



Synbiotics of *Pediococcus pentosaceus* and Fructooligosaccharide enhances the growth rate, enzymatic digestion, feed efficiency, hematological profiles, and lysozyme activity of Golden Rabbitfish (*Siganus guttatus*)

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ABSTRACT

A 60-day dietary trial was conducted in twelve fiberglass tanks, each with a capacity of 300 L and stocked at a density of 50 fish tank⁻¹, to investigate the influence of different combinations of synbiotics between *Pediococcus pentosaceus* and Fructooligosaccharide (FOS) on the growth rate, feed efficiency, digestive enzyme activities, hematological profiles and lysozyme activity of rabbitfish. Fingerlings weighing 5.10±0.28 g were fed with synbiotics containing FOS alongside varying masses of *P. pentosaceus* cells, including 10⁷ CFU g⁻¹ (NT1), 10⁸ CFU g⁻¹ (NT2), and 10⁹ CFU g⁻¹ (NT3). Meanwhile, a control group received a basic diet containing 0.5 % FOS without *P. pentosaceus* supplementation. The trial was performed in triplicates. After 60 days, the addition of synbiotics significantly improved the growth rate, feed efficiency, digestive enzyme activities, hematological profiles, and lysozyme activity of Golden Rabbitfish across all dietary treatments. The results indicate that supplementing the diet of golden Rabbitfish with synbiotics containing *P. pentosaceus* and FOS, particularly *P. pentosaceus* at a concentration of 10⁸ CFU g⁻¹, is advisable to enhance the overall growth rate, feed efficiency, and health status of the fish.

1. Introduction

The golden Rabbitfish (*Siganus guttatus*) is a herbivorous, euryhaline, and marine fish species (Simora et al., 2015). This fish is renowned for its high market value and popularity and its exceptional nutritional value (Tabugo et al., 2012). Golden Rabbitfish are commonly raised in brackish water areas where natural foods are abundant (Gorospe and Demayo, 2013) due to their wide adaptability to salinity levels ranging from 1 ppt to seawater (Binh et al., 2022). The golden Rabbitfish is also recognized as a relatively fast-growing species (Gonzales et al., 2018) with simple cultivation methods. It can be cultivated at high densities and can also be fed on formulated feed (Juario et al., 1985; Saoud et al., 2007; Syah et al., 2020). This fish is particularly well-suited for aquaculture farming due to its high consumer preference (Tabugo et al., 2012) and delicious white meat (Syah et al., 2020). These advantages suggest that rabbitfish is an important species for aquaculture farming

from a view of economic perspective.

In Vietnam, rabbitfish can be found along the coastal areas in the Central region, especially in Binh Dinh, Quang Nam, and Thua Thien Hue provinces (Duy et al., 2017). The fish has emerged as a distinctive local aquatic species in the central region of the country, especially in the Tam Giang - Cau Hai lagoon area. In Thua Thien Hue province, rabbitfish is primarily polycultured with either tiger shrimp (*Penaeus monodon*) or white shrimp (*Litopenaeus vannamei*) in earthen ponds at densities ranging from 0.3 to 1 fish m⁻² (Duy et al., 2017). Occasionally, rabbitfish is also monocultured in earthen ponds, net cages, or even cement tanks. Unfortunately, rabbitfish fingerlings for aquaculture in Vietnam are still predominantly collected from the wild (Duy et al., 2017), which does not meet the demand for fingerlings for cultivation (MARD, 2016). Annually, natural rabbitfish larvae often appear at the estuary or sea mouth from July to October (Phu, 2021). Thus, local farmers have to rear seeds throughout the extreme climatic conditions

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(during the rainy, cold, and flood months) with sudden and high fluctuations of salinity and temperature. This is considered the main reason for the mortality of rabbitfish fingerlings. A failure in rearing natural seeds has an extreme effect on the productivity plans of rabbitfish farming.

So far, many studies have been conducted to figure out effective approaches that can improve the survival, health condition, and quality of rabbitfish fingerlings. Among the identified strategies, the application of either probiotics or synbiotics is feasible and reliable. This is supported by the attention they have garnered in aquaculture following their successful implementation of animal feeding practices (Mariyam et al., 2021). Probiotics comprise live, dead microbial cells and are considered beneficial bacteria for the host, including aquatic animals (Pandiyan et al., 2013; El-Saadony et al., 2021). Once probiotics are administered via either feed or rearing water, they aid the host animal and contribute in direct or indirect ways to shield the host against harmful pathogens (Martínez et al., 2012; Pandiyan et al., 2013). Probiotics enhance disease resistance, nutrient uptake, improve growth performance, enhance tolerance responses, influence hematological indices, modulate intestinal microflora, and promote overall health status (El-Saadony et al., 2021; Martínez et al., 2012; Ringø et al., 2020; Yilmaz et al., 2022). Mariyam et al. (2021) reported that probiotics compete with various environmental stressors and promote host growth by enhancing feed value, inhibiting pathogenic microorganisms, facilitating enzymatic digestion, boosting immune responses, and exhibiting anti-mutagenic properties. Numerous studies have demonstrated the positive effects of probiotics on fish species living in brackish and marine environments. For instance, Nguyen et al. (2018) documented the beneficial influence of *Lactococcus lactis* WFLU12 in helping Olive Flounder (*Panaeolus olivaceus*) fight against *Streptococcus parauberis*, thereby improving fish growth performance and food conversion. Linh et al. (2018) asserted that *L. lactis* K-C2 positively influences the growth rate and intestinal microbial community and changes the amino acid of amberjack (*Seriola dumerili*). The application of probiotics significantly enhanced the growth and survival rates, microbial community, feed conversion, digestive enzyme activity, and immune system of seabass (*Lates calcarifer*) (Anna et al., 2019; Sokooti et al., 2022). Meanwhile, the simultaneous use of probiotics and non-digestible food ingredients, such as fructooligosaccharide (FOS) and mannan oligosaccharide (MOS) has been reported to yield beneficial effects on the host via synergistic effects, hence referred to as "synbiotics" (Knipe et al., 2021; Li et al., 2018). The addition of synbiotics into fish diets increase the population of beneficial bacteria (probiotics) within the fish's intestine. This stimulates the production of exogenous enzymes and boosts the immune system, ultimately improving digestion, enhancing nutrient absorption, and preventing pathogens (Cerezuela et al., 2011). These combined effects lead to an improvement in the growth rate and disease resistance of fish. Synbiotics (*Bacillus* spp. and *Enterococcus* spp. along with MOS and FOS) have been documented to exhibit synergistic effects on the growth and survival rates, intestinal microbial community, digestive enzyme activity, and disease resistance of rainbow trout (*Oncorhynchus mykiss*), Japanese flounder (*Panaeolus olivaceus*), yellow croaker (*Larimichthys polyactis*), and cobia (*Rachycentron canadum*) (Villumsen et al., 2020; Cerezuela et al., 2011; Huynh et al., 2017). Moreover, synbiotics have also been utilized as alternatives to antibiotics. For instance, synbiotic (Lacto Forte) can serve as a surrogate for antibiotics in controlling *Pseudomonas fluorescens* in tilapia (*Oreochromis niloticus*) (Ismail et al., 2019).

Recently, several studies have highlighted the positive effects of *Pediococcus pentosaceus* on aquatic animals. *P. pentosaceus* inhibits various pathogenic bacteria, including antibiotic-resistant *Staphylococcus aureus*, *Escherichia coli*, and *Vibrio* spp. (Thao et al., 2021). It has also been proven as a potential probiotic, promoting immune response, disease resistance, and growth rate, as well as showing positive effects on the body composition and intestinal microflora of aquatic animals (El-Dakar et al., 2021; Moslehi, 2016; Thao et al., 2021; Wanna et al.,

2023; Won et al., 2020). Furthermore, the combined use of *P. pentosaceus* and FOS has been shown to have synergistic effects in improving the growth performance, immune response, and *Vibrio parahaemolyticus* resistance of whiteleg shrimp (*Litopenaeus vannamei*) (Hong et al., 2022). However, studies on the beneficial effects of *P. pentosaceus* combined with FOS on marine fish species are still limited. Therefore, it is hypothesized that supplementing *P. pentosaceus* and FOS to diets can improve the growth rate, feed efficiency, digestive enzyme activities, hematological profiles, and lysozyme activity in Golden Rabbitfish.

2. Material and methods

2.1. Rabbitfish fingerlings

Fingerlings of rabbitfish were delivered from a local hatchery in Thuan An ward, Hue City, Vietnam. The initial fingerling size was 5.10 ± 0.28 g and 4.60 ± 0.36 cm. Upon arrival at the laboratory, the fingerlings were maintained for two weeks to adapt to the experimental conditions (temperature 23–30 °C; salinity 15–20 ppt; pH 7–7.5; and dissolved oxygen 5–6 mg L⁻¹).

2.2. Probiotic preparation

The probiotic bacteria (*P. pentosaceus* HN10) used in this study were obtained from the Laboratory of Enzyme and Protein Technology at the Institute of Biotechnology, Hue University. *P. pentosaceus* HN10, isolated from fermented *Solanum macrocarpon* (a homemade fermented food), has been identified, and its biochemical characteristics were studied (Thao et al., 2021).

The probiotic was prepared according to the protocol outlined by Thao et al. (2021). In brief, *P. pentosaceus* HN10 was cultured on De Man, Rogosa & Sharpe (MRS) agar medium (Merck, Darmstadt, Germany) and aerobically incubated at 30 °C for 24 h. A single colony of *P. pentosaceus* HN10 was inoculated in 5 mL of sterile MRS broth at 30 °C in a shaking incubator (Kuhner shaker, ISF-1-W, Switzerland) at 180 rpm for 24 h. The culture solution was transferred to a 250 mL Erlenmeyer flask, consisting of 50 mL of sterile MRS broth, and further incubated for 24 h at 30 °C at 180 rpm shaking speed. Subsequently, the bacterial cells were harvested by centrifugation at 14,000 rpm (Sanyo NSE Mistral 2000R, Japan) for 10 min. The obtained pellet was washed twice in sterile phosphate buffered saline (PBS). Finally, the cell pellets were re-suspended in PBS to achieve an OD_{600 nm} of 1.0, equivalent to 1×10^9 CFU mL⁻¹ which was confirmed through the enumeration of viable cells technique described by Cappuccino and Welsh (2017).

2.3. Experimental diet preparation

The basal diet was designed to maintain iso-lipidic (100 g kg⁻¹ crude lipid) and iso-nitrogenous (360 g kg⁻¹ crude protein) based on recommended nutrient values for rabbitfish (Saoud et al., 2010; Ghanawi et al., 2011). The control diet was prepared by supplementing the basal diet with 0.5 % FOS (Table 1).

The preparation and storage of the diets were conducted as methods mentioned by Adel et al. (2017) and Hong et al. (2022). In brief, the ingredients were measured, milled to pass through a 250-mm-mesh screen, and thoroughly mixed in a blending machine. Then, fish oil and water were supplemented until a cohesive dough was formed. Subsequently, each diet was subjected to a laboratory pelleting machine (3 A, Vietnam), which resulted in pellets with a diameter ranging from 0.5 to 1 mm. The pellets were dried under ambient temperature for 48 hours to reduce a moisture level below 12 %. To prepare the different synbiotic supplementation diets, *P. pentosaceus* cells initially stocked at a concentration of 1×10^9 CFU mL⁻¹ were diluted with PBS solution to achieve a concentration of 1×10^8 CFU mL⁻¹. Subsequently, the diluted *P. pentosaceus* cells were sprayed onto the basal diet at a ratio of 1:9

Table 1
Ingredients and proximate composition of the basal diet for Golden Rabbitfish.

| Ingredients | % |
|--|----------|
| Fish meal (FM) | 34.0 |
| Soybean meal (SM) | 26.0 |
| Corn starch | 17.0 |
| Rice bran | 15.4 |
| Fish oil (cod liver oil) ^a | 6.0 |
| Vitamin premix ^b | 1.44 |
| Mineral premix ^c | 0.05 |
| Binder | 0.06 |
| Fructooligosaccharide (FOS) ^d | 0.05 |
| Compositions | % |
| Dry matter (DM) | 88.6 |
| Crude lipid (EE) | 10.2 |
| Crude protein (CP) | 35.8 |
| Ash | 7.4 |

^a The Moller's Tran, Ltd., Nauy.

^b Vitamin and mineral premix (diet kg⁻¹): vitamin A, 2,000 IU; vitamin D3, 1,200 IU; vitamin E, 21 mg; vitamin B1, 5 mg; vitamin B2, 5 mg; vitamin B6, 5 mg; vitamin B12, 0.025 mg; vitamin K3, 2.5 mg; niacinamide, 25 mg; inositol, 60 mg; biotin, 0.05 mg; folic acid, 1.3 mg; pantothenic acid calcium, 20 mg; ascorbic acid (35 %), 110 mg.

^c Mineral premix (diet kg⁻¹): Co, 1.5 mg; FeSO₄, 105 mg; CuSO₄, 12.5 mg; KI, 1.0 mg; NaCl, 165 mg; KCl, 95 mg; MnSO₄, 10 mg; MgSO₄, 10 mg; Na₂SeO₃, 1.0 mg.

^d The Wako Pure Chemical Industries, Ltd., Japan.

(10 mL of diluted *P. pentosaceus* cells mixed with 90 g of the basal diet) to achieve a concentration of 1×10^7 CFU g⁻¹ of feed. Similarly, the diet containing *P. pentosaceus* at a concentration of 1×10^8 CFU g⁻¹ was obtained by spraying 10 mL of the original 1×10^9 CFU mL⁻¹ *P. pentosaceus* cell suspension onto 90 g of the basal diet. Finally, to obtain the diet with *P. pentosaceus* at a concentration of 1×10^9 CFU g⁻¹, the *P. pentosaceus* cells were concentrated to a final suspension of 1×10^{10} CFU mL⁻¹ and then mixed with the basal diet at a ratio of 1:9. The control diet (CT) was prepared by spraying 10 mL of PBS alone on the basal diet. The prepared feed was stored in the refrigerator at 4°C until utilization and fresh feed batches were prepared biweekly to maintain the viability of *P. pentosaceus*.

2.4. Feeding trial

A total of 600 rabbitfish fingerlings (size of 5.10 ± 0.28 g) were randomly allocated into twelve 300 L fiberglass tanks, with three tanks per dietary treatment, at a density of 50 fish in each tank. Continuous aeration was maintained at high levels (≥ 5 mg L⁻¹) using an air blower, Model GB-200, Taiwan) and the environmental parameters in tanks were controlled to ensure optimal conditions (temperature 25–28 °C; salinity 15–20 ppt; pH 7–7.5; dissolved oxygen 5–6 mg L⁻¹ and NH₃ ≤ 0.1 mg L⁻¹). The fingerlings were fed with synbiotics at different probiotic concentrations: 1×10^7 CFU g⁻¹ (NT1), 1×10^8 CFU g⁻¹ (NT2), and 1×10^9 CFU g⁻¹ (NT3). Additionally, in the control treatment (CT), fish were fed with a basal diet containing 0.5 % FOS without *P. pentosaceus* supplementation for 60 days. The experimental water was prepared by filtering seawater through a sand filter and pumping it into a 10 m³ container tank. Subsequently, seawater in the tank was mixed with tap water to achieve a salinity range of 15–20 ppt. The light intensity was consistently maintained at 600 lux through fluorescent lamps, following a 12 h light/dark regime. Golden Rabbitfish fingerlings were hand-fed the experimental diets twice daily at 07:0 and 17:0 h. Feeding rates were initiated at 5 % of the total fish weight daily and adjusted based on fish satiation. Feed amounts were then adjusted biweekly according to biomass estimates obtained from bulk weight measurements. The tank bottoms were cleaned daily by siphoning, and the discharged water (approximately 5 % of the water) was

compensated. Additionally, a 30 % water exchange was conducted weekly in all tanks to maintain water quality.

2.5. Evaluation of growth and feed utilization

Fish were collected biweekly (30 fish per treatment) and were anesthetized using 0.25 mL L⁻¹ of Aqui-S. Subsequently, fish biomass was measured. The growth rate, feed efficiency, and condition factor (K), including final body weight (FBW), final body length (FBL), daily growth rate (DGR), weight gain (WG), survival rate (SR), and feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and lipid efficiency ratio (LER), were calculated for each treatment using formulas described by Chelema Dezfoulnejad and Molayemraftar (2022), Sokooti et al. (2022), and Mohammadi et al. (2021).

Daily weight gain (DWG, g day⁻¹)

$$DWG \text{ (g day}^{-1}\text{)} = (W_i - W_o)/t$$

Weight gain (WG, %)

$$WG \text{ (%) = } (W_f - W_o)/W_o$$

Where: W_o, W_i, and W_f represent the live weights of the fish at the beginning, on day i, and on harvested day, respectively, and t represents the experiment duration.

Feed intake (FI, g fish⁻¹)

$$FI \text{ (g fish}^{-1}\text{)} = FI/SF$$

Feed conversion ratio (FCR)

$$FCR = FI/WG$$

Protein efficiency ratio (PER)

$$PER = WG/PI$$

Lipid efficiency ratio (LER)

$$LER = WG/LI$$

Where: FI (dry matter) represents the amount of feed intake, SF stands for the number of surviving fish, WG is the weight gain of fish, PI and LI represent the protein and lipid intake, respectively.

Condition factor

$$K = (100 \times W)/L^3$$

Where: W and L represent the body weight and length of the fish, respectively, and K represents the condition factor that was used to assess the health condition of the fish.

Survival rate (SR, %):

$$SR \text{ (%) = } (N_t/N_o) \times 100$$

Where: N_o represents the number of fish on the initial day of the experiment, and N_t represents the final number of fish on the harvested day.

2.6. Blood and gut sampling

Blood and gut samples were collected following the previous studies by Molayemraftar et al. (2022) and Sokooti et al. (2022). In brief, nine fish per treatment were harvested and immediately anesthetized using Aqui-S solution (0.25 mL L⁻¹). Blood samples were then withdrawn from the caudal vein using a 2.5 mL sterile syringe. The fish intestine was also removed and pooled to determine intestinal enzyme activities and bacterial concentration. Intestinal samples were stored at -40°C for further analysis.

2.7. Digestive enzymatic assay

2.7.1. Protease assay

The intestinal samples were thawed and homogenized using a buffer solution consisting of 50 mM potassium phosphate at pH 7. Subsequently, 100 μL of the extracted sample was combined with 400 μL of a buffer containing 50 mM potassium phosphate and 0.65 % casein (w/v) at pH 7. The mixture was thoroughly vortexed and incubated at 37°C. After 10 minutes, 500 μL of TCA (Trichloroacetic acid) reagent was added to each tube to halt the reaction. The solutions were then further incubated at 37°C for an additional 30 minutes. Following incubation, the reaction mixture underwent centrifugation at 10,000 rpm for 10 minutes, and the resulting supernatant was carefully collected into new Eppendorf tubes. Next, 200 μL of the supernatant was mixed with 500 μL of a 500 mM Na_2CO_3 solution. Subsequently, 100 μL of Folin reagent was added, thoroughly mixed, and maintained at 37°C for 30 minutes. The protease activity was measured at 660 nm, following the method described by [Cupp-Enyard \(2008\)](#).

2.7.2. Amylase assay

The amylase activity was assessed by quantifying the amount of reducing sugar generated from soluble starch ([Bernfeild, 1988](#)). The mixture reaction contained 100 μL of enzyme and 400 μL of 50 mM phosphate buffer containing 1 % soluble starch (w/v) at pH 7.0. The mixture was incubated at 40°C for 30 minutes in a shaking water bath. After incubation, the reaction was stopped by adding 500 μL of a 1 % 3, 5-Dinitrosalicylic acid solution. Subsequently, the tubes were boiled for 15 minutes and then allowed to cool to room temperature. The absorbance value was recorded at a wavelength of 540 nm using a spectrophotometer. A standard curve was generated using glucose. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μmol of glucose per minute under the given experimental conditions.

2.7.3. Lipase assay

To qualify lipase activity, 900 μL of a solution buffer (50 mM sodium phosphate buffer, 0.5 % (v/v) Triton X-100, and 150 mM NaCl pH 7.2) was mixed with 100 μL of the samples and incubated at 37°C for 10 minutes. Subsequently, 10 μL of a 50 mM p-nitrophenyl butyrate (pNPB) solution was added, and the mixture underwent additional incubation at room temperature for 5 minutes. The absorbance value was then measured at 400 nm using a spectrophotometer ([Eckel and Robbins, 1984](#)).

2.8. Determination of water parameters and intestinal bacterial concentration

Water parameters including pH, dissolved oxygen (DO), temperature, and salinity were daily measured using a pH meter (Hanna Model HI98190, Rumani), a portable DO meter (Hanna Model HI-9146, Rumani), a thermometer, and a refractometer (Atago Model 2491-master's, Japan), respectively. Additionally, water samples were collected weekly at 09:00 h for the analysis of total ammonia nitrogen (TAN, $\text{NH}_4^+\text{-N}$), nitrite nitrogen ($\text{NO}_2\text{-N}$), and nitrate nitrogen ($\text{NO}_3\text{-N}$) using multi-spectrophotometers (Hanna Model-HI83099, Romania).

To analyze intestinal microbial concentration, gut samples including the middle and posterior parts were spiced, pooled, and homogenized using a tissue grinder in sterile normal saline solution (1:10 w/v). The intestine suspension was serially diluted, and 25 μL of each dilution was spread onto a MRS agar plate containing De Man Rogosa and Sharpe agar (MRS) for probiotic bacteria or lactic acid bacteria, Tryptic Soy Agar (TSA, Hi Media-India) for total aerobic heterotrophic bacteria, and Thiosulphate Citrate Bile Salt agar (TCBS, Hi Media-India) for *Vibrio* spp. After 48 h of incubation at 30°C, the colony-forming units (CFUs) were counted.

2.9. Hematological parameters and lysozyme activity

2.9.1. Erythrocytes cell counts

The blood sample was diluted 200 times by pipetting 10 μL of blood into an Eppendorf tube containing 1990 μL of Natt and Herrick solution. The mixture was gently shaken to ensure thorough mixing. Erythrocyte cell density was determined using an erythrocyte cell counting chamber and counted under an optical microscope at 40 \times magnification. Four large boxes (each large box containing 25 small boxes) at the four corners of the counting chamber and one box in the center of the counting chamber were counted. Erythrocyte cell density was calculated according to the formula described by [Natt and Herrick \(1952\)](#).

$$\text{EC (cells mm}^{-3}\text{)} = C \times 10 \times 5 \times 200$$

Where: EC is the density of erythrocyte cells; C is the total number of erythrocyte cells in the five counting areas; 10 (the distance between the lame and the counting chamber is 0.1 mm); 5 (the area of each counting area is 0.2 mm^2); and 200 (the number of dilution).

2.9.2. Leukocyte cell counts

The blood samples were fixed on lamens and stained with Wright and Giemsa solution ([Hrubec et al., 2000](#)). The number of leukocytes in the blood was determined using the formula:

$$\text{LC (cell mm}^{-3}\text{)} = (\text{Number of leukocyte cells in 1.500 cells} \times \text{R/Number of erythrocytes in 1.500 cells}) \times 100$$

Where: LC is the density of leukocyte cells; R is the density of erythrocyte cells on the counting chamber (cell mm^{-3}).

2.9.3. Serum lysozyme activity

Serum lysozyme activity was determined using an ion-exchange chromatography kit (Bangalore Genei, India). In brief, serum samples were diluted in phosphate buffer (pH 7.4) to achieve a final concentration of 0.33 mg mL^{-1} . Then, 50 μL of the diluted serum sample was combined with 3 μL of *Micrococcus luteus* suspension (Bangalore Genei, India) in the same buffer (with an absorbance at 450 nm of 0.5 – 0.7). The mixture was thoroughly mixed for 15 seconds and then measured using a spectrophotometer at 450 nm. The absorbance values were then compared with the lysozyme standard curve. Lysozyme activity was expressed as Unit mg^{-1} protein ([Ellis, 1990](#)).

2.10. Biochemical composition analysis

To analyze the biochemical compositions of the tested fish, nine fish from each trial were collected and anesthetized in an ice bath. Additionally, 200 g of formulated feed was collected to analyze its composition. The dry matter (DM) content was analyzed following AOAC standard 930.15, 1990 ([AOAC, 1999](#)). Total nitrogen was analyzed following AOAC standard 930.15, 1990, and crude protein (CP) was calculated by 6.25 \times N (the nitrogen content). Ether extracts (EE) were determined according to AOAC 930.15, 1990, while total ash (Ash) was measured following to AOAC 942, 1990.

2.11. Data analysis

The data normality was confirmed using the Kolmogorov-Smirnov test. Subsequently, one-way analysis of variance (ANOVA) was conducted on the data using SPSS version 22 software. Duncan's test was then employed to identify significant differences among the various experimental treatments. Additionally, bacterial counts and digestive enzyme activity between the two sampling times (day 30 and day 60) were compared using the independent Student's t-test, with differences considered significant at $p < 0.05$.

3. Results

3.1. Monitoring water quality

Water quality parameters, including temperature (ranging from 23.2 to 30.8°C), salinity (15–17 ppt), and dissolved oxygen (4.80–6.81 mg L⁻¹) throughout the experimental period remained stable and were not influenced by the synbiotics ($p > 0.05$). However, pH, TAN, and NO₃-N concentrations were affected by the synbiotic diets ($p < 0.05$) (Table 2). Specifically, a notable trend was observed in the concentrations of pH, TAN, and NO₃-N, with the synbiotic treatments showing a significant decrease compared to the control group. Nevertheless, no significant difference was found among the synbiotic treatments. Interestingly, the concentration of NO₂-N was not affected by the synbiotics ($p > 0.05$).

3.2. Growth rate, feed efficiency, condition factor, and survival of Golden Rabbitfish

The growth rate of Golden Rabbitfish, including final body length (FBL), final body weight (FBW), weight gain (WG), and daily growth rate (DGR), significantly increased in the synbiotics-supplemented diets compared to the control (Table 3). The highest values for these parameters were observed in NT2 (FBW: 20.54 ± 0.21 g, FBL: 11.96 ± 0.12 cm, WG: 302.32 ± 4.67 %, DGR: 0.26 ± 0.05 g day⁻¹), and these values were significantly different from the other treatments ($p < 0.05$). Similarly, feed efficiency was statistically influenced by the synbiotics-supplemented diets. Feed intake (FI) varied among the experimental treatments, whereas fish consumed highest in NT2 (26.01 ± 0.26 g fish⁻¹), which was significantly different from the other treatments ($p < 0.05$). However, NT1 and NT3 trials did not exhibit significant difference ($p > 0.05$). Notably, fish fed with the synbiotics-supplemented diets exhibited significantly improved feed conversion ratio (FCR) compared to the control. However, the NT2 trial resulted in the lowest FCR (1.69 ± 0.02), which was significantly different from the other trials. Additionally, fish fed with the synbiotics-supplemented diets showed significant enhancements in lipid efficiency ratio (LER) and protein efficiency ratio (PER) compared to the control diets ($p < 0.05$).

Similarly, the synbiotics-supplemented diets exhibited a significant effect on the condition factor (K). The highest K value was in the control group (CT) (1.44 ± 0.04), which was significantly different from the other treatments. There was also a significant difference between NT2

Table 2
Effect of synbiotics use on water quality parameters.

| Parameters | Treatment | | | | P-value |
|--|--|--------------------------|--------------------------|--------------------------|---------|
| | CT | NT1 | NT2 | NT3 | |
| Temperature (°C) | 25.30 ± 1.75 | 25.26 ± 1.77 | 25.28 ± 1.78 | 25.29 ± 1.77 | 0.187 |
| | 15.35 ± 0.58 | 15.26 ± 0.45 | 15.24 ± 0.47 | 15.30 ± 0.49 | |
| pH | 7.45 ± 0.12 ^b | 7.37 ± 0.11 ^a | 7.35 ± 0.13 ^a | 7.34 ± 0.14 ^a | 0.015 |
| | 5.43 ± 0.33 | 5.42 ± 0.27 | 5.43 ± 0.25 | 5.41 ± 0.27 | |
| TAN (mg L ⁻¹) | 0.51 ± 0.11 ^b | 0.46 ± 0.10 ^a | 0.45 ± 0.12 ^a | 0.46 ± 0.13 ^a | 0.022 |
| | 0.28 ± 0.06 | 0.27 ± 0.08 | 0.26 ± 0.05 | 0.26 ± 0.06 | |
| NO ₂ -N (mg L ⁻¹) | 2.04 ± 0.37 ^b | 1.96 ± 0.25 ^a | 1.94 ± 0.27 ^a | 1.95 ± 0.28 ^a | 0.015 |
| | NO ₃ -N (mg L ⁻¹) | | | | |

The data are presented as mean ± standard deviation. Different letters (a, b, c) in the same row indicate significant statistical differences ($p < 0.05$).

CT (control, basal diet without *P. pentosaceus*), NT1 (basal diet supplemented with *P. pentosaceus* at a dose of 10⁷ CFU g⁻¹), NT2 (basal diet supplemented with *P. pentosaceus* at a dose of 10⁸ CFU g⁻¹), and NT3 (basal diet supplemented with *P. pentosaceus* at a dose of 10⁹ CFU g⁻¹).

Table 3

Effect of synbiotics on growth rate, feed efficiency, condition factor (K), and survival of Golden Rabbitfish (*S. guttatus*) reared for 60 days.

| Parameters | Treatment | | | | P-value |
|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|---------|
| | CT | NT1 | NT2 | NT3 | |
| IBW (g) | 5.10 ± 0.26 | 5.10 ± 0.25 | 5.10 ± 0.28 | 5.10 ± 0.23 | 0.686 |
| | 16.82 ± 0.10 ^a | 19.40 ± 0.14 ^c | 20.54 ± 0.21 ^d | 18.76 ± 0.20 ^b | |
| FBW (g) | 16.82 ± 0.10 ^a | 19.40 ± 0.14 ^c | 20.54 ± 0.21 ^d | 18.76 ± 0.20 ^b | 0.001 |
| | 4.60 ± 0.38 | 4.60 ± 0.27 | 4.60 ± 0.36 | 4.60 ± 0.34 | |
| IBL (cm) | 4.60 ± 0.38 | 4.60 ± 0.27 | 4.60 ± 0.36 | 4.60 ± 0.34 | 0.415 |
| | 10.55 ± 0.11 ^a | 11.59 ± 0.08 ^c | 11.96 ± 0.12 ^d | 11.31 ± 0.10 ^b | |
| FBL (cm) | 10.55 ± 0.11 ^a | 11.59 ± 0.08 ^c | 11.96 ± 0.12 ^d | 11.31 ± 0.10 ^b | 0.000 |
| | 229.61 ± 2.13 ^a | 280.29 ± 2.32 ^c | 302.32 ± 4.67 ^d | 268.15 ± 4.04 ^b | |
| WG (%) | 229.61 ± 2.13 ^a | 280.29 ± 2.32 ^c | 302.32 ± 4.67 ^d | 268.15 ± 4.04 ^b | 0.001 |
| | 0.20 ± 0.01 ^a | 0.24 ± 0.08 ^c | 0.26 ± 0.05 ^d | 0.23 ± 0.06 ^b | |
| DWG (g day ⁻¹) | 0.20 ± 0.01 ^a | 0.24 ± 0.08 ^c | 0.26 ± 0.05 ^d | 0.23 ± 0.06 ^b | 0.001 |
| | 21.24 ± 0.14 ^a | 24.88 ± 0.35 ^b | 26.01 ± 0.26 ^c | 24.14 ± 0.39 ^b | |
| FI (g fish ⁻¹) | 21.24 ± 0.14 ^a | 24.88 ± 0.35 ^b | 26.01 ± 0.26 ^c | 24.14 ± 0.39 ^b | 0.001 |
| | 1.81 ± 0.01 ^c | 1.74 ± 0.05 ^b | 1.69 ± 0.02 ^a | 1.77 ± 0.08 ^b | |
| FCR | 1.81 ± 0.01 ^c | 1.74 ± 0.05 ^b | 1.69 ± 0.02 ^a | 1.77 ± 0.08 ^b | 0.015 |
| | 1.74 ± 0.03 ^a | 1.81 ± 0.05 ^b | 1.87 ± 0.02 ^c | 1.78 ± 0.04 ^b | |
| PER | 1.74 ± 0.03 ^a | 1.81 ± 0.05 ^b | 1.87 ± 0.02 ^c | 1.78 ± 0.04 ^b | 0.000 |
| | 6.11 ± 0.02 ^a | 6.36 ± 0.05 ^b | 6.56 ± 0.05 ^c | 6.26 ± 0.06 ^b | |
| LER | 6.11 ± 0.02 ^a | 6.36 ± 0.05 ^b | 6.56 ± 0.05 ^c | 6.26 ± 0.06 ^b | 0.001 |
| | 1.44 ± 0.04 ^c | 1.25 ± 0.05 ^{ab} | 1.20 ± 0.03 ^a | 1.30 ± 0.06 ^b | |
| K | 1.44 ± 0.04 ^c | 1.25 ± 0.05 ^{ab} | 1.20 ± 0.03 ^a | 1.30 ± 0.06 ^b | 0.001 |
| | 86.00 ± 1.00 | 89.00 ± 2.00 | 91.00 ± 1.00 ^a | 87.00 ± 2.00 | |
| SR (%) | 86.00 ± 1.00 | 89.00 ± 2.00 | 91.00 ± 1.00 ^a | 87.00 ± 2.00 | 0.181 |
| | | | | | |

The data are presented as mean ± standard error (n = 3). Different letters (a, b, c) in the same row indicate significant statistical differences ($p < 0.05$).

CT (control, the basal diet without *P. pentosaceus*), NT1 (basal diet supplemented with *P. pentosaceus* at a dose of 10⁷ CFU g⁻¹), NT2 (basal diet supplemented with *P. pentosaceus* at a dose of 10⁸ CFU g⁻¹), and NT3 (basal diet supplemented with *P. pentosaceus* at a dose of 10⁹ CFU g⁻¹).

Abbreviations: IBW, initial body weight; FBW, final body weight; IBL, initial body length; FBL, final body length; WG, weight gain; DWG, daily weight gain; FI, feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; LER, lipid efficiency ratio; K, condition factor; and SR, survival rate.

and NT3 ($p < 0.05$). However, no significant difference occurred in NT1 as compared to NT2 and NT3 groups ($p > 0.05$). Contradictorily, the survival rate (SR) of *S. guttatus* varied from 86.0 % to 91.0 % and was not influenced by the synbiotics-supplemented diets ($p > 0.05$).

3.3. Digestive enzyme activity

In general, the enzyme activities including protease, lipase, and amylase, in the fish guts fed with synbiotics were significantly higher compared to those of the control group (Fig. 1). These values also showed remarkable improvement between the two different sampling times. On day 30 of the experiment, there was a significant difference in the protease, lipase, and amylase activities of *S. guttatus* in all the synbiotics trials compared to the control. Among them, NT2 was the most effective treatment in enhancing the activity of lipase and amylase in the digestive system of *S. guttatus* (5.38 ± 0.72 U g⁻¹ and 3838 ± 227 U g⁻¹, respectively). In the NT3 treatment with the highest *P. pentosaceus* supplementation, amylase activity tended to decrease, with only 4.96 ± 0.62 U g⁻¹ remaining. Similar results were also observed for lipase activity, with 3321 ± 116 U g⁻¹ at day 30 in diets supplemented with bacteria at a density of 10⁹ CFU g⁻¹. However, the digestive system in the NT3 treatment exhibited the best protease activity, reaching 307.58 ± 30.11 U g⁻¹ after 30 days.

The protease, lipase, and amylase activities of *S. guttatus* on day 60 in the synbiotics diets were also significantly higher than those in the control diet (by 33.59 % for amylase and 22.56 % for lipase in diet NT2, and 26.27 % for protease in diet NT3). Thus, using synbiotics in the diet of *S. guttatus* has increased the level of enzyme activity necessary for food digestion in fish, such as amylase, lipase, and protease.

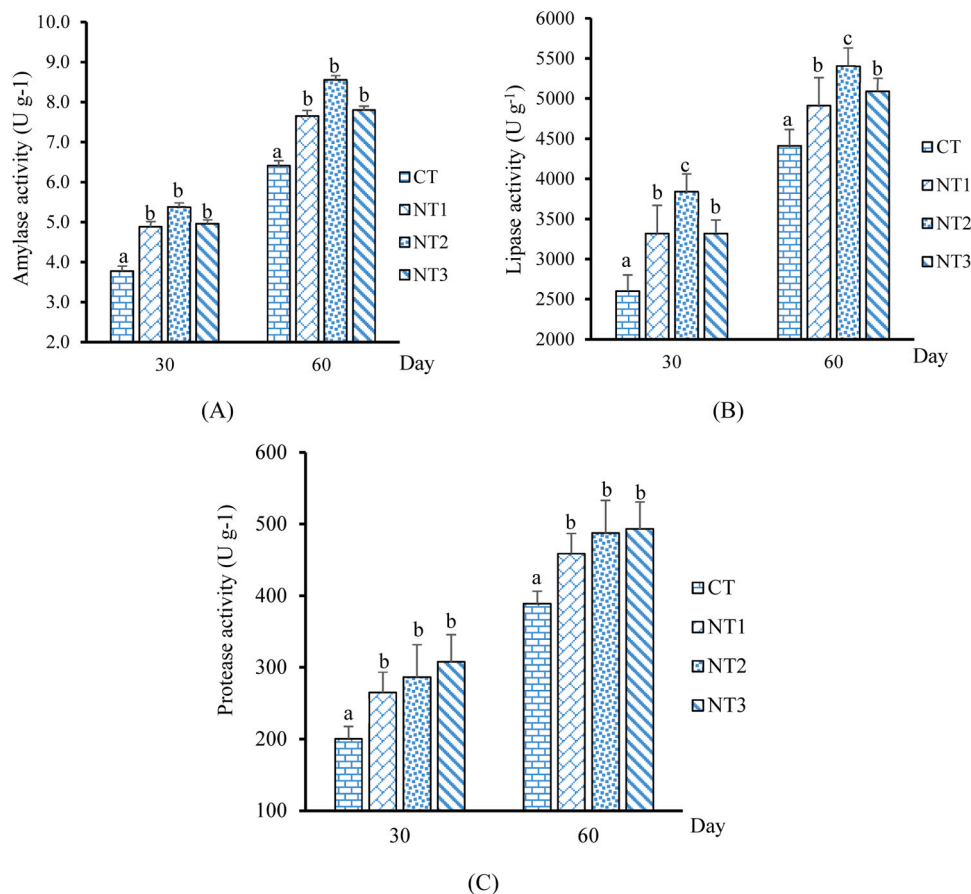


Fig. 1. Protease activity (A), lipase activity (B), and amylase activity (C) of Golden Rabbitfish reared for 30 days and 60 days. Different letters on each bar (mean \pm standard deviation, $n=9$) represent statistical differences ($p < 0.05$).

3.4. Intestinal bacterial counts

The intestinal bacterial community of Golden Rabbitfish (*S. guttatus*) was influenced by the synbiotics diets (Table 4). After 30 days of the experiment, there was a noticeable increase in the counts of total aerobic heterotrophic bacteria on TSA plates and lactic acid bacteria on MRS plates, which correlated with the higher concentrations of probiotics in

Table 4

Effect of synbiotics use on intestinal bacterial counts of *S. guttatus*.

| Parameters | Treatment | Day-30 | Day-60 |
|--|-----------|----------------------------------|----------------------------------|
| Total aerobic heterotrophic bacteria (TSA count $\times 10^6$ CFU mL^{-1}) | CT | 2.49 \pm 0.33 ^{A, a} | 2.81 \pm 0.24 ^{A, a} |
| | NT1 | 5.24 \pm 1.14 ^{A, b} | 7.09 \pm 0.47 ^{B, b} |
| | NT2 | 6.87 \pm 0.52 ^{A, c} | 7.99 \pm 0.32 ^{B, c} |
| | NT3 | 6.99 \pm 0.27 ^{A, c} | 7.92 \pm 0.43 ^{B, c} |
| Vibrio bacteria (TCBS count $\times 10^4$ CFU mL^{-1}) | CT | 10.51 \pm 0.35 ^{A, c} | 10.48 \pm 0.39 ^{A, c} |
| | NT1 | 9.21 \pm 0.78 ^{B, b} | 7.55 \pm 0.28 ^{A, b} |
| | NT2 | 7.77 \pm 0.33 ^{A, a} | 7.48 \pm 0.33 ^{A, ab} |
| | NT3 | 7.16 \pm 0.16 ^{A, a} | 6.88 \pm 0.26 ^{A, a} |
| Lactic acid bacteria (MRS count $\times 10^7$ CFU mL^{-1}) | CT | 2.31 \pm 0.16 ^{A, a} | 2.43 \pm 0.16 ^{A, a} |
| | NT1 | 8.12 \pm 0.47 ^{A, b} | 9.67 \pm 0.56 ^{B, a} |
| | NT2 | 10.08 \pm 0.55 ^{A, c} | 10.49 \pm 0.46 ^{A, c} |
| | NT3 | 10.60 \pm 0.33 ^{A, c} | 10.65 \pm 0.54 ^{A, c} |

The data are presented as mean \pm standard deviation ($n=9$). Different lowercase letters (a, b, c) in the same column indicate statistically significant differences ($p < 0.05$) among the trials. Different uppercase letters (A, B) in the same row indicate statistically significant differences ($p < 0.05$) between collection times. CT (control, basal diet without *P. pentosaceus*), NT1 (basal diet supplemented with *P. pentosaceus* at a dose of 10^7 CFU g^{-1}), NT2 (basal diet supplemented with *P. pentosaceus* at a dose of 10^8 CFU g^{-1}), and NT3 (basal diet supplemented with *P. pentosaceus* at a dose of 10^9 CFU g^{-1}).

the synbiotic diets. The highest TSA and MRS counts were found in NT3 (6.99×10^6 CFU mL^{-1} and 10.60×10^7 CFU mL^{-1} , respectively), and there were significant differences compared to those in CT and NT1 ($p < 0.05$). However, no significant difference was found between NT3 and NT2 ($p > 0.05$). Interestingly, TCBS count decreased significantly with the increased concentrations of probiotic in the synbiotics diets. A lower concentration of TCBS count was found in NT3 (7.16×10^4 CFU mL^{-1}) compared to CT and NT1 ($p < 0.05$). However, no significant difference was found between NT3 and NT2 ($p > 0.05$). Similarly, the highest value of MRS count and the lowest value of TCBS count on day 60 were also found in NT3 (10.65×10^7 CFU mL^{-1} and 6.88×10^4 CFU mL^{-1} , respectively). Meanwhile, the highest value of TSA count was in NT2 (7.99×10^6 CFU mL^{-1}); no significant difference was detected between NT2 and NT3. In comparison, concentrations of TCBS and MRS counts among treatments were not significantly different between the sampling times (day 30 and 60), except for NT1, which had a higher concentration of MRS count and a lower concentration of TCBS count on day 60 compared to those on day 30. Contradictorily, the concentration of TSA count among the synbiotics treatments was significantly different between the sampling times (day 30 and day 60), except for CT, which was not significantly different between day 30 and day 60 ($p > 0.05$).

3.5. Hematological parameters and lysozyme activity

The hematological parameters and lysozyme activity of *S. guttatus* fed with different concentrations of probiotics in the synbiotics diets showed significant improvements in erythrocyte count (EC), leukocyte count (LC), and lysozyme activity compared to the control (Fig. 2). These parameters were the highest in NT2 and were significantly different compared to CT, NT1, and NT3. However, no significant

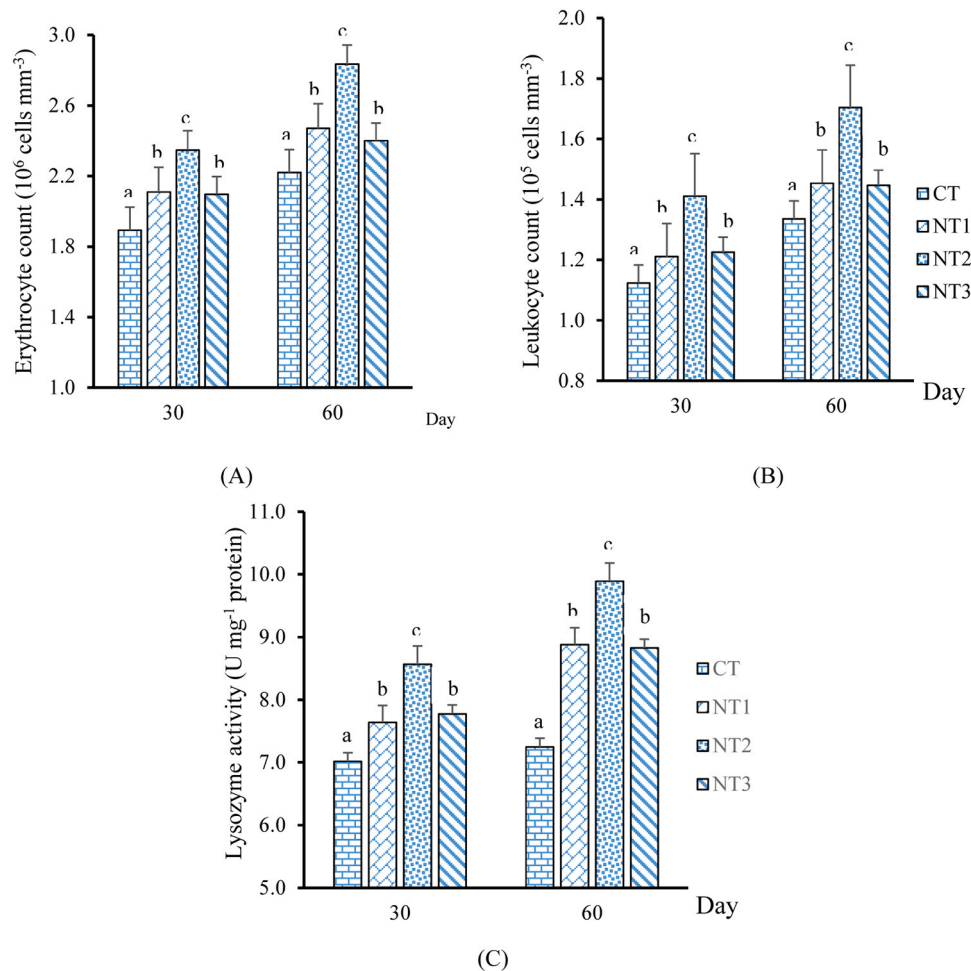


Fig. 2. Erythrocyte (A), leukocyte (B), and lysozyme activity (C) counts of Golden Rabbitfish reared for 30 days and 60 days. Different letters on each bar (mean \pm standard deviation, $n = 9$) represent statistical differences ($p < 0.05$).

difference in hematological parameters and lysozyme activity was found between NT1 and NT2. Furthermore, by day 60 of the trial, there was an increase in the values of EC, LC, and lysozyme activity in Golden Rabbitfish, all of which were significantly higher than those recorded on day 30. Thus, it could be concluded that the hematological parameters and lysozyme activity of *S. guttatus* remarkably increased corresponding to the feeding time.

3.6. Proximate composition

The whole-body proximate chemical composition of *S. guttatus* was significantly influenced by the diets supplemented with synbiotics (Table 5). Analysis data revealed notable changes in key components such as moisture and protein contents. Interestingly, fish received the synbiotics-supplemented diets exhibited an increase in total protein, while reducing the moisture (M) content compared to those fed with the control diet. The lowest value of M was in NT2 (70.20 %) and was significantly different compared to the control. However, no significant differences occurred among the synbiotics-supplemented treatments. A higher value of total protein (CP) was found in NT3 (65.50 %) and was significantly different compared to both the CT and NT1. However, no significant difference in CP levels between NT3 and NT2 was observed ($p > 0.05$). Meanwhile, total lipid (EE) and ash contents were more or less the same among the experimental treatments and were not affected by the synbiotics-supplemented diets ($p > 0.05$).

Table 5

Effect of synbiotics use on the proximate composition (% dry matter) of Golden Rabbitfish (*S. guttatus*).

| Parameters | Treatment | | | | P-value |
|-----------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|---------|
| | CT | NT1 | NT2 | NT3 | |
| Moisture (M, %) | 73.33 \pm 0.46 ^b | 71.08 \pm 0.69 ^a | 70.20 \pm 0.44 ^a | 70.86 \pm 0.55 ^a | 0.048 |
| Crude protein (CP, %) | 61.72 \pm 0.43 ^a | 63.00 \pm 0.24 ^b | 65.23 \pm 0.36 ^c | 65.50 \pm 0.55 ^c | 0.001 |
| Crude lipid (EE, %) | 22.05 \pm 0.18 | 21.85 \pm 0.16 | 21.91 \pm 0.13 | 21.79 \pm 0.16 | 0.691 |
| Ash (%) | 17.87 \pm 0.10 | 17.90 \pm 0.20 | 17.86 \pm 0.11 | 17.93 \pm 0.15 | 0.962 |

The data are presented as mean \pm standard deviation ($n=9$). Different letters (a, b, c) in the same row indicate significant statistical differences ($p < 0.05$).

CT (control, basal diet without *P. pentosaceus*), NT1 (basal diet supplemented with *P. pentosaceus* at a dose of 10^7 CFU g^{-1}), NT2 (basal diet supplemented with *P. pentosaceus* at a dose of 10^8 CFU g^{-1}), and NT3 (basal diet supplemented with *P. pentosaceus* at a dose of 10^9 CFU g^{-1}).

4. Discussion

4.1. Water quality

Good water quality is critical in aquaculture farming. Throughout this study, changes in water parameters remained relatively narrow, consistently falling within the optimum range recommended for farming

Golden Rabbitfish. Water temperatures varied from 23.2 to 30.8°C. Carlo Mendoza et al. (2020) highlighted a notable improvement in the survival rates of *S. guttatus* fingerlings when reared at lower temperatures ranging from 22 to 26°C. In terms of salinity, dissolved oxygen (DO), and pH, which slightly fluctuated from 15.0 to 17.0 ppt, 4.80–6.81 mg L⁻¹, and 7.00–7.80 respectively. Golden Rabbitfish exhibits a remarkable tolerance to wide salinity concentrations ranging from 1 to 35 ppt (Gorospe and Demayo, 2013; Binh et al., 2022). This adaptability allows the fish well-suited for diverse aquatic environments, particularly thriving in brackish water conditions. Syah et al. (2020) documented that values of 4.50 mg L⁻¹ and higher are considered suitable DO levels for rabbitfish survival and growth. The optimum values of pH for culturing *S. guttatus* range from 6 to 8.5 (Carlo Mendoza et al., 2020). A significant difference in pH values ($p < 0.05$) was observed among the experimental treatments, whereas the control treatment exhibited higher pH levels compared to the synbiotics treatments. The decrease in pH observed across all synbiotics treatments could be attributed to the proliferation of lactic acid bacteria and the concurrent fermentation process (Table 4). This process leads to the generation of organic acids (such as acetic, butyric, and propionic acids), which contribute to the pH reduction within the tanks (Okey et al., 2018; Sokooti et al., 2022). El-sayed et al. (2022) and Narmatha et al. (2017) had comparable results. Likewise, concentrations of TAN and NO₃-N were considerably reduced in the synbiotics treatments ($p < 0.05$). The reduction in TAN and NO₃-N concentrations could be attributed to the introduction of synbiotics into the aquatic environment, which appears to accelerate the decomposition rate of organic matter. This process facilitates the removal of undesirable waste substances, including ammonia, carbon dioxide, and sulfide, from the tanks (Okey et al., 2018). Similarly, many studies reported that the concentrations of TAN and NO₃-N decreased significantly in the probiotics-treated groups (Banerjee and Ray, 2017; El-Sayed et al., 2022; Okey et al., 2018).

4.2. Growth performance

In the current study, supplementing the basal diet with *P. pentosaceus* resulted in enhanced growth rates (FBW, FBL, WG, and DGR) and improved feed efficiency (FI, FCR, PER, and LER) in Golden Rabbitfish (Table 3). The increase in growth rate and improved feed efficiency could be attributed to the improved digestibility and availability of nutrients for the fish. Both synbiotics and probiotics have been extensively studied for their pivotal role in enhancing feed digestion in fish by increasing digestive enzyme production or modification of the gut environment which leads to harmful microorganisms (Cavalcante et al., 2020; Merrifield et al., 2010). Moreover, synbiotics establish a conducive environment in the gut, fostering the growth and activity of beneficial bacteria (Okey et al., 2018). Consequently, these contributions improve feed digestion and absorption, resulting in an enhanced overall fish growth rate. This is corroborated by data on digestive enzyme activity (lipase, protease, and amylase), where fish-fed synbiotic diets exhibited 1.5–2 times higher than those in the control group (Fig. 1). And the increase in bacterial profiles, particularly lactic acid bacteria, in the intestinal gut of the fish fed on synbiotic diets exhibited a notable four to five folds increasing compared to those in the control group (Fig. 2). This phenomenon may also be linked to the organic acids (e.g. lactic acid) fermentation of *Pediococcus*. Lactic acid bacteria (LAB) including *Pediococcus* are recognized as promising probiotics in aquaculture due to their potential to enhance the growth performance, feed efficiency, and overall health of aquatic animals (Keereeleng et al., 2022).

The enhancement growth rates of fish in the current study are consistent with many previous studies. For example, El-Dakar et al. (2007) found that rabbitfish (*Siganus rivulatus*) fed feeds with commercial Biogen (synbiotics) had better FBW, specific growth rate (SGR), protein ratio (PR), and FCR compared to those without Biogen.

Additionally, adding synbiotics (*L. plantarum*, *S. boulardii*, *L. acidophilus*, and MOS) and biofloc meal to Nile tilapia (*Oncorhynchus mykiss*) diet is an effective strategy for promoting growth rate and improving gut microbiota and body composition of the fish (Hersi et al., 2023). Moreover, diet supplementation with synbiotics (Biomim IMBO) significantly increased the growth performance, survival rate, and feeding efficiency of rainbow trout (*O. mykiss*) fingerlings (Mehrabi et al., 2012). Furthermore, probiotics such as *P. pentosaceus* have been reported to have beneficial effects on the growth performance and feed utilization of fish. For instance, diet supplementation with *P. pentosaceus* enhanced the growth rate and digestive enzyme activities of common carp (*Cyprinus carpio*) (Ahmadifar et al., 2020). The supplementation of the diet with *P. pentosaceus* improved both the growth rate and overall health conditions of red sea bream (*Pagrus major*) juveniles (Dawood et al., 2016). These findings collectively support the efficacy of synbiotics and probiotics in improving the growth rate and feed utilization of various fish species. Conversely, the lower values of K observed in all the synbiotics treatments compared to those in the control could be attributed to better growth in the synbiotics treatments, resulting in higher biomass. This increase in biomass might lead to stress in the fish. However, in this study, the obtained values of K (ranging from 1.20 to 1.44) were within the suitable range for the growth of Golden Rabbitfish. Bennet (1970) reported that a value of $K \geq 1$ is considered good for the survival and growth of fish with a body form similar to that of rabbitfish, tilapia, and similar species. Hence, it can be concluded that K did not interfere with the growth and survival rates of the fish.

4.3. Digestive enzyme activity

The findings revealed that the digestive enzyme activities (lipase, protease, and amylase) obtained in Golden Rabbitfish fed with synbiotics supplementation (*P. pentosaceus* and FOS) were higher than those in fish fed without *P. pentosaceus* supplementation (the control) (Fig. 1A, B, C). Moreover, synbiotics-supplemented diets showed a time-dependent of the digestive enzyme activities. Sokooti et al. (2022) documented that probiotics and synbiotics increase the secretion of digestive enzymes, including lipase, protease, and amylase, representing the intestinal villi wall by inducing polyamines production, resulting in higher amounts of these enzymes in the intestinal system of fish. The presence of these enzymes with endogenous digestive enzymes leads to improving the digestive process and enhancing the bioavailability of essential nutrients (Assan et al., 2022). The increasing digestive enzyme activity in the host enhances the fish's capacity for improved digestion, absorption, and increased feed efficiency, and consequently boosts the growth rate (Assan et al., 2022; Ghanei-Motlagh et al., 2021; Sokooti et al., 2022). This finding is in agreement with the study result of Yousefi et al. (2023), which reported that dietary supplementation of synbiotics significantly improved the amylase and lipase activities of rainbow trout (*O. mykiss*). Synbiotic supplementation improved digestive enzyme activity, leading to increase digestive system efficiency, and the growth of common carp (*Cyprinus carpio*) fingerlings (Ghasempour et al., 2015).

4.4. Bacterial representation

The bacterial community within the fish intestines plays a pivotal role in vital bodily functions, including digestion and the regulation of various diseases (Sokooti et al., 2022). The findings from the current study demonstrate that diets incorporating synbiotics express a stimulatory effect on the intestinal bacterial community of Golden Rabbitfish and a time-effect response in the lactic acid bacteria counts. Conversely, synbiotics-supplemented diets had an antagonistic influence on the reducing *Vibrio* spp. Both probiotics and synbiotics have demonstrated the ability to alter the gut microbiota (El-Saadony et al., 2021; Ringø et al., 2020; Sokooti et al., 2022). The mixture of prebiotics and probiotics has been reported to improve gut microbiota (Huynh et al., 2017). A reduction in the number of *Vibrio* spp counts might be

attributed to the probiotic *P. pentosaceus*, which was reported to lower the intestinal pH through organic acids fermentation, resulting in a reduction of *Vibrio* population in the shrimp intestinal tract (Thao et al., 2021). Furthermore, synbiotics consisting of *Bacillus* spp, lactic acid bacteria, and yeast have been employed either as probiotics or in combination with prebiotics due to their antagonistic effects on *Vibrio* spp. (Austin and Day, 1990). Similar to our findings, Sokooti et al. (2022) reported that sea bass (*Lates calcarifer*) fed with probiotics-supplemented diets significantly increased the numbers of total bacterial and lactic acid bacteria in the intestines of the fish. Dietary supplementation of synbiotics markedly improved total bacteria in the intestinal gut of *O. mykiss* (Yousefi et al., 2023).

4.5. Hematological parameters and lysozyme activity

In this study, EC, LC, and lysozyme activity of Golden Rabbitfish were influenced by dietary synbiotics supplementation and sampling times (days 30 and 60) (Fig. 2A, B, C). According to our findings, the hematological parameters and lysozyme activity of Golden Rabbitfish *S. guttatus* were dramatically increased in either synbiotics groups or sampling times. In particular, it also indicates that EC, LC, and lysozyme activity showed the highest values with the administration of synbiotics (at a *P. pentosaceus* dose of 10^8 CFU g^{-1}). The results of this study highlight the potential benefits of using synbiotics, especially *P. pentosaceus* and FOS, as dietary supplementation for fish in the context of immune stimulation. According to Okey et al. (2018), the application of prebiotics and probiotics, individually or in combination as synbiotics, generates immunostimulants that boost the immune system. The flora from synbiotics might migrate across the gut cell wall and activate the immune system by secreting various antigens (Isolauri et al., 2002). Many studies are consistent with these findings, as Sewaka et al. (2019) reported that juveniles of red tilapia (*Oreochromis* spp.) fed synbiotic-supplemented diets exhibited significantly higher lysozyme activity than those in the control. The synbiotics consisting of *Solanum ferox* and *Lactobacillus casei* improved the function of the immune system and enhanced resistance ability against bacterial infections such as *Pseudomonas fluorescens* and *Aeromonas hydrophila* in catfish (*Clarias gariepinus*) (Hardi et al., 2022). The addition of synbiotics has the potential to enhance antioxidant response and improve the health of seabass (*Lates calcarifer*) (Siddik et al., 2022). According to Harpeni et al. (2021), EC, along with hematocrit and hemoglobin, plays an important role in the anemia symptoms in fish. In this study, values of EC fluctuated from $1.89 - 2.83 \times 10^6$ cells mm^{-3} . Thus, the results of EC showed the fish were not anemic. Normally, EC levels in marine teleostei vary as in *Catla* ranged from 0.82 to 1.30×10^6 cells mm^{-3} (Rajesh et al., 2015), brown-marbled grouper (*Epinephelus fuscoguttatus*) $0.88-1.90 \times 10^6$ cells mm^{-3} (Harpeni et al., 2021), and striped hybrid bass (*Morone chrysops* \times *Morone saxatilis*) $3.66-4.96 \times 10^6$ cells mm^{-3} (Hrubec et al., 1997). Meanwhile, leukocytes (LC) play a critical role in non-specific immunity, primarily responding to antigens neutralization through the process of phagocytosis (Okey et al., 2018). The levels of LC are also varied among different fish species. For instance, brown-marbled grouper (*Epinephelus fuscoguttatus*) had LC $1.4-1.8 \times 10^5$ cells mm^{-3} (Harpeni et al., 2021) while in striped hybrid bass (*Morone chrysops* \times *Morone saxatilis*) ranged from 0.32 to 1.15×10^5 cells mm^{-3} (Hrubec et al., 1997). Saurabh and Sahoo (2008) reported that lysozyme serves as a non-specific humoral immune defense against bacteria by activating the complement cascade and facilitating phagocytosis. The levels of lysozyme activity in our study were in agreement with the results of Sallam et al. (2020) who reported that the levels of lysozyme activity of rabbitfish (*S. rivulatus*) fluctuated from 6.5 to 12.0 U mg^{-1} protein.

4.6. Proximate composition

At the end of the trial, the proximate chemical analysis of the whole fish body revealed a notable reduction in moisture content in the

synbiotics groups as compared to the control. Conversely, the recent results revealed that synbiotics-supplemented diets significantly increased fish protein content. According to our findings, it is evident that dietary synbiotics supplementation has an improving effect on the body composition of Golden Rabbitfish (*S. guttatus*). Changes in the protein content of the fish body are likely attributed to the presence of synbiotics, particularly the probiotic *P. pentosaceus* in the digestive tract, resulting in enhanced digestibility and utilization of nutrients, coupled with alterations in the synthesis and deposition rate of proteins in fish muscles (Bahnasawy et al., 2020). Similarly, Hersi et al. (2023) reported a significant improvement in the body composition of Nile tilapia (*O. niloticus*) feeding with synbiotics and biofloc meal diets. Protein levels in cobia (*R. canadum*) fed with synbiotics were significantly higher than those of the control fish (Mehrabi et al., 2012). Conversely, Rodriguez-Estrada et al. (2009) reported no significant differences in moisture, crude ash, crude lipids, and crude protein contents found in rainbow trout (*O. mykiss*) fed with either single or combined supplementation of *E. faecalis*, Mannan Oligosaccharide, and Polyhydroxybutyrate Acid. Similar to our findings, Sri et al. (2023) reported that the body composition of rabbitfish (*S. guttatus*) feeding with a locally formulated diet had levels of CP ranging from 58.63 % to 65.47 %; EE 18.51–23.36 %; and ash 12.79–14.31 %. The carcass composition of rabbitfish (*S. rivulatus*), such as CP ranged from 53.10 % to 57.37 %; EE from 21.41 % to 29.32 %; and ash from 17.43 % to 21.04 % (El-dakar et al., 2010). The body composition of spinefoot rabbitfish (*S. rivulatus*), including CP varied from 47.95 % to 62.02 %; EE from 11.78 % to 30.80 %; and ash from 13.70 % to 24.03 % (Abdel-Aziz et al., 2022).

5. Conclusions

The current study demonstrates that employing synbiotics (*P. pentosaceus* and FOS) notably enhanced water quality, growth rate, feed efficiency, digestive enzyme activity, hematological profiles, and lysozyme activity in Golden Rabbitfish. The findings suggest that supplementing the diet of Golden Rabbitfish with synbiotics containing *P. pentosaceus* and FOS, particularly *P. pentosaceus* at a concentration of 10^8 CFU g^{-1} , in the cultivation is advisable to optimize the overall growth rate, feed efficiency, and health status of the fish.

Ethical approval

This experiment was conducted with the approval of the Animal Ethics Committee of Hue University (Approval No. HUVN0029, October 2023).

CRedit authorship contribution statement

Ton That Chat: Writing – original draft, Visualization, Validation, Project administration, Methodology, Funding acquisition, Conceptualization. **Peter Bossier:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis, Conceptualization. **Hoang Nghia Manh:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Truong Thi Hoa:** Writing – original draft, Methodology, Formal analysis, Data curation. **Le Tan Phat:** Methodology, Formal analysis, Data curation. **Le Thi Nhat Anh:** Methodology, Formal analysis, Data curation. **Nguyen Duc Huy:** Writing – review & editing, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no financial interests or personal relationships that could have influenced the present work.

Data availability

Data will be made available on request.

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