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**EFFECT OF DIETARY *SACCHAROMYCES BOULARDII* ON
GROWTH PERFORMANCE AND HEAT TOLERANCE IN
GUPPIES.**

By

NANDAKUMARAN A/L RAJANDRAN

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ABSTRACT

Guppies (*Poecilia reticulata*), are a major export in the ornamental fish industry. Challenges associated with intensive farming practices, suboptimal growth performance and reduced tolerance to elevated water temperatures. *Saccharomyces boulardii*, a well-documented probiotic yeast in food fish, remains relatively underexplored in ornamental fish species. Therefore, this study was conducted to evaluate the effects of dietary *S. boulardii* supplementation on growth performance, feed efficiency, heat tolerance, and reproductive output in guppies. A total of six experimental groups were used, comprising three control replicates (G0) and three probiotic replicates (G1), with 10 fish per tank. Fish were fed for four weeks, during which G0 received a commercial diet, while G1 was fed a diet supplemented with *S. boulardii* at 1×10^7 CFU/g feed. Growth parameters, including weight gain and feed conversion ratio (FCR), were assessed at the end of the feeding trial, while survival was monitored throughout the experiment. Following the feeding period, all groups were subjected to a 48-hour heat tolerance challenge at 32°C to evaluate thermal resilience. Guppies fed the probiotic-supplemented diet exhibited significantly higher mean weight gain (0.24 g; $p < 0.05$) and improved FCR (1.187; $p < 0.05$) compared to the control group. Survival rates during the feeding trial were comparable between groups. During the heat tolerance challenge, the probiotic group exhibited a 100% survival rate, slightly higher than that of the control group (96.67%), suggesting improved physiological resilience to thermal stress; however, the difference was not statistically significant. Additionally, a higher frequency of offspring production was observed in the probiotic group during the feeding period. Overall, this study demonstrates that dietary *S. boulardii* supplementation can improve feed efficiency, promote modest growth enhancement, enhance heat tolerance, and potentially stimulate reproductive output in guppies, highlighting its potential application in sustainable ornamental fish culture.

Keywords: *Poecilia reticulata*, probiotic supplementation, growth performance, feed conversion ratio (FCR), heat tolerance, reproductive performance

ABSTRAK

Ikan gupi (*Poecilia reticulata*) merupakan antara spesies eksport utama dalam industri ikan hiasan. Walau bagaimanapun, industri ini berdepan pelbagai cabaran yang berkaitan dengan amalan penternakan intensif, termasuk prestasi pertumbuhan yang tidak optimum serta toleransi yang rendah terhadap peningkatan suhu air. *Saccharomyces boulardii*, iaitu yis probiotik yang telah didokumentasikan dengan baik dalam ikan makanan, masih kurang dikaji penggunaannya dalam spesies ikan hiasan. Oleh itu, kajian ini dijalankan bagi menilai kesan penambahan *S. boulardii* dalam diet terhadap prestasi pertumbuhan, kecekapan penggunaan makanan, toleransi haba dan pengeluaran reproduktif ikan gupi. Sebanyak enam kumpulan eksperimen digunakan, terdiri daripada tiga replikasi kumpulan kawalan (G0) dan tiga replikasi kumpulan probiotik (G1), dengan 10 ekor ikan bagi setiap tangki. Ikan diberi makan selama empat minggu, di mana G0 menerima diet komersial, manakala G1 diberi diet yang diperkaya dengan *S. boulardii* pada kepekatan 1×10^7 CFU/g makanan. Parameter pertumbuhan, termasuk pertambahan berat badan dan nisbah penukaran makanan (Feed Conversion Ratio, FCR), dinilai pada akhir tempoh pemberian makanan, manakala kadar kelangsungan hidup direkodkan sepanjang eksperimen. Selepas tempoh pemberian makanan, semua kumpulan menjalani ujian toleransi haba selama 48 jam pada suhu 32°C bagi menilai ketahanan terhadap tekanan haba. Ikan gupi yang diberi diet probiotik menunjukkan purata pertambahan berat badan yang lebih tinggi secara signifikan (0.24 g; $p < 0.05$) serta FCR yang lebih baik (1.187; $p < 0.05$) berbanding kumpulan kawalan. Kadar kelangsungan hidup sepanjang tempoh pemberian makanan adalah setara antara kedua-dua kumpulan. Semasa ujian toleransi haba, kumpulan probiotik mencatatkan kadar kelangsungan hidup sebanyak 100%, sedikit lebih tinggi berbanding kumpulan kawalan (96.67%), yang menunjukkan peningkatan ketahanan fisiologi terhadap tekanan suhu, walaupun perbezaan ini tidak signifikan secara statistik. Selain itu, kekerapan penghasilan anak yang lebih tinggi turut diperhatikan dalam kumpulan probiotik sepanjang tempoh kajian. Secara keseluruhan, kajian ini menunjukkan bahawa penambahan *S. boulardii* dalam diet berpotensi meningkatkan kecekapan penggunaan makanan, merangsang peningkatan pertumbuhan sederhana, meningkatkan toleransi haba, serta berpotensi merangsang prestasi reproduktif ikan gupi, sekali gus menonjolkan potensinya dalam amalan penternakan ikan hiasan yang mampan.

Kata kunci: *Poecilia reticulata*, suplemen probiotik, kadar pertumbuhan, nisbah penukaran makanan (FCR), toleransi haba, kadar kemandirian, prestasi pembiakan

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LIST OF ABBREVIATIONS

CFU	-	Colony Forming Unit.
DOF	-	Department of Fisheries
FCR	-	Feed conversion ratio
FPV	-	Faculty of Veterinary Medicine
g	-	Gram
R	-	Replicates
G0	-	Control group
G1	-	Intervention group
<i>S. baoulardii</i>	-	<i>Saccharomyces Boulardii</i>
UMK	-	Universiti Malaysia Kelantan
WG	-	Weight gain
AWG	-	Average weight gain

CHAPTER 1

1.0 INTRODUCTION

The ornamental fish sector is one of the most economically profitable sectors in fish farming activities globally. Malaysia's ornamental fish industry is a significant subsector of the country's aquaculture, playing a crucial role in the national economy. This industry is currently experiencing rapid growth (Hoseinifar et al., 2023; Othman et al., 2017). Apart from that, Malaysia is also a major exporter of ornamental fish to international markets, making it a significant player in the global ornamental fish trade. By 2030, Malaysia is predicted to produce 352 million ornamental fish, valued at RM550 million. In 2023, the production of ornamental fish was 241 million, with a value of RM373 million, of which 21% was for export purposes (Department of Fisheries, 2023).

Furthermore, numerous types of ornamental fish are produced globally, and they can be further classified into several categories. The live-bearing category of ornamental fish is one of the most popular among fish hobbyists and entrepreneurs due to its vibrant colors, hardiness, and ease of breeding, especially in the case of guppies (*Poecilia reticulata*). Guppies are among the top ornamental fish exported worldwide, particularly from Southeast Asia (Casey, 2016). The demand for fancy tail guppies and competition grade strains, also known as show guppies, has further increased in the global ornamental fish trade due to their extremely diverse color and fin shapes, such as Albino Red, Moscow Blue, Dumbo Ear, and Cobra guppy, which was obtained from selective breeding (Alex, 2021). These strains command higher prices in aquatic exhibitions and international markets. However, high density of selective breeding and intensive farming practices, in the effort to cope with high market demand, have reduced their hardiness and productivity. Two of the challenges faced in intensive ornamental freshwater fish farming are poor tolerance to elevated water temperatures, as well as poor growth performance and a high feed conversion ratio (Sonia & Victor, 2019; Islam et al., 2020).

Prolonged exposure of fish to elevated water temperatures, which is more than 30°C, can reduce their feed intake, growth rates, reproductive performance, and increase mortality due to low tolerance to elevated heat (Breckels & Neff, 2013). In addition, most ornamental fish produced in

high-density breeding setups also exhibit poor growth rates and feed efficiency (Nica et al., 2020). As an alternative, the use of beneficial bacteria, also known as probiotics, is believed to overcome these issues in ornamental freshwater fish, as their effectiveness in food fish that are being produced in ponds has been proven. Probiotics, particularly *Saccharomyces boulardii*, a beneficial yeast, have garnered attention for their role in enhancing growth performance and mitigating environmental stressors in food fish, such as tilapia and carp (Mashhadizadeh et al., 2024). However, the application of these probiotics in ornamental freshwater fishes, especially live-bearing fishes, remains underexplored.

1.1 RESEARCH PROBLEM STATEMENT

Prolonged exposure to elevated water temperature can significantly affect the growth, immunity, reproductive performance, and survival of tropical ornamental fish such as guppies. Fishes that are being produced through high-density intensive farming are also prone to poor growth performance and feed efficiency. The use of probiotics, specifically *Saccharomyces boulardii*, in feed is not commonly practiced in ornamental fish production, but their effects have been widely studied in food fish. There is no research on the use of these probiotics to enhance growth performance and tolerance to elevated temperatures in guppies. Understanding the role of these dietary probiotics may offer a sustainable solution to enhance the resilience and performance in ornamental aquaculture under climate-related stress.

1.2 RESEARCH QUESTIONS

Is dietary supplementation with *Saccharomyces boulardii* able to enhance the growth performance?

Is dietary supplementation with *Saccharomyces boulardii* able to enhance heat tolerance in guppies?

1.3 RESEARCH HYPOTHESIS

H₀: Dietary supplementation with *Saccharomyces boulardii* has no significant effect on the growth or heat tolerance of guppies.

H_A: Dietary supplementation with *Saccharomyces boulardii* will exhibit significantly better growth performance and heat tolerance compared to the control group.

1.4 RESEARCH OBJECTIVE

1. To evaluate the effects of *Saccharomyces boulardii* on the growth rate of guppies.
2. To assess the heat tolerance and survival rate in guppies fed with dietary *Saccharomyces boulardii*



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CHAPTER 2

2.0 LITERATURE REVIEW

2.1 ORNAMENTAL FISH INDUSTRY IN MALAYSIA

The ornamental fish industry in Malaysia is a significant and rapidly growing sector within the country's agricultural industry. It holds significant economic potential by substantially contributing to both the local economy and international trade. Malaysia is a major global exporter in the ornamental fish market, contributing 9% to the global trade and ranking second after Singapore (Anjur et al., 2021). Apart from that, the ornamental fish industry is also providing a source of income for the breeders, whether it is a small holder or large-scale breeding, with the production projected to reach 352 million fish valued at RM 550 million by 2030 (DOF, 2023). According to the Department of Fisheries statistics for 2023, Johor led in ornamental fish production with a value of RM 261 million, followed by Perak at RM 87 million, Penang at RM 8 million, and Sarawak at RM 3.5 million. Due to their high commercial value for international trade, ornamental fish have recently gained rapid importance as a source of foreign exchange and employment (Anjur et al., 2021). In regions like the Klang Valley, the sale of ornamental fish, mostly alien species, including livebearers, significantly contributes to the income of pet store owners (Saba et al., 2021). Among the ornamental fish with commercial value, the guppy recorded one of the highest sales values at RM 11 million (BERNAMA, 2024).

2.2 GUPPIES (*Poecilia reticulata*)

Guppies are one of the most common freshwater ornamental fish in the aquarium hobby, and they are often viewed as beginner-friendly, as they have been around for a long time. During the early days of fish aquaculture, there were limited options for freshwater ornamental fish production, and only small and hardy fish could be transported. This is when guppies became famous in the international ornamental fish trade (Alex, 2021). Initially, guppies were discovered twice, first by German naturalist and explorer Wilhelm Peters in Venezuela in 1859, and then two years later by Italian zoologist Filippo De Filippi, who independently discovered the same fish in Barbados. This fish was initially known by two names: *Poecilia reticulata* and *Lebistes*

poecilioides, but it is more commonly known as the guppy, after Robert John Lechmere Guppy, who introduced it to the western world (Alex, 2021).

Originally, guppies are known to come from many different freshwater bodies in South America; however, today, almost all guppies sold in ornamental fish markets are globally produced in captivity (Alex, 2021). Guppies are known for their hardiness and high production rate. The recommended water parameters are a temperature range of 20°C to 26°C and a pH range of 6.5 to 8.0 (Alex, 2021). However, despite their adaptability, guppies are susceptible to sub-lethal effects of elevated temperatures, which can lead to reduced growth, compromised immunity, and impaired reproductive performance (Breckels & Neff, 2013). Guppies exposed to thermal stress exhibit oxidative damage and behavioral disruptions, making them ideal candidates for research on stress mitigation (Moniruzzaman et al., 2018).

2.3 SACCAROMYCES BOULARDII IN AQUACULTURE

The use of antibiotics in ornamental fish to boost production has become a major concern, as this can lead to antimicrobial resistance and make the fish susceptible to various diseases. Probiotics have emerged as beneficial microbial supplements in aquaculture, known to improve gut health, immunity, and stress tolerance in fish. This can help eradicate dependence on antibiotics, focusing more on maintaining and improving the quality of the fish being produced (Organica Biotech, 2024). Among all probiotics, *Saccharomyces boulardii* is a famous probiotic that is being used in the production of food fish. *Saccharomyces boulardii*, a non-pathogenic yeast, has been widely studied for its ability to enhance nutrient absorption and intestinal health in fish (Moreira et al., 2012; GHOSH et al., 2008). Apart from that, Moreira et al. (2012) highlighted the successful application of *Saccharomyces boulardii* in silver catfish (*Rhamdia quelen*) larvae, reporting enhanced survival and digestive enzyme activity. Furthermore, a recent study found that feeding Nile tilapia with *Saccharomyces boulardii* in combination with other probiotics results in enhanced growth performance, strengthened antioxidant defenses, improved immune responses, and significantly increased resistance to heat stress (Mashhadizadeh et al., 2024). While various studies support the use of probiotics in freshwater food fish, a comprehensive examination of the use of *Saccharomyces boulardii* in ornamental fish, such as guppies, remains lacking. This research seeks to fill that gap by evaluating growth performance, survival, and heat

stress resilience in guppies, thereby contributing to sustainability and resilience in ornamental aquaculture.

2.4 GROWTH PERFORMANCE INDICATORS IN ORNAMENTAL FISH

Growth performance in ornamental and cultured fish can be evaluated using certain quantitative indicators that can express the ability of fish to grow and to utilize the feed under certain rearing conditions. Weight gain and specific growth rate (SGR) can be used to determine the increase in fish body weight over time compared to their initial weight, which will give an indication of the efficiency of the diets and rearing conditions. Feed conversion ratio (FCR) is used to determine the efficiency of the feed intake comparing the weight gained to the amount of feed consumed, and a lower FCR is more desirable. Survival rate gives an indication of the percentage of fish that survive the entire experimental period, which gives an indication of the stress tolerance and health status of the fish. These indicators are very effective in monitoring the growth performance but can be affected by factors such as water quality, feeding regimes, and physiological status (Abdel-Tawwab & Wafeek, 2014).

2.5 THERMAL STRESS AND HEAT TOLERANCE IN FRESHWATER FISH

Temperature is one of the most important environmental components that has a significant effect on fish physiology, growth, and survival. When freshwater fish are subjected to water temperatures above the optimal range, they tend to show reduced feed efficiency and growth performance because their energy is used to deal with the metabolic stress caused by the high temperatures instead of using it for the growth of the fish (Abdel-Tawwab et al., 2025). High temperatures can also affect the enzymatic processes, digestion, and nutrient absorption, leading to decreased growth performance in fish. Moreover, high temperatures can affect fish immune systems, making them more vulnerable to pathogens and increasing mortality from prolonged exposure (Abdel-Tawwab et al., 2025). Experiments on goldfish have shown that elevated water temperatures increase the feed conversion ratio and induce physiological stress, including oxidative stress and hormonal imbalances. (Bagnyukova et al., 2007). Therefore, heat tolerance and thermal stress management are essential in ornamental and aquaculture industries to ensure optimal growth, health, and survival of freshwater fish.

2.6 PHYSIOLOGICAL AND METABOLIC RESPONSES OF FISH TO HEAT STRESS

Fish may undergo drastic physiological and metabolic alterations due to heat stress as they try to cope with the high temperatures by maintaining homeostasis. The rise in water temperature accelerates the metabolic rate, thereby increasing the utilization of oxygen and the need for energy. This makes it difficult for plants and animals to survive and reproduce (Abdel-Tawwab et al., 2025). Prolonged exposure to heat stress affects the endocrine system, particularly the levels of cortisol and thyroid hormones, resulting in stunted growth and metabolic disorders. On a cellular level, heat stress can trigger oxidative stress due to the overproduction of reactive oxygen species (ROS), leading to damage to lipids, proteins, and DNA if the antioxidant system is overwhelmed (Zabidi et al., 2021). Moreover, heat stress reduces the metabolic rate due to the impact of heat on the efficiency of mitochondria and enzymatic activity. Moreover, the immune system is often suppressed, making fish more vulnerable to diseases in high temperatures (Abdel-Tawwab et al., 2025).

2.7 EFFECTS OF PROBIOTIC SUPPLEMENTATION ON GROWTH PERFORMANCE IN FISH

Supplementation of probiotics has been well documented to improve growth rates and feed efficiency in fish by promoting gut health and nutrient absorption. Probiotics like *Saccharomyces boulardii*, *Lactobacillus*, and *Bacillus spp.* have the ability to regulate the intestinal microbiota, resulting in improved activity of digestive enzymes and better utilization of dietary nutrients, ultimately resulting in increased weight gain and specific growth rate (SGR) (Hai, 2015; Abdel-Tawwab et al., 2022). Moreover, probiotics have been proven to improve feed conversion ratio (FCR) by improving feed efficiency and minimizing nutrient losses. Improved immune responses and decreased physiological stress in probiotic-supplemented fish also help in increased growth rates, as the energy expenditure on stress and disease resistance is minimized (Ringo et al., 2018). Thus, supplementation of probiotics has been recognized as a sustainable nutritional approach to improve growth performance, health, and survival rates of ornamental as well as commercial fish species (Hai, 2015; Ringo et al., 2018).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 ETHICAL CONSIDERATION

The animal ethics application was approved by the Institutional Animal Care and Use Committee (IACUC) to conduct the research titled "Effect of Dietary *Saccharomyces boulardii* on Growth Performance and Heat Tolerance in Guppies," under approval code UMK/FPV/ACUE/FYP/010/2025.

3.2 EXPERIMENTAL DESIGN

An experiment investigating the effect of *Saccharomyces boulardii* on the growth performance and heat tolerance in guppies (*Poecilia reticulata*) was conducted in the Aquatic Laboratory, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Pengkalan Chepa, Kelantan. Sixty healthy juvenile guppies, 4 to 5 weeks old with uniform size and weight, were selected for the experiment. All the guppies were purchased from the same breeder and subsequently transferred to the Aquatic Laboratory at FPV, UMK. All the fish used in this research had not been exposed to any antibiotics or probiotics prior to this study. Two days before the arrival of the guppies, six (2 feet x 1 feet) aquariums were prepared with 24 liters of freshwater and proper aeration. A randomised control trial (RCT) was used in this experimental study, where the guppies were randomly allocated into two groups: the control group (G0), to which no probiotic was given, and the experimental group (G1), which was provided with *Saccharomyces boulardii*-supplemented feed. Ten fish were placed in each tank, with three replicates for each group. Then the fish underwent a one-week acclimatisation period to adapt to the new environment and feed. During this acclimation period, the fish was observed for any visible deformities or signs of illness. After the acclimation period, the water quality was assessed, and the experiment using the probiotic feed was initiated. After the feed trial, each group was exposed to an elevated temperature (32–34°C) to evaluate the effect of the probiotic on the fish's heat tolerance by monitoring the survival rate.

3.3 STUDY POPULATION

The guppies were randomly divided into 2 groups which are the control group (G0) and the experiment group (G1).

3.4 FEED PREPARATION AND FEEDING

3.4.1 COMMERCIAL GUPPY FEED

In this experiment, commercially prepared guppy feed (Marubeni Nisshin, Japan) with a size of 0.3 to 0.5 mm was used. This feed does not contain any probiotics. The feed contains Vitamin A, Vitamin D3, Vitamin E, Vitamin B1, Vitamin B6, Vitamin B12, Biotin, Nicotinic acid, Pantothenic acid, Inositol, Vitamin C, Manganese, Ferrous fumarate, Colt, Calcium iodide, Magnesium, Choline, Calcium lactate, Potassium phosphate, Emulsifier, and Ethoxyquin. The commercial feed was stored in a ziplock bag and placed in a dry area. Table 1 shows the feed composition analysis value.

Table 1. Feed composition analysis value

Composition	Crude protein	Crude fat	Crude fiber	Crude ash	Crude calcium	Total phosphorus
Value (%)	≥ 51	≥ 8	≤ 3	≤ 16	≥ 2	≥ 1.5

3.4.2 PROBIOTIC FEED PREPARATION

Saccharomyces boulardii (Xi'an Cuiyuan Biotechnology Co., China) with a CFU count of 10 billion per gram (1×10^{10}) was used in this study. In order to prepare the probiotic feed, the *Saccharomyces boulardii* raw material was mixed with the commercial guppy feed to achieve the target CFU per gram feed (1×10^7 /g). For 10g of feed, the target CFU count will be (1×10^8 /g). The required amount of *Saccharomyces boulardii* raw material was calculated using the formula:

$$\text{Required raw material} = \text{Target CFU count per g} / \text{CFU per gram of raw material}$$

$$= (1 \times 10^8) / (1 \times 10^{10}) = \mathbf{0.01g} / 10g \text{ of feed}$$

The required raw material was diluted using 1-2 ml of 0.85% NaCl. Then, the diluted probiotic was evenly pipetted onto the commercial guppy feed on a petri dish, while gently mixing the feed to achieve a uniform coating. After thoroughly mixing the probiotic in the feed, the prepared feed was dried in the incubator at 34°C for 4 hours. Then the prepared probiotic feed was put into a ziplock bag and stored in the refrigerator at 9°C. The feed experiment was started the next day.

3.4.3 FISH FEEDING

Each group of guppies will be fed 5% of their body weight every day, which is 0.1g of feed per day. The fish will be fed twice a day, in the morning and evening, with 0.05g of feed each time. For the experiment group, the probiotic feed was thawed at room temperature for 5 to 10 minutes before feeding the fish. During the feeding period, the behaviour of the fish was also monitored for any abnormalities. The probiotic feeding was done for 4 weeks, and the average body weight of each group was measured (Table 2) before and after the feeding trial to calculate the average weight gain at the end of the experiment. The average weight gain and FCR of each group were measured using the formula from Table 2.1. In addition, during the feeding period, the water quality of each group was checked every 2 weeks using a water parameter test kit. All feces, excess feed, and sediment were removed every week, along with a 10% water change. Aeration was provided continuously throughout the experiment. Weight gain (WG) and Feed Conversion Ratio (FCR) were calculated using the stated formula :

Weight gain (WG) = final weight - initial weight

Feed Conversion Ratio (FCR) = Total feed given (g) / Total weight gain

3.5 HEAT TOLERANCE TEST

24 hours after the feeding trials, the water temperature was gradually elevated by 2°C every two hours until it reached 32°C, using an aquarium water heater. The water temperature was maintained for 48 hours. The initial temperature of the water in each tank was recorded before the temperature was elevated. The behavior of the fish was observed during this period and the survival rate was recorded after 48 hours (Table 7). At the end of the heat test, the survival rate of

each group was measured using the formula shown below. After the heat test, the water temperature was gradually reduced. Survival rate was calculated using the stated formula:

$$\text{Survival Rate (\%)} = (\text{Number of fish at the beginning of the experiment} / \text{Number of fish at the end of the experiment}) \times 100$$

3.6 STATISTICAL ANALYSIS

After collecting all the data at the end of the experiment, statistical analysis was done. A paired t-test was conducted to compare the initial and final body weights of guppies in each replicate within both groups. In addition, an independent t-test was conducted to compare the mean weight gain between the control and probiotic groups. An independent t-test was also conducted to determine whether the difference in FCR (Table 6) and survival rate between G0 and G1 is statistically significant.

CHAPTER 4

4.0 RESULTS

4.1 AVERAGE WEIGHT GAIN IN GUPPIES

The average weight gain and feed conversion ratio (FCR) of guppies from the control group (G0) and treatment group (G1) across three replicates are presented in the table below (Table 2). All groups had a similar initial average body weight of 0.20 g, indicating uniform starting conditions. The final average weight ranged from 0.42 to 0.43 g in G0 and 0.43 to 0.44 g in G1. The average weight gain was slightly higher in the treatment group (0.23 – 0.24 g) compared with the control group (0.22 – 0.23 g). In addition, G1 exhibited lower FCR values (1.17 – 1.22) compared to G0 (1.22 – 1.27), indicating more efficient feed utilization in the treated fish. Overall, these results indicate that the treatment enhanced growth performance and feed efficiency in guppies compared to the control group.

Table 2. Average weight gain and FCR of each group

No.	Group	Replicates	Initial average weight (g)	Final average weight (g)	Average weight gain (g)	FCR
1	G0	1	0.20	0.42	0.22	1.27
2	G0	2	0.20	0.43	0.23	1.22
3	G0	3	0.20	0.42	0.22	1.27
4	G1	1	0.20	0.43	0.23	1.22
5	G1	2	0.20	0.44	0.24	1.17
6	G1	3	0.20	0.44	0.24	1.17

4.1.1 WEIGHT GAIN IN CONTROL GROUP

A paired t-test was conducted to compare the initial and final body weights of guppies in the control group. The results showed a significant increase in body weight from the initial

measurement (M = 0.20 g) to the final measurement (M = 0.42 g) in the control group of guppies. Since $p < 0.05$, the null hypothesis (H_0) was rejected

Control Group (G0R1, G0R2, G0R3)

Table 3. Statistical analysis of G0 AWG

		Paired Samples Statistics			
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	FinalBW	.4233	3	.00577	.00333
	initialBW	.2000	3	.00000	.00000

		Paired Samples Test					Significance			
		Paired Differences			95% Confidence Interval of the Difference		t	df	One-Sided p	Two-Sided p
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper				
Pair 1	FinalBW - initialBW	.22333	.00577	.00333	.20899	.23768	67.000	2	<.001	<.001

4.1.2 WEIGHT GAIN IN INTERVENTION GROUP

A paired t-test was conducted to compare the initial and final body weights of guppies in the intervention group. The results showed a significant increase in body weight from the initial measurement (M = 0.20 g) to the final measurement (M = 0.44 g) in the intervention group of guppies.

Since $p < 0.05$, the null hypothesis (H_0) was rejected.

Intervention Group (G1R1, G1R2, G1R3)

Table 4. Statistical analysis of G1 AWG

		Paired Samples Statistics			
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	FinalBW	.4367	3	.00577	.00333
	initialBW	.2000	3	.00000	.00000

Paired Samples Test										
Pair 1	FinalBW - initialBW	Paired Differences					t	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				One-Sided p	Two-Sided p
					Lower	Upper				
		.23667	.00577	.00333	.22232	.25101	71.000	2	<.001	<.001

4.1.3 AVERAGE WEIGHT GAIN BETWEEN GROUPS

An independent t-test was conducted to compare the mean weight gain between the control and probiotic groups. Since $p = 0.024$ ($p < 0.05$), the null hypothesis (H_0) was rejected. There is a statistically significant difference in weight gain between the two groups. The probiotic group (*S. boulardii*) gained significantly more weight (0.24 g) than the control group (0.22 g) after the 4-week feeding trial.

Table 5. Statistical analysis of AWG between G0 & G1

Group Statistics										
Group	N	Mean	Std. Deviation	Std. Error Mean						
WG control group	3	.2233	.00577	.00333						
intervention group	3	.2367	.00577	.00333						

Independent Samples Test											
		Levene's Test for Equality of Variances		t-test for Equality of Means							
		F	Sig.	t	df	Significance		Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						One-Sided p	Two-Sided p			Lower	Upper
WG	Equal variances assumed	.000	1.000	-2.828	4	.024	.047	-.01333	.00471	-.02642	-.00025
	Equal variances not assumed			-2.828	4.000	.024	.047	-.01333	.00471	-.02642	-.00025

4.1.4 FEED CONVERSION RATIO

An independent t-test was conducted to compare the mean feed conversion ratio (FCR) between the control and probiotic groups. Since $p = 0.024$ ($p < 0.05$), there is a statistically significant difference in the FCR between the two groups. The probiotic group (*S. boulardii*) has a better FCR (1.19) than the control group (1.25) after the 4-week feeding trial.

Table 6. Statistical analysis of FCR between G0 & G1

Group Statistics											
Group		N	Mean	Std. Deviation	Std. Error Mean						
FCR	control group	3	1.2533	.02887	.01667						
	intervention group	3	1.1867	.02887	.01667						

Independent Samples Test											
		Levene's Test for Equality of Variances		t-test for Equality of Means							
		F	Sig.	t	df	Significance		Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						One-Sided p	Two-Sided p			Lower	Upper
FCR	Equal variances assumed	.000	1.000	2.828	4	.024	.047	.06667	.02357	.00123	.13211
	Equal variances not assumed			2.828	4.000	.024	.047	.06667	.02357	.00123	.13211

4.2 SURVIVAL RATE AFTER HEAT TOLERANCE TEST

The survival rate of guppies from the control group (G0) and treatment group (G1) across three replicates after the heat test is presented in the table below (Table 7). All replicates had the same number of fish at the beginning and end of the heat test, except for R1 from the control group. The control group (G0) exhibited a slight mortality in one replicate, resulting in a survival rate of 90%. In contrast, the remaining two replicates for G0, along with all three replicates for the supplemented group (G1), achieved a perfect 100% survival rate. The consistent 100% survival across all G1 replicates suggests that dietary supplementation may have contributed to the advantage in heat stress tolerance under these experimental conditions, although overall high survival is observed in both groups.

An independent t-test was conducted to compare the mean survival rate between the control and probiotic groups after the heat tolerance test.

Since $p = 0.211$ ($p > 0.05$), the null hypothesis cannot be rejected. The difference in survival rates between the two groups is not statistically significant.

However, the intervention group showed a higher mean survival rate (100%) than the control group (96.67%), which is biologically meaningful, although not statistically significant due to the small sample size ($n = 3$ per group).

Table 7. Survival rate after heat tolerance test

No.	Group	Replicates (R)	Initial number of fish	final number of fish	Survival rate (%)
1	G0	1	10	9	90%
2	G0	2	9	9	100%
3	G0	3	9	9	100%
4	G1	1	9	9	100%
5	G1	2	9	9	100%
6	G1	3	10	10	100%

Table 8. Statistical analysis of the survival rate between G0 & G1

Group Statistics

Group	N	Mean	Std. Deviation	Std. Error Mean
survival control group	3	96.67	5.774	3.333
intervention group	3	100.00	.000	.000

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				95% Confidence Interval of the Difference			
		F	Sig.	t	df	Significance One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
survival	Equal variances assumed	16.000	.016	-1.000	4	.187	.374	-3.333	3.333	-12.588	5.921
	Equal variances not assumed			-1.000	2.000	.211	.423	-3.333	3.333	-17.676	11.009

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CHAPTER 5

5.0 DISCUSSION

5.1 GROWTH PERFORMANCE

The primary objective of this study was to evaluate the effect of dietary *S. boulardii* on the growth performance of guppies. The results of this study demonstrate a clear, moderate, and statistically significant positive impact of dietary *S. boulardii* on the growth performance of guppies. Both the control (G0) and probiotic-supplemented groups (G1) started the feed trial with the same average initial weight of 0.20 g, ensuring that any changes observed at the end of the experiment were due to the dietary treatment rather than baseline differences. At the end of the 4-week feeding trial, G1 achieved a significantly higher average weight gain and a lower Feed Conversion Ratio (FCR) compared to G0. Although the differences in growth were modest, they were consistent across the three replicates, and it is biologically relevant for small-bodied fish like guppies, where increases in growth are naturally modest.

An independent t-test was used to compare the mean weight gain between the control and intervention groups because these groups consisted of independent samples receiving different treatments, making the test appropriate for determining whether the treatments resulted in statistically significant differences. The independent t-test revealed a statistically significant difference in weight gain between the control and intervention groups, $t(4) = -2.828$, $p = 0.024$, with the intervention group showing higher mean weight gain (0.24 g) compared to the control group (0.22 g). This indicates that *S. boulardii* supplementation significantly enhanced growth performance in guppies. Other than that, a paired t-test was conducted to compare the initial and final weights within each group, because the measurements were taken from the same fish replicates at two different time points, and also to ensure the growth over the 4 weeks is significant. In the control group, the comparison between initial and final body weight showed a consistent and significant difference in body weight ($p < 0.001$). A similar pattern was observed in the probiotic group, where initial and final weights also differed significantly ($p < 0.001$). This confirms that both groups experienced normal and healthy growth, validating the effectiveness of the feeding protocol.

Feed conversion ratio (FCR) is a key indicator of nutritional efficiency. The improved feed conversion ratio (FCR) in G1 also indicates the ability of *S. boulardii* to enhance nutrient absorption and gut function. An independent t-test was conducted to determine whether there was a significant difference in the mean Feed Conversion Ratio (FCR) between the G0 and G1. The test revealed a statistically significant improvement in FCR in the probiotic group compared to the control group, with $p = 0.024$, indicating that *S. boulardii* supplementation enhanced feed efficiency and allowed guppies to convert feed into biomass more effectively. This finding strongly supports the alternative hypothesis (H_a) and confirms the growth-promoting potential of this probiotic in ornamental aquaculture.

The enhanced growth in G1 can be due to several well-documented mechanisms of action of *S. boulardii*. As a live yeast, it is known to improve gut health and function. It can secrete digestive enzymes, such as phosphatases, proteases, and amylases, that support the breakdown of complex dietary components, which in turn increases the nutrient availability for the host (GHOSH et al., 2008; Moreira et al., 2012). Furthermore, *S. boulardii* has been shown to strengthen the intestinal mucosal barrier and positively modulate the gut microbiota, creating an environment more favourable for efficient digestion and absorption (Galliano Zanello et al., 2009; Terciolo et al., 2019; McFarland, 2010). The significantly improved FCR in G1 is a key economic indicator, demonstrating that guppies supplemented with probiotics were able to convert feed into body mass more efficiently. This means less feed is wasted, leading to lower production costs and reduced organic waste in the aquaculture system of ornamental fish. This result aligns with previous studies in food fish species like Nile tilapia and silver catfish, where *S. boulardii* can significantly improve growth parameters and FCR by enhancing gut health, nutrient absorption, and digestive enzyme activity (Mashhadizadeh et al., 2024). The success of this probiotic in guppies suggests that these benefits are transferable to high-value ornamental species, providing a viable strategy to improve productivity in intensive farming systems where optimal growth is critical.

5.2 SURVIVAL DURING FEEDING TRIAL

Survival of fish during the 4-week feeding trial provides an important indication of the safety, tolerability, and potential effects of *S. boulardii*. In this study, both groups displayed high survival rates, approaching 100% before the heat tolerance test. This indicates that the

experimental conditions, including water quality, tank management, and the commercial diet itself, were excellent and provided a low-stress environment for the guppies. Water quality was monitored regularly, proper aeration was maintained at all times, feeding was done consistently, and stocking density was appropriate. All these factors can minimize external stressors and may contribute to the overall high survival rates.

Other than that, this high survival rate also demonstrates that the incorporation of *S. boulardii* into the feed at the administered dosage (1×10^7 CFU/g feed) was safe and well-tolerated by the guppies. The probiotic did not cause any toxicity, stress, or adverse effects, as supported by the absence of behavioral abnormalities during daily feeding and monitoring sessions. The few deaths recorded were minimal, random, and not clustered in any particular tank, suggesting that they were likely due to natural variability rather than treatment effects. In many aquaculture trials, introducing a new dietary supplement may lead to digestive or physiological stress (Merrifield et al., 2010). However, in this study, the acceptance and tolerance of the probiotic-coated feed by the guppies show that *S. boulardii* does not negatively affect appetite, metabolism, or overall fish health.

Overall, the findings from this study indicate that *S. boulardii* is safe for continuous daily feeding in guppies and does not negatively impact the survival of the fish under normal rearing conditions. This supports its potential for long-term use in ornamental aquaculture, where safety and compatibility with routine feeding practices are crucial.

5.3 HEAT TOLERANCE

The heat tolerance test was conducted to assess the potential of *S. boulardii* to enhance the tolerance of guppies to elevated water temperature, which is a common stressor in intensive aquaculture. The results showed that the group supplemented with *S. boulardii* (G1) exhibited a 100% survival rate after 48 hours at 32 - 34 °C. The control group (G0) also exhibited a high survival rate (96.67%), although a single mortality occurred in one of the replicates. Although the numerical difference is small and may not be statistically significant, it can be meaningful because thermal stress typically causes rapid physiological changes in guppies, making even a mild improvement biologically significant.

An independent t-test was used to compare the mean survival rates between the control and intervention groups upon exposure to elevated water temperatures, to determine whether the intervention resulted in statistically significant differences in the survival rates. The independent t-test revealed no statistically significant difference in survival between the control and probiotic groups ($p = 0.211$), although the probiotic-treated group exhibited higher survival (100%), indicating a biologically meaningful improvement in heat tolerance.

Probiotics such as *S. boulardii* are known to modulate several physiological pathways that influence stress resilience. One possible mechanism is the enhancement of antioxidant capacity. Heat stress induces oxidative stress by increasing the production of reactive oxygen species (ROS). Previous studies have demonstrated that *S. boulardii* supplementation enhances the activity of antioxidant enzymes, including superoxide dismutase and glutathione peroxidase, thereby helping fish counteract oxidative damage (Mashhadizadeh et al., 2024). Behaviourally, the probiotic-fed fish displayed more stable activity levels and fewer signs of thermal distress, like erratic swimming, lethargy, and gasping near the surface, compared to the control group during the heat challenge. This qualitative observation supports the quantitative findings and suggests better physiological stability under stress in G1. Other than that, the difference in survival rate also suggests that *S. boulardii* may support metabolic homeostasis of the fish during stress. Heat increases metabolic rate in ectothermic animals and elevates the oxygen demand. Fish with higher metabolic efficiency, which is supported by better digestion and nutrient absorption, may sustain longer physiological functions during heat exposure.

The results of this study are consistent with those of previous studies on other fish species. For example, studies in tilapia and carp have shown that *S. boulardii* supplementation improves thermal resilience by reducing cortisol levels, enhancing immune gene expression, and maintaining intestinal microflora balance under heat stress (Mashhadizadeh et al, 2024; Moreira et al., 2012). Although such studies have mostly focused on food fish, the present study demonstrates that ornamental guppies exhibit similar physiological responses. But the influence of the probiotic on the cortisol levels, immune gene expression, and intestinal microflora balance was not investigated in this study.

Overall, the findings strongly support the hypothesis that *S. boulardii* improves heat tolerance in guppies. This has important implications for ornamental aquaculture, particularly in tropical

climates where water temperatures frequently exceed optimal ranges. Even a small increase in thermal resilience can significantly reduce mortality during heat waves, making probiotics a valuable management tool.



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CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

In conclusion, this study successfully demonstrated that dietary supplementation with *S. boulardii* offers multiple beneficial effects for guppies, contributing to improved growth performance, feed efficiency, heat tolerance, and reproductive activity. Although the improvement in growth parameters, such as weight gain and FCR, was modest, the consistent trend across all replicates indicates that *S. boulardii* supports more efficient nutrient utilization and digestive function. The probiotic was well tolerated by the guppies, with no negative effects on survival during the feeding period, confirming its safety and compatibility with routine ornamental fish diets. Furthermore, although not statistically significant, the probiotic group showed 100% survival at elevated water temperature, compared with slightly lower survival in the control group. This suggests that *S. boulardii* enhances physiological resilience to thermal stress, a trait of increasing importance in tropical aquaculture, where water temperature fluctuations are common due to climate change. Additionally, the increased reproductive activity observed in the probiotic group indicates potential benefits for breeding performance, which is valuable for the ornamental fish industry. Overall, the study supports the use of *S. boulardii* as a beneficial dietary supplement for guppies, highlighting its potential to enhance sustainability, productivity, and resilience in ornamental aquaculture.

6.2 RECOMMENDATION

This research on *S. boulardii* supplementation in guppies can be further improved in the future by extending the feeding trial beyond four weeks, as a longer experimental duration may reveal clearer and more substantial improvements in growth performance, physiological health, and overall probiotic efficacy. Other than that, to identify the optimal concentration of probiotics required to achieve the optimal growth rate and greatest benefits in aquaculture, multiple probiotic dosages, such as 10^6 , 10^7 , and 10^8 CFU/g feed, can be tested. This will help the farmer use probiotics in a safer manner, without causing microbial imbalance in fish, and avoid unnecessary costs from overdosing. Furthermore, future studies should also investigate the

influence of probiotics on fish reproduction by incorporating a comprehensive reproductive assessment. This assessment should include quantifying fry production, gestation frequency, and female body condition to validate the reproductive advantages observed qualitatively in this research. Additionally, evaluating immune parameters, including antioxidant enzyme levels and gut histology, would provide deeper insight into the physiological improvements and mechanisms that lead to enhanced growth rates and heat tolerance in fish supplemented with *S. boulardii*. Apart from that, increasing the number of fish and replicates per group is also recommended to enhance statistical power and reduce variability, which ensures more robust conclusions. All of these suggested recommendations will contribute to a more comprehensive understanding of the probiotic's role and its potential application in the ornamental aquaculture industry.

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8.0 APPENDICES



Figure 1: Acclimation of the guppies before releasing them into the aquarium.



Figure 2: Aquarium set up with clean water and aerator for the experiment.



Figure 3: Measuring the average body weight of each group before the feeding trial.



Figure 4: Measuring the amount of *Saccaromyces boulardii* raw material needed to be mixed into the feed.



Figure 5: Measuring the amount of commercial feed needed to prepare probiotic feed.



Figure 6: Drying the prepared probiotic feed at room temperature.



Figure 7: Daily measurement of the amount of feed to be fed to each group at a time.



Figure 8: Measuring the average body weight of each group at the end of the feeding trial.

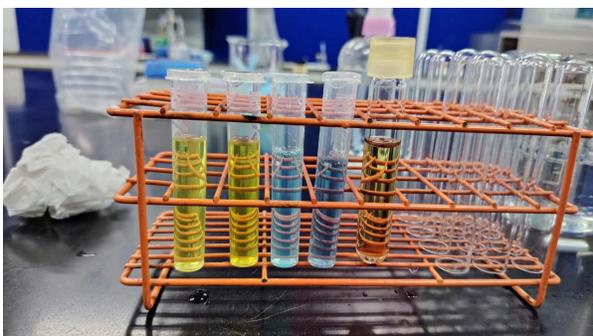


Figure 9: Checking the water parameters before starting the feed trial and heat tolerance test.

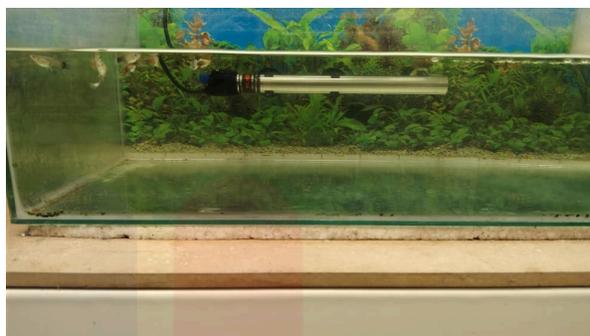


Figure 10: Aquarium water heater was used to gradually increase the water temperature.



Figure 11: The water temperature was frequently measured during the heat tolerance test.