarily targets major bacterial diseases such as motile Aeromonas septicemia, vibriosis, and streptococcosis (Ridzuan et al., 2022). Advancing more effective and accessible vaccination strategies is crucial for supporting the sustainable growth of the Malaysian aquaculture sector.

Fish vaccines are frequently regarded as expensive due to their complicated, labour- intensive, and time-consuming production methods (Du et al., 2022). The regulatory framework controlling aquaculture vaccines has also grown more demanding, with strict adherence to Good Manufacturing Practices (GMP) identical to those required for vaccines intended for terrestrial animals (Martins et al., 2022). Although the aquaculture vaccine market is tiny, the cost of satisfying GMP requirements is equivalent to that of human and animal vaccines, which adds considerably to the entire cost. Furthermore, the small market size limits economies of scale in vaccine development, with just around 30 commercial fish vaccines accessible worldwide, as opposed to over 760 vaccines for human illnesses (Ma et al., 2021). As a result, fish vaccinations are frequently more expensive than alternative disease control measures, such as probiotic supplements, which are widely regarded as more cost-effective and accessible to fish farmers, particularly in low- and middle-income countries.

Despite its proven benefits, the practical application of vaccination in aquaculture is often constrained by complex regulatory requirements, labour-intensive administration methods, and strain-specific efficacy. These limitations are particularly evident in developing aquaculture sectors such as Malaysia, where access to affordable and locally produced vaccines remains limited. Consequently, increasing attention has been directed toward alternative and complementary disease management strategies that are more cost-effective, practical, and environmentally sustainable. Among these, probiotics have emerged as a promising functional feed-based approach for enhancing fish health, improving immune responses, and reducing disease susceptibility. Therefore, probiotic application in aquaculture warrants further discussion as a potential strategy for sustainable disease mitigation and productivity enhancement.

2.6 Probiotic Application in Aquaculture

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host. The term "probiotic" is derived from the Greek word "biotic," which means "bios" or "life," and the Latin preposition "pro," which means "for," (Saha et al. 2022). Lactic acid bacteria (LAB) are probiotics that are commonly employed in food industry to provide numerous benefits for the user. *Lactobacillus*, *Streptococcu*s, and *Enterococcus* are the common lactic acid bacteria that are well known as having a number of health benefits for both humans and animals (Gopal and Dhanasekaran, 2021). In aquaculture, probiotics can be introduced into the rearing culture system through different methods and pathway including via water additions, feed additives, or injection to optimize their colonization and functionality within the host (Muhammed et al., 2025). The direct addition of probiotic into the rearing water allows immediate interaction with the fish's skin and gills, however the incorporation into formulated feeds or via dietary feedings are the common method that being applied in today aquaculture activity. This method may allow consistent ingestion and delivery along the gastrointestinal tract where the probiotic can exert its beneficial effect to the host efficiently (Afonso et al., 2021). In addition, probiotic inclusion via feeding also becoming the most broadly utilized in this industry because of its simplicity, cost-effectiveness, and proven efficiency in facilitating the establishment of beneficial microbes in the intestinal microbiota of fish (Tachibana *et al.,* 2020). Table 2.1 summarized the potential benefits of probiotic supplementation to the fish in aquaculture.

Table 2.2

Benefits of Dietary Probiotic Supplementation in Fish Aquaculture

Aspect	Benefits	Examples	Previous study	References
Growth performance	Improves feed conversion ratio and promotes faster weight gain due to enhanced nutrient absorption	Increased growth rate in tilapia fed with *L. plantarum* for a better protein utilization	Nile tilapia fed *L. plantarum* showed improved specific growth rate (~3.12 % day ^{1}*)* and FCR (~1.23) with microencapsulated probiotics	Bahrami et al., 2023
Survival rate	Enhances survival under stress and during pathogen exposure by improving immune response	Higher post-challenge survival in *Oreochromis* spp. against pathogens.	MLCA-fed tilapia had highest survival 83 % survival against *S. agalactiae*	Bahrami et al., 2023
Gut microbiota modulation	Promotes beneficial microflora and inhibits Pathogenic bacteria in the gut.	Increased lactic acid bacteria counts and lower pathogen load in gut	Probiotics reshape microbiome in tilapia causing reduction of *Proteobacteria* in gut after probiotic treatment	Madhulika et al., 2025
Digestive enzyme activity	Enhances secretion of enzymes for a better digestion and nutrient	Higher intestinal enzyme activity in probiotic-treated fish	Probiotic produce digestive enzymes and vitamins, improving feed utilization	Madhulika et al., 2025

Water quality improvement	Reduces organic waste by improving feed utilization and reducing ammonia excretion.	Lower ammonia and nitrite levels in probiotic-fed tanks	Mixed *Bacillus* probiotics decreased nitrogenous waste in pond systems	Zabidi et al., 2021
tolerance	Improves tolerance to environmental stressors (temperature, salinity, handling).	Higher survival during salinity stress in red tilapia	Cortisol levels were significantly lower in the probiotic-fed group (3.6 ± 0.36 ng/g) compared to controls (5.1 ± 0.47 ng/g).	Zabidi et al., 2021
Environment sustainability	antibiotic use, reducing risk of resistance and residues; supports eco-friendly production	Provide more safe and environmental- friendly approach compared to antibiotics	Probiotics provide disease control without antibiotics therefore promote sustainable aquaculture practices	Sarmah and Sarma, 2025

The enhancement of fish health through probiotic supplementation provides far reaching benefits that extend beyond individual production systems. This improvement not only elevates farm level productivity and profitability but also reinforces the resilience and sustainability of the aquaculture sector, thereby contributing to national food security and supporting long term economic development (Zorriehzahra et al. 2022). The integration of these probiotic strategies into aquaculture practices aligns with the global shift toward more sustainable and eco-conscious aiming to minimize the use of antibiotics, improve disease resistance, and enhance overall farm productivity and environmental stewardship (Mohammed et al. 2025). The most important part is the probiotic supplementation is a comparatively low- cost strategy when compared to antibiotics and vaccines, requiring minimal infrastructure changes and reducing the long-term expenses associated with disease management and water treatment. For instance, *Bacillus* probiotics have been shown to deliver improved growth and immune responses at minimal input cost, helping to offset the investment in probiotic application and reduce reliance on expensive antibiotics and disease treatments (Calcagnile et al., 2024). These economic benefits, coupled with increasing market demand for fish products labeled as “antibiotic free” or “naturally produced,” reinforce probiotics as a financially viable solution for small scale and commercial farmers alike.

Despite providing the aquaculture sector with a low-cost method in dealing with disease, probiotic supplementation considered as a cost-effective method compare to vaccine and antibiotic. This is because, the promising efficacy of probiotics arises from their remarkable ability to withstand harsh environmental fluctuations, including variations in pH, bile salts, and salinity encountered both in the rearing water and within the gastrointestinal tract of the host fish (Zhou et al., 2020). This resilience exceeds that of antigens employed in traditional fish vaccines, which often require strict handling conditions and may elicit variable immune responses (Cowan et al. 2016). Plus, the ability of the antigens to stay viable along feeding pathway might be challenged by the harsh environment in the fish gut. By surviving these challenging conditions, probiotics are able to colonize the intestinal mucosa, where they confer multifaceted benefits such as enhancing nutrient assimilation, modulating immune responses, and ultimately reducing the need for antibiotic interventions (Mohapatra et al., 2019). Such attributes position probiotics as a cornerstone in advancing sustainable and bio- secure aquaculture practices.

Moreover, probiotics are environmentally safe and sustainable, posing minimal risk to aquatic ecosystems. Therefore, it become highly demanded to be fully utilized in culturing aquatic organism nowadays by fish farmers. In recent years, numerous fish and shrimp hatcheries across Malaysia that have begun implementing early-stage probiotic supplementation immediately after stocking (Zabidi et al. 2021). Unlike antibiotics, probiotic strains do not disrupt native microbial communities, accumulate in tissues, or promote antimicrobial resistance (Hasan et al. 2023). The emergence of antibiotic resistance from antibiotic treatment also urged this alternative for a safer culturing practice. Many probiotic microorganisms including *Lactobacillus*, *Bacillus*, and *Saccharomyces* spp. can reduce organic waste, nitrogenous compounds, and pathogen load in rearing systems, thus improving water quality and ecological balance. As a result, probiotic use aligns with the goals of sustainable aquaculture by reducing chemical inputs, enhancing biosecurity, and supporting long term fish production with minimal environmental impact (Turlybek et al. 2025).

2.6.1 *Lactiplantibacillusplantarum* and *Lacticaseibacillusparacasei*

In aquaculture, the commercial probiotic supplements usually used by the small-scale and large-scale fish farmers may consist of single or multiple strains of probiotics. The commercial probiotics are easy to purchase by farmers, and they are commonly sold in powders, liquid solutions, capsules, granules, and tablets. Liquid and powder forms are the most common usage by fish farmers in hatcheries across Malaysia due to the flexibility and effectiveness for large-scale use. However, the selection in choosing the right probiotic strain before applied into the aquaculture system is very important since different probiotic strains have varying effectiveness in response to environmental factors such as fish species itself, the fish's gut conditions and aquatic environments (Hancz, 2022). Figure 2.5 shows the probiotic strains with its distinct characteristics and function which commonly applied as dietary supplementation in aquaculture.

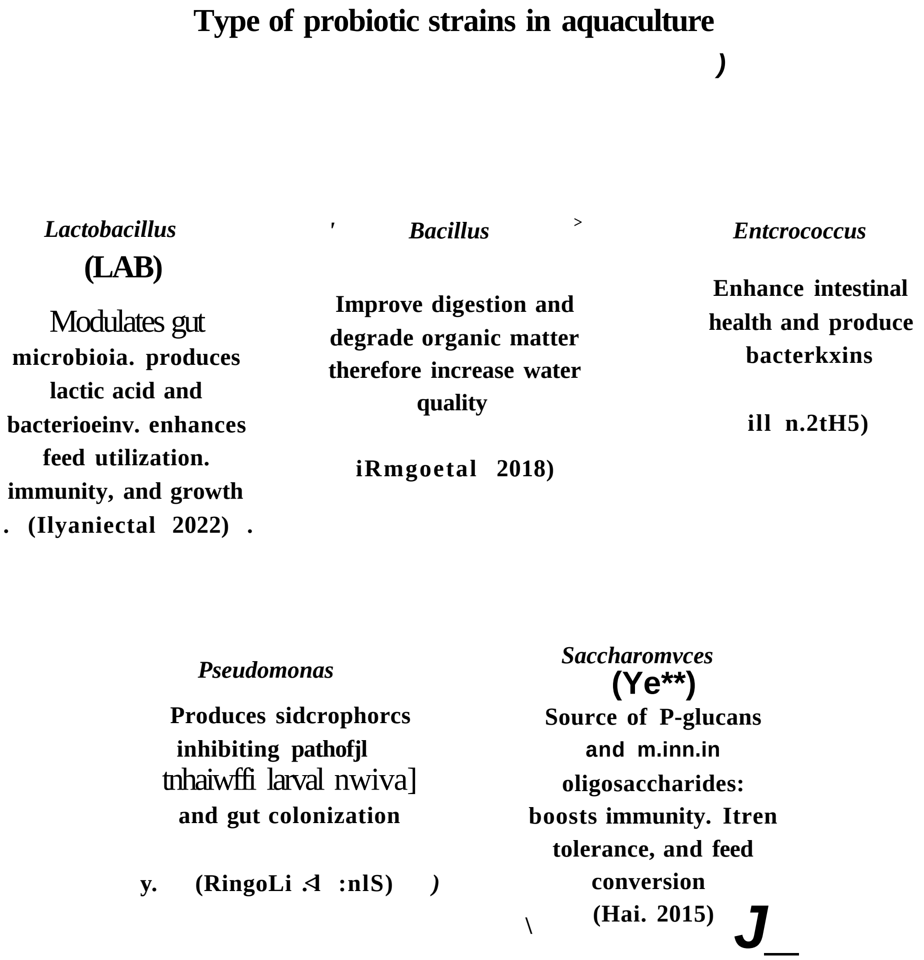


Figure 2.5 Type of Probiotic Strains Applied as Treatment in Aquaculture Industry

Both *L. plantarum* and *L. paracasei* belong to the group of lactic acid bacteria (LAB), a diverse class of Gram-positive, rod-shaped, catalase-negative, and non- spore-forming bacteria known for producing lactic acid through carbohydrate fermentation (Latif et al., 2023). These species are known as facultative anaerobes, allowing them to thrive in both oxygen-rich and oxygen-deprived environments, which is critical for survival in fluctuating aquaculture systems (Dempsey and Corr, 2022). This adaptability is especially advantageous in aquaculture environments, where oxygen levels may fluctuate. Based on Figure 2.5, LAB represent a type of probiotic groups that is extensively studied in aquaculture due to its physiological flexibility which enhances their survivability and functional stability in diverse aquatic conditions, making them as a promising candidate for sustainable probiotic applications in aquaculture (Dempsey and Corr, 2022).

Despite the multiple beneficial properties attributed by probiotic, the efficacy in aquaculture is not universally consistent across all studies. Recent reviews have emphasized that the predictability of probiotic effects can vary depending on strain characteristics, host species, environmental conditions, and administration protocols (Elsegeny, 2025; Rahayu, 2024). For example, inconsistent results have been observed in growth performance and immune responses, with some trials reporting minimal or non-significant improvements under certain salinity regimes or feeding strategies, suggesting that dose, duration, and host compatibility should be carefully optimized (Elsegeny, 2025). Additionally, the absence of standardized dosing strategies and species-specific response data remains a major challenge for commercial probiotic adoption in aquaculture systems, which may partly explain why some studies report variable outcomes (Elsegeny, 2025). These discrepancies underscore the importance of tailoring probiotic selection and application according to strain origin, host factors, and environmental parameters rather than assuming universal efficacy based solely on genus or species identity. Therefore, in this study, both local isolates, *L. plantarum* BE7 and *L. paracasei* BUM6, and their delivery dosages were evaluated through in vitro assessments under harsh conditions reflecting the actual stresses of the gastrointestinal and aquaculture environments. A comparative summary of strain-specific probiotic performance and dosage ranges reported in recent aquaculture studies is presented in Table 2.2.

Table 2.3

Strain-Specific Probiotic Effects and Dosage Ranges in Aquaculture

Probiotic strain	Host species	Dosage range (CFU)	Administration	Key outcomes	Key reference
Lactobacillus plantarum	Nile tilapia	10^7–10^9 CFU/g feed	Oral	Improved growth rate, immune response, disease resistance	Rahayu et al., 2024
L. plantarum (host-derived)	Hybrid grouper	10^8 CFU/g feed	Oral	Higher immune and growth responses vs non-host strains	Han et al., 2024
Lactobacillus paracasei	Common carp	10^7–10^9 CFU/g feed	Oral	Enhanced gut microbiota and immune gene expression	Elsegeny, 2025
Bacillus subtilis	Goldfish	10^6-10^{10} CFU/mL	Water/Feed	Optimal performance at 10^8 CFU	Du et al., 2022
Bacillus amyloliquefaciens	White shrimp	10^7–10^9 CFU/g feed	Oral	Higher survival and resistance to Vibrio	Turlybek et al., 2025
L. plantarum + B. subtilis	Nile tilapia	10^8 CFU/g feed	Oral	Synergistic immune and feed efficiency effects	Hasan et al., 2023

Lactic acid bacteria are widely distributed in nature and are commonly associated with carbohydrate rich environments where fermentation occurs. They are frequently isolated from a variety of fermented foods and beverages, including dairy products such as yogurt and cheese, plant based products like sauerkraut and kimchi, and fermented fish products such as budu and pekasam, which are widely consumed in Malaysia. In aquatic environments, LAB is naturally present in the gastrointestinal tract of freshwater and marine fish, where they contribute to nutrient digestion and immune modulation (Giri et al., 2019). They are also found in biofilms, sediments, and the surfaces of aquatic plants within aquaculture ponds (Lulijwa et al., 2022). LAB represent a promising group of probiotics for aquaculture, offering a wide range of beneficial properties that may not be as pronounced in other probiotic strains. This type of strain also known for its potential in enhancing gut microbiota diversity and improving nutrient absorption, leading to better overall health and growth performance in aquatic organisms.

The success of probiotic-based disease prevention strategies in aquaculture is largely governed by strain-specific characteristics that determine their functional performance in the host. Although *Bacillus* spp. are widely used in commercial probiotic formulations due to their spore-forming ability, which provides high resistance to processing, storage, and delivery conditions, LAB are generally considered more effective for pathogen control as they can actively colonize the gastrointestinal tract and exert antagonistic effects through competitive exclusion and antimicrobial compound production (Cutting, 2011). As an example, *L. plantarum* produces bacteriocins, hydrogen peroxide, and organic acids such as lactic acid, which lower the pH and inhibit the growth of a variety of fish pathogens, including *A. hydrophila* and *S. agalactiae*. Recent studies have also highlighted its potential to modulate host immune responses by stimulating innate immunity and promoting the expression of immune-related genes, further reinforcing its role as a functional probiotic candidate for disease prevention in aquaculture (Awad et al., 2025). *L. paracasei* demonstrates strong adhesion capability to intestinal mucus, high bile and acid tolerance, and immunomodulatory effects, particularly the stimulation of pro-inflammatory cytokines that enhance fish immune response (Latif et al., 2023). This strain is known for its ability to adhere to the intestinal epithelial cells, promoting a healthier gut environment and helping in the prevention of pathogen colonization.

In comparing stress tolerance traits relevant to red hybrid tilapia culture, LAB strains such as *L. plantarum* BE7 and *L. paracasei* BUM6 demonstrate quantifiably superior resilience to key environmental stressors associated with gastrointestinal transit and aquaculture environment conditions. For instance, many LAB strains isolated from aquatic environments have shown >70–90% viability at pH 3–4 for 2–3 hours in vitro, whereas several commercial probiotic preparations containing non-LAB strains including *Bacillus* spp. and generic *Lactobacillus* formulations often exhibit sharply reduced viability <50% survival under similar acidic conditions (Li et al., 2024; Hasan et al., 2025). In terms of bile salt tolerance, robust aquatic LAB isolates typically maintain >60–80% survival at 0.5–1.0% bile concentrations, levels approximating those encountered in fish intestines, while some commercial LAB products demonstrate only moderate tolerance at lower bile levels 0.3% and suffer viability loss as concentrations increase (Rahayu, 2024; Li et al., 2024). Regarding salinity tolerance, LAB strains sustained >50–80% survival even at 2–3% NaCl, a range consistent with variable aquaculture environments, in contrast to many terrestrial commercial probiotics that show steep viability declines beyond ~1–1.5% NaCl (Han, 2025; Che et al., 2024). Overall, these in vitro findings for *L. plantarum* BE7 and *L. paracasei* BUM6 demonstrate promising strain-specific traits that support their potential suitability for probiotic application as dietary supplements in cultured red hybrid tilapia.

2.7 Chapter Summarization

It is well known that applying probiotics, especially lactic acid bacteria, can boost immune responses, increase growth performance, and lessen disease outbreaks in aquaculture. Despite being isolated from fermented aquatic products that are readily available in the area and exhibiting potential probiotic characteristics, there is still lack of research especially focused on *L. plantarum* BE7 and *L. paracasei* BUM6. Given the significant economic worth of red hybrid tilapia farming in Malaysia and the ongoing difficulties caused by bacterial pathogens like *S. agalactiae* and *A. hydrophila*, which are significant causes of high mortality and monetary losses in intensive culture systems, this disparity is crucial. Furthermore, developing sustainable and economical alternatives is necessary due to the rising worry over antibiotic resistance and the high price of vaccines. Given these considerations, the current study was conducted to determine the probiotic strains' ability to survive in gastrointestinal stress situations, their impact on the growth and survival of red hybrid tilapia, and their effectiveness in protecting against *S. agalactiae* and *A. hydrophila*. The next chapter reviews the methodological parts that consist in vitro screening of probiotic strains candidates, *L. plantarum* BE7 and *L. paracasei* BUM6 and in vivo application to the red hybrid tilapia.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research Flow Chart

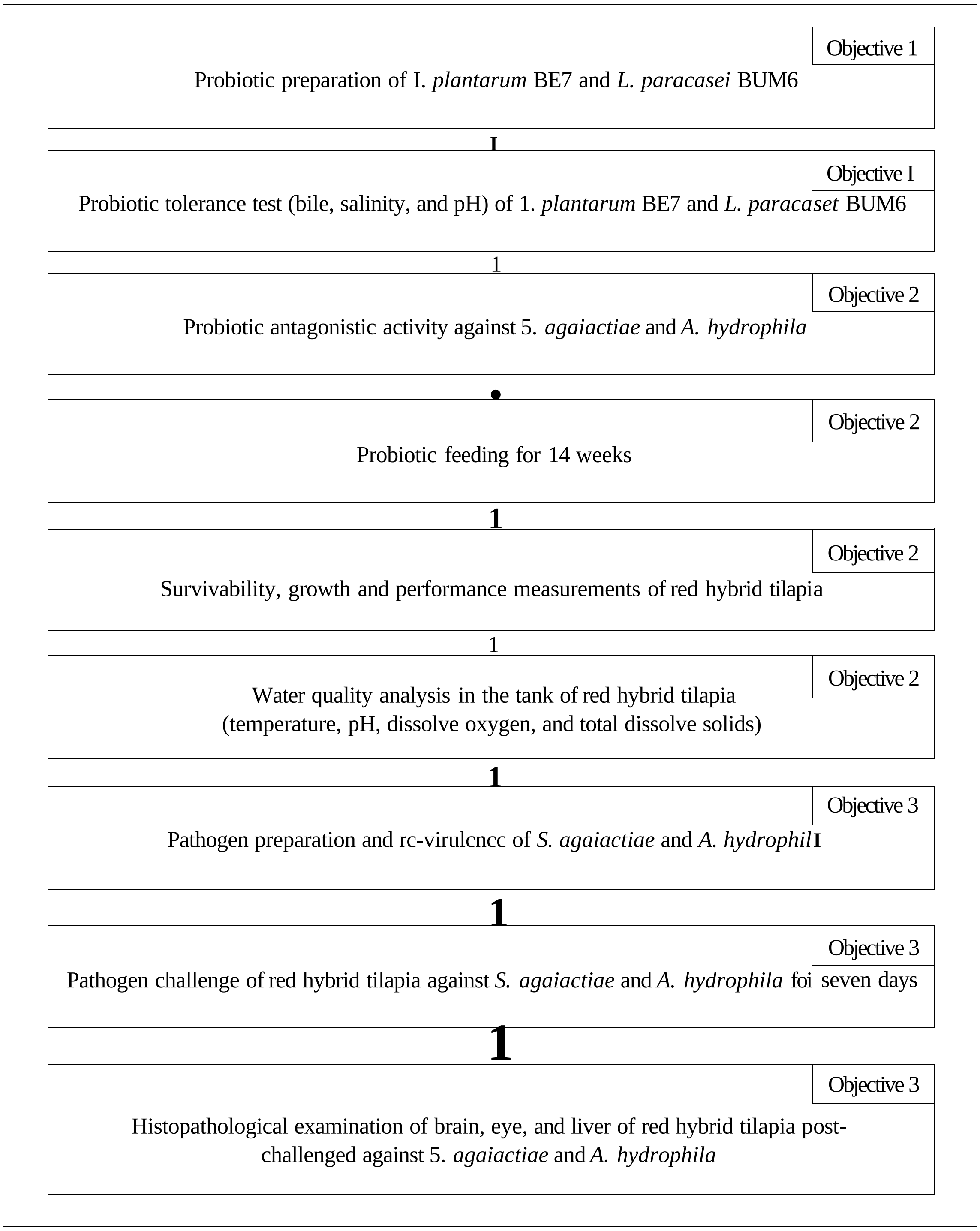


Figure 3.1 Research Flow Chart

3.2 Probiotic Origin

Both probiotic strains were locally isolated from traditional Malaysian fermented foods. *L. plantarum* BE7 was isolated from *belacan*, a fermented shrimp or krill (*udang geragau*) product prepared by mixing with salt, followed by sun-drying and pressing into blocks. *L. paracasei* BUM6 was isolated from *budu*, a traditional fermented fish sauce produced from salted anchovies (*ikan bilis*).

3.3 Probiotic Preparation

Pure culture of *L. plantarum* BE7 and *L. paracasei* BUM6 were obtained from Universiti Sains Islam Malaysia and stored in chiller at 4°C for storage (Ilyanie et al., 2022; Ilyanie et al., 2023). The probiotic strains were inoculated into De Man, Rogosa and Sharpe (MRS) broth and incubated overnight at 37°C. The overnight culture was centrifuged at 5,000 × g for 1 minute at 4°C. After that, the supernatant was removed, and the pellet was resuspended using phosphate buffer solution. The overnight culture was diluted with phosphate buffer solution until the concentration of probiotic reach 1 x 10^9 CFU/mL, which the recommended concentration in aquaculture studies to support sufficient gut colonization and ensure treatment efficacy to the host (Wanguyun et al., 2019).

3.4 Probiotic Tolerance Test

The tolerance of *L. plantarum* BE7 and *L. paracasei* BUM6 to bile salts, salinity, and pH was evaluated. For the bile tolerance test, the probiotic strains were inoculated into MRS broth supplemented with 0% (control), 0.5%, 1%, and 2% (w/v) bile salts, representing bile concentrations commonly present in the fish gut, and incubated at 30°C for 24 hours (Nakharuthai et al., 2023). The optical density (OD) of the cultures was measured using a spectrophotometer. For the salinity tolerance test, the strains were inoculated into MRS broth supplemented with 0% (control), 0.05%, 1%, 2%, and 3% NaCl to simulate salinity conditions of freshwater (0.05–1%) and brackish water (1–3%) (Musie and Gonfa, 2018). Cultures were incubated at 30°C for 24 hours prior to OD measurement. For the pH tolerance test, MRS broth was adjusted to pH 2, 4, 6.2 (control), and 8 to reflect acidic conditions in the fish stomach and basic conditions in the intestine. The cultures were incubated at 30°C for 3 hours, corresponding to the average gut transit time during which ingested feed remains in the stomach before passing through the digestive tract (Ilyanie et al., 2022). After incubation, probiotic viability was assessed using the plate count method on MRS agar, with plates incubated at 30°C for 48 hours (Nakharuthai et al., 2023).

3.5 Probiotic Antagonistic Assay

The antagonistic activity of the probiotic cultures against the fish pathogens *S. agalactiae* and *A. hydrophila* was assessed using the agar spot test (Mezaal and Chelab, 2024). Overnight cultures of the probiotic strains grown in MRS broth (10 CFU/mL) were spotted onto MRS agar plates, air dried for 30 minutes, and incubated at 37 °C for 24 hours. Following incubation, both pathogens were prepared at a concentration of 10 CFU/mL (refer to Section 3.7.1). A 1% inoculum of each pathogen was mixed with 10 mL of molten Tryptone Soy Agar (TSA) and overlaid onto the MRS agar plates containing the probiotic spots. After the TSA solidified, the plates were re incubated at 37 °C for 24 hours, and zones of inhibition (mm) surrounding the probiotic spots were measured to determine the antagonistic effect of the isolates (El Ahmadi et al., 2025). Inhibition zones 20 mm were considered strong, 10–19 mm moderate, and < 10 mm weak inhibitory activity, as commonly applied in probiotic antagonistic studies (Ringø et al., 2020; Hoseinifar et al., 2023).

3.6 Fish Study

This section involves the methodological part regarding the in vivo feeding assessment on the red hybrid tilapia which was conducted after the in vitro screening of probiotic candidates.

3.6.1 Fish Experimental Design

A total of 270 healthy juveniles of red hybrid tilapia (*Oreochromis* spp.) with an average weight of 2–2.5 g was obtained from a local fish farmer in Parit Tinggi, Kuala Pilah, Negeri Sembilan. The fish were randomly allocated into three groups: a control group, T1 (fed with *L. plantarum* BE7-supplemented feed), and T2 (fed with *L. paracasei* BUM6- supplemented feed). Each group was further divided into three replicates, with 30 fish stocked per 150 L transparent polypropylene tank containing aerated, dechlorinated freshwater maintained at 24 °C. The experimental tank layout for the fish feeding trial was shown in Figure 3.2. Prior to the feeding trial, all fish underwent a seven-day acclimatization period under identical rearing conditions without probiotic supplementation. During acclimatization, fish were fed a basal commercial diet (GOLD COIN 988: min 30% protein, max 6% fibre, max 4% fat, max 13% moisture) at 5% body weight, twice daily at 9:30 a.m. and 3:30 p.m. (Cadorin et al., 2022). Water quality parameters, including temperature, dissolved oxygen (DO), and pH, were monitored daily to ensure stable conditions. Below is the formula of the feeding rate calculation that was applied throughout the research:

Feeding Calculation Used in the In Vivo Trial

$$\text{Daily Feed (g)}=(Nx\backslash y)^{x}\ 0.05$$

$$\text{Feed per Feeding (g)}= (N*W)\ \text{x}\ 0.05/2$$

Where:

N = number offish per tank (30 fish)

W = mean body weight offish (g)

Feeding frequency = twice daily (morning and evening)

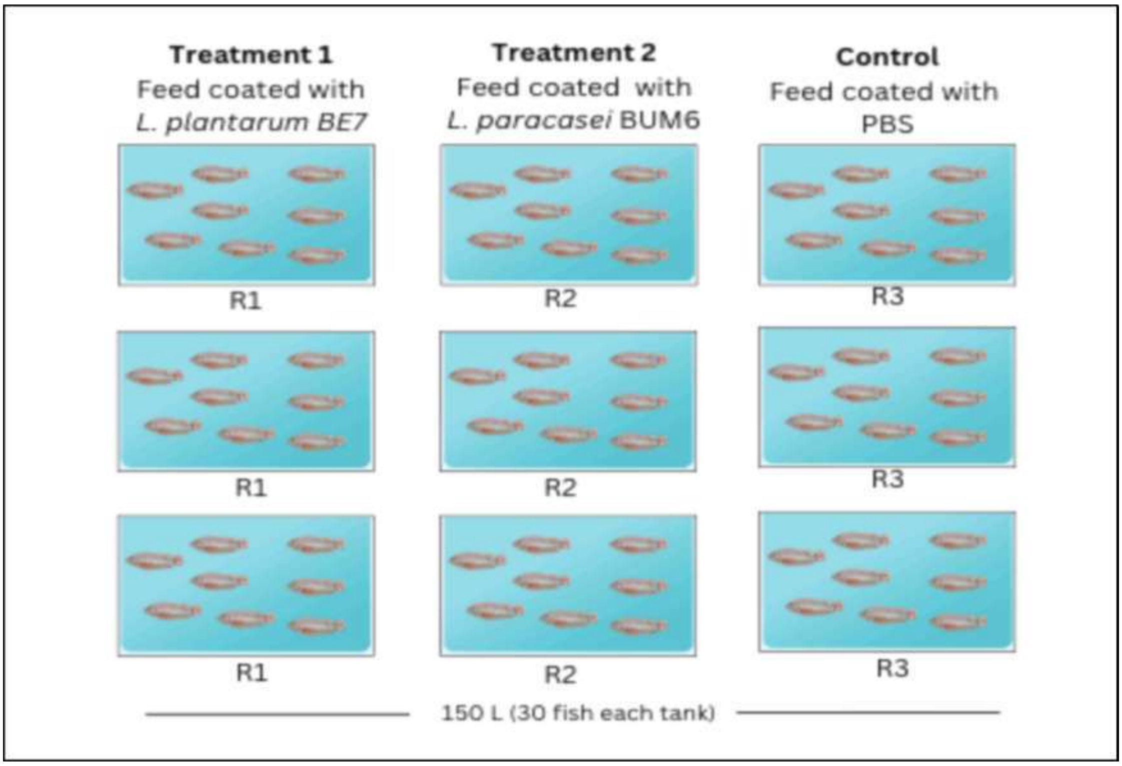


Figure 3.2 Schematic Layout of Experimental Tank Showing The Randomized Block Design for Red Hybrid Tilapia Feeding Trials.

3.6.2 Probiotic Feeding

Following acclimatization, a 14-week feeding trial was conducted. The general feeding schedule and tank conditions described in Section 3.5.1 were maintained throughout the trial. Probiotic preparation was performed daily prior to feed coating, following the procedure described in Section 3.2 (Wanguyun et al., 2019), to obtain fresh overnight cultures of *L. plantarum* BE7 and *L. paracasei* BUM6. For treatment groups T1 and T2, the feed was coated with the fresh overnight cultures at a ratio of 1 mL/g (1 × 10 CFU/mL). The control group feed was coated with phosphate buffer solution at the same ratio. All coated feeds were air-dried at room temperature for 1 hour before being administered. One hour after feeding, the uneaten feed was collected, dried, and weighed to determine feed intake (Deyab and Hussein, 2015)

3.6.3 Growth Performance Measurements

Red hybrid tilapia growth performance was evaluated throughout the 14 weeks culture period to determine the impact of probiotic treatment on every week. 15 fish were randomly chosen from each group to measure the body weight, length, and width. To minimise stress during handling, the fish were sedated with a commercial anaesthetic (Transmore, Nika Trading) at a concentration of around 200 ppm (Noor et al., 2019) prior measurement. The survival rate of fish, specific growth rate (SGR), absolute growth rate (AGR) of weight, length, and width, and feed conversion ratio (FCR) were measured for growth performance analysis using following calculations:

$$\text{Survival rate } (\%) = Nt / N0 \times 100$$

Where Nt and N0 are final and initial number of the juvenile.

$$SGR = (\text{In } Wt - \text{In } W0) \times 100 / t$$

$$\text{AGR of Weight/Length/Width} = (Wt/Lt/wt - W0/L0/w0) / t$$

Where Wt/Lt/wt and W0/L0/w0 are final and initial weight/length/width of the juvenile, and t is the number of feeding period.

$$FCR = \text{Weight of feed consumed (g)} / \text{ weight gain of fish (g)}$$

3.7 Water Quality Analysis

The water quality in each tank was measured weekly during the 14 weeks of probiotic administration. The water parameter such as the dissolved oxygen (DO) was measured using a Digital Dissolved Oxygen Meter DO9100, while the pH, temperature, and total dissolved solids (TDS) were measured using the PH-686 Water Quality Tester. Measurements were conducted consistently in the morning to reduce variability due to diurnal fluctuations. These parameters were monitored to ensure they remained within optimum levels and controlled environment for red hybrid tilapia to grow and survive including temperature (25 to 32°C), pH (6.5 to 9), dissolve oxygen (>5 mg/L), and total dissolve solids (250 to 1000 ppm) (Dauda et al. 2022). Any deviations from recommended levels were recorded and corrective actions were taken to maintain a stable aquatic environment throughout the study period.

3.8 Pathogen Challenge

In this study, a pathogen challenge assay was conducted using *S. agalactiae* and *A. hydrophila* to evaluate the protective effects of dietary probiotics against the bacterial infections. This procedure involved the preparation and re-virulence of the pathogens prior to intraperitoneal injection into the experimental fish.

3.8.1 Pathogen Preparation and Re-virulence

Streptococcus agalactiae and *A. hydrophila* were obtained from Universiti Putra Malaysia for pathogen challenge (Amal et al., 2022). The pathogens were cultured in Tryptone Soy Broth (TSB) and incubated overnight at 30°C for 20 h. The overnight cultures were diluted to a concentration of 10 CFU/mL, which was used as the median lethal dosage for pathogenicity testing (Amal et al., 2022). To ensure the virulence of these isolates, following Koch’s postulates, the fish were injected intraperitoneally with 10 CFU/mL of *S. agalactiae* and *A. hydrophila* (Mohd Ali et al., 2023). Freshly dead fish that showed signs and symptoms of *S. agalactiae* and *A. hydrophila* infection were dissected, and the pathogens were re-isolated onto TSB agar. On the following day, the pathogens from the agar were inoculated into TSB medium and incubated overnight at 30°C. The overnight cultures were then prepared to a concentration of 10 CFU/mL for the pathogen challenge assay.

3.8.2 Pathogen Challenge

After 14 weeks of probiotic feeding administration, six red hybrid tilapia from each group (T1, T2, and control) were randomly selected for the pathogen challenge experiment against *Streptococcus agalactiae* and *Aeromonas hydrophila*. The fish were first sedated using a commercial anesthetic (Transmore) at a concentration of 200 ppm to reduce handling stress during injection (Noor et al., 2019). Subsequently, fish from each replicate group (T1, T2, and control) were injected intraperitoneally with *S. agalactiae* and *A. hydrophila* at a concentration of 10 CFU/mL, at a dosage of 1 mL of inoculum per gram of fish body weight (Nur Nazifah et al., 2011; He et al., 2017). Mortality and clinical signs, including fin hemorrhages, erratic swimming, lethargy, and exophthalmia (pop-eye), were observed for seven days post-challenge (Laith et al., 2017). Organs from deceased fish in both challenged and unchallenged groups were re-isolated and inoculated onto selective media for pathogen identification, in order to confirm that mortality during the challenge period was caused by the respective pathogens

3.8.3 Histopathological Examination

Freshly dead red hybrid tilapia fish were dissected to retrieve the organs such as brain, eye, and liver (Laith et al., 2017). The organs were placed into a container containing with 10% neutral buffered formalin solution for preservation (Legario *et al.*, 2020). All fixed tissue samples were outsourced to Histopathology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) for standard histological processing, including dehydration, paraffin embedding, sectioning and haematoxylin and eosin (H&E) staining. The obtained longitudinal tissue of brain, eye, and liver tissue sample of the fish fed with probiotic, *L. plantarum* BE7, *L. paracasei* BUM6, and without probiotic were examined under light microscope with different magnifications including 40X, 100X, and 400X to observe the lesions produced by infection of *S. agalactiae* and *A. hydrophila*. The lesions in each tissue samples were scored using a semi-quantitative lesion scoring with an ordinal scale of (0 = unchanged, 1 = mild, 2 = moderate, 3 = severe) to compare the severity lesions between probiotic-fed and the untreated fish (Landmann et al., 2021).

3.9 Statistical Analysis

The administration of probiotic treatment to the red hybrid tilapia experiment were carried out in triplicate and the results were analyzed using one-way analysis of variance (ANOVA) with the Statistical Package for Social Sciences (SPSS) for Windows. Statistically significant differences ($p < 0.05$) was examined using Tukey’s post hoc test and the results were reported as mean ± standard error.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter presents the outcomes of both in vitro screening and in vivo evaluation of two lactic acid bacteria, *L. planta*rum BE7 and *L. paracasei* BUM6, investigated as potential probiotic candidates for red hybrid tilapia. The results are organized according to the predefined objectives, including bacterial tolerance to pH, bile, and salinity, the effects of dietary supplementation on growth performance and survival, and the protective efficacy against *S. agalactiae* and *A. hydrophila* infections. The findings are supported by relevant statistical analyses and interpreted in comparison with previously reported studies

4.1 Probiotic Tolerance test

4.1.1 Bile Tolerance Test

The percentage survivability of *L. plantarum* BE7 and *L. paracasei* BUM6 grew in MRS broth medium adjusted with the given concentration were recorded higher than 70% following the incubation period at 37 C for 24 hours as shown in Figure 4.1. Despite both probiotic strains showed successful survivability, their survival rate was recorded decreased as the concentration of the bile salts increased. In this research, the bile salt concentrations of 0.5%, 1%, and 2% are the range readings of the actual bile salt conditions in the fish's guts, and it is very crucial for both probiotic strains to show great resistance before they reach the intestine to exert the beneficial effects of probiotics on the red hybrid tilapia (Nayak et al., 2010). The viability of *L. plantarum BE7* was observed higher than *L. paracasei* BUM6 as it showed higher survival rate in all tested bile salt concentration. The survivability trends for both strains were shown to be dose-dependent, with higher bile salt concentration causing lower ability of the probiotics to survive. The percentage survivability was not significantly different when both probiotics were inoculated in MRS broth added with 1% bile salt.

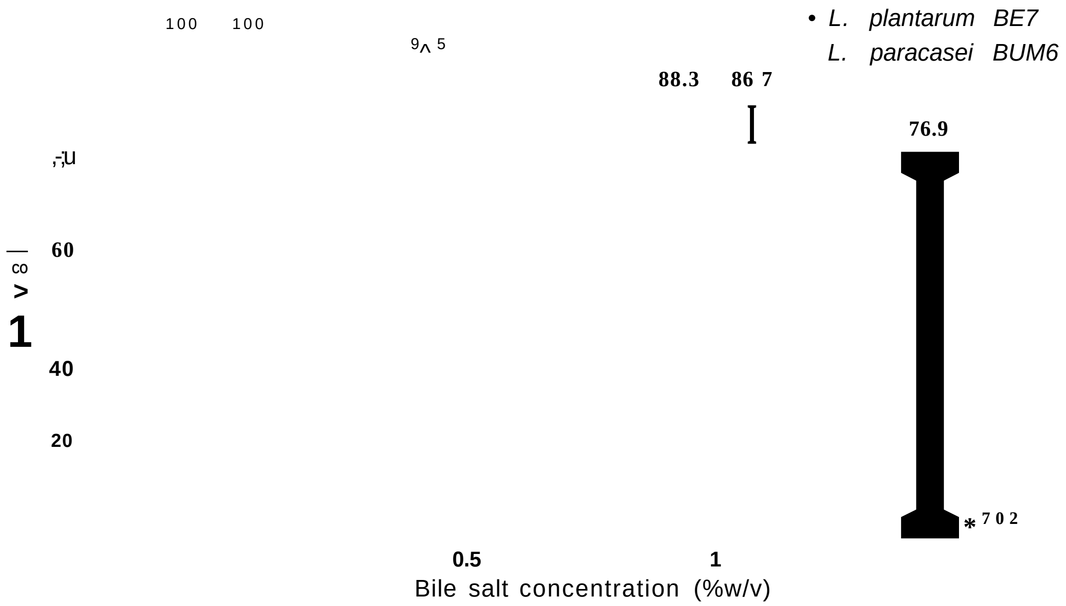


Figure 4.1 The Percentage Survivability of *L. plantarum* BE7 and *L. paracasei* BUM6 on Different Bile Salt Concentration (0%(Control), 0.5%, 1%, And 2%) At 37°C for 24 Hrs. Note: * Shows Significance Difference between Group (p<0.05)

Bile tolerance is a critical criterion in the selection of effective probiotic strains for aquaculture, as it directly influences a probiotic's ability to survive and function within the host's gastrointestinal tract. In fish, the gastrointestinal environment poses several challenges, including exposure to bile salts, which are secreted by the liver and stored in the gallbladder to aid in lipid digestion. While essential for metabolic processes, bile salts possess antimicrobial properties that can disrupt bacterial cell membranes and inhibit the growth of non-resistant strains. Therefore, only probiotics with robust bile tolerance can endure gastrointestinal transit, successfully colonize the gut, and exert their beneficial effects. These effects include modulation of the intestinal microbiota, enhancement of nutrient absorption, stimulation of immune function, and increased resistance to pathogenic infections (Ghiasi et al. 2018). Selecting bile-tolerant strains thus ensures the probiotic's viability and functional efficacy, supporting the health, growth, and overall resilience of aquaculture species.

The ability of *L. plantarum* BE7 to show greater viability compared to *L. paracasei* BUM6 maybe because *L. plantarum* BE7 had higher resistance toward the harsh bile salts concentration. Most of the *Lacticaseibacillus* and *Lactiplantibacillus* species could survive at bile salt concentrations of 0.3% reflecting the actual bile salts concentration in the human gut (Khushboo *et al,* 2023). Different in thickness and composition of the exopolysaccharides (EPS) in each strain maybe becoming the factor different resistance between both strains towards 0.5%, 1%, and 2% bile salts concentration reflecting the actual condition in the fish's guts (Melchior *et al.,* 2020). EPS is formation of layer that act as a protective barrier on the cell surface, protecting bacteria from the detrimental effects of bile salts (Khushboo et al., 2023). *L. plantarum* is known for generating a large amount of EPS with a variety of structural characteristics. It also generates larger and more resilient biofilms that contain EPS layers resulting in greater bile salt resistance and adherence to intestinal surfaces (Werning et al., 2022). *L. paracasei*, on the other hand, also generates EPS, however, the amount and structural complexity maybe lower than *L. plantarum*, resulting in a lower survival rate in the fish's intestines under severe bile salt conditions (Lee et al., 2022).

In this study, the survival patterns observed was align closely with previous findings, confirming the higher bile salt tolerance of *L. plantarum* compared to *L. paracasei* (Lee et al., 2022). Chen et al. (2022) examined the bile salt tolerance of *Lactobacillus* strains, including *L. plantarum* and *L. paracasei* showed that *L. plantarum* strains had a wide range of bile salt tolerance, with some strains sustaining growth rates exceeding 90% in the presence of 1.2% bile salts. In contrast, *L. paracasei* strains demonstrated generally lesser tolerance, with lower growth rate under the same circumstances. Huang et al. (2022) found that *L. plantarum* strains, including BBE7, demonstrated higher survival rates under bile salt concentrations of 0.075% and 0.1%, with some strains sustaining over 70% survivability. On the other hand, Sriphannam and Kummasook (2020) examined that while *L. paracasei* strains demonstrate some bile salt tolerance, they are not as resilient as *L. plantarum* BE7, especially at high bile concentrations.

When compared with probiotic strains commonly used in commercial supplements, the bile salt resistance demonstrated by *L. plantarum* BE7 and *L. paracasei* BUM6 appears comparable or even superior in certain aspects. For instance, *Lactobacillus rhamnosus* GG, one of the most strain widely used in commercial probiotics, has been reported to survive bile salt concentrations of up to 0.3–0.5%, with significant reductions in viability observed at higher concentrations (Zhang et al., 2019). Similarly, *Lactobacillus acidophilus*, another common probiotic species, typically shows optimal survival at bile concentrations below 0.5%, with decreased growth under more extreme bile conditions (Sánchez et al., 2017). In contrast, several *L. plantarum* strains have been shown to tolerate bile salt concentrations exceeding 1.0%, highlighting their superior adaptability to harsh gastrointestinal environments (Archer et al., 2018). This suggests that the local isolate, *L. plantarum* BE7, exhibits bile tolerance levels that are at least comparable to, and potentially more robust than those of strains currently used in commercial probiotic formulations.

4.1.2 Salinity Tolerance Test

According to Figure 4.2, *L. plantarum BE7* and *L. paracasei BUM6* were able to grow in all of the given salinity concentration with a survival rate higher than 60%. Both probiotic strains can tolerate in the given range of salinity however higher salinity levels corresponded with reduced survival of the probiotics, demonstrating a clear dose-dependent trend for both strains. *L. plantarum* BE7 was observed had a higher and better salinity tolerance compare to *L. paracasei BUM6* resulting in higher survival rate. The percentage survivability was not significantly different when both probiotics were inoculated in MRS broth with the salinity concentration of 0.5% and 1%.

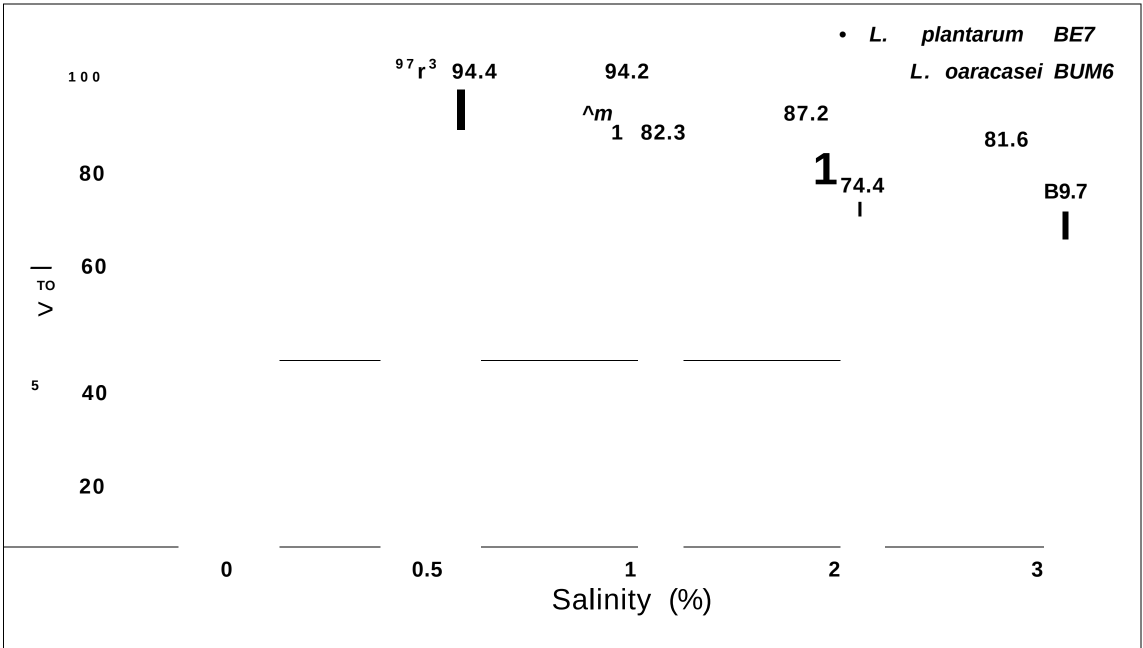


Figure 4.2 The Percentage Survivability of *L. plantarum* BE7 and *L. paracasei* BUM6 on Different Salinity Concentration (0%(control), 0.5%, 1%, 2%, and 3%) at 37°C for 24 hours. Note: * Shows Significance Difference between Group ($p<0.05$)

The salinity tolerance test is used for assessing the type of probiotic strains that suitable for application in red hybrid tilapia, a species commonly cultured in both freshwater and brackish water environments (Fuadi et al., 2021). Given the potential for fluctuating salinity levels due to environmental changes such as tidal influence, seasonal variation, or water exchange practices, it is essential that selected probiotics to remain its viability and functional properties across a range of osmotic conditions. Probiotic strains that are sensitive to salt stress may experience reduced survival and diminished ability to confer health benefits, ultimately compromising their effectiveness in promoting gut health, immune response, and growth performance (Amoah et al. 2023). Therefore, evaluating salinity tolerance ensures the robustness and adaptability of probiotic candidates, reinforcing their practical application and reliability in dynamic aquaculture systems where environmental salinity is not constant.

Generally, most bacteria cannot survive in high-salinity environments because elevated salt concentrations create osmotic pressure that draws water out of the cell, leading to dehydration and cellular stress (Wennerström and Oliveberg, 2021). The absence of water inside of the bacteria may disable the function of bacterial protein causing mortality. Bacteria that could survive in high saline environment is known as halophilic bacteria and halotolerant bacteria. In this study, it was very important for the probiotic strains *L. plantarum* BE7 and *L. paracasei BUM6* to survive in the given range of salinity (0.5%, 1%, 2%, and 3%) since it is the salinity of water reflecting the freshwater and brackish water were favours for red hybrid tilapia to live. Even though the survival rates for both probiotics decreased as the salinity concentration of the broth increased, excellent percentage survivability for both strains were recorded even in the highest salinity concentration which was 3%. Previous salt tolerance test results on different strains of Lactobacillus demonstrated that *Lactobacillus acidophilus* CM1 and *Lactobacillus delbrueckii* OS1 could live at 4 and 6% NaCl levels, significantly (Khushboo et al., 2023). Furthermore, *L. paracasei* isolated from practically all sources demonstrated considerable diversity in adaptations to osmotic stress (Reale et al., 2015). Therefore, both probiotics strains used in this study were demonstrated by previous study able to survive within the applied concentration.

According to previous findings, commercially available strains like *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* GG can withstand NaCl concentrations between 0.5% and 2.0%, with growth and viability clearly declining at salinity levels above 2% (Terpou et al., 2019). Similar to this, *Bacillus subtilis*, a spore-forming probiotic that is frequently used in aquaculture feed, shows adequate survivability at moderate salinity (up to 2.5% NaCl), while increased osmotic stress reduces the metabolic activity of its vegetative cells (Cutting, 2011). On the other hand, the current study showed that both local isolates, *L. plantarum* BE7 and *L. paracasei* BUM6, retained good survivability even at 3% salinity, indicating better halotolerant properties than a number of commercially available probiotic strains.

Lactiplantibacillus plantarum and *L. paracasei* could survive in the given range of salinity because they might have genes and metabolic pathways that may allow them to adapt towards fluctuating conditions of osmolarity. According to Liang et al., (2013), *L. plantarum* may possesses genes that help it store proline, which is required for primary metabolism under salt stress. Proline is an essential amino acid that helps microbes survive under extreme circumstances, especially salt stress. Therefore, it helps the probiotic to maintain cellular homeostasis, protects proteins, and scavenges reactive oxygen species (ROS) ensuring its viability even in high saline condition (Chanalia et al., 2018). It also has genes implicated in multi-component binding-protein-dependent transport systems of glycine, betaine, and carnitine. In addition, *L. paracasei* possesses many sugar transport systems and metabolic pathways, allowing it to digest a wide range of carbohydrates (Cui and Qu, 2021). This allows it to adapt to varied settings using various carbohydrates. Therefore, the application of local isolates, *L. plantarum* and *L. paracasei* as probiotic inclusion into feed additives in probiotic treatment of fish in aquaculture become very promising. The viability of both strains was able to be compromised during the pathway to the reach to the fish guts and exerts its beneficial effect without worrying with its viability during administration.

4.1.3 pH Tolerance Test

Figure 4.3 showed an excellent survivability for both probiotic strains, *L. plantarum* BE7 and *L. paracasei* BUM6 within the given pH adjusted medium. Both of the probiotic strains were able to survive in all pH with a greater than 50% survival rate. The result demonstrated even in harsh acidic condition which was pH 2 and slightly alkalic condition which was pH 8, both probiotic strains were able to maintain its viability about more than 50%. The percentage survivability was significantly different between both probiotics when inoculated into the MRS broth in those pH level. Plus, since lactic acid bacteria prefers a medium which is lightly acidic, both *L. plantarum* BE7 and *L. paracasei* BUM6 showed the highest percentage survivability compare to other pH. Overall, *L. plantarum* BE7 was recorded showing a higher survival rate compare to *L. paracasei* BUM6 indicating a better resistance within the pH adjusted medium.

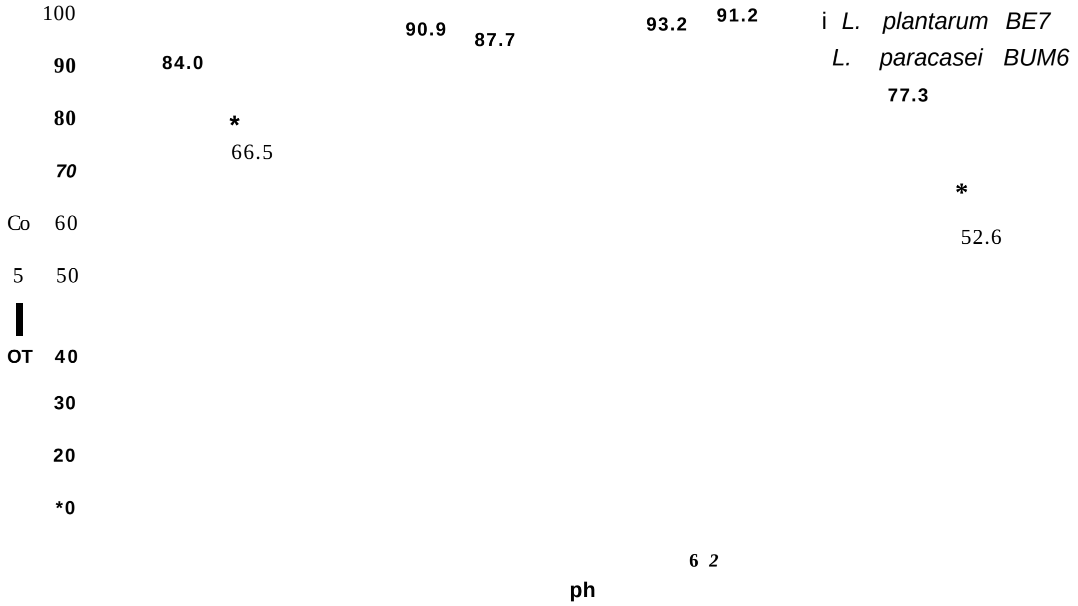


Figure 4.3 The Percentage Survivability of *L. plantarum BE7* and *L. paracasei BUM6* on Different Range of pH 2, 4, 6.2 (Control) and 8 At 37°C. Note: * Shows Significance Difference between Group ($p<0.05$).

The pH tolerance test is an important factor in selecting successful probiotic strains for red hybrid tilapia since it determines the bacteria's ability to survive in the acidic environment of the fish's gastrointestinal system. After the feed consumption by fish, probiotics must travel through the stomach, where pH values can decrease dramatically, particularly after digestion. A high survival rate in low-pH environments guarantees that probiotics may colonize the gut, increase digestive enzyme function, influence the immune system, and compete with harmful bacteria. Thus, assessing pH tolerance is critical for ensuring the stability, functioning, and overall efficiency of probiotic therapies in enhancing the health and performance of red hybrid tilapia.

Although the favorable pH for probiotic growth is slightly acidic, the highly acidic conditions of the fish stomach (approximately pH 2–4) may reduce the viability of probiotic strains, particularly during feed administration (Merrifield et al., 2010). Besides, the probiotic strains used in aquaculture also must be able to withstand at slightly alkalic condition which is at pH 8 reflecting the actual pH of the fish's intestine. Therefore, the benefits from probiotic to the host can be utilized if the strain used for this application can sustain and remain highly viable within the pH range between pH 2, 4, and 8. Both of the probiotics were incubated for 3 hours at 37 C reflecting the actual time constraint for the feed to stay in the fish stomach (Hai et al., 2015).

Liang et al., (2013) mentioned that the presence of proton pumps, such as F F -ATPase systems, which actively remove protons from the bacterial cell to maintain internal pH homeostasis, was may be the main reason of strong tolerance demonstrated by the probiotics in pH 2 and pH4- adjusted media. Furthermore, in slightly alkalic condition like pH 8, the probiotic's survival approach requires regulating intracellular pH through sodium/proton (Na /H) antiport systems, which may assist remove excess hydroxide ions and preserve cytoplasmic pH stability (Sionek et al., 2023). Overall, these adaptive systems highlight the importance of cellular pH regulation mechanisms in determining probiotic robustness. Strains exhibiting efficient proton and ion transport systems are more likely to survive gastrointestinal conditions and exert long-term beneficial effects on fish health

4.2 Probiotic Antagonistic Assay

According to Table 4.1, *L. plantarum* BE7 showed a very strong inhibition zone (22.1 ± 1.9 mm) compared to *L. paracasei* BUM6 (18 ± 2 mm) against *S. agalactiae*. On the other hand, *L. paracasei* BUM6 was observed to yield a stronger inhibition zone (22.7 ± 1.2 mm) compared to *L. plantarum* BE7 (19 ± 2 mm) against *A. hydrophila*. No significance different between the inhibition zone produced by both probiotic strains compare to the antibiotics. Therefore, it reveals that *L. plantarum* BE7 exhibited a greater inhibitory effect toward *S. agalactiae* compared to *L. paracasei* BUM6, whereas *L. paracasei* BUM6 exhibited greater antagonistic properties against *A. hydrophila* compared to *L. plantarum* BE7. The inhibition zones produced by both probiotics were slightly smaller than the antibiotics had produced against fish pathogens, *S. agalactiae* and *A. hydrophila*.

Table 4.1
Antagonistic Effect Zone of Inhibition (ZOI) of *L. plantarum* and *L. paracasei* against *S. agalactiae* and *A. hydrophila*

	Tested bacterial strains (ZOI in mm)	
LAB	***S. agalactiae***	***A. hydrophila***
L. plantarum BE7	22.1 ± 1.9	19 ± 2
L. paracasei BUM6	18 ± 2	22.7 ± 1.2
Ampicillin	23.5 ± 2	-
Tetracycline	-	23 ± 2.3

Note: -: Not tested to specific pathogen. Ampicillin and Tetracycline used as positive controls. Inhibition zones 20 mm were considered strong, 10–19 mm moderate, and < 10 mm weak inhibitory activity

The application of probiotic inclusion into dietary feeding of aquatic organisms in the aquaculture sector has been widespread globally to overcome losses caused by fatal disease infections during outbreaks. *S. agalactiae* and *A. hydrophila* are the common pathogens that cause serious infections and mortalities in global rearing culture. Since the emergence of antibiotic resistance happened from the misuse antibiotic treatment into the industry. The probiotic treatment becomes a better alternative to the antibiotic treatment as the good bacteria has relatively no negative impact on human and environmental health. Therefore, probiotic antagonism activity was performed to observe the *L. plantarum* BE7 and *L. paracasei* BUM6 antimicrobial properties towards *S. agalactiae* and *A. hydrophila*.

Notably, the diameter of the inhibition zones produced by both probiotic strains was quite similar to those generated by the commercial antibiotics used as positive controls which were ampicillin and tetracycline. The antimicrobial activity exhibited by the probiotics was classified as very strong, indicating their effectiveness. These findings highlight the promising potential of *L. plantarum* BE7 and *L. paracasei* BUM6 as viable alternatives to conventional antibiotics. As environmentally friendly and sustainable agents, these probiotics offer a safer approach with minimal adverse impacts on both consumers and the environment. Overall, *L. plantarum* BE7 demonstrated stronger antimicrobial activity against *S. agalactiae*, whereas *L. paracasei* BUM6 was more effective against *A. hydrophila*.

According to the result, the efficiency of the probiotic treatment during administration might vary depending on the different selection of probiotic strains used. Not all probiotics have good and similar antimicrobial properties against diseases in aquaculture. The application of the correct strain to the cultured fish will yielded a better production while helping this sector to mitigate disease at the same time. Thi et al., (2023) mentioned that lactic acid bacteria have inhibitory effects on *S. agalactiae* and *A. hydrophila*, lowering its pathogenicity. *L. plantarum* BE7 and *L. paracasei* BUM6 have a great inhibitory effect against the fish pathogens, *S. agalactiae* and *A. hydrophila* may be due to the production of antimicrobial compounds, creating competitive exclusion, and causing changes in pathogen adhesion properties (Hu et al., 2019). These factors might be giving an ability for *L. plantarum* BE7 to produce large inhibition zone during antimicrobial assay.

Lactiplantibacillus plantarum BE7 and *L. paracasei* BUM6 strains might produce organic acids and bacteriocins which may prevent or lower the *S. agalactiae* and *A. hydrophila* growth and development in agar during agar spotted assay (Hu et al., 2019). Plus, the synthesis of organic acids, such as lactic acid and acetic acid, lowers the pH of the environment, making it unsuitable for many pathogenic bacteria to grow. Bacteriocins, or tiny antimicrobial peptides, can also precisely target and inhibit closely related or broad-spectrum infections by damaging their cell membranes (Zhou et al., 2020). This dual method of acidification and bacteriocin synthesis improves the probiotic's competitive edge while also protecting the host organism in aquaculture settings for further treatment usage (Akter et al., 2020). The tilapia fed with *L. plantarum* strains showed greater survival rates after being challenged with *S. agalactiae* because the probiotic was able to suppress pathogen colonization via organic acid synthesis and gut regulation (Hu et al., 2019). Lastly, LAB strains exhibited obvious inhibitory zones against *A. hydrophila*, with *L. paracasei* suppressing the pathogen by acid generation and bacteriocin release (Akter et al., 2020). Due to the antimicrobial properties of probiotics against fish pathogens, the Nile tilapia had significantly reduced in mortality, fewer clinical signs, and better immunological responses than control groups (Newaj-Fyzul et al., 2014).

4.3 Growth and Performance Measurements

The effects of dietary probiotic supplementation on the physiological development and production efficiency of red hybrid tilapia were evaluated using growth performance indicators. Growth indicators such as weight gain, specific growth rate (SGR), absolute growth rate (AGR), and feed conversion ratio (FCR) are commonly used as key performance indices in aquaculture, as they reflect the ability of fish to efficiently utilize nutrients for somatic growth. Improvements in these metrics are frequently linked to improved nutritional absorption, improved digestion, and the fish’s general health. Consequently, assessing growth performance offers crucial information about the functional role of probiotics as dietary supplements and their potential to increase aquaculture sustainability and productivity.

4.3.1 Survival Rate

Figure 4.4 shows the survival rate of red hybrid tilapia fed withZ. *plantarum* BE7 (Tl), *L. paracasei* BUM6 (T2) and without probiotic (control) for 14 weeks with a feeding rate of 5% of the fish bodyweight. The survival rates of red hybrid tilapia in all groups exhibited a rapid decline from the 1st to the 4th week of the feeding administration period. Thereafter, from the 4th to the 14th week, a continued decline in survival was observed across all groups, however, the rate of decline was markedly slower compared to the initial four weeks. Highest survival rate was recorded in fish administered with *L. plantarum* BE7, showing a statistically significant difference compared to fish fed with *L. paracasei* BUM6 and the control group ($p < 0.05$). This happens might be because of the probiotic supplementation *L. plantarum* BE7 and *L. paracasei* BUM6 that may enhance the fish's resilience towards internal and external stressors during the culture period resulting in higher survival rate compare to the fish in the control group.

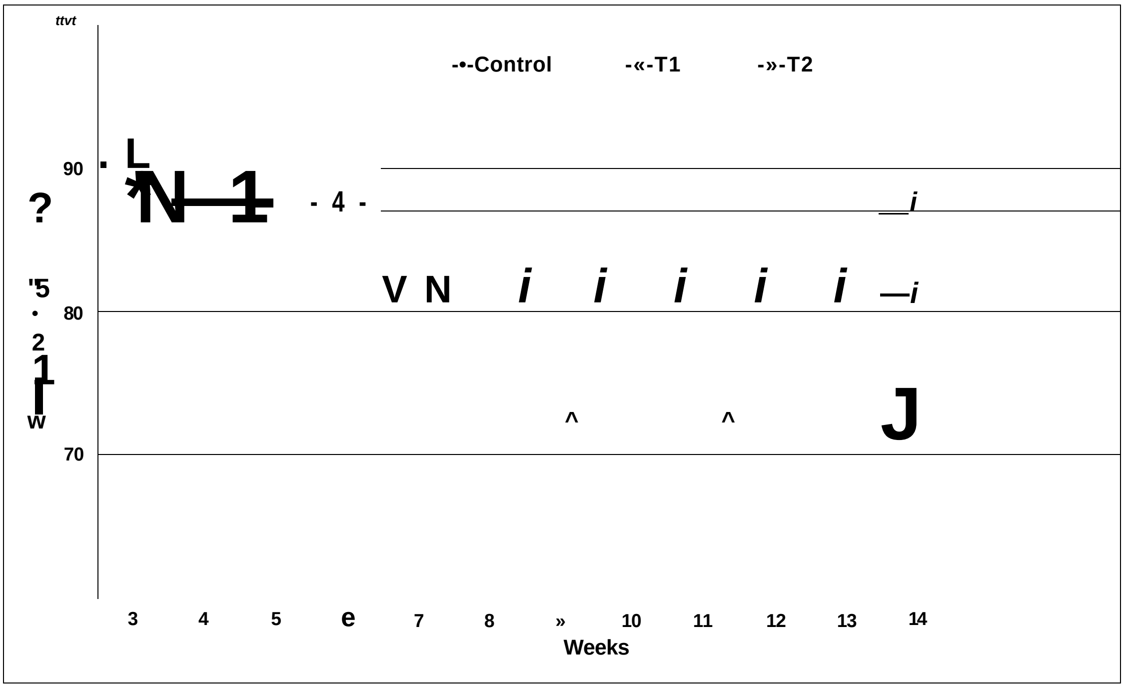


Figure 4.4. The Survival Rate of Red Hybrid Tilapia Fed with Probiotic-Supplemented Feed with *L. plantarum* BE7 (Tl), *L. paracasei* BUM6 (T2), and without Probiotic (control) for 14 weeks. * Shows Significance Difference between Group ($p<0.05$)

The survival rate of fish is an important parameter that directly affects aquaculture economic viability, sustainability, and total production (Saha et al., 2022). In aquaculture, high survival rates imply an excellent management strategies and ideal environmental conditions, which are critical for increasing output and profitability. A better survival rate results in more biomass, which increases marketable production and profitability for aquaculture operations. Few factors that might affecting the survivability of the fish in rearing cultures including the disease resistance against fish pathogen, overstocking that may leads to stress, and poor feeding efficiency (Wanja et al., 2020). in the present study, survival rate was therefore used as a key biological indicator to evaluate the effectiveness of probiotic supplementation on fish health and stress resilience under controlled rearing conditions

The early decline in survival observed in the control group during the initial weeks of the feeding trial may be attributed to increased exposure to environmental and physiological stressors in the absence of probiotic supplementation. Potential internal and external stress factors during the experimental period include noise disturbance, light exposure, competition for feed, limited space due to stocking density, and the possible presence of subclinical pathogens in the rearing tanks (Kusku et al., 2022). These stressors are known to activate the hypothalamus–pituitary–interrenal (HPI) axis in fish, leading to elevated cortisol levels. Prolonged cortisol secretion can negatively affect immune function by diverting energy away from growth and immune defence mechanisms (Lemos et al., 2023). Tort (2011) further reported that sustained high cortisol levels suppress the expression of key immune-related genes such as interleukin-1 beta (IL-1β), tumour necrosis factor-alpha (TNF-α), and immunoglobulin M (IgM), which may reduce the ability of fish in the control group to resist opportunistic infections and environmental challenges. In contrast, probiotic-supplemented groups (T1 and T2) exhibited higher survival rates, suggesting that probiotic inclusion may have enhanced stress tolerance and immune resilience, thereby reducing early mortality. This pattern is consistent with the survival trend observed in this study, where probiotic-treated groups maintained higher survival rates compared to the control group throughout the feeding period.

Notably, no mortality was recorded during the acclimatization period, indicating that fish were initially healthy and well adapted to the rearing environment prior to the feeding trial. The onset of mortality only occurred after the experimental diets were introduced, suggesting that the feeding phase itself may have introduced additional stress factors. These may include changes in feed composition and texture, increased competition during feeding, handling disturbances, and physiological stress associated with dietary transition. Such factors are known to induce stress responses in fish and may contribute to increased vulnerability to subclinical infections or environmental challenges. Although a decline in survival was observed in all groups, the more pronounced mortality in the control group indicates that probiotic supplementation may have mitigated the negative effects of these stressors. This suggests that probiotics did not eliminate mortality entirely but likely enhanced stress tolerance and immune resilience, resulting in comparatively higher survival in the treated groups.

Du et al., 2022 stated that probiotic supplementation, *L. plantarum* BE7 and *L. paracasei* BUM6 has been shown to mitigate stress in red hybrid tilapia by enhancing physiological resilience and immune function. These probiotics contribute to a balanced gut microbiota, which not only improves nutrient absorption but also strengthens the intestinal barrier, thereby reducing susceptibility to pathogenic invasions (Liang et al., 2022). Furthermore, they stimulate the innate immune system, elevating parameters such as lysozyme activity and white blood cell counts, that may strengthen the fish's defense mechanisms against stress-induced immunosuppression. Previous study done by Hasan et al., 2023 stated that the dietary inclusion of *L. plantarum* has been reported to alleviate oxidative stress and inflammation in Nile tilapia exposed to environmental stressors, further underscoring its role in stress mitigation. These findings align with the present study, supporting the hypothesis that *L. plantarum* BE7 and *L. paracasei* BUM6 may contribute to improved survival by enhancing physiological and immunological stability in red hybrid tilapia

4.3.2 Specific Growth Rate

The Specific Growth Rate (SGR) is an important parameter in aquaculture that measures production efficiency and environmental sustainability. Based on Figure 4.5, the SGR of fish in all groups showed an increase trendline, indicating that the growth and development rates of the fish improved throughout the feeding administration period. The SGR offish fed with both probiotics was observed to be higher than that of fish fed without probiotics. The highest SGR was recorded in fish fed with *L. plantarum* BE7, with a significant difference compared to fish fed with *L. paracasei* BUM6 and the fish in the control group ($p<0.05$). At the 14th week, the fish fed with *L. plantarum* BE7 grew at a rate of 3.52% per day, followed by fish fed with *L. paracasei* BUM6 (3.38% per day), and fish in the control group (2.6% per day). A higher SGR was recorded in fish fed with *L. plantarum* BE7 compared to fish fed with *L. paracasei* BUM6. Therefore, probiotic supplementation with *L. plantarum* BE7 and *L. paracasei* BUM6 may improve daily growth rates in red hybrid tilapia.

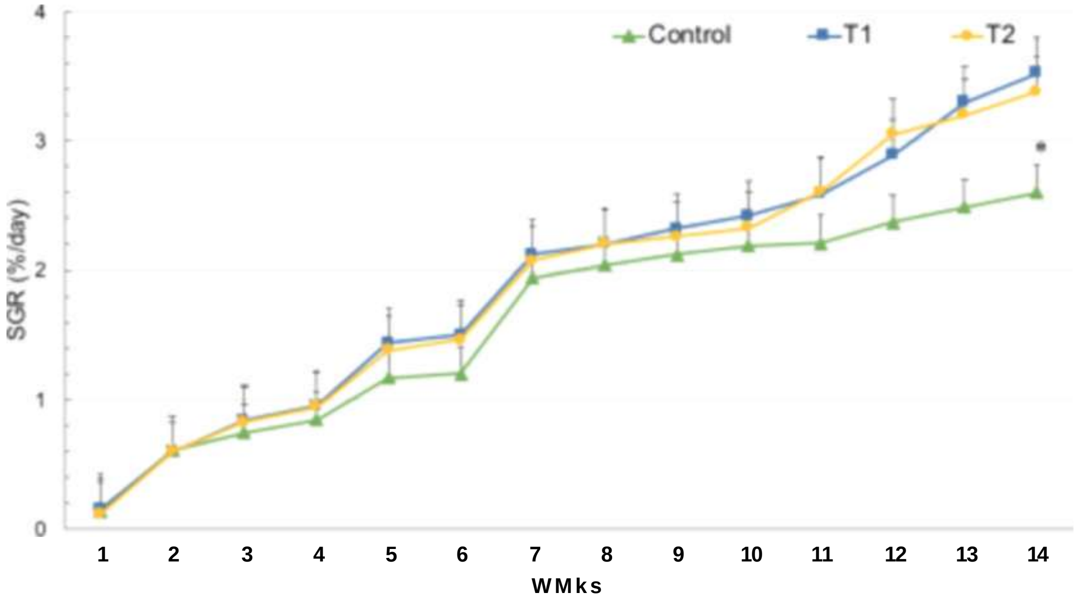


Figure 4.5 The Specific Growth Rate (%/day) of Red Hybrid Tilapia Fed with Probiotic- Supplemented Feed with *L. plantarum* BE7 (Tl), *L. paracasei* BUM6 (T2), and without Probiotic (control) for 14 weeks. * Shows Significance Difference between Group ($p<0.05$)

The enhanced of weight gain and daily growth rates among the probiotic-fed red hybrid tilapia may happened due to the gut microbiota modulation which may improve the digestion efficiency, nutrient uptake, and immune system. Zhang et al. (2025) reported that *L. plantarum* had a positive impact on the gut microbiota which promoted the proliferation of beneficial bacteria in the fish gut, leading to improved digestion and nutrient absorption from the feed. The high availability of nutrient uptake directly from the feed may result in better growth and development, as higher levels of macronutrients, minerals, and vitamins can be absorbed efficiently to support cellular functions in the fish. Therefore, this situation may help the fish to utilize all of the nutrients which can directly convert into pure body mass, resulting in better growth and development among both probiotic-fed fish groups.

These findings are consistent to previous studies. Giri et al. (2021) reported that fish fed with *L. plantarum* exhibited significantly higher net weight gain and specific growth rates compared to control groups, with optimal results observed at higher concentrations (e.g., 10^9 CFU/g). Plus, Cazorla et al. (2018) revealed that fish fed *L. paracasei* had a 12% higher biomass than control groups, demonstrating a definite growth performance advantage. Torres-Maravilla et al. (2024) showed that probiotic strain also may improve fish immune responses, resulting higher growth by lowering stress and energy loss caused by sickness (Torres-Maravilla et al., 2024). Muhammad et al., (2023) demonstrated that tilapia treated with *L. plantarum* had an SGR of around 2.94% each day, suggesting strong growth performance observed in the present study

4.3.3 Absolute Growth Rate

The absolute growth rate (AGR) and specific growth rate (SGR) are important metrics commonly used to evaluate fish growth performance in aquaculture. AGR measures the overall gain in weight, length, and width over a certain period, typically expressed as grams per day (g/day) for weight and centimeters per day (cm/day) for size dimensions. It provides a direct assessment of the fish's development in absolute terms, making it useful for observing physical growth across different parameters. In contrast, SGR represents the growth rate as a percentage of the fish's body weight over time, offering a relative measure of growth efficiency that allows for comparison between treatment and control groups (Márquez et al., 2024). Studies have emphasized that using both AGR and SGR together provides a more complete picture of fish development and can aid in optimizing aquaculture practices (Giri et al., 2021). By integrating these measurements, researchers and farmers can better understand fish growth dynamics and adjust feeding strategies, stocking densities, and management practices to maximize production efficiency and fish health.

Table 4.2
Absolute Growth Rate of Weight, Length, and Width of Red Hybrid Tilapia Fed with Commercial Fish Feed Coated with *L. plantarum* BE7 (T1), *L. paracasei* BUM6 (T2), and Phosphate Buffer Solution (Control) for 14 weeks.

Absolute growth rate	Control	T1	T2
Weight (g/day)	0.27 ± 0.09^a	0.71 ± 0.21^b	0.59 ± 0.19^b
Length (cm/day)	0.06 ± 0.02	0.09 ± 0.03	0.09 ± 0.03
Width (cm/day)	0.04 ± 0.01	0.06 ± 0.02	0.06± 0.02

Note: Values are presented as mean ± standard deviation (n = X). Different superscript letters (a, b) within rows indicate significant differences ($p < 0.05$)

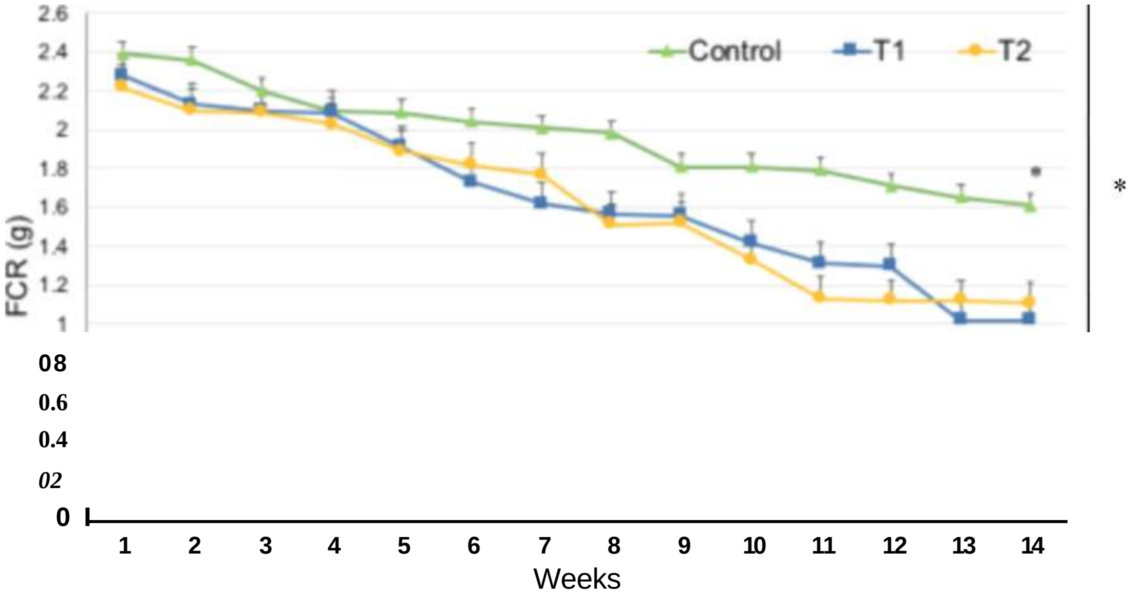
According to the Table 4.2, higher AGR of weight were recorded in group of fish fed with *L. plantarum* BE7 (0.71g ± 0.21 g/day) and *L. paracasei* BUM6 (0.59g ± 0.19 g/day) with a significance difference value compare to the group of that fed without probiotic (0.27 ± 0.09 g/day) ($p<0.05$) as indicated by the different superscript letters. In contrast, no significant differences were observed among treatments for length and width growth rates, although both probiotic-treated groups showed numerically higher values than the control. These findings suggest that probiotic supplementation primarily enhanced somatic weight gain rather than linear or lateral growth, indicating improved feed utilization and nutrient assimilation in the treated groups.

Even the previous studies about the effect of *L. plantarum* BE7 and *L. paracasei* BUM6 specifically toward the AGR of weight, length, and width of fish in aquaculture is very limited, any strain of probiotic applied as dietary supplementation was proved able to impact the metabolic processes, intestinal health, and nutrient absorption of the fish in a good way. Pacheco et al. (2022) revealed that fish supplemented by probiotic supplements displayed greater numbers of lactic acid bacteria and lower levels of pathogenic bacteria, which may provide a better gut environment that support growth and development at an optimum rate. In addition, Ferdous et al., (2025) also discovered that fish treated with probiotics had increased expression of growth hormone (GH) and insulin-like growth factors (IGFs), which further supported growth performance.

Probiotics are known to increase intestinal absorption efficiency and digestive enzyme activity, which promotes muscle accretion and energy storage (Nguyen et al., 2019). Therefore, the selective increase in somatic weight gain without significant changes in length or width may be attributed to improved nutrient assimilation and protein deposition rather than accelerated skeletal growth. Similar results have been shown in tilapia and other farmed fish, where probiotic administration mainly increased body mass through improved metabolic efficiency and feed consumption rather than linear growth (Yusoff et al., 2021; Ferdous et al., 2025). Increased somatic weight gain alone is considered a reliable indicator of probiotic efficacy, as probiotics primarily enhance digestive efficiency and nutrient absorption rather than skeletal growth, a pattern commonly reported in probiotic-fed fish (Nguyen et al., 2019;

4.3.4 Feed Conversion Ratio

According to the Figure 4.6, it can be observed that the trendline of FCR value for fish in all groups dropped from the 1st week until the 14th week of feeding administration with probiotic and without probiotic. The FCR for the fish fed with *L. plantarum* BE7 and *L. paracasei* BUM6 were recorded significantly lower than the FCR offish in the control group ($p<0.05$). However, between probiotic treated groups, the fish fed with *L. plantarum* BE7 was recorded have a lower FCR value than the fish fed with *L. paracasei* BUM6. The result showed that fish fed with *L. plantarum* BE7 had the highest feeding efficiency followed by the fish fed with *L. paracasei* BUM6, and the fish in the control group. Along the feeding administration for 14 weeks, significant difference of FCR between all group can be observed occurred starting at 6th week of experiment.



"igure 4.6 FCR of Red Hybrid Tilapia Fed with Probiotic- Supplemented Feed with *L. plantarum* BE7 (Tl), *L. paracasei* BUM6 (T2), and without Probiotic (Control) for 14 Weeks. * Shows Significance Difference Between Group ($p<0.05$)

The aquaculture industry has identified feed conversion ratio as an essential requirement for attaining efficient production to satisfy the expanding global population since it is highly connected to the feeding quality and feeding behavior of the farmed fish. With a better feeding efficiency, fish farmers in aquaculture can strategize the entire feeding system such as the brand of fish feeds, the feeding time and the most crucial aspect in feeding administration is the feeding rate. In this study, the feeding rate selection was 5% of the total bodyweight of the fish. According to research, this feeding rate is a good option for aquaculture procedures as it achieves an optimal balance between growth and resource consumption. Studies suggest that a 5% feeding rate considerably boosts growth performance in red tilapia, with ideal results found in numerous trials (Deyab and Hussein, 2015). For instance, tilapia fingerlings fed at this rate displayed increased feed conversion ratios and body composition compared to lesser rates (Rahman et al., 2023). Fish health is not endangered by this feeding approach, as evidenced by the good survival rates of tilapia at various feeding rates, including 5% (Deyab and Hussein, 2015). Because it corresponds with the fish's metabolic requirements, feeding at 5% of body weight promotes improved nutritional absorption and development. Additionally, this rate optimizes feed utilization and reduces waste, both of which are critical for sustainable aquaculture practices.

Fish in the probiotic-treated group demonstrated lower FCR readings compared to the fish that were fed without probiotics might be because of the presence of the beneficial effects offered by the probiotic inclusion in their dietary feedings. Probiotic strains like *L. plantarum* and *L. paracasei* have been shown to improve the feed conversion ratio (FCR) in tilapia through various mechanisms. First and foremost, probiotics as dietary supplementation may enhance the activity and efficiency of digestive enzymes such as protease, amylase, and lipase in the gut tracts, hence improving feed digestion and enhance nutrient absorption directly from the feed given (Wangkahart et al., 2024). Furthermore, the administration of probiotics also may modify the gut microbiota composition, which may increase the numbers of beneficial bacteria that can outcompete the pathogens for the attachment site along the fish’s gut lining, supporting a healthy gut environment for optimal digestion activity (Hossain et al., 2022). Plus, probiotics may enhance the innate immune response, resulting in lower stress levels and enhanced health, which can contribute to greater feed consumption (Nguyen et al., 2019). The combination of improved gut health and reduced stress levels among the cultured fish supported optimal feeding behavior, more efficient feed digestion, and enhanced nutrient absorption, resulting in a lower amount of feed required for body mass gain. These findings indicate that fish in both probiotic-treated groups utilized the supplied feed more efficiently, as reflected by their lower feed conversion ratio (FCR) values compared to fish in the untreated control group.

In this study, several controlled feeding methods were applied to ensure that the feed conversion ratio (FCR) accurately reflected actual feeding efficiency. During the feeding period, fish were fed at a fixed feeding rate of 5% of total body weight over a 14-week cultivation period to ensure consistent and controlled feed input. Feeding was conducted twice daily (morning and evening), with the daily ration divided equally between the two feeding events and spaced approximately six hours apart to allow sufficient digestion time, promote optimal nutrient absorption, and prevent excessive feed intake in a single feeding session. This feeding strategy minimized feed residue accumulation that could otherwise deteriorate water quality in the culture system. In addition, fish were fed using the apparent satiation method, whereby feed was administered gradually to allow active consumption, and feeding was terminated once feeding activity slowed or uneaten pellets began to sink. This approach minimized the residence time of uneaten feed in the water, thereby reducing nutrient leaching and pellet disintegration prior to removal, and improving the accuracy of FCR estimation

In aquaculture, lower FCR value in aquaculture is making the culturing system become more cost-effective. Rearing culture with a lower FCR value indicates a better feed utilization and less feed is used for the fish to convert into body mass, therefore it will reduce the feeding cost needed. A culturing system with a lower FCR value only requires a lesser amount of feed to produce an output volume. The uneaten feeds are one of the biggest factors that pollutes the optimum water quality in a rearing culture. By improving the feeding efficiency, it will reduce the volume of organic matter from the feed that may pollute the water through the decomposition process; thus, better water quality will be obtained. It can be concluded that, lower FCR value not only benefits the economic viability side, it also gives a huge positive impact toward the environmental sustainability. Therefore, the probiotic inclusion of both local strains, *L. plantarum* BE7 and *L. paracasei* BUM6 as supplementary diet of the fish were demonstrated able to improve the feeding efficiency among the cultured fish.

4.4 Water Quality Analysis

Water has become one of the most important value in culturing fish activities. The water quality play huge role for an effective culturing system because it may impact the cultured fish overall development, health, and total production (Devi et al., 2017). Maintaining ideal water conditions is critical for avoiding stress development and disease infection in fish, which can result in considerable economic losses in aquaculture. Among the fish farmers, few parameters that are frequently checked on during maintaining water quality are the temperature, pH levels, dissolved oxygen, ammonia, and total dissolve solids. The highlighted parameters are very vital because it have a direct impact on fish performance and environmental sustainability (Zhang et al., 2025). By ensuring a good water setting in a rearing culture system, it may guarantee the overall welfare of cultured fish while promising a better productivity at the same time (Devi et al., 2017). In this study, the water quality parameters of red hybrid tilapia fed with probiotics and without probiotics were observed every week for 14 weeks to investigate either the presence of probiotic in the fish diets may impact the optimum water quality for the fish to grow.

4.4.1 Temperature

Based on Figure 4.7, all groups showed an average water temperature of 25°C starting from the 1st week until 14th week of feeding administration. The water temperature of red hybrid tilapia in all groups were recorded on the lower limit of the optimum temperature for the red hybrid tilapia to grow which is 25°C to 32°C (Tran et al., 2022). No fluctuation of temperature was recorded during 14 weeks of feeding regimens, which indicated that the inclusion of probiotics as dietary supplementation for the probiotic-fed fish group did not affect the optimal water temperature for the red hybrid tilapia to live. No significance difference was recorded for the water temperature offish in all groups ($p>0.05$). Pandit and Nakamura, 2020 studied that the growth rates of red hybrid tilapia fish peak between 30°C and 31°C, with considerable gains in feed conversion and survival rates reported in this temperature range and the tilapia fish exhibit the fastest growth at 31°C. In contrast, Al-Harbi et al., (2016) had stated that while higher temperatures can enhance growth, they may also elevate stress and disease risks with high temperatures around 33°C increase susceptibility to infections like *S. agalactiae.*

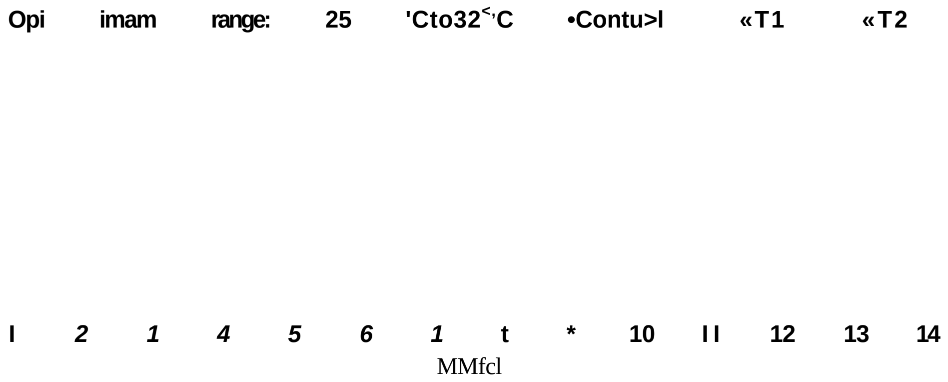


Figure 4.7 Temperature of Red Hybrid Tilapia Fed with *L. plantarum* BE7 (Tl), *L. paracasei* BUM6 (T2), and Control for 14 weeks.

Temperature is an important environmental component that affects the development, metabolism, immunity, and overall health of red hybrid tilapia. Red hybrid tilapia, a tropical freshwater fish, thrives at water temperatures ranging from 25°C to 32°C (Tran et al., 2022). Deviations from this ideal range might cause stress, decreased feed intake, slower development rates, and an increased susceptibility to illness. Therefore, it is very crucial to maintain the consistency of an ideal temperature throughout cultivation process to ensure higher output, fish health and preventing economic losses due to disease outbreaks in red hybrid tilapia farming.

An optimal temperature ranges differ between fish species, influencing metabolic processes, gene expression, and physiological features (Mekonnen et al., 2025). According to the findings, incorporating both probiotic strains, *L. plantarum* BE7 and *L. paracasei* BUM6, into the fish diet did not alter the optimal water temperature throughout the feeding period, indicating that their inclusion may exert a positive influence on maintaining stable thermal conditions for the cultured fish. Previous studies showed that the probiotics supplementation in the diet of fish might help them to survive in unfavorable temperature changes by strengthening the digestive health of the fish. Dawood *et al.,* 2020 stated that the inclusion of probiotic into the gastrointestinal tract of the fish may boost the digestive enzyme activity, which is critical for higher nutritional absorption especially at lower temperatures where digestive efficiency might be dropped. Furthermore, Torres-Maravilla et al., 2024 stated that probiotics such as *L. paracasei* may help reduce fish stress responses during temperature fluctuations. Lower stress levels can contribute to more stable metabolic activity, which may indirectly help maintain consistent internal and environmental conditions.

4.4.2 pH

Based on Figure 4.8, the pH of water in all groups were recorded within the optimum pH for red hybrid tilapia to grow which in a range between 6.5 to 9.0. However, on 7th week of feeding administration, the water pH of fish in the control group tank (highlighted in the red box) was recorded dropped lower than the average reading throughout the week maybe because of the increased in water acidity from nitric acid production that may come from the decomposition process (Datta, 2012). Aside from that, no fluctuation of pH occurred between all groups during the feeding period resulting in no significance difference for the pH reading. According to Fabay et al., 2020, keeping a pH within an optimum value is critical for the proper cultivation for red hybrid tilapia species because it enhance optimal physiological function, efficient oxygen uitilization, reduced ammonia toxicity, and promote stable microbial ecosystem.

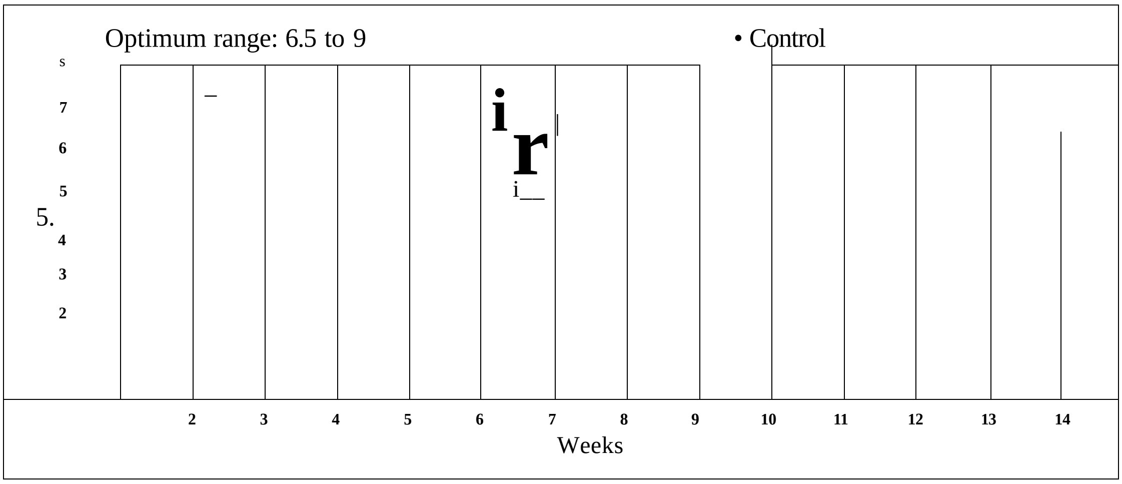


Figure 4.8 pH of Red Hybrid Tilapia Fed with *L. plantarum* BE7 (Tl), *L. paracasei* BUM6 (T2), and Control for 14 Weeks.

In aquaculture, the pH reading of water culture system is critical for sustaining overall water quality and general health of cultured fish. Red hybrid tilapia is known as a resilient species that can handle a pH range of 6.5 to 9.0, although best development and survival occur in the narrower range of 7.0 to 8.0 (Heba et al., 2013). When water pH goes outside of this range, it can induce physiological stress, disrupt osmoregulation, lower feed efficiency, and weaken the immune system, leaving the fish more prone to illness (Abdel-Tawwab et al., 2019). Hence, monitoring optimum water pH may promote healthy growth and development and increase feeding efficiency in rearing culture.

In this study, the addition of both probiotic strains in the dietary feedings of fish in both treatment groups was observed did not cause any significant changes to the optimum pH levels for the red hybrid tilapia to grow and survive through feeding period thus showing that the probiotics may give potential benefits towards the water pH instead. The fluctuation of pH may be affected by few factors including the ammonia level in water rearing culture. Parvathy et al., (2023) stated that the accumulation of ammonia and organic matters from the uneaten feeds and feces are detrimental for aquatic life as it changed the optimum pH balance for the fish. Moreover, ammonia accumulation from waste may cause intoxication to the cultivated fish which may cause convulsions, coma, and death to occur if the poor water quality is not properly treated. This is most likely due to elevated levels of NH4 , which can depolarize neurons and displace K ions, leading to the activation of NMDA-type glutamate receptors. This activation results in an excessive influx of Ca^{2} , ultimately causing neuronal cell death in the central nervous system (Chapra et al. 2021).

Hence, by applying probiotic into the fish diet, it may break down the toxic substances which is the accumulated ammonia into amino acids and other nitrogenous compounds through fermentation process which is safer and more degradable compare to ammonia (Parvathy et al. 2023). Furthermore, Lili and Permana (2022) stated that this may happened because the probiotics are able to generate enzymes like proteases and cellulases, which may help break down complex harmful nitrogenous substances. However, supplementation of red hybrid tilapia with *L. plantarum* BE7 and *L. paracasei* BUM6 did not directly improve the water quality. Unlike probiotic from genus *Bacillus* which known as nitrifying bacteria that able to directly degrade ammonia and nitrate, *L. plantarum* BE7 and *L. paracasei* BUM6 may enhance the water quality by different approach. Since *L. plantarum* BE7 and *L. paracasei* BUM6 are both probiotic strains that classified under LAB, primarily gut probiotics that colonize the intestinal tract of the red hybrid tilapia, they enhance the water quality of the rearing culture through feed digestion and nutrient uptake improvement. Nguyen et al, (2018) demonstrated that supplementing Nile tilapia with *L. plantarum* did not cause any significant impact on the optimal level of pH and ammonia due to better feed efficiency and nutrient uptake. Abdelhamid et al. 2020 also mentioned that *L. paracasei* inclusion improved intestinal villi morphology and upregulated digestive enzyme activity thereby maintain the water quality through nutritional and immune modulation.

4.4.3 Dissolve Oxygen

Based on Figure 4.9, DO value of water in all group were showed within a healthy range that may support fish growth and development which was above than 5 mg/L. Deyab and Hussein, (2015), stated that red hybrid tilapia require right DO levels, usually greater than 5.5 mg/L, to sustain respiration, digesting, and immunological function. No significant difference was recorded between the DO value of water in all groups, indicating that the inclusion of probiotics into the dietary feedings in the probiotic-fed fish group did not cause any significant impact on the DO value. Throughout the feeding period, a rise in water temperature maybe reduce the DO in water as oxygen molecules travel quicker and disperse into the atmosphere, leaving less oxygen in the water for fish to respire (Boyd, 2019). Plus, the decomposition of decaying fish may deplete oxygen in the water itself, limiting the quantity of oxygen accessible to the fish (Chapra et al., 2021). These two factors might be the strong reason there were differences in DO value between each group.

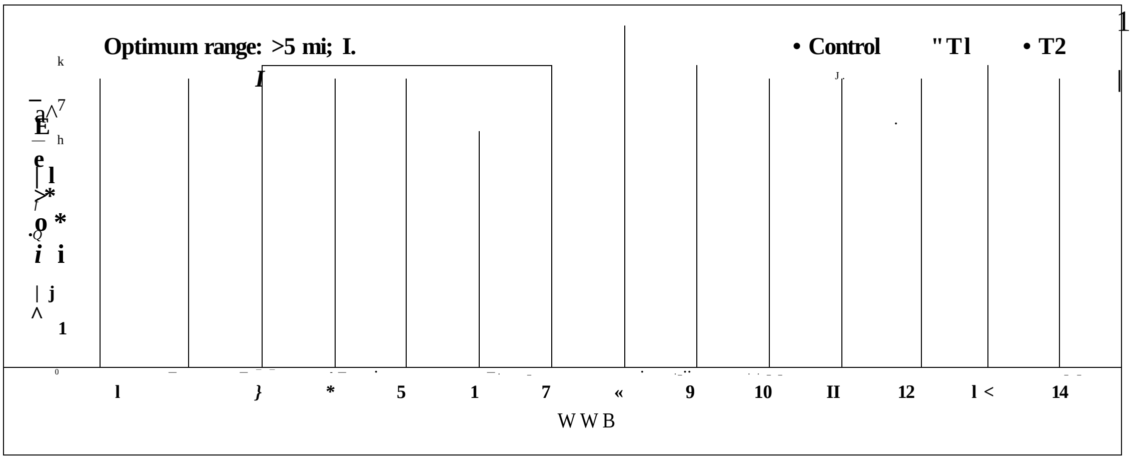


Figure 4.9 Dissolved Oxygen of Red Hybrid Tilapia Fed with *L. plantarum* BE7 (Tl), *L. paracasei* BUM6 (T2), and Control for 14 Weeks.

Dissolved oxygen (DO) is also among the important water quality indicators in aquaculture, influencing the survival, growth, and metabolic efficiency of red hybrid tilapia. During culturing fish, an insufficient oxygen levels can cause stress, decreased feeding activity, slower development rates, and an increased susceptibility to illness and death. In severe circumstances, hypoxic conditions can result in massive deaths, particularly in highly packed systems or at night when oxygen use exceeds production. Thus, maintaining appropriate dissolved oxygen levels through effective aeration and water management is critical for sustaining high output and healthy stock in red hybrid tilapia aquaculture.

Throughout the experiment, inadequate amount of DO also can be detected through observing the fish behavior itself despite using specific DO meter. The fish in tank that have lower DO water value can be observed were struggled to breath and gasp on the surface of the water. Bulbul Ali and Mishra (2022) highlighted that this situation maybe occurred because the fish tried to require more oxygen above the surface of the water since the amount of oxygen inside the tank insufficient and depleted. Monitoring an optimal DO level in aquaculture setting is very crucial since higher DO levels correspond with decreased stress levels in fish, which is critical for their general health and growth performance while lower DO levels can lead to stress and mortality in cultured species, affecting profitability in aquaculture. For example, DO levels below 3 mg/L have been proven to impair feed intake and growth in Nile tilapia (Sriyasak et al., 2015). A few studies showed that probiotic treatment able to improve the quality of the dissolve oxygen in the fish rearing culture.

Nathanailides et al. 2021 stated that since the probiotics may improve the feeding efficiency of cultured fish, there may be less waste and uneaten feeds in the water setting, which might consume oxygen to decompose resulting in lower DO. Bahnasawy *et al.,* 2020 studied that adding 200 ppm of *L. plantarum* increased dissolved oxygen levels to 9.02 mg/L and decreased dangerous ammonia levels of Nile tilapia culture. Furthermore, Surkatti et al., 2022 showed that inclusion of lactic acid bacteria into the water may help to break down organic molecules therefore reduced the biochemical oxygen demand (BOD) in wastewater environments. *L. paracasei* specifically may reduce nitrite concentrations in aquaculture systems, which can deplete oxygen levels and impair aquatic life (Thom and Bai., 2024). However, no sign of evidence showing that the probiotics in dietary feedings of fish in probiotic-fed group did not cause changes in optimum DO value for the fish to grow, thereby proves that the utilization of probiotics for culturing fish is safe and recommended.

4.4.4 Total Dissolve Solids

Based on Figure 4.10, the TDS values of water in all groups were showed below than 140 ppm. In aquaculture, maintaining an ideal TDS value in water for red hybrid tilapia fish cultivation is very important for growth development and survivability. Tadeo and Malaya (2021) mentioned that TDS values between 250 and 1000 mg/L are appropriate for controlled systems and up to 2000 mg/L for pond cultures. These ranges assist guarantee optimal osmoregulation, metabolic efficiency, and overall well-being for the fish. Based on the result, it showed that the TDS value for each group throughout the experimental period were below than the optimal value for the red hybrid tilapia to grow and survive in a controlled setting. Unlike TDS, lower TDS value of water do not cause negative impact towards the health of cultured organism. This is because red hybrid tilapia is well known as a tough fish species capable of surviving in low TDS water, particularly in freshwater settings where minerals are still present in low levels (Mohamad et al., 2021). However, according to Chen (2022), monitoring TDS within an optimum range is highly recommended since a TDS value much lower than 100 mg/L may reduce the fish performance in aquaculture systems due to insufficient minerals in the water.

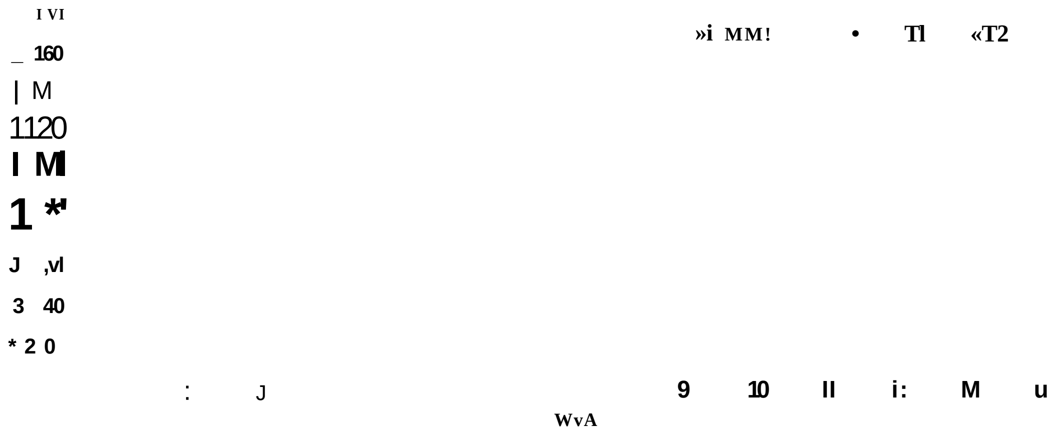


Figure 4.10 Total Dissolve Solids of Red Hybrid Tilapia Fed with *L. plantarum* BE7 (Tl), *L. paracasei* BUM6 (T2), and Control for 14 Weeks.

TDS are the concentrations of inorganic salts and tiny organic matter dissolved in water, which include calcium, magnesium, sodium, potassium, bicarbonates, chlorides, and sulphates. Maintaining adequate TDS levels during red hybrid tilapia growth is critical for guaranteeing consistent water quality and good physiological performance. Tilapia are slightly adaptable to a wide range of TDS, although high or low concentrations can disturb osmoregulation, stress the fish, and impair growth performance. High TDS levels can also impair gill function, restrict oxygen intake, and enhance the toxicity of specific chemicals like ammonia and heavy metals. There are few factors contributing to the fluctuated TDS reading during fish cultivation period such as the accumulation of waste from uneaten feeds and through excretion process.

Uneaten feed decomposes over time, releasing organic and inorganic particles into the water, whilst fish metabolism generates waste products such as ammonia, urea, and other soluble components may intoxicated the fish. According to Tucker and Boyd (2012), these processes not only increase TDS, but they may also degrade water quality if not effectively controlled, compromising fish health, growth performance, and system stability. Hence, the healthy reading of TDS was controlled by replacing 20 to 30% of the total volume of water twice a week with a new aerated dechlorinated water to ensure survivability and fish optimal feeding (Timmons and Ebeling, 2010). Through the observation, as the water become cloudy the feeding become slower resulting in lots of uneaten food on the surface of the water that may contributed to poor TDS value. Therefore, by changing the water regularly, it may prevent the fish from stress by removing the water odour and discoloration, maximizing oxygen intake and replenishing used minerals.

The inclusion of probiotics in the diet of fish treatment groups did not cause any significant change towards the TDS value therefore it can be proved that probiotic able to maintain the optimum TDS value and did not cause any negative impact towards the parameter since no significance difference between TDS of water from all groups. According to Yu et al., (2021), probiotic as feed additives may able to increase gut health, therefore enhancing digestion and nutrient intake. This may happen maybe because, better feeding efficiency may result in lower amount of uneaten feeds produced therefore decrease the TDS levels. Since probiotic was able to improve fish immunity, it may give the fish with a stronger resistance and adaptation with any fluctuate changes in water quality that may cause mortality to easily occur (Timmons and Ebeling, 2010). According to Yu et al., (2021), high mortality increased the rate of decomposition process resulting in higher TDS value that may lower the water quality for the cultured organism to grow efficiently.

4.5 Pathogen Challenge

The pathogen challenge is an important tool for assessing the efficacy of probiotics in improving disease resistance in fish species, notably against common aquaculture pathogens. In this research, the survival rate of fish post-challenge was an important sign of the protective effects of probiotic supplements. Clinical signs and symptoms such as erratic swimming, skin lesions, exophthalmia, and haemorrhages were used to determine the severity of the illness. Furthermore, histopathological examination of critical organs such as the brain, eye and liver were performed to detect internal tissue damage and inflammatory reactions caused by the infection. Comparing the outcomes of probiotic-fed and non-probiotic-fed fish revealed important information on the role of probiotics in increasing immunological defence, reducing pathological changes, and improving overall survival during pathogenic stress.

4.5.1 Survival Rate

The survival rate of red hybrid tilapia against fish pathogens is a crucial indicator of fish health and disease susceptibility in aquaculture. Monitoring survival rates helps to determine the effectiveness of disease prevention and treatment strategies, as well as the overall resilience of fish to pathogenic stress. Factors such as immune response, environmental conditions, and management practices strongly influence survival outcomes in aquaculture systems. Therefore, evaluating the survival performance of red hybrid tilapia following pathogenic challenges provides valuable insights for developing more sustainable and productive aquaculture practices. Thus, survival rate analysis is essential for ensuring the long-term health and performance of this economically important species.

According to Figure 4.11, the survival rate of fish in all groups decreased throughout the seven days post-challenge with *S. agalactiae*. However, the onset of mortality differed among treatments. The survival rate of fish fed with *L. plantarum* BE7 began to decline on the fourth day of pathogen challenge, later than fish fed with *L. paracasei* BUM6 and those in the control group, which showed a decline as early as the second day of exposure. At the end of the challenge period, the highest survival rate was recorded in fish fed with Z. *plantarum* BE7 (61.1%), followed by fish fed with Z. *paracasei* BUM6 (44.4%) and the control group (33.3%). These results indicate that dietary supplementation with Z. *plantarum* BE7 in Treatment 1 enhanced disease resistance and provided better protection against *S. agalactiae* infection compared to Z. *paracasei* BUM6.

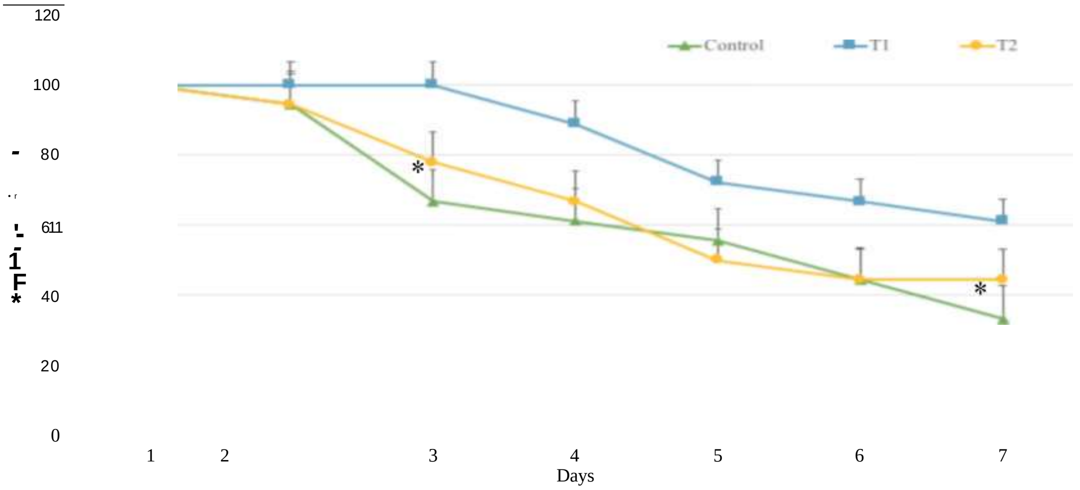


Figure 4.11 Survival Rate of Red Hybrid Tilapia Fed with *L. plantarum* BE7, Z. *paracasei* BUM6, and Fish Feed Only (Control) Challenged with *S. agalactiae* for Seven Days. * Shows Significance Difference between Group ($p<0.05$)

The improved survival observed in fish fed with *L. plantarum* BE7 may be attributed to the successful colonisation of this probiotic strain in the fish gut, which likely contributed to enhanced immune modulation and inhibition of pathogen proliferation. Effective gut colonisation by probiotics allows beneficial bacteria to compete with pathogens for adhesion sites and nutrients, strengthen intestinal barrier function, and stimulate host immune responses, thereby improving the ability offish to resist infections and increasing survival rates.

The enhanced disease resistance provided by *L. plantarum* BE7 may also be related to its strong antimicrobial properties and immune-stimulating effects. The probiotic antagonistic assay showed thatZ. *plantarum* BE7 produced a larger inhibition zone against *S. agalactiae* compared to *L. paracasei* BUM6, indicating stronger antagonistic activity. This suggests that gut colonisation by *L. plantarum* BE7 may suppress pathogen growth within the host, thereby improving survivability. Kumaree et al. (2015) reported thatZ. *plantarum* exhibits strong antimicrobial activity against *S. agalactiae* and other pathogens such as *Salmonella* and *E. coli.* In addition, Zhang et al.

(2019) highlighted thatZ. *plantarum* is increasingly used as a sustainable alternative to antibiotics in aquaculture. These findings indicate that *L. plantarum* BE7 has strong potential as a probiotic candidate for controlling *S. agalactiae* outbreaks and enhancing fish survival in aquaculture systems.

Figure 4.12 shows that survival rates in all groups decreased over the seven-day challenge period with *A. hydrophila.* Mortality began on the third day post-infection, and noticeable differences in survivability were observed among treatments from day three until day seven. At the end of the challenge, the highest survival rate was recorded in fish fed with *L. paracasei* BUM6 (55.6%), followed by fish fed with *L. plantarum* BE7 (44.4%)) and the control group (38.9%>). Overall, both probiotic-fed groups exhibited higher survival rates than the non-probiotic control group, indicating that probiotic supplementation enhanced disease resistance against *A. hydrophila.* These results suggest that dietary inclusion of *L. paracasei* BUM6 in Treatment 2 improved the protective capacity offish and contributed to higher survival.

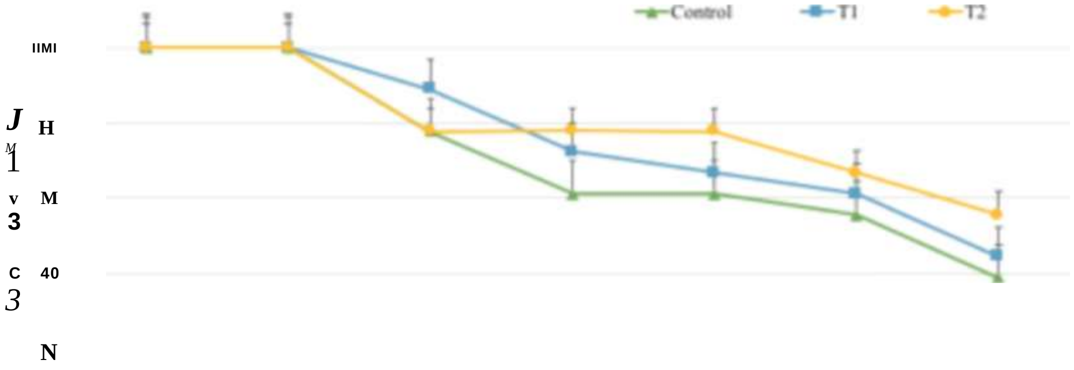


Figure 4.12 Survival Rate of Red Hybrid Tilapia Fed with *L. plantarum* BE7, *L. paracasei* BUM6, and Fish Feed Only (Control) Challenged with A *hydrophila* for Seven Days. * Shows Significance Difference between Group (p<0.05)

Although studies on *L. paracasei* BUM6 in aquaculture are limited, previous research on similar *L. paracasei* strains supports these findings. Van Doan et al. (2021) reported significantly higher survival rates in Nile tilapia fed with *L. paracasei* l61-27b during *A. hydrophila* exposure, particularly at higher probiotic concentrations. Their results demonstrated survival rates of 63.33%, 66.67%, 80.00%, and 83.33% at concentrations of 10 , 10 , 10 , and 10 CFU/mL, respectively. Although the experimental period was shorter, these findings support the results of this study, indicating that supplementation with *L. paracasei* BUM6 at 10 CFU/mL enhanced disease resistance and improved survival in red hybrid tilapia.

The beneficial effects of *L. paracasei* BUM6 may also be associated with its ability to colonise the intestinal tract and modulate host immunity. The probiotic antimicrobial assay showed that *L. paracasei* BUM6 produced a larger inhibition zone against *A. hydrophila* compared to *L. plantarum* BE7, suggesting stronger antagonistic activity. Nayak (2010) reported that *L. paracasei* enhances innate immune responses by increasing phagocytic activity, lysozyme production, and immune-related gene expression. Furthermore, *L. paracasei* improves intestinal barrier function by reducing pathogen adhesion and colonisation while promoting a balanced gut microbiota (Merrifield et al., 2010). These combined mechanisms indicate that effective gut colonisation by *L. paracasei* BUM6 likely contributed to enhanced resistance against *A. hydrophila*, reduced tissue damage, and ultimately higher survival rates during pathogen challenge (Newaj-Fyzul et al., 2014).

4.5.2 Clinical Signs and Symptoms

Based on the result, several clinical signs were observed among f fish in all groups during the challenged period *with S. agalactiae* and *A. hydrophila*, including an abnormal swimming pattern, loss of buoyancy, and isolation from schooling groups. For the clinical symptoms, less severe lesions were observed among the fish in group of fish fed with probiotic compare to the fish in control group. Clinical symptoms shown by the fish after being challenged including skin discoloration, haemorrhages around fins, mouth and operculum area, and corneal opacity. Kumar et al., (2025) reported that the fish that suffered from erratic swimming pattern and loss of buoyancy might be occur as an indicator of pathogenic infection into the central nervous system that might causing the motor neurons unable to support the normal locomotive function. Consequently, the infected fish were observed struggling to swim normally and the ability to float while feeding become poor (Turner et al., 2023).

The observation and reporting of clinical signs and symptoms in red hybrid tilapia following pathogen exposure is critical in aquaculture disease investigations because it provides early markers of illness onset, severity, and progression. Clinical signs such as abnormal swimming behaviour, exterior lesions, haemorrhages, and organ swelling represent the fish's physiological and immunological state, allowing researchers to examine the pathogenic influence and efficacy of nutritional or therapeutic treatments. Careful evaluation of these clinical indicators not only assists in the diagnosis of specific illnesses, but also leads to a better understanding of host-pathogen interactions in experimental settings.

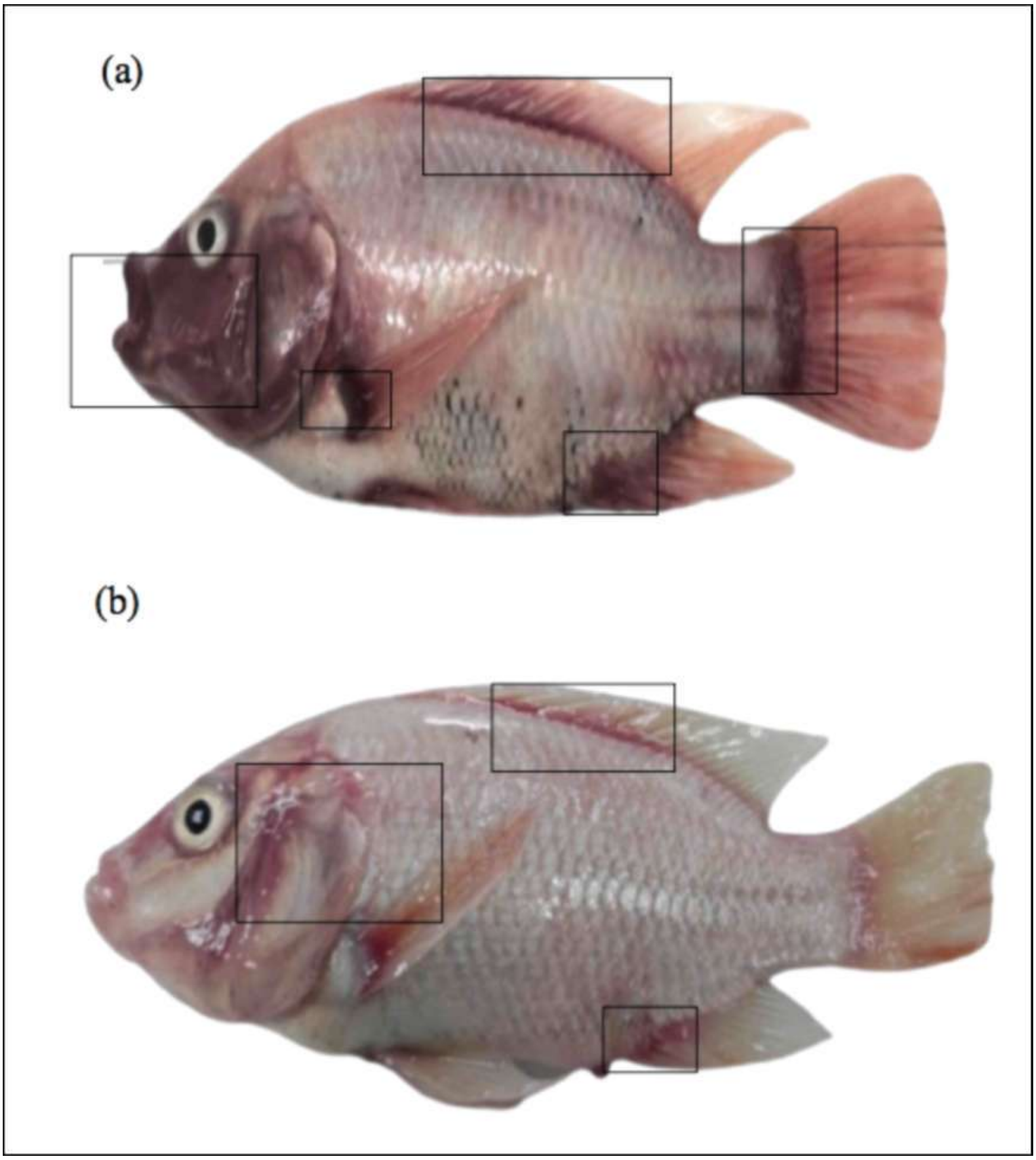


Figure 4.13 (a) Representative of Red Hybrid Tilapia from Control Group Challenged with *S. agalactiae* Showing Severe Hemorrhage Around Multiple Areas Around Body, Mouth, Operculum, Gills, and Fins Area (Box), (b) Representative of Red Hybrid Tilapia Fed with *L. plantarum* BE7 Challenged with *S. agalactiae* Showing Mild Hemorrhage Around Operculum and Fins Area (Box).

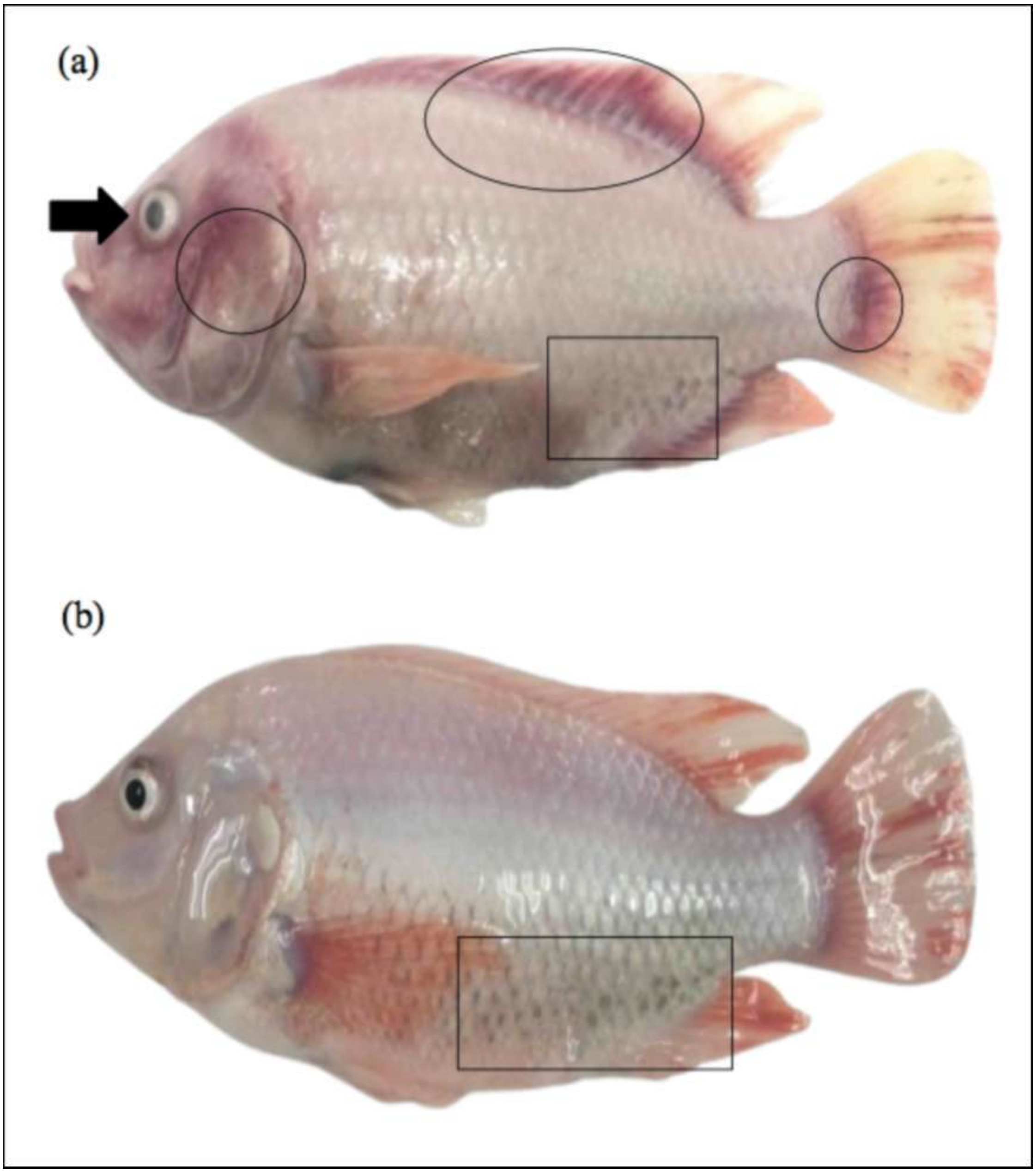


Figure 4.14 (a) Representative of Red Hybrid Tilapia from Control Group Challenged with *A. hydrophila* Showing Hemorrhage Around Operculum, Dorsal, Anal, and Caudal Fin (Circle), Detachment of The Scales (Box), and Corneal Opacity (Thick Arrow), (b) Representative of Red Hybrid Tilapia Fed with *L. paracaseiBUM6* Challenged with *A. hydrophila* Only Showing Skin Darkening on The Ventral Side of The Fish Body Between Anal Fin and Pelvic Fin (Box).

Each fin in fish anatomy serves a specialized function that collectively supports efficient and stable swimming. The dorsal fins play a crucial role in aiding rapid turns, sudden stops, preventing body rolling, and maintaining overall balance (Kumar et al., 2025). Alongside the dorsal fins, the anal fins act as stabilizers, minimizing excessive rolling and pitching to help the fish maintain orientation and swimming accuracy (Giammona, 2021). Recent findings further highlight the importance of coordinated fin movements, where synchronized activity among the dorsal, anal, and caudal fins enhances propulsion and maneuverability (Howard, 2019). The caudal fin, in particular, is the principal propulsive structure responsible for generating thrust and forward motion (Du et al., 2022). When these fins are compromised, such as through haemorrhages or tissue damage caused by pathogenic infections, fish may experience a significant loss of locomotor efficiency. This impairment in fin function is a major factor contributing to the abnormal swimming behavior and reduced mobility observed in infected fish.

Severe hemorrhages around the operculum, mouth, and fins signify a systemic infection with serious pathological changes. The bacterium's virulence components, specifically its capsular polysaccharide might be contributed to its pathogenicity, causing widespread tissue damage and inflammation in the fish body. According to the previous histological assessments, severe haemorrhages are the visible signs of damaged tissues that exhibit dilated blood vessels, necrosis, and infiltration of inflammatory cells (Bisai *et al.,* 2025). Furthermore, corneal opacity also is one of the lesions at the eye area that were visible among the fish in control group. Corneal opacity is a condition where the eye appear as cloudy or white affecting the vision of the fish. This symptom was visible among the infected fish with *S. agalactiae* because of the fluid from the tissue leaks to the area of the eyeball. Ghetas *et al.,* (2021) mentioned that during the challenge period, the fish that suffered with this symptom showed reduce in appetite and tend to be isolated from the schooling group. Studies prove a significant infiltration of inflammatory cells in the meninges and ocular tissues, which contributes to ocular opacity.

Greater survival rate was observed among the red hybrid tilapia is likely attributed to the immunomodulatory effects of probiotic supplementation with *L. plantarum* BE7 and *L. paracasei* BUM6. Recent studies have shown that microencapsulated *L. plantarum* significantly boosts innate immune responses, including increased lysozyme activity and respiratory burst, leading to higher survival rates in Nile tilapia challenged with *S. agalactiae* (Torres-Maravilla et al., 2024). Additionally, probiotics demonstrated inhibitory activity against both *A. hydrophila* and *S. agalactiae*, suggesting their potential as natural alternatives to antibiotics in aquaculture (Nawawi et al., 2024). Furthermore, certain probiotic strains can disrupt quorum sensing mechanisms of *A. hydrophila*, reducing its virulence and enhancing fish survival rates during infection challenges (Abdullah et al., 2023). Last but not least, dietary inclusion of Lactobacillus species has also been associated with elevated levels of immune markers such as immunoglobulin M (IgM), lysozyme activity, and acid phosphatase, indicating strengthened immune responses crucial for combating infections during disease outbreaks (Torres-Maravilla et al., 2024).

Despite exhibiting clinical signs such as skin discoloration, hemorrhages, and erratic swimming during pathogen challenges, fish in the probiotic-treated groups demonstrated significantly higher survival rates and more rapid recovery compared to those in the control group. This enhanced resilience is attributed to the immunomodulatory effects of probiotics, which bolster the fish's immune response, improve gut health, and facilitate faster healing processes (Gupta et al., 2023). The application of probiotics in aquaculture not only reduces mortality rates but also minimizes the need for antibiotics, leading to cost savings and sustainable farming practices (El-Saadony et al., 2021). Consequently, incorporating probiotics into aquaculture systems can mitigate economic losses associated with disease outbreaks, ensuring consistent production and profitability for aquaculture businesses (Hasan et al., 2023).

Overall, the reduced severity and frequency of clinical signs observed in the probiotic-treated groups suggest that probiotic supplementation may play a protective role in mitigating disease manifestations in red hybrid tilapia. Fish receiving probiotic supplementation often exhibit milder clinical symptoms, reduced haemorrhages, improved swimming behaviour, and faster recovery compared to non-supplemented controls (Hasan et al., 2023). These findings support the present observations that dietary inclusion of *L. plantarum* BE7 and *L. paracasei* BUM6 may contribute to reduced clinical severity by enhancing immune resilience and physiological stability during bacterial infection challenge

4.5.3 Histopathological Examination

Histopathological study of the brain, eye, and liver in red hybrid tilapia infected with *S. agalactiae* and *A. hydrophila* is critical for determining the severity and pattern of tissue damage induced by bacterial infections. Microscopic examination of these organs allows for the discovery of pathological abnormalities such as neuronal degeneration, ocular inflammation, hepatocellular necrosis, and vascular congestion, all of which indicate systemic disease development. Infections with *S. agalactiae* frequently target the nervous system and eyes, but *A. hydrophila* regularly affects several internal organs, including the liver, therefore investigating these specific tissues may give vital insights into pathogen-specific disease processes. In addition to qualitative findings, semi-quantitative lesion scoring is important for assessing the severity of histological damage which range from (0 = unchanged, 1 = mild, 2 = moderate, 3 = severe) lesion. The information is critical for determining the severity of illness and assessing the efficacy of probiotic supplementation during bacterial challenge.

Figure 4.15 shows the histological structure of brain tissue of the fish post-challenged with *S. agalactiae.* Based on the brain histology of red hybrid tilapia in both probiotic-fed group and control group, few similarities in formation of lesions with varying degree of severity were observed between each group. Few lesions including brain edema, spongiosis, brain necrosis and infiltration of inflammatory cells were observed within the brain tissue. First, the significant lesion that can be observed was brain edema. Brain edema was formed due to an inflammatory response of host cell towards the invasion of *S. agalactiae* into the host central nervous system. The pathogen infection may had caused an initial inflammatory response, resulting in elevated levels of pro- inflammatory cytokines such as IL- 1β and TNF-α in the brain (Palang et al., 2020). Therefore, the infiltration of inflammatory cells into the brain might increase the permeability of vascular including blood-brain barrier and disrupt the normal flow of cerebrospinal fluid leading towards accumulation of fluid within the brain tissue (Palang et al., 2020). Based on the histological view, the indication of brain edema can be observed based on signs of swelling and vacuolation in the extracellular spaces of the brain histology. Weis et al., (2019) mentioned that the build-up of fluid increased intracranial pressure, which might impair blood flow and lead to ischemia where it is a condition of lack of oxygen supply to the brain tissue. Plus, prolonged absence of oxygen and nutrition can induce cell death, resulting in brain necrosis.

Brain tissue of fish from control group had significantly showed severe brain necrosis with a score lesion of 3. Due to the combination of direct bacterial invasion and inflammatory response of host cell towards *S. agalactiae*, brain necrosis that led to massive destruction of the brain architecture had occurred (Palang et al., 2020). However, in the brain tissue of fish from both treatment groups Figure 4.15 (T1) and Figure 4.15 (T2), no brain necrosis was observed. Vijayaram et al. (2025) stated that this may happen, maybe because of the presence of probiotics that might boost the neurotransmitters including serotonin and dopamine, which are essential for brain health and function against pathogen infection. Siripaopradit et al., (2024) mentioned that the probiotics inclusion may diminish the neuroinflammation by regulating cytokine levels, lowering pro-inflammatory indicators that may worsen the vacuolization formation and raising anti- inflammatory markers to prevent further damage of neurons. Vijayaram et al. (2025) stated that since the probiotic enhanced the gut microbiome of fish, which is critical for maintaining the gut- brain axis, increased microbiome diversity can lead to improved neuroprotection and cognitive outcomes.

Furthermore, the histological examination of brain also was showing localized spongiform changes. This lesion described as the structure of brain appear spongy under the microscope during histological analysis. In Figure 4.16 (C), the severity of brain spongiform in brain histology of fish in control group was observed severe with a lesion score of 3. However, less severe lesion was observed in brain tissue of fish fed with *L. plantarum* BE7 with a lesion score of 1 and *L. paracasei* BUM6 with a lesion score of 2. Based on the observation, the lining layer of the dura mater of the brain appeared loose and disintegrated compared to the brain tissue of fish in treatment groups, especially T1; the entire lining of the dura mater looks more visible and in a good structure under 400X magnification. The difference in severity of brain spongiosis in brain tissue of fish in both groups occurred maybe because of the probiotics may help the gut-brain axis by fostering a healthy gut microbiota, which contributes to structural integrity of the blood-brain barrier, lowering permeability and preventing neuroinflammation (Elbahnaswy et al., 2024). Bock et al., (2024) stated these probiotics may also modify the makeup of the gut microbiota, resulting in favorable metabolic changes that reduce brain spongiosis condition.

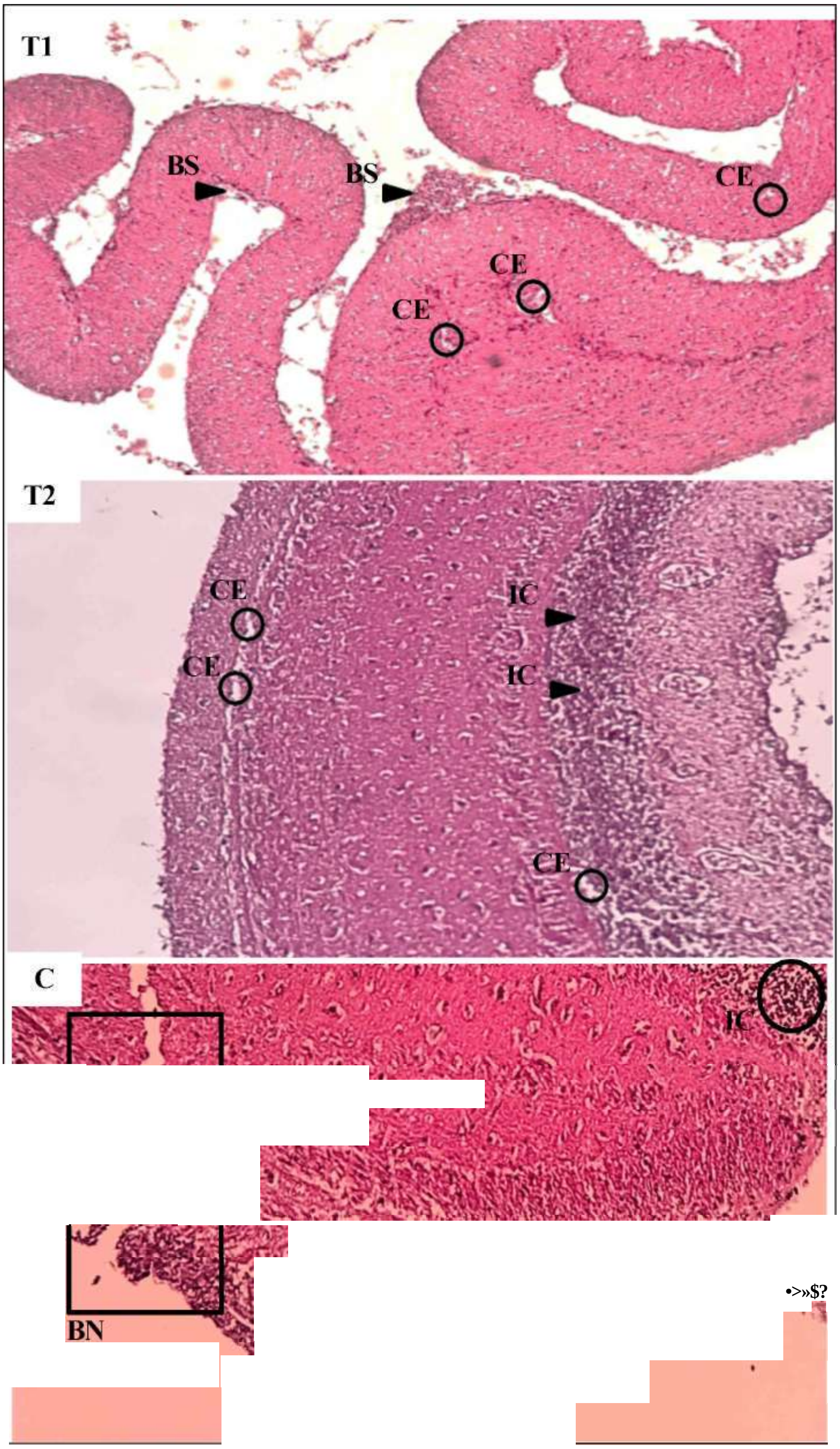


Figure 4.15 Histological Structure of Brain of Red Hybrid Tilapia Challenged with *S. agalactiae* (Tl) Brain Section Showing (CE) Cerebral Edema (Circle) and (BS) Brain Spongiosis (Head Arrow) (40X). (T2) Cerebral Edema (Circle) and Infiltration of (IC) Inflammatory Cells (Head Arrow) (100X). (C) Severe Necrosis (box), Severe Brain Spongiosis (Thick Arrow), and Infiltration of Inflammatory Cells (Circle) (100X).

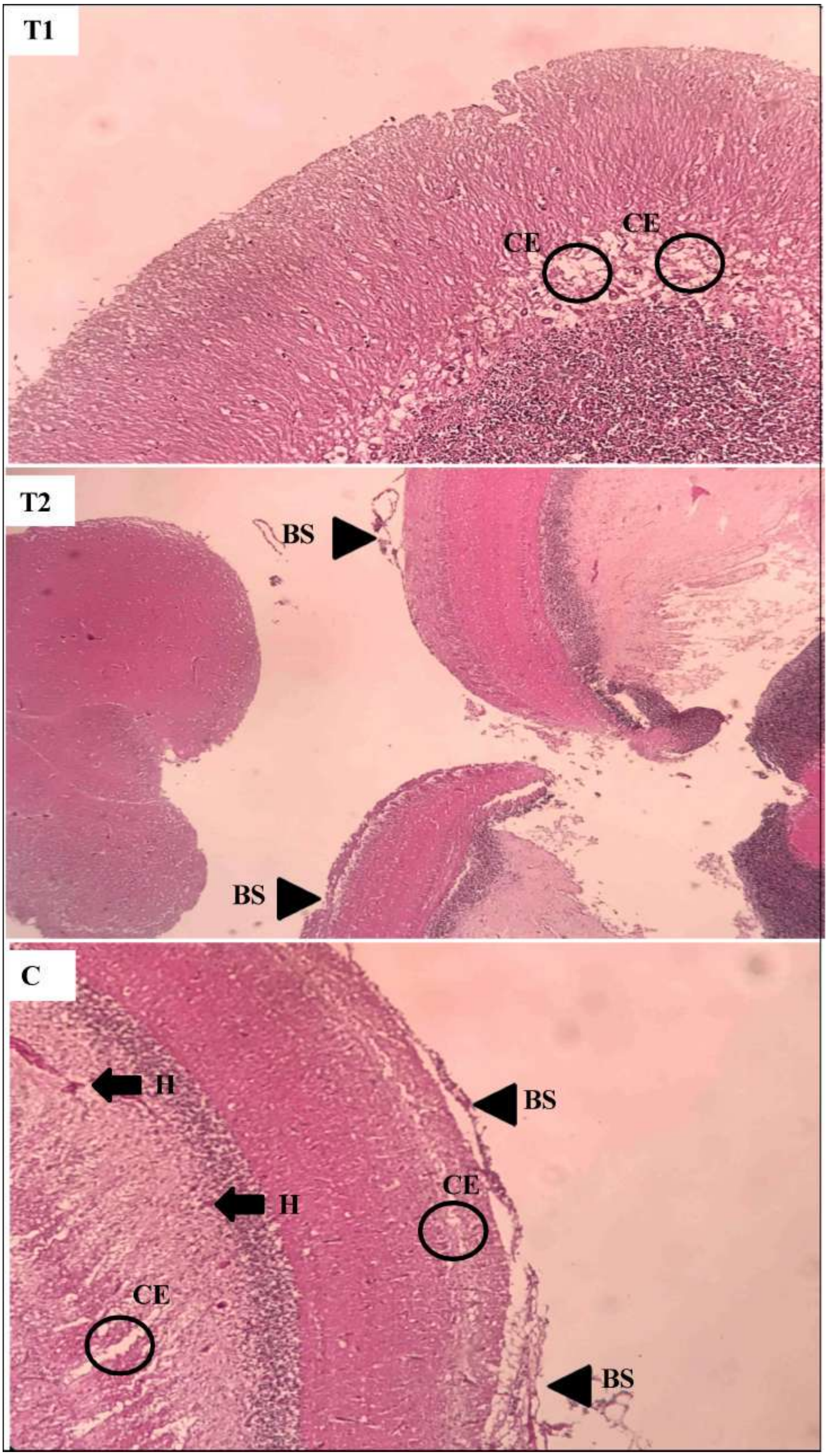


Figure 4.16 Histological structure of brain of fish challenged with A *hydrophila.* (T1) (CE) Cerebral edema (circle) (100X). (T2) (BS) Brain spongiosis (thick arrow) (40X). (C) (H) Hemorrhage (thick arrow), cerebral edema (circle) and brain spongiosis (head arrow) (100X).

In eye histopathological examination, lens capsular damage was observed with a contrast degree in severity in ocular tissue of fish from both probiotic-fed groups and the control group. The severity of the lesion was scored according to the entire shape and structure of lens tissue and the capsular, which were the important indications of the disease's influence on ocular health under the microscopic view. Based on Figure 4.17, most of the lens capsular of the ocular tissues of fish fed with probiotics, *L. plantarum* BE7, *L. paracasei* BUM6 and control group were observed damaged. However, the histological examination revealed that the general ocular structure of fish fed with the probiotics were still in a good form under the microscopic view as compared to the ocular tissue of the control group, which showed a complete loss of shape. This showed that the inclusion of probiotic supplementation into dietary feedings of fish during pathogen challenge may gave the fish with a stronger protection ability against bacterial infection which preventing the lesions on the lens from becoming more severe. Since *L. plantarum* BE7 and *L. paracasei* BUM6 have the ability to compete for nutrients and adhesion sites on mucosal surfaces against the pathogens on the fish guts, this may prevent the pathogen from developing a serious injury on eye tissue of fish in the treatment groups (Hosseini et al., 2025).

Moreover, eye edema is another lesion that can be observed on ocular tissue of the fish. Eye edema is a type of ocular injury that mainly cause by inflammation (Laith et al., 2017). The lesion is symbolized by the development of vacuoles in the cytoplasm of affected cells, which may cause cell lysis and contribute to the overall tissue damage. Vacuolation in the cytoplasm of affected ocular cells, contribute to the enlargement noticed in the eyes (Filik et al. 2025). Plus, vacuoles in histology samples also can be used as a marker to determine the degree of tissue damage. Based on Figure 4.18, the eye edema on ocular tissue of fish treated with *L. plantarum BE7* (Figure 4.17 (T1)) was scored as mild lesion (Score 1). Meanwhile, no eye edema on ocular tissue of fish treated with *L. paracasei* BUM6 (Figure 4.17 (T2)). However, there were lots of vacuolation in the lens, indicates severe eye edema (Score 3) on ocular tissue of fish from the control group (Figure 4.17 (C)) which caused by the presence of *S. agalactiae* that produced cytotoxins causing the host cells to vacuolate (Laith et al., 2017). Kumaree *et al.,* (2015) mentioned that less severe edema was observed in eye tissue of probiotic-fed fish maybe because the inhibitory effect offered by the probiotic strain against *S. agalactiae* which may lower inflammatory reactions.

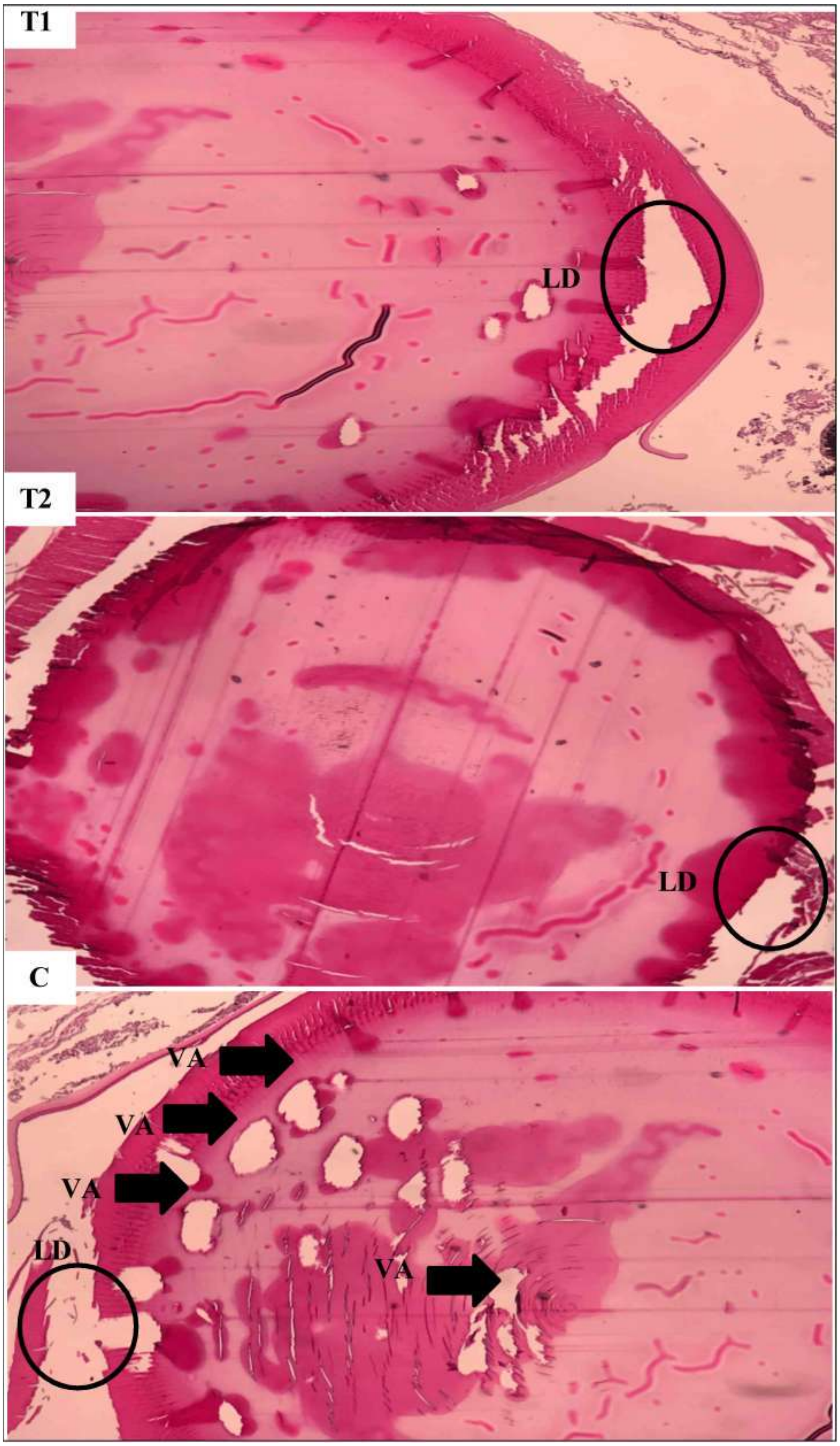


Figure 4.17 Histological structure of eye offish challenged with *S. agalactiae.* (Tl) Eye section showing mild (LD) damage of lens capsule (circle) (100X). (T2) Damage of the lens capsule (circle) (100X). (C) Severe (VA) vacuolization of the eye tissue (thick arrow) and damage of the lens capsule (circle) (100X).

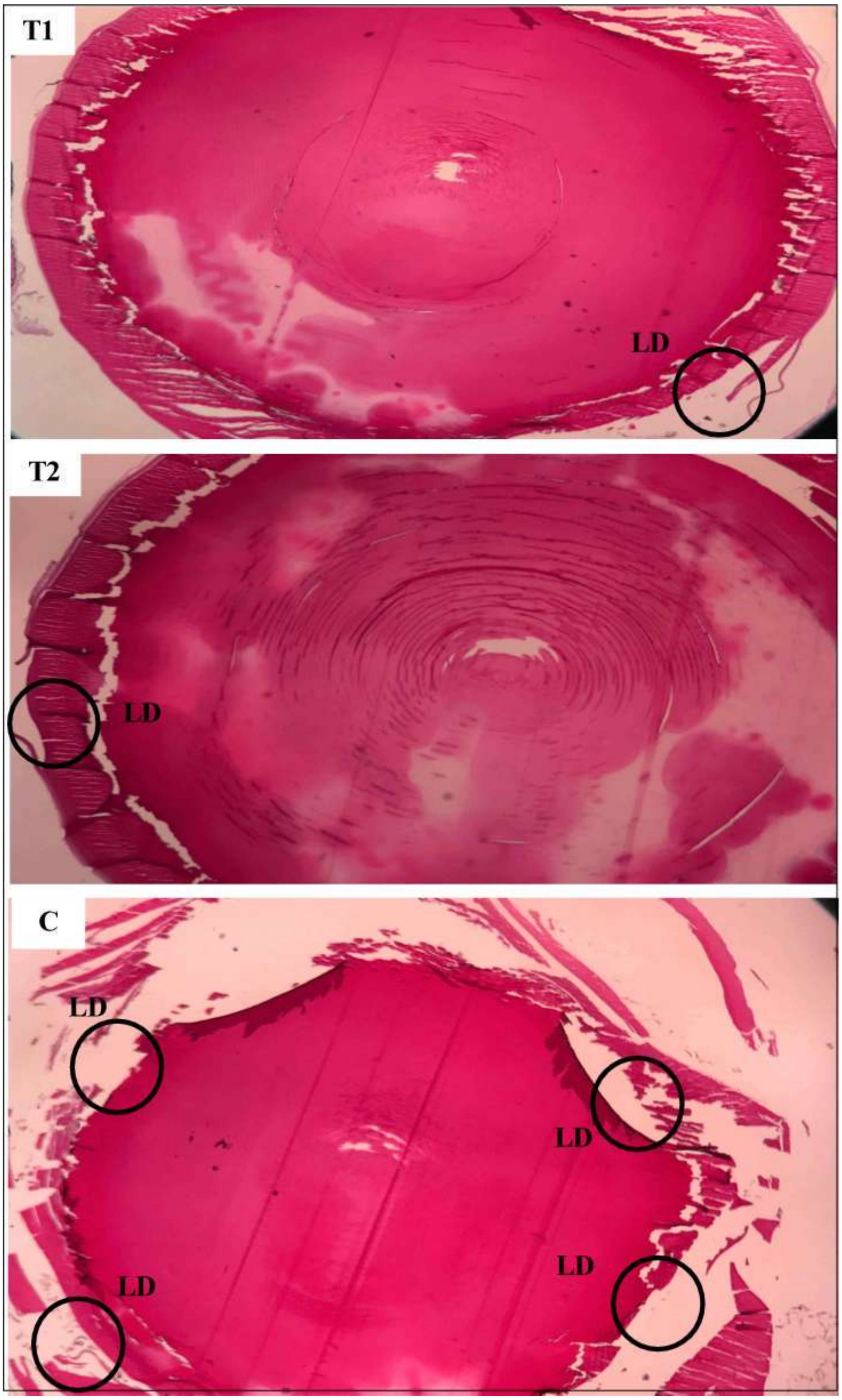


Figure 4.18 Histological structure of eye offish challenged with *A. hydrophila*. (Tl) Mild (LD) damage of the lens capsule (circle) (100X). (T2) Mild damage of the lens capsule showing the eye structure remain intact (circle) (100X). (C) Severe damage of the lens capsule causing loss of the lens structure (circle) (100X).

The liver tissues based on Figure 4.19 and 4.20 show lesions including hepatocyte necrosis and infiltration of inflammatory cells. However, the differences between each lesion can be compared based on the severity in each tissue samples representing each group. The liver hepatocyte is a vital cell type in the liver's parenchymal tissues that is involved in several liver activities, including detoxification, glucose metabolism, lipid metabolism, albumin secretion, clotting factors, and complements. The combination of direct bacterial invasion and host's inflammatory response were the main cause of tissue damage and further degeneration observed on the liver histology of fish (Laith et al., 2017). According to the Figure 4.19 (C) and Figure 4.20 (C) which represent the liver histology of fish from control group challenged with *S. agalactiae* and *A. hydrophila*, severe congestion that led to the loss of liver architecture were observed. This lesion was caused by severe degeneration of hepatocytes and congestion in the sinusoid that was blocking the blood flow between the tissues, initiating the necrosis process among the hepatocytes. The loss of limitless amounts of liver tissue led the cell arrangement to seem loose and unpacked with viable hepatocyte. In addition, the Figure 4.20 (C) also showed hemorrhages among the hepatocyte an indication sign of *A. hydrophila* infection during the challenge period.

The hepatocyte in liver tissue of fish also showed vacuolar degeneration indicating cellular distress. The exposure of pathogen to the fish during pathogen challenge was causing liver congestion, exacerbating hypoxic conditions in hepatocytes (Wang et al. 2025). The liver hepatocytes were displayed ballooning degeneration which caused the cytoplasm to fill with transparent vacuoles, indicating disturbed cellular compartmentalization and mitochondrial enlargement (Keppler, 2012). The infections caused an immediate systemic inflammatory response, resulting in further cellular damage and degeneration effects on liver tissue. In Figure 4.20 (T1) and Figure 4.20 (T2), the liver tissue of fish fed with *L. plantarum* BE7 and *L. paracasei* BUM6 were observed with a presence of vacuoles, however the vacuolation was less severe compare to the liver tissue of fish fed without probiotic under Figure 4.20 (C). The difference in severity maybe occur because of the presence of probiotic in the dietary feedings of fish in both treatment groups which may reduce the inflammatory response and increase the lipid metabolic function in the liver tissue at the same time. As the pro-inflammatory cytokines become reduced, the inflammation-related to liver damage become lowered resulting in less severe deterioration of liver hepatocytes observed

(Long et al., 2022).

Histological analysis of red hybrid tilapia tissues revealed that *L. plantarum* BE7 and *L. paracasei* BUM6 provided significant protection against bacterial pathogens. Despite the existence of clinical symptoms and lesions such as brain spongiosis, eye oedema, and liver necrosis, fish on probiotic-supplemented diets showed less histological abnormalities than those fed a control diet, particularly in important tissues such as the brain, eyes, and liver. This research emphasises the importance of probiotics in improving host immune defence, lowering inflammatory responses, and preserving tissue integrity during pathogen stress. Notably, fish in the treatment groups not only shown greater tissue resilience, but they also survived until the end of the pathogen challenge and recovered faster than fish in the control group. These results emphasise the potential of probiotic supplementation as a viable preventative technique in aquaculture to limit disease impact, minimise mortality, and ultimately prevent financial loss caused by infection-related setbacks.

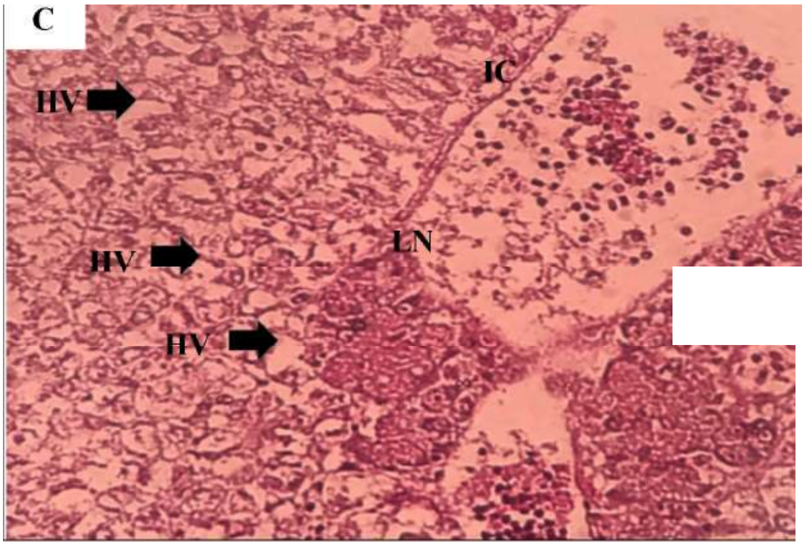


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Figure 4.19 Histological structure of liver of fish challenged with *S. agalactiae*. (T1) Liver section showing the (IC) infiltration of inflammatory cells (circle) (400X). (T2) Necrosis of hepatocytes (box) infiltration of neutrophils (thick arrow) (400X). (C) Increase in (HV) vacuolization in hepatocytes (thick arrow), (LN) liver necrosis (box), and infiltration of inflammatory cells (circle) (400X).

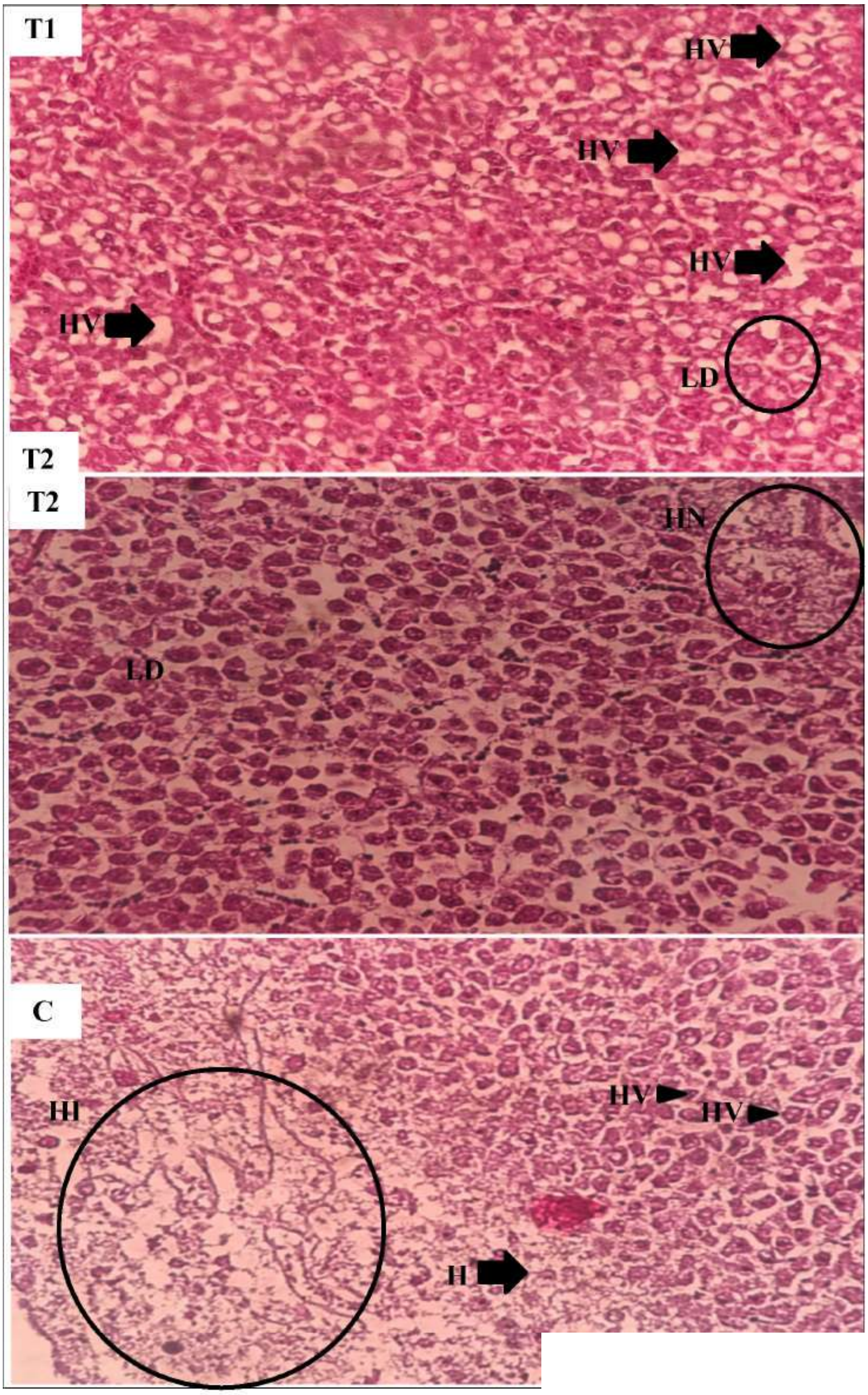
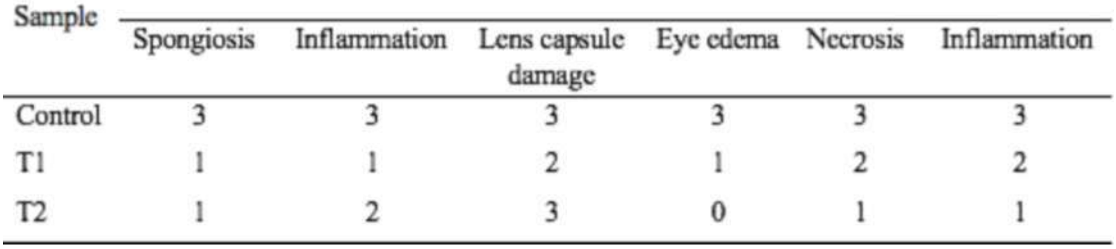


Figure 4.20 Histological structure of liver offish challenged with A *hydrophila.* (T1) Increase in (HV) vacuolization of hepatocytes (thick arrow) (400X). (T2) (HN) Hepatocyte necrosis (circle) (400X). (C) Liver section of red hybrid from control group challenged with A *hydrophila* showing (H) hemorrhage (thick arrow), hepatic infarction causing loss of liver architecture (circle) and cytoplasmic vacuolization in hepatocyte (head arrow) (400X).

Table 4.3

Tissue s Lesion Scoring of Red Hybrid Tilapia Challenged with *S. Agcilcicticie*



Sample	Brain lesion score		Eye lesion score		Liver lesion score	
	Spongiosis	Inflammation	Lens capsule damage	Eye edema	Necrosis	Inflammation
Control	3	3	3	3	3	3
T1	1	1	2	1	2	2
T2	1	2	3	0	1	1

Note: Level of histological damage which range from no lesion to severe lesion (0 = unchanged, 1 = mild, 2 = moderate, 3 = severe) (Landmann et al, 2021)

Table 4.4

Tissue’s Lesion Scoring of Red Hybrid Tilapia Challenged with *A. Hydrophila*

	Brain lesion score		Eye lesion score		Liver lesion score	
Sample	Spongiosis	Inflammation	Lens capsule damage	Eye edema	Necrosis	Inflammation
Control	3	3	3	:	3	3
n	1	2	0	:	0	1
T2	2	3	0	:	1	2

Note: Level of histological damage which range from no lesion to severe lesion (0 = unchanged, 1 = mild, 2 = moderate, 3 = severe) (Landmann et al, 2021)

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In conclusion, the present study demonstrates that both locally isolates, *L. plantarum* BE7 and *L. paracasei* BUM6 exhibit promising probiotic characteristics under both in vitro and in vivo conditions relevant to red hybrid tilapia culture. Both strains showed high survival rates (>60%) and strong tolerance to bile salts, low pH, and varying salinity, indicating their ability to withstand harsh gastrointestinal and environmental stresses during feed administration. Among the two strains, *L. plantarum* BE7 consistently exhibited higher tolerance across all stress parameters, suggesting superior physiological adaptability.

In terms of growth performance, dietary supplementation with *L. plantarum* BE7 and *L. paracasei* BUM6 resulted in significantly improved survival rate, specific growth rate, absolute weight gain, and feed conversion ratio over the 14-week feeding period compared to the control group ($p < 0.05$), indicating a positive influence on growth efficiency and nutrient utilization among the red hybrid tilapia.

Furthermore, probiotic-supplemented fish exhibited higher survival and milder clinical signs following experimental challenge with *S. agalactiae* and *A. hydrophila*, accompanied by reduced histopathological lesion severity relative to the control group. Overall, this study successfully fulfilled all objectives by demonstrating the environmental tolerance, growth-promoting effects, and pathogen protection ability of both probiotic strains, particularly *L. plantarum* BE7, highlighting its suitability for practical application in sustainable tilapia aquaculture.

Based on the comparative performance of both strains, *L. plantarum* BE7 is recommended as the primary probiotic candidate for red hybrid tilapia culture due to its superior tolerance to gastrointestinal and environmental stresses, consistent enhancement of growth performance parameters (SGR, AGR, FCR), and stronger protective effects against *S. agalactiae*. In contrast, *L. paracasei* BUM6 demonstrated higher survival rates during *A. hydrophila* challenge, indicating a more specific antagonistic potential against motile Aeromonas infections. Importantly, both probiotic strains significantly reduced histopathological lesion severity, suggesting systemic tissue protection. Therefore, *L. plantarum* BE7 may be prioritised as a general-purpose probiotic for growth and streptococcosis prevention, while *L. paracasei* BUM6 may serve as a targeted probiotic for *A. hydrophila* outbreaks or be applied in combination strategies for broader pathogen control.

Several limitations of this study should be acknowledged. The primary limitation was the insufficient sample size for histopathological examination, as only one representative organ sample from each experimental group was evaluated. Consequently, differences in lesion severity among organs in both probiotic-fed and control groups were assessed descriptively, limiting the statistical power of the findings. In addition, this study did not include an assessment of the stability and viability of *L. plantarum* BE7 and *L. paracasei* BUM6 following feed coating and prior to administration, which may influence the actual dose delivered to the fish. Furthermore, the stress tolerance and probiotic properties were evaluated primarily under in vitro conditions, which may not fully represent the complex physiological and microbial interactions occurring in vivo within the gastrointestinal tract of red hybrid tilapia.

Future studies should therefore incorporate larger and statistically robust sample sizes for histopathological analysis to enable meaningful quantitative comparisons across treatment groups. In addition, detailed validation of the feed coating process should be performed, including assessment of probiotic viability before and after coating, as well as uniformity checks through sampling multiple feed pellets and quantifying viable cell counts. Furthermore, combining the local probiotic strains could enhanced the treatment efficacy due to possible synergistic interaction. Moreover, extended experimental durations and in vivo investigations focusing on immune-related biomarkers, gut microbiota profiling, and host–microbe interactions are also recommended to better elucidate the mechanistic basis of probiotic effects. Such improvements would enhance the reliability, reproducibility, and translational relevance of probiotic application in aquaculture systems.

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Muhammad Harith Haqeem bin Zunaide obtained his Bachelor of Science (Hons.) in Biology from Universiti Teknologi MARA (UiTM) Kuala Pilah in 2025. His academic background has equipped him with a strong foundation in biological sciences, particularly in microbiology and aquatic biology. His current research focusing on the application of probiotics in aquaculture which investigates the potential of beneficial bacteria to enhance fish growth and disease resistance. His research interests include aquaculture, microbial biotechnology, and sustainable fish farming practices. This work contributes to the development of environmentally friendly alternatives to improve aquaculture productivity and health management.

LIST OF PUBLICATION:

Haqeem, M. H. Z., Ilyanie, H.Y., Ida Muryany, M.Y., NurHasyimah, R. and Izzati, A. (2025). Effects of dietary probiotic supplementation, *Lactiplantibacillus plantarum* strain Be7 on growth and survival of *Oreochromis niloticus*. *Food Research*. *9*(4). 10- 16. https://doi.org/10.26656/fr.2017.9(4).127.