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Research article

Dietary effects of *Saccharomyces cerevisiae* and *Bifidobacterium longum* probiotics cocktail on the growth and health of Mozambique tilapia (*Oreochromis mossambicus*) in aquaculture

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Abstract

Probiotics provide a cost-effective and eco-friendly solution as feed additives for enhancing fish growth and immunity. A wide variety of commercial probiotics, of microalgae, bacteriophages, and yeast extracts, are widely used in aquaculture. However, the use of a concoction probiotic premix as a single feed additive remains underexplored. This study aimed to evaluate the effects of *S. cerevisiae and Bifidobacterium longum* probiotic mixtures on the growth performance, feed utilisation, serum biochemistry, and intestinal morphometry of Mozambique tilapia. A 60-day feeding trial was conducted using fingerlings (4.5 ± 1.55 g) distributed into three replicate tanks across four treatment groups receiving probiotic mixtures at 0.00 g/kg (control), 0.45 g/kg, 0.9 g/kg, and 1.35 g/kg feed. Higher inclusion levels (1.35 g/kg feed) significantly improved survival probability (0.92), final weights, and percentage weight gain ($P < 0.05$). Feed conversion ratio, specific growth rate, and protein efficiency ratio were significantly better in (1.35 g/kg) probiotics ($P < 0.001$). Higher probiotic doses (1.35 g/kg) significantly improved haemoglobin, MCV, MCH, MCHC, ALP, albumin, globulin, glucose, and total protein ($P < 0.05$), while AST and ALT significantly improved ($P < 0.05$). Intestinal histomorphology: villus width, length, crypt depth, wall thickness, and goblet cell were significantly enhanced ($P < 0.05$). This study concludes that a concoction of S. *cerevisiae* and *B. longum* significantly enhances growth, survival, health, and feed efficiency. This study recommends 0.9–1.35 g/kg feed of probiotics mix as fish feed additives for tilapia. Further research and policy support are essential to commercialise this probiotic blend, leveraging its global availability and affordability to boost aquaculture productivity.

Keywords: Aquaculture, Antioxidant, Bacterial Probiotics, Fish Innate immunity, Mozambique tilapia, Yeast probiotics.

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INTRODUCTION

Global aquaculture has experienced exponential growth over the last decade compared to capture fisheries, making it one of the most lucrative industries in food production. This rapid expansion has positioned aquaculture as an essential subsector in efforts to meet the rising global demand for fish as an affordable protein source (Fao, 2024; Kaminski et al., 2024; Syanya et al., 2024). The industry's growth has been driven by advancements in aqua feed production through natural feed additives derived from plants, animals, or microbes (Abd El-Naby et al., 2024; Ashraf et al., 2024; Youssef et al., 2024), improved hatchery systems for fingerling production (Syanya et al., 2024), and efficient transportation and market systems, all of which have enhanced its viability. However, fish feed constitutes over 70% of the variable cost for producing 1 kg of fish, significantly reducing profitability (Maundu et al., 2024). Therefore, the efficiency, palatability, and quality of fish feeds as additives (Ersainovich et al., 2025) are critical for improved microbiota resulting in improved digestibility and feed utilization for better profitability in aquaculture. Probiotics, particularly *Saccharomyces cerevisiae* (Opiyo et al., 2019; Islam et al., 2021) and *Bifidobacterium longum*,(Sholihuddin et al., 2020; Todorov et al., 2024) offer a cost-effective and eco-friendly solution as feed additives. Probiotics have effectively been used to promote growth and fish health in Nile tilapia (*Oreochromis niloticus*) (Cano-Lozano et al., 2022) common carp (*Cyprinus carpio*)(Jinendiran et al., 2021; Li et al., 2023) white leg shrimp (*Penaeus vannamei*)(Reyes et al., 2024) giant freshwater prawn (*Macrobrachium rosenbergii*) (Mujeeb Rahiman et al., 2010).These probiotics not only improve growth performance and feed utilisation but also reduce production costs, contributing to the sustainability and economic viability of aquaculture. Probiotics enhance fish growth by improving gut health, boosting immune responses, and preventing diseases in Nile tilapia (*O. niloticus)* (Redhwan et al., 2024). They improve feed conversion ratios (FCR), increase feed palatability, and enhance nutrient availability by stimulating digestive enzyme activity in shrimp farming (Krummenauer et al., 2014). Probiotics are broadly classified into bacterial probiotics, bacteriophages, microalgae, and yeast (Assefa and Abunna, 2018; Chauhan and Singh, 2019). Among yeast-based probiotics, *S. cerevisiae* has shown significant potential in tilapia and shrimp farming. Yeast supplementation modulates the intestinal microbiota and structure, positively influencing cultured fish's overall health and productivity, especially in Nile tilapia (*O. niloticus*) (Islam et al., 2021). Baker's yeast (*S. cerevisiae) has* shown significant benefits in various aquaculture species, including Nile tilapia (*O. niloticus*) (Opiyo et al., 2019b ; Mohammady et al., 2023) and Rohu carp (*Labeo rohita)* (Jahan et al., 2021). These probiotics promote growth, enhance immunity, and improve water quality. For instance, *S. cerevisiae* improved growth, gut health, and immunity in Nile tilapia (Ferreira et al., 2019; Abd El-Naby et al., 2024) and boosted productivity in freshwater prawns (Taguemount et al., 2024). Additionally, probiotic yeast controls pathogens by creating a healthier gut environment, reducing disease outbreaks (del Valle et al., 2023). Its benefits extend beyond aquaculture, improving fillet yield and shelf life in poultry by lowering pH and slowing spoilage (El Asuoty et al., 2024; Thuy, 2025). Probiotics bacteria are also widely used in aquaculture, with examples like *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus* aiding digestion and immunity (Aly et al., 2008; El-Saadony et al., 2021; Tanveer et al., 2024). *Nitrosomonas* and *Nitrobacter* facilitate nitrification, (Asha et al., 2024) while *Lactobacillus spp.* and *Pseudomonas spp.* inhibit pathogens and degrade organic matter (Mohapatra et al., 2013; Krummenauer et al., 2014; Majeed et al., 2025; Thuy, 2025). , and *Bifidobacterium longum* promotes growth in fish and crustaceans (Todorov et al., 2024). A mix of *Lactobacillus paracasei* and *B. longum* enhances growth, immunity, and disease resistance in shrimp (Huang et al., 2022). Probiotics also improve reproductive health, including gonadal development, in zebrafish (Valcarce et al., 2019) Although various studies highlight the effectiveness of baker's yeast (*S. cerevisiae*) and *B. longum* probiotics, each independently

showing positive effects on growth, immunity, and gut health in different fish species, no study has evaluated the efficacy of combining these two probiotics in Mozambique tilapia (*O. mossambicus*). This study, therefore, aims to assess the effectiveness of a dietary mixture of baker's yeast (*S. cerevisiae*) and *B. longum* on the growth performance of Mozambique tilapia at different dosage levels. Additionally, it seeks to evaluate the impact of this probiotic combination on immune response, gut health, and survival rates in an aquaculture setting.

MATERIAL AND METHODS

Experimental Fish and Culture Conditions

Mozambique tilapia (*O. mossambicus*) fingerlings, with an average weight of 4.5 ± 1.55g, were harvested from a nursery tank at the Hatchery Complex, School of Industrial Fisheries, Cochin University of Science and Technology, Kerala, India. A total of 260 fingerlings were sorted and transferred to 700-litre fibreglass tanks containing 500 litres of water for one week of acclimatization. During this period, they were fed a control basal diet twice daily at 8% of body weight. Water quality was maintained through partial replacement of approximately 50% of the water, while the excreta and unconsumed feeds were syphoned daily.

Experimental scheme

A mixture of a probiotic consisting of baker's yeast (S. *cerevisiae*) and *Bifidobacterium longum* in a 1:1 ratio was incorporated into a basal diet at four different treatment inclusion levels: 0.00 g/kg feed (PB0 - Control), 0.45 g/kg feed (PB1), 0.9 g/kg feed (PB2), and 1.35 g/kg feed (PB3). These dosages were determined based on the prior study by Abdel-Ghany et al. (2020); Islam et al. (2021) and Huang et al. (2022). These probiotics *(S. cerevisiae*) and *B. longum,* considered commercial aquaculture multi-strain probiotics, were procured from the local Aquafeed shop in Ernakulam district Kerala, India. The experimental treatment groups followed a completely randomised design with four treatment levels: PB0 (control), PB1 (0.45 g/kg), PB2 (0.9 g/kg), and PB3 (1.35 g/kg), each conducted in triplication. Following acclimatisation, twenty fingerlings were randomly allocated to each treatment group in triplicate over a 60-day treatment period. The fish were fed twice daily at 8% of their body weight for the duration of the eight weeks. Feed amounts were adjusted every two weeks based on the bulk body weight measurements of the fish from each group. Water quality was rigorously maintained through continuous aeration and daily partial water exchanges. Feed residues and fish excreta were removed daily to maintain cleanliness and water levels were regularly topped up. Fish growth was monitored biweekly, measuring weight, total length, and standard length. Growth performance and feed utilisation were assessed using specific formulas. The proximate composition of the formulated basal diets provided included crude protein 44.24%, moisture content 34.913%, ash content 12.363%, and lipid 2.820%.Table 1: These were determined following the procedure outlined by AOAC (2005) and Horwitz and AOAC International (2000).

Table 1 Ingredients and Approximate Composition of Basal Diet Feeds.

Basal feed Ingredient compositions	Probiotic conglomerate inclusion levels in the diet g/Kg of feeds			
	Control PB0 (0.00) g	PB1 (0.45) g	PB2 (0.9) g	PB3 (1.35 g
Fish meal	25.27	25.27	25.27	25.27
Wheat bran	19.34	19.34	19.34	19.34
Soya bran	22.45	22.45	22.45	22.45
Corn glutten	18.36	18.36	18.36	18.36
Rice polishings	8.2	8.2	8.2	8.2
sunflower oil	1	1	1	1
Viatamin premix	3	3	3	3
Mineral premix	2	2	2	2
Cassava flour binder	4	4	4	4
Cod liver oil	1	1	1	1
Proximate composition				
Crude protein (%)	44.24 ± 100	44.24 ± 100	44.24 ± 100	44.24 ± 100
Moisture content (%)	34.913 ± 0.589	34.913 ± 0.589	34.913 ± 0.589	34.913 ± 0.589
Ash content (%)	12.363 ± 0.488	12.363 ± 0.488	12.363 ± 0.488	12.363 ± 0.488
Lipid content (%)	2.820± 0.359	2.820± 0.359	2.820± 0.359	2.820± 0.359

Survival, growth and feed utilization

At the end of the 60-day treatment period, all fish were weighed, as well as during each sampling interval. The daily feed consumption for each treatment group was determined by calculating the difference in weight of the food containers before and after feeding. Several growth parameters, including weight gain (WG; % WG), specific growth rate (SGR), hepatosomatic index (HSI), and viscerosomatic index (VSI), were recorded. Additionally, feed utilisation indices, such as feed conversion ratio (FCR) and protein efficiency ratio (PER), were calculated using standard formulas adopted from (Benedito-Palos et al., 2007; Mişe Yonar, 2019). Growth performance and feed utilization were calculated as follows:

Fish condition factor (C.F) = Final weight of the fish / (Total length $)^3$x100

Weight gain (WG) g = (Final weight of fish – Initial weight)

Percentage Weight gain (PWG) % = (Final weight)- (Initial weight)/ (Initial weight) x 100

Feed conversion ratio (FCR) = Feed consumed / Weight gained by the fish.

Specific growth rate (SGR) % = (log Final body weight of fish)- (log Initial body weight of fish)/ (Duration of fish culture) x 100

Protein efficiency ratio (PER) g = Mean weight gain of fish/ crude protein intake.

Feed efficiency Ratio (FER) g = Weight gain / total feeds consumed.

Gonadosomatic index = weight of the gonads $\div$ total weigf thefish $\times 100$

Hepatosomatic index = Liver weight $\div$ total weightofthe fish $\times 100$

Visceral somatic index = Total visceral weight $\div$ total weight of fish $\times 100$

Automatic Haematological and serum biochemistry parameters analysis

Blood samples were collected from six fish per treatment group (two per replicate) after anaesthetising them with oregano essential oil. Blood was drawn from the caudal vein using a 5 ml syringe with a 24-gauge needle. Samples for complete blood count (CBC) analysis were placed in EDTA tubes to prevent clotting, while glucose testing samples were stored in sodium fluoride tubes to inhibit glucose oxidation. Blood for liver function tests and hormone profiling were collected in serum tubes. Haematological profiles were assessed within one hour using an automated haematology analyser (HeCo Vet C, SEAC, Florence, Italy) as

per (Fazio et al., 2012; Fazio, 2019). The analyser measured red blood cell count (RBC), haematocrit (Hct), haemoglobin (Hgb), white blood cell count (WBC), platelets, and indices such as MCV, MCH, and MCHC. Impedance analysis and specialised software excluded nucleated RBCs from WBC counts. A lysing reagent (SEAC) containing potassium cyanide, quaternary ammonium salts, and surfactants was used to ensure accurate results. Human blood with stabilised platelets served as a control for instrument calibration. Samples were diluted (1:8) with an isotonic solution and tested using a "pre-diluted" setting per Fazio et al. (2024) for Nile tilapia (*O. niloticus*). Serum samples were centrifuged at 3,500 × g for 15 minutes to separate the supernatant. Biochemical analyses included total protein, liver enzymes (ALT, AST), globulin, albumin, and cholesterol. Total protein was measured via the biuret method, while ALP activity was assessed using para-nitrophenyl phosphate, recording the absorbance at 450 nm as described by Adham et al., (2002) and Abo-Taleb et al. (2024).

Histomorphology analysis

At the end of a 60-day treatment period, histological and histometric analyses were performed on the intestines of the fish. Three fish per treatment group were euthanised, and intestinal and liver tissue samples were collected, fixed in 10% formalin for 24 hours, and labelled. The fixed tissues were dehydrated in graded alcohol baths (70%, 95%, and 100%), cleared in xylene, and infiltrated with molten paraffin wax (58–60°C). After embedding, the tissue blocks were sectioned into 5 µm slices, floated on warm water, and mounted on slides.

The sections were de-waxed, rehydrated, stained with haematoxylin, differentiated in acid alcohol, and counterstained with eosin. Slides were then dehydrated, cleared in xylene, and mounted with DPX, producing six slides per treatment group for analysis. Histological evaluation was conducted using a compound microscope equipped with a NIKON digital camera (Model: DS-Fi1). Images were captured and analysed in ImageJ software, with a scale of 10 pixels equivalent to 1 µm for precise cell measurements. Histometric parameters, including villus height, width, intestinal muscle thickness, and goblet cell counts, were measured at 100× magnification. Six slides (two per fish) were prepared, with six random images per slide. Each slide was analysed in three zones, and 10 random grid fields per zone were measured, with final data based on an average of nine measurements per slide. Goblet cell counts were determined using a 300-point grid in ImageJ across a 400 µm² area, following protocols by Palladino et al. (2023) and Loureiro Paschoalini et al. (2024)

Liver anti-oxidant enzyme analysis process.

Liver samples were collected from one fish per treatment group, and the tissue was rinsed in ice-cold 1% sodium chloride (NaCl) solution. The liver was homogenised in an ice-cold buffer (150 mM Tris-HCl, 0.1 mM EDTA, and 0.1% Triton X-100, pH 7.8). Homogenates were centrifuged at 10,000 × g for 10 minutes at 4°C to obtain supernatants for further analysis. Samples were prepared in triplicate to assess antioxidant enzyme activity, with enzyme assays conducted according to the method outlined by (Eissa et al., 2024). Malondialdehyde (MDA) content was measured spectrophotometrically using the Thiobarbituric Acid Reactive Substances (TBARS) method. The supernatant was reacted with TBARS reagent and incubated at 45°C for 30 minutes. After cooling, the mixture was centrifuged at 20,000 × g for 5 minutes, and absorbance was measured at 450 nm, 530 nm, and 650 nm. MDA content was calculated using the formula: 6.45 × (OD650 – OD450) – 0.559 × OD530, adapted from Zheng (2023) and Abo-Taleb et al. (2024). Total Antioxidative Capacity (T-AOC) was assessed following Sitjà-Bobadilla et al. (2005), by incubating 0.03 mL of homogenate with 0.6 mL hydrogen peroxide (H_2O_2) buffer at 37°C for 25 minutes. After adding 3,5-dichloro-2-hydroxybenzenesulfonate, the remaining hydrogen peroxide was quantified calorimetrically at 530 nm. Catalase activity was determined by monitoring

hydrogen peroxide (H_2O_2) reduction at 450 nm, as described by (Mişe Yonar, 2019). The reaction was carried out with 5 mL of 0.05% H_2O_2 solution and 0.2 mL tissue homogenate for 20 minutes, followed by the addition of ammonium heptamolybdate to stop the reaction. One unit of catalase activity was defined as the reduction of 1 µmol of H_2O_2 per minute. Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide anion production in the xanthine/xanthine oxidase system, with one unit of activity defined as the amount of enzyme required to reduce colour formation by 50%, measured at 550 nm (Mansour and Esteban, 2017; Huang et al., 2022; Wigraiboon et al., 2024). Glutathione reductase (GR) activity was assessed spectrophotometrically by monitoring NADPH consumption, as described by Abo-Taleb et al. (2023). The reduction of oxidised glutathione (GSSG) to reduced glutathione (2GSH) in the presence of NADPH was quantified by the decrease in absorbance at 530 nm. One unit of GR activity was defined as the amount of enzyme required to reduce 1 µmol of NADPH per minute.

Statistical analysis

Statistical analyses were conducted using SPSS software (version 24). Data normality was assessed with the Kolmogorov-Smirnov test. One-way ANOVA, followed by Tukey's HSD test, was used to evaluate growth performance, liver enzyme activities, antioxidant properties, blood parameters, body indices, and intestinal histopathological changes at $P < 0.05$. Results are presented as mean ± standard error (SE). Morphometric parameters, including total and standard lengths and body weight, were analysed to assess the effects of probiotic inclusion levels on *O. mossambicus* growth. Kaplan–Meier analysis was applied to survival rates across treatment groups, identifying significant differences using Tukey's pairwise comparisons.

RESULTS

Survival rate of *O. mossambicus* fingerlings.

The study demonstrated that varying inclusion levels of a *S. cerevisiae* and *B. longum* probiotic mixture significantly influenced the survival of *O. mossambicus*. Kaplan-Meier survival plots showed lower probabilities in the control (PB0, 0.83) and PB2 (0.85, 0.9 g/kg feed) groups. Higher inclusion levels (PB3, 1.35 g/kg feed) significantly improved survival to 0.92. PB1 (0.45 g/kg feed) resulted in a slightly higher survival probability of 0.87. These findings indicate enhanced survival with increased probiotic inclusion (Figures 1a and 2c-d).

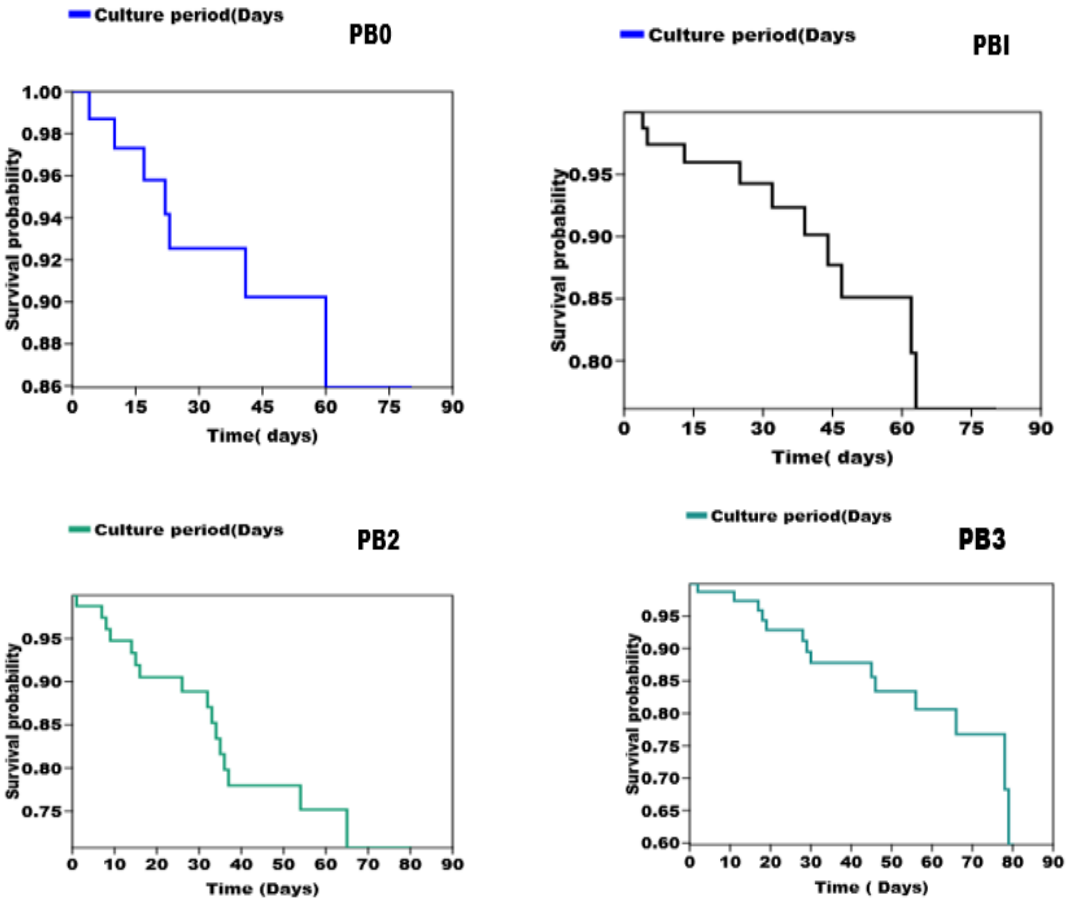

Figure 1 Kaplan-Meier survival probability plot across different (*S. cerevisiae*) and *B. longum* probiotic conglomerate inclusion levels: **PB0** - Survival probability control group (Control 0g/Kg feed), **PB1**-Survival probability at (0.45 g/kg feed), **PB2-** Survival probability at (0.9 g/Kg feed) and **PB3** - Survival probability at (1.35 g/kg feed).

Growth and feed utilisation

The inclusion of *S. cerevisiae* and *B. longum* probiotic mixture in fish diets significantly improved growth parameters and feed utilisation. Fish fed higher probiotic mixture inclusion of PB2 (0.9 g/kg) and PB3 (1.35 g/kg) diets showed significantly higher final weights (14.69 g and 16.286 g) and percentage weight gain (275.742% and 323.357%), respectively, compared to control ($P < 0.05$) Table 2. The feed conversion ratio (FCR) significantly improved in fish from higher probiotic mix inclusion PB3 (1.280) compared to other inclusion levels and control ($P < 0.001$). Specific growth rate (SGR) and protein efficiency ratio (PER) were significantly higher in the PB2 (0.9 g/kg feed) inclusion treatment level. Fish condition factor (K) was significantly improved in PB2 (0.9 g/Kg feed) inclusion treatment level; however, a higher probiotic mixture in PB3 (1.35 g/Kg feed) reduced fish condition factor (Table 2).

Table 2 Effects of different inclusion levels of *S. cerevisiae* and *B. longum* probiotic mixture on fish growth parameters and feed utilisation

Fish growth Parameters	*S. cerevisiae* and B. *longum* probiotic mixture inclusion level in fish diet			
	Control: PB_0 (0.00g/Kg of feeds)	PB_1 (0.45g /Kg of feeds)	PB_2 (0.9g /Kg. of feeds)	PB_3 (1.35g /Kg of feeds)
Initial weight (g)	4.735 ±0.243	4.436 ± 0.231	4.797 ± 0.202	4.605 ± 0.183
Final Weight (g)	11.87± 0.387 [a]	12.014± 0.372 [a]	14.69± 0.499 [b]	16.286 ± 0.291 [b]
Percentage weight gain (%)	253.693± 15.991[b]	163.573± 18.774 [a]	275.742± 16.417 [b]	323.357± 15.419[b]
Fish Condition Factor	1.642± 0.041 [a]	1.434 ± 0.063 [b]	1.731± 0.063 [c]	1.399± 0.063 [b]
Feed conversion Ratio	2.864± 0.154 [a]	2.490± 0.068 [b]	1.832± 0.110 [a]	1.280± 0.068 [a]
Feed Conversion Efficiency	0.365± 0.028 [a]	0.287± 0.006 [b]	0.358± 0.013 [a]	0.306± 0.006 [a]
Specific growth rate (%)	0.680± 0.053 [a]	0.659± 0.043 [a]	0.949± 0.034 [b]	0.840± 0.033 [b]
Protein efficiency ratio	0.221± 0.014 [a]	0.227± 0.017 [a]	0.370± 0.010 [b]	0.315± 0.015 [b]

Note: The growth Parameters are presented in Mean ± standard error **(S.E)** within three determinations. Where one-way ANOVA was significant (p<0.05), Turkey's pairwise test was performed. Means with different superscript letters in each row indicate significant differences at (P-<0.05)

Haematology and Serum Biochemistry

The inclusion of probiotics conglomerate of *S. cerevisiae* and *B. longum* significantly influenced the haematology and serum biochemistry of the fish. Haemoglobin (Hb) levels were significantly higher in fish from the PB1 (0.45 g/kg) and PB3 (1.35 g/kg) treatment groups compared to the control ($P < 0.001$). However, Hb level was lower in the PB2 (0.9 g/kg feed treatment group) Table 3. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were significantly higher in fish from PB1 (0.45 g/kg feed) and PB3 (1.35 g/kg feed) inclusion treatment levels. (P<0.05). Haematocrit (Hct) levels did not show significant differences across all treatment groups ($P > 0.05$).

Table 3 Effects of different inclusion levels of *S. cerevisiae* and *Bifidobacterium longum* probiotic mixture on haematology of *O. mossambicus.*

Complete blood counts (CBC)	*S. cerevisiae* and B. *longum* probiotic mixture inclusion level in fish diet			
	Control: PB_0 (0.00g/Kg of feeds)	PB_1 (0.45g /Kg of feeds)	PB_2 (0.9g /Kg. of feeds)	PB_3 (1.35g /Kg of feeds)
Haemoglobin (g/dl)	6.783± 0.235 [a]	8.086 ± 0.213 [b]	6.781 ± 0.314 [c]	8.128 ± 0.106 [b]
Haematocrit (%)	28.231 ± 5.276	26.568 ± 0.248	23.845 ± 0.427	25.705 ± 0.270
Mean Corpuscular Volume (g/dl)	145.25 ± 1.422 [a]	148.633 ± 0.994 [a]	140.648 ± 0.868 [b]	149.025 ± 0.354 [a]
Mean Corpuscular Haemoglobin	35.255 ± 1.462 [a]	46.09 ± 1.417 [b]	34.991 ± 0.543 [a]	41.881 ± 0.648 [b]
Mean Corpuscular Haemoglobin Concentrations (%)	28.368 ± 0.477 [a]	30.15 ± 0.276 [b]	27.788 ± 0.514 [a]	29.698 ± 0.255 [b]

Note: The haematology parameters are presented in Mean ± standard error (S.E). Where one-way ANOVA was significant (p<0.05), the Turkey's pairwise test was performed and Means with different superscript letters in each row indicate significant differences at (P-<0.05)

Liver enzyme activities showed reduced aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in fish from higher probiotic cocktails of PB2 (0.9 g/kg feed) and PB3 (1.35 g/kg feed) treatment groups compared to the control and lower inclusion level ($P < 0.05$). However, alkaline phosphatase (ALP) was significantly higher in fish from the higher probiotics mix PB3 (1.35 g/kg feed) inclusion treatment level ($P < 0.05$) (Table 4). Albumin (ALB), globulin (GLO), and total protein (TP) levels were significantly enhanced in fish from the PB2 (0.9 g/kg feed) and PB3 (1.35 g/kg feed) treatment groups ($P < 0.05$). serum cholesterol was significantly lower in fish from lower probiotics mixtures PB1 (0.45 g/kg) and PB2

(0.9 g/kg feed). Glucose level was significantly enhanced in fish from PB3 (1.35 g/kg feed) ($P < 0.05$) (Table 4).

Table 4 Effects of different inclusion levels of *S. cerevisiae* and *B.longum* probiotic mixture serum Biochemistry of *O. mossambicus.*

Serum Biochemistry	*S. cerevisiae* and B. *longum* probiotic mixture inclusion level in fish diet			
	Control: PB_0 (0.00g/Kg of feeds)	PB_1 (0.45g /Kg of feeds)	PB_2 (0.9g /Kg. of feeds)	PB_3 (1.35g /Kg of feeds)
Aspartate Amino transferase (µ/L)	47.758 ± 0.254 [a]	45.72 ± 0.290 [a]	42.348 ± 0.459 [b]	40.171 ± 0.294 [c]
Alanine Aminotransferase (µ/L)	27.085 ± 0.163 [a]	26.2 ± 0.192 [a]	24.391 ± 0.183 [b]	21.928 ± 0.197 [c]
Alkaline phosphate (µ/L)	23.956 ± 0.285 [d]	24.778 ± 0.189 [d]	25.015 ± 0.435 [e]	27.585 ± 0.226 [f]
Albumin (mg/dl)	1.47 ± 0.063 [a]	2.236 ± 0.090 [b]	2.418 ± 0.072 [b]	2.35 ± 0.096 [b]
Globulin (mg/dl)	3.376 ± 0.127 [c]	6.013 ± 0.208 [d]	6.608 ± 0.191 [d]	6.671 ± 0.207 [d]
Serum Cholesterol (mg/dl)	98.241 ± 0.148 [a]	95.783 ± 0.307 [b]	96.78 ± 0.334 [b]	97.431 ± 0.442 [a]
Glucose (mg/dl)	81.321 ± 0.450 [g]	94.25 ± 0.229 [f]	87.515 ± 0.593 [g]	97.063 ± 0.474 [f]
Total protein (g/dl)	4.557 ± 0.123 [a]	5.514 ± 0.128 [a]	5.985 ± 0.079 [b]	6.571 ± 0.211 [b]

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Note: The serum biochemistry parameters are presented in Mean ± standard error (S.E) within three determinations. Where one-way ANOVA was significant ($p<0.05$), the Turkey's pairwise test was performed and Means with different superscript letters in each row indicate significant differences at (P-<0.05)

Body Somatic Indexes and Liver enzyme Antioxidant Activities and innate immunity.

The inclusion of *S. cerevisiae* and *B. longum* probiotics conglomerate in the diet of O. mossambicus significantly impacted liver oxidant enzyme activities and physiological indices in fish (Table 5). Malondialdehyde (MDA), a marker of lipid peroxidation, was significantly higher in fish from higher inclusion levels PB2 (0.9 g/kg) and PB3 (1.35 g/kg) treatment groups compared to the control ($P < 0.0001$). Superoxide dismutase (SOD) and catalase, key antioxidant enzymes, were significantly increased in fish from higher probiotic mixture PB2 (0.9 g/kg) and PB3 (1.35 g/kg) treatment groups ($P < 0.05$). Glutathione reductase (GR) levels were also significantly elevated in fish from the PB2 (09 g/Kg) and PB3 (1.35 g/Kg) treatment groups ($P < 0.001$) (Table 5). Total antioxidative capacity was higher in higher probiotic mixture-treated groups, especially PB2 (0.9 g/kg) and PB3 (1.35 g/kg) ($P < 0.0001$). Nitric oxide synthase (NOS) activity was significantly higher in fish from the PB2 (0.9 g/kg) and PB3 (1.35 g/kg feed) treatment groups ($P < 0.05$). Among physiological indices, visceral somatic index (VSI) and stomach index (SI) were significantly increased in fish from higher probiotics mixture PB2 (0.9 g/kg) and PB3 (1.35 g/kg feed) treatment groups ($P < 0.05$). Conversely, the gonadosomatic index (GSI) declined significantly in fish from higher probiotics mixture PB2 (0.9 g/kg) and PB3 (1.35 g/kg feed) treatment groups ($P < 0.001$) (Table 5).

Table 5 Effects of different inclusion levels of *S. cerevisiae* and *B. longum* probiotic mixture on body somatic indexes and Liver Anti-oxidant Enzyme Activities of *O. mossambicus*

Liver Oxidant Enzymes activities	*S. cerevisiae* and *B. longum* probiotic mixture inclusion level in fish diet			
	Control: PB0 (0.00g/Kg of feeds)	PB1 (0.45g /Kg of feeds)	PB2 (0.9g /Kg. of feeds)	PB3 (1.35g /Kg of feeds)
Malondialdehyde	5.666 ± 0.310 [a]	6.466 ± 0.210 [a]	7.2 ± 0.110 [b]	8.2 ± 0.105 [b]
Superoxide dismutase	29.253 ± 0.374 [a]	30.26 ± 0.561 [a]	38.763 ± 0.230 [b]	41.45 ± 0.559 [b]
Catalase enzyme	26.383 ± 0.563 [a]	28.096± 0.262 [b]	28.273 ± 0.429 [b]	29.14 ± 0.427 [b]
Glutathionine reductase	36.503 ± 4.493 [a]	39.753 ± 1.761 [a]	57.48 ± 0.439 [c]	64.3 ± 2.586 [b]
Total antioxidative capacity	1.903 ± 0.018 [d]	1.943 ± 0.118 [d]	3.023 ± 0.0409 [c]	3.046 ± 0.040 [c]
Nitric oxide synthase	4.22 ± 0.135 [a]	5.126 ± 0.139 [a]	7.203 ± 0.204 [b]	8.11 ± 0.037 [b]
Viscral somatic index	6.632± 0.248 [a]	6.747± 0.286 [a]	9.002± 0.350 [b]	8.156± 0.476 [b]
Heptasomatic index	0.903± 0.023 [b]	1.034± 0.066 [a]	1.069± 0.057 [a]	1.094± 0.021 [a]
Stomach index	2.430± 0.212 [a]	2.565± 0.299 [a]	3.200± 0.381 [b]	4.352± 0.316 [b]
Gonadosomatic index	0.326 ± 0.021[a]	0.278 ± 0.012 [a]	0.214 ± 0.013[b]	0.17 ± 0.007[b]

Note: The fish parameters are presented in Mean ± standard error (S.E). Where one-way ANOVA was significant ($p<0.05$), the Turkey's pairwise test was performed and Means with different superscript letters in each row indicate significant differences at (P-<0.05).

Histo morphometry of the fish intestine at different probiotics inclusions

Various histological parameters, including villus length, width, area, crypt depth, and intestinal wall thickness, resulting from the dietary administration of a probiotic mixture of *S. cerevisiae* and *B. longum*, are presented in Figure 2 (a) (gut morphology of fish from control) Figure 2(b) gut morphology of fish from 0.45g/Kg feed treatment groups. Figure 3(a) gut morphology of fish from 0.9g/Kg feed treatment and Figure 3 (b) gut morphology of fish from 1.35g/Kg feed treatment group. Intestinal villus width and villus length were significantly greater in fish from PB2 (0.9 g/kg feed S. *cerevisiae* and *B. longum* probiotic mixture inclusion treatment levels compared to control and other groups at 131 um and 316 um, respectively ($P < 0.05$). (Figure 4a and b) respectively. However, crypt depth and thickness of walls significantly increased with an increase in probiotics mixture dosage level, with PB3 (1.35 g/kg feed) showing the highest crypt depth and thickness wall, 76.2 um and 34.5 um, respectively ($P < 0.05$). (Figure 5a and b) *respectively.* The number of goblet cells and villus areas was significantly higher in S. *cerevisiae* and *B. longum* probiotic mixture inclusion levels (PB3, 1.35 g/kg feed) compared to the control and other groups ($P < 0.05$). (Figure 6 a and b) respectively.

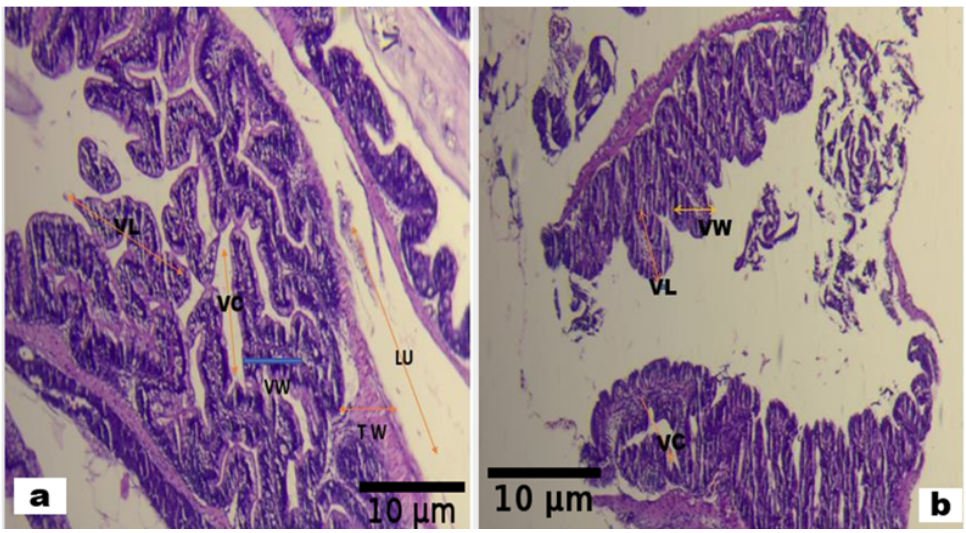

Figure 2 The overview of the anterior section of the intestines at [a](0.0g/Kg Control), [b]-(0.45g/Kg feed) ,Note: **VW** (Villus width), **VL** (Villus length), **VC** (Villus crypts) **LU** (Lumen), **MV** (Microvilli),**TW** (Thickness of walls) **LU** (Lumen), **MV** (Microvilli) distribution.

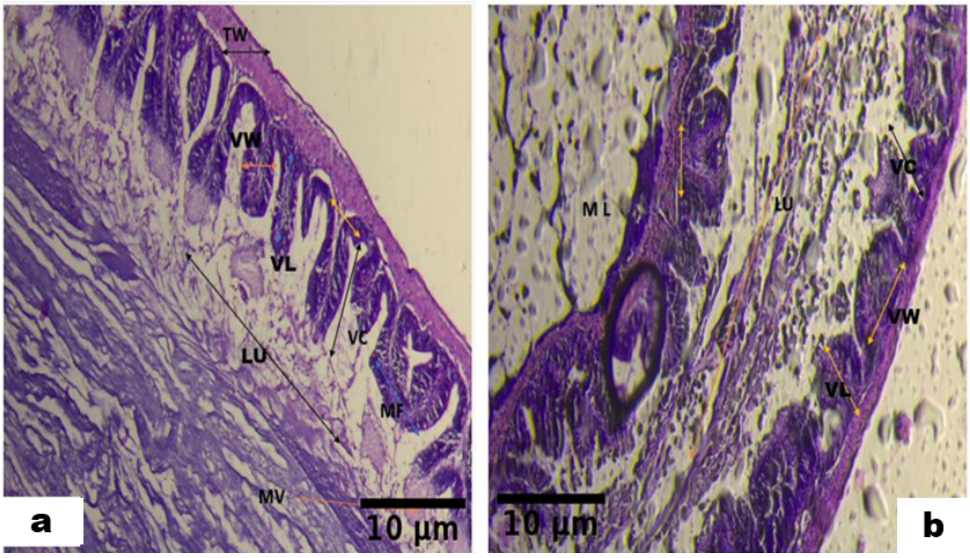

Figure 3 [a] - (0.9g/Kg feed) and **[b]**- (1.35g/Kg feed) S. *cerevisiae* and *B. longum* probiotic mixture in *O. mossambicus*.. Note: **VW** (Villus width), **VL** (Villus length), **VC** (Villus crypts) **LU** (Lumen), **MV** (Microvilli),**TW** (Thickness of walls) **LU** (Lumen), **MV** (Microvilli) distribution.

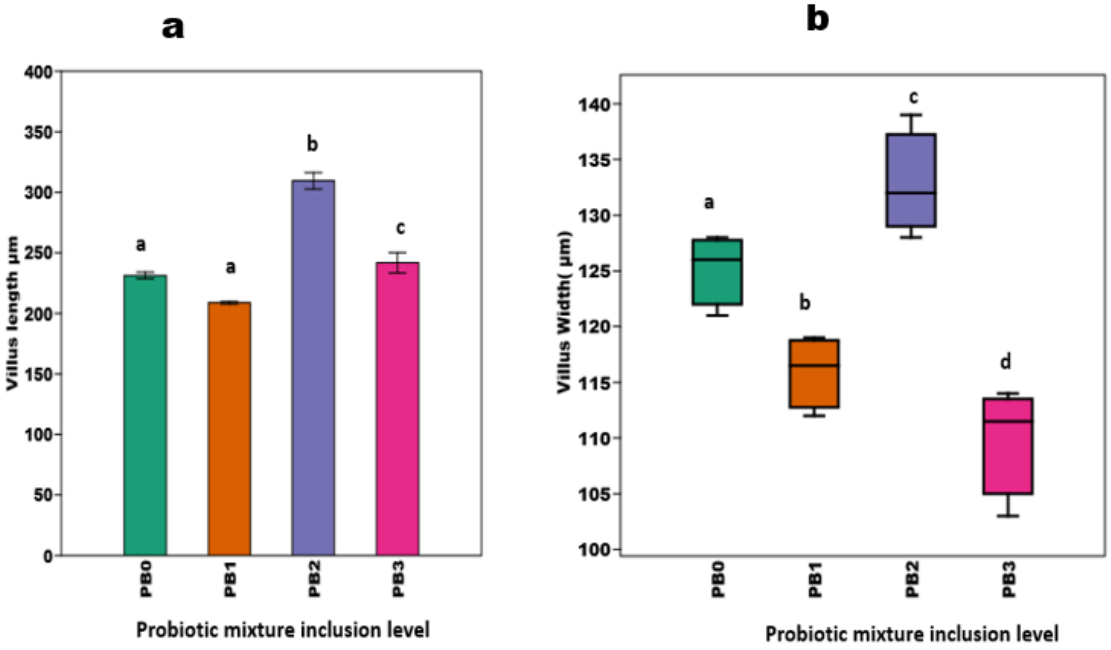

Figure 4 Histomorphometry of Villus length (a) and Villus width (b) at different inclusions of *S. cerevisiae* and *B. longum* probiotic mixture in *O. mossambicus*.

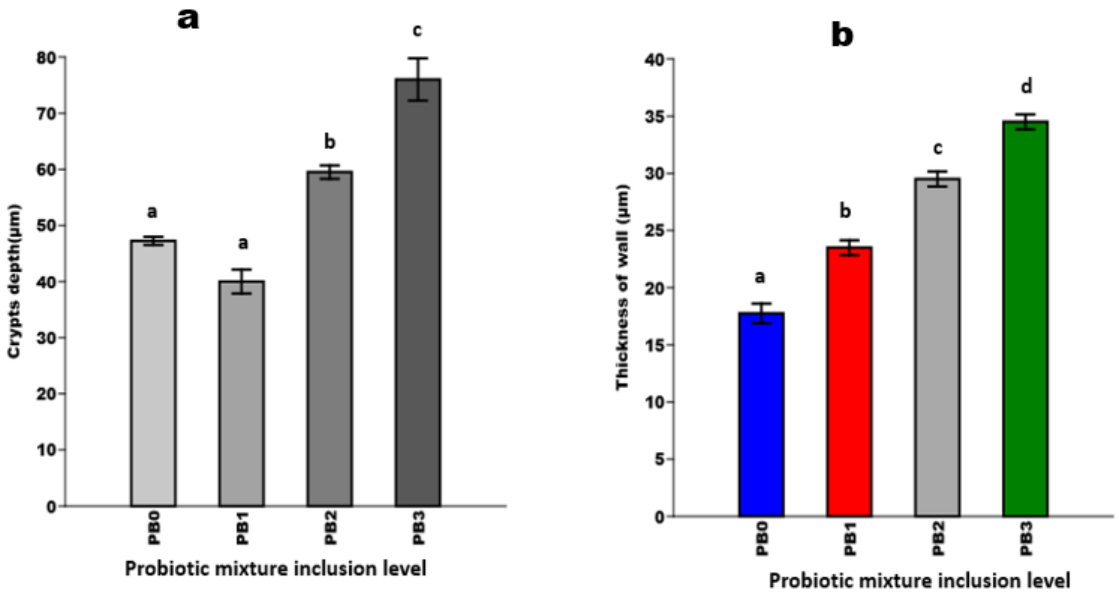

Figure 5 Histomorphometry of Crypts depth(a) and Thickness of wall(b) at different inclusions of *S. cerevisiae* and *B. longum* probiotic mixture in *O. mossambicus*.

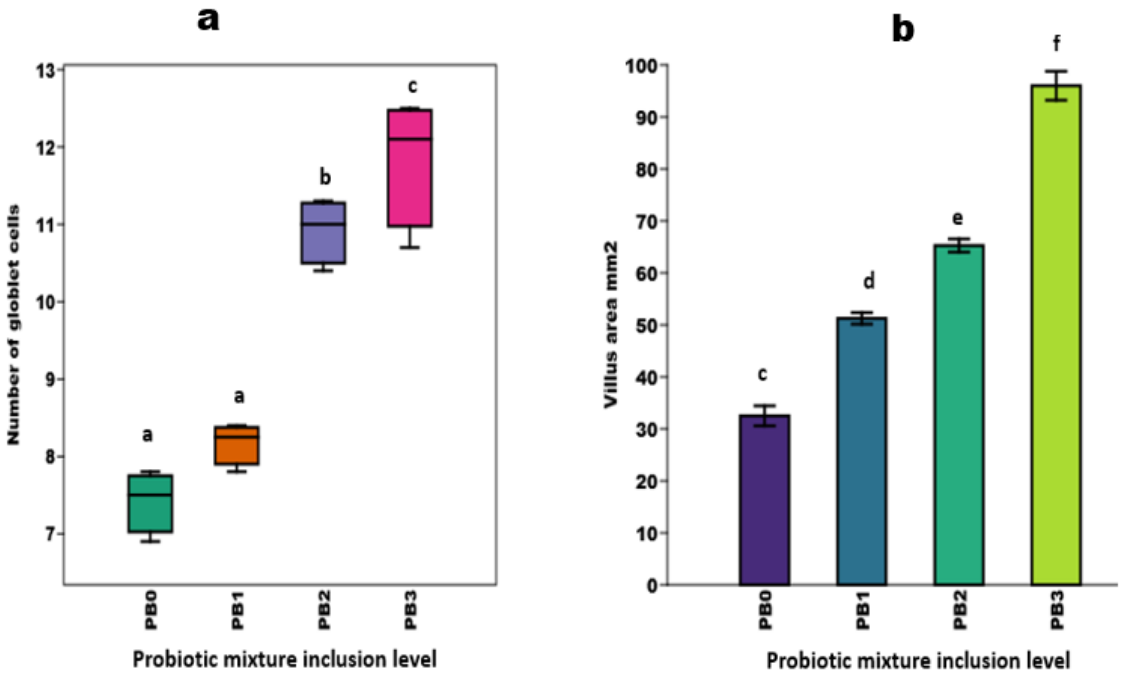

Figure 6 Histomorphometry of the number of goblet cells (a) and Villus area(b) at different inclusions of *S. cerevisiae* and *B. longum* probiotic mixture in *O. mossambicus*.

DISCUSSION

Probiotics positively influence the growth and immunity of fish. A wide range of commercial probiotics from bacteria, microalgae, bacteriophages, whole yeast, or yeast extracts are widely used in aquaculture (Irianto and Austin, 2002; Mujeeb Rahiman et al., 2010; Mohammady et al., 2023; Kumar et al., 2024). Most of these probiotics originate from the host or outside of the host species, where they are identified, characterised, and isolated for use as fish feed additives or added to the water of the culture system. Different probiotics in aquaculture used as fish feed additives have been reported to boost productivity (Ntakirutimana et al., 2023; Syanya et al., 2023). Among these probiotics, baker's yeast *S. cerevisiae* is increasingly applied in aquaculture systems as an effective growth promoter with the capability of immunity function enhancement and influences on fish gut morphology (Opiyo et al., 2019; Taguemount et al., 2024). Equally Probiotic bacteria are also widely used in aquaculture, with examples like *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus* aiding digestion and immunity (Aly et al., 2008; El-Saadony et al., 2021; Tanveer et al., 2024).*Nitrosomonas* and *Nitrobacter* facilitate nitrification, (Asha et al., 2024), while *Lactobacillus spp.* And *Pseudomonas spp.* inhibit pathogens and degrade organic matter (Mohapatra et al., 2013; Krummenauer et al., 2014; Majeed et al., 2025; Thuy, 2025).. Above all, *Bifidobacterium longum has* been effectively used to promote growth in fish and crustaceans (Todorov et al., 2024). Similarly, a mix of *Lactobacillus paracasei* and *B. longum was* reported to enhance growth, immunity, and disease resistance in shrimp (Huang et al., 2022).In the current study, a mixture of S. *cerevisiae* and *B. longum* probiotics in fish diets significantly improved growth parameters and feed utilisation. This is due to enhanced gut microbiota and enzymatic activity, as was reported in Nile tilapia (*O. niloticus*) fed dietary fermented *S. cerevisiae* extract (Hilyses) supplementation (Abd El-Naby et al., 2024). Increased final weights and percentage weight gain in higher probiotic conglomerate inclusions at PB2 (0.9 g/kg) and PB3 (1.35 g/kg) treatments are indications of improved nutrient

absorption and metabolism due to higher probiotic mixture inclusion in the fish diet. These findings are in tandem with those of del Valle et al. (2023) and Huang et al. (2022) who noted probiotics improve nutrient absorption, metabolism, and growth performance in fishes and crustaceans . The significantly lower FCR in fish from a higher *S. cerevisiae* and *B. longum* probiotics mixture inclusion in the diet PB3 (1.280) indicates better feed efficiency, consistent with Islam et al. (2021), Gule and Geremew (2022) who reported probiotics reduce feed wastage and enhance growth performance in Nile tilapia (*O. niloticus*). Moreover, dietary baker yeast and *B. longum* have independently been shown to significantly improve the growth, immune response, and disease resistance of Nile tilapia and shrimp (*Litopenaeus vannamei)* (Huang et al., 2022; Mohammady et al., 2023). Similarly, the current study reveals that the enhanced growth, FCR, and SGR of *O. mossambicus* fed higher levels of *S. cerevisiae* and *B. longum* probiotic mixtures result from improved nutrient utilisation. This increased growth performance can be attributed to the positive effects of *S. cerevisiae* supplementation in the fish diet, which enhances nutrient availability, digestion, and absorption by stimulating the secretion and activity of key digestive enzymes such as amylases, lipases, and proteases. Increased inclusion levels of *S. cerevisiae* and *B. longum* probiotics mixture significantly improved the survival of *O. mossambicus* with PB3 (1.35 g/kg feed) exhibited the highest survival probability (0.92). These results suggest that higher probiotic mixture inclusion enhances survival, potentially by improving immune function, gut health, and disease resistance, consistent with earlier findings on the role of probiotics in strengthening host resilience and promoting overall health in aquaculture (Islam et al., 2021a; Huang et al., 2022; Amenyogbe, 2023; Syanya et al., 2023; Redhwan et al., 2024).*S. cerevisiae* and *B. longum* probiotic mixtures in the diet of *O. mossambicus* influenced the haematological and serum biochemistry parameters. Higher haemoglobin (Hb) levels, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) in fish from PB3 (1.35 g/kg) inclusions suggest enhanced oxygen transport capacity, which is associated with improved iron bioavailability or erythropoiesis. Similar effects have been reported in Rohu *(Labeo rohita*), where yeast probiotics supported haematological parameters and immunity (Jahan et al., 2021). Elevated MCV and MCH values suggest larger, haemoglobin-rich red blood cells, due to increased erythropoiesis and changes in cell membrane permeability. A similar finding was reported in rainbow trout (*Oncorhynchus mykiss*), where probiotics enhance red cell metrics through improved nutrient absorption and metabolic function (Pooramini et al., 2014). Liver enzymes, AST and ALT, decreased with higher probiotic mixture doses, especially in PB2 (0.9 g/kg) and PB3 (1.35 g/kg), indicating reduced hepatocellular stress. This may imply a hepatoprotective role, as probiotics modulate oxidative stress and inflammatory responses (Dawood et al., 2020). However, ALP levels were elevated with increased *S. cerevisiae* and *B. longum* probiotic mixtures in the fish diet, potentially reflecting increased liver activity or bone turnover associated with higher metabolic rates. Our findings are in tandem with Tanveer et al. (2024), who reported enhanced growth, haematological profile, blood biochemistry, and antioxidant capacity of *Clarias batrachus* fingerlings fed on dietary multi-strain probiotics. In our study, albumin (ALB) and globulin (GLO) levels increased in higher S. *cerevisiae* and *B. longum* probiotic mixtures inclusions, pointing to improved protein synthesis and immune response, possibly through probiotics' influence on gut microbiota and immune cell proliferation. The findings concur with Abdel-Ghany et al. (2020), Abd El-Naby et al. (2024) where the supplementation of fermented *S. cerevisiae* extract in the diet of Nile tilapia improved immunity, haematology, antioxidant activity, and intestinal health. Our findings also reveal that higher inclusion levels of *S.cerevisiae* and *B. longum* probiotics mixtures significantly improved various body somatic indexes and liver antioxidant enzyme activities in *O. mossambicus*. Notably, the Stomach Index (SI) was markedly higher in fish from the PB2 (0.9 g/kg) and PB3 (1.35 g/kg) probiotics inclusion groups, suggesting enhanced feeding and nutrient absorption associated with probiotic-induced gut health, as corroborated

by Hossain et al. (2024), who linked probiotic supplementation with improved gonadal development in Nile tilapia (*O. niloticus*). Puri et al. (2022) also reported improved gastrointestinal morphology in finfish aquaculture. Similarly, the increase in the Hepatosomatic Index (HSI) with increased *S. cerevisiae* and *B. longum* probiotic mixtures treatments implies improved liver efficiency, aligning with findings by Omar et al. (2024) on probiotics enhancing liver function in Nile Tilapia (*O. niloticus*).

Antioxidant enzymes, such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT), play a crucial role in the fish's defence mechanism, providing essential protection against oxidative stress in fish (Sitjà-Bobadilla et al., 2005; Mohammadi et al., 2020; Abd El-Naby et al., 2024). Our findings indicate that catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) levels significantly increased at higher inclusion levels of *S. cerevisiae* and *Bifidobacterium longum* probiotic mixtures PB3 (1.35 g/kg). This suggests that the probiotic mixture plays a key role in strengthening antioxidative defences. A significant increase in total antioxidative capacity (TAOC) and nitric oxide synthase (NOS) further demonstrates enhanced oxidative protection and immune modulation. These findings are in line with, Gobi et al. (2018), where dietary supplementation with the probiotic *Bacillus licheniformis* Dahb1 enhanced growth performance, boosted mucus and serum immune parameters, and increased antioxidant enzyme activity in Mozambique tilapia (*O. mossambicus). Varying* levels of *S. cerevisiae* and *B. longum* probiotic mixtures significantly impacted the fish's intestinal morphology and structure. Histomorphometric analysis revealed that villus width and length increased significantly with higher probiotic inclusion. The widest villi were observed in fish fed the highest dose of the probiotic mixture, PB3 (1.35 g/kg), due to enhanced gut health and nutrient absorption facilitated by this probiotic combination. This aligns with the findings of Islam et al. (2021), who reported enhanced villus width and length in Nile tilapia (O. *niloticus), thereby* increasing intestinal surface area and nutrient absorption efficiency in fish fed on a probiotic-supplemented diet. The significant increase in the number of goblet cells, particularly in the PB3 treatment group, suggests an enhancement in mucin production, which is crucial for protecting the intestines from pathogenic organisms and improving gut function. This finding is consistent with research by de Moraes et al. (2022) , who found that probiotics such as *Saccharomyces cerevisiae* stimulate mucin secretion, which improves intestinal immunity and barrier function and acts as a tool to enhance gut health and performance in aquaculture.

CONCLUSIONS

The study concludes that while *S. cerevisiae* (baker's yeast) and *B. longum* have been independently used as effective probiotics in aquaculture, their combination in the right proportions offers a superior alternative to stand-alone applications. The findings demonstrate that a diet enriched with *S. cerevisiae* and *B. longum* probiotic mixtures significantly improves growth, feed utilisation, immunity, and gut health in *O. mossambicus*. Higher inclusion levels enhanced growth performance, survival rates, and feed efficiency. Optimal dosages, particularly PB2 (0.9 g/kg) and PB3 (1.35 g/kg), maximised final weight, feed conversion ratio (FCR), and nutrient absorption. Improved gut morphology, such as increased villus size, facilitated better nutrient uptake. Additionally, elevated haematological indices (Hb, MCV, and MCH) reflected improved health. Enhanced liver enzyme activity and reduced AST and ALT levels in PB3(1.35g/Kg feed) indicate protective effects against liver stress, while elevated ALB and GLO levels suggest enhanced protein synthesis and immune function. The probiotic mixture also boosted antioxidant defences, evidenced by increased SOD, GPx, and CAT levels, indicating strong antioxidative capabilities. Increased goblet cell density and

mucin production further strengthened intestinal immunity and function. This study recommends the use of *S. cerevisiae* and *B. longum* mixtures at 0.9–1.35 g/kg feed as additives for *O. mossambicus* and other tilapia species. This approach is cost-effective, offering higher net returns and cumulative benefits that surpass the individual effects of standalone probiotics. Adopting this mixture in fish diets could enhance yields, improve feed utilisation, and promote economic sustainability in aquaculture. Further research and policy support are essential to standardise and commercialise this probiotic blend, leveraging its global availability and affordability to boost aquaculture productivity.

CREDIT AND AUTHORSHIP CONTRIBUTION

FJS, ARNH, and PM M: Conceptualisation, data methodology, data curation, analysis, writing original draft,
HM: Supervision, guiding formal data validation, and final editing and review.
WMM and PM: Reviewing, English proofreading, and final editing

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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