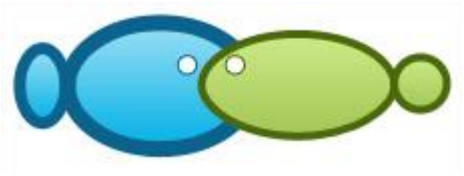

Enhancing growth performance and feed efficiency in red tilapia (*Oreochromis niloticus*) by predigesting artificial feed with probiotic bacteria and herbal additives

[1]Ediwarman Ediwarman, [1]Titin Kurniasih, [1]Novita Panigoro, [1]Ena Sutisna, [1]Adang Saputra, [2]Paino, [3]Yuliana Fitria, [4]Eko Harianto, [5]Donwill Panggabean, [5]Kasful Anwar, [1]Brata Pantjara, [6]Moh Mustakim, [7]Waryat, [8]Saptana

[1] Research Center for Freshwater Aquaculture, National Research and Innovation Agency, Cibinong; [2] Center of Freshwater Aquaculture Development, Sungai Gelam, Jambi, Indonesia; [3] Department of Fisheries Resources Utilization, Jakarta Technical University of Fisheries, Jakarta, Indonesia; [4] Batanghari University, Jl. Slamet Riyadi Broni Jambi 36122, Indonesia; [5] Fisheries Management Study Program, Postgraduate School, Indonesia Open University, Jl. Cabe Raya, Kota Tangerang Selatan 15437, Indonesia; [6] Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Mulawarman University, Samarinda, Kalimantan Timur, Indonesia 75129; [7] Research Center for Agroindustry, National Research and Innovation Agency (BRIN), Jalan Raya Bogor KM. 47 Cibinong, Nanggewer Mekar, Bogor, Indonesia 16912; [8] Research Center for Cooperative, Corporation, and People's Economy, National Research and Innovation Agency (BRIN), Gedung Widya Graha, Jl. Gatot Subroto Kav. 10 Jakarta Selatan 12710, Indonesia. Corresponding author: T. Kurniasih, titin.kurniasih@brin.go.id

Abstract. Fermented feed for red tilapia (*Oreochromis niloticus*), enriched with probiotics and herbs, can affect fish growth and health. This study sought to evaluate the growth performance and feed efficiency of red tilapia administered *Lactobacillus* spp. probiotic fermented feed augmented with herbal components. The artificial feed underwent a 12-hour fermentation process utilizing *Lactobacillus* spp. bacteria enriched with herbs prior to being administered to the fish. Feeding occurred bi-daily at 5% of the fish biomass weight. The research employed a fully randomized design featuring various probiotic dosage interventions. Control feed A was unfermented, treatment B was fermented with a 10 mL kg^{-1} feed dosage, treatment C was fermented with a 20 mL kg^{-1} feed dosage, and treatment D was fermented with a 30 mL kg^{-1} feed dosage. A total of 1,200 juvenile red tilapia, each with an initial weight of approximately 25.16±0.490 g, were reared in 12 net cages at a stocking density of 100 juveniles per net cage. The study demonstrated that during the 60-day rearing period, feed fermented with *Lactobacillus* spp. bacteria and enhanced with herbs can enhance the survival rate and growth performance of red tilapia. The findings indicated an elevation in the mean final weight of the fish to 70.28 g in treatment A, 79.39 g in treatment B, 76.10 g in treatment C, and 76.66 g in treatment D. The survival rate rose from 90.00% to 93.00%. Specific growth rate varied between 1.44% and 1.62% day^{-1}, with a feed conversion ratio between 1.83 and 2.22, and a feed conversion efficiency ranging from 74.10% to 86.24%. The analysis concluded that for optimal specific growth rate in red tilapia cultivation, the incorporation of probiotic bacteria into artificial feed should be no less than 25 mL per kg of feed, achieving a minimal feed conversion ratio of 1.85.
Key Words: feed conversion efficiency, feed conversion ratio, *Lactobacillus* spp., probiotic fermented feed, specific growth rate, survival rate.

Introduction. The enhancement of growth performance and feed efficiency in aquaculture, especially in species like red tilapia (*Oreochromis niloticus*), represents a crucial attention, particularly despite the increasing global demand for sustainable aquaculture approaches. The integration of probiotic bacteria and herbal additives into

artificial feed represents an innovative strategy for enhancing these parameters. Live microorganisms known as probiotics provide health benefits to the host and have demonstrated improvements in growth performance, feed efficiency, and immune responses across different fish species, such as tilapia (Samidjan et al 2022; Yones et al 2019). Herbal additives like noni (*Morinda citrifolia*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), aromatic ginger (*Zingiber zerumbet*), galangal (*Alpinia galanga*), Javanese galangal (*Alpinia javanica*), and pineapple (*Ananas comosus*) have demonstrated potential benefits in enhancing the health and growth of various fish species, including red tilapia (*Oreochromis niloticus*) (Ali et al 2020; Siregar 2023). The bioactive compounds found in these herbs are recognized for their ability to enhance immune responses, promote digestion, and display antimicrobial properties, positioning them as promising options for feed supplementation in aquaculture.

Herbal additives are acknowledged for their ability to improve fish health and promote growth. Turmeric contains the active compound curcumin, recognized for its anti-inflammatory and antioxidant properties, which may enhance the immune response in fish (Singh et al 2022). In a similar way, ginger has demonstrated the ability to boost digestive enzyme activity, resulting in improved nutrient absorption and growth performance in tilapia (Ahmed et al 2023). Recent investigations into the noni fruit have highlighted it's potential as an immunostimulant, with findings suggesting it can improve the immune response and general health of fish (Ali et al 2020). The exploration of noni fruit in aquaculture has been conducted, especially regarding its immunostimulatory effects. Noni possesses a high concentration of antioxidants and has demonstrated the ability to improve the immune response in fish, which may result in enhanced growth rates and increased disease resistance (Ali et al 2020; Muahiddah 2023). Furthermore, the application of aromatic ginger and galangal has been documented to exhibit antimicrobial properties, which may assist in managing pathogenic bacteria within aquaculture systems (Rabelo-Ruiz et al 2023).

Studies show that probiotics can significantly enhance the gut microbiota of fish, leading to improved nutrient absorption and growth performance (Samidjan et al 2022). The use of *Saccharomyces cerevisiae* in tilapia diets has been linked to enhanced growth rates and better blood profiles (Sutthi & Thaimuangphol 2020). Furthermore, research indicates that probiotic strains, including *Lactobacillus* and *Bacillus*, can improve the immune response of tilapia, thereby increasing their resistance to diseases (Yones et al 2019). However, although the advantages of probiotics in aquaculture are extensively recorded, there is still a lack of comprehension regarding the combined effects of integrating probiotics with herbal additives on the growth performance and feed efficiency of red tilapia. This research presents an opportunity to investigate the combined effects of probiotic bacteria enriched with herbs on the predigestion of artificial feed and its subsequent impact on tilapia growth performance and feed utilization.

Combining probiotic bacteria with herbal enhancements to develop a predigested artificial feed designed specifically for tilapia. This novel method seeks to improve the digestibility and nutritional quality of the feed, which could result in better growth rates and enhanced feed efficiency. This study aimed to evaluate the effects of predigesting artificial feed using probiotic bacteria enriched with herbs, focusing on the synergistic effects of these components on the growth performance and feed efficiency of red tilapia (*Oreochromis niloticus*). The goal is to contribute to the development of more effective and sustainable feeding strategies in aquaculture. The ongoing challenges of antibiotic resistance and environmental sustainability in the industry highlight the importance of exploring innovative feeding strategies for the future of aquaculture.

Material and Method

Preparation of experimental fish. All animal experiments were performed in accordance with the ethical standards for the care and use of experimental animals, and the protocol was reviewed and supervised by the Freshwater Aquaculture Development Center (FADC), Sungai Gelam, Jambi, Indonesia. The study complied with the national guidelines for animal experimentation issued by the Ministry of Marine Affairs and Fisheries of the Republic of

Indonesia (Regulation No. 6/PERMEN-KP/2020). The test fish used in this study were juvenile red tilapia (*Oreochromis niloticus*), measuring 8–12 cm in length. A total of 1,200 fish were obtained from the Freshwater Aquaculture Development Center, Sungai Gelam, Muaro Jambi Regency, Jambi Province, after being selected for uniform size, good health, activeness, and absence of disease. To adapt them to the feed and rearing environment, the fish were acclimated for one week. After acclimation, they were fasted for one day to eliminate the effect of previous feed metabolism. They were randomly divided into four dietary treatments and stocked into 12 net cages, with 100 juveniles per cage. The initial weight was then recorded, averaging 25.16 ± 0.490 g per individual.

Probiotic enrichment preparation. The probiotic enrichment was prepared using a mixture of herbal and non-herbal ingredients (Table 1). The herbal components (turmeric, ginger, aromatic ginger, galangal, Javanese galangal, noni, and pineapple) were included for their bioactive properties, such as improving digestion, immune response, and antimicrobial activity in fish. Non-herbal ingredients, including fish meal, yeast, and molasses, were used mainly to provide nutrients that support the growth and metabolic activity of *Lactobacillus* spp. during fermentation. All ingredients were cleaned, ground, and mixed thoroughly with 10 liters of water, followed by inoculation with *Lactobacillus* spp. culture (1×10^{10} CFU). The mixture was placed in a sealed container for 10–12 days of fermentation, with stirring every three days. Although both herbal and non-herbal components were used, the enrichment is referred to as "herb enrichment" throughout the manuscript to emphasize the role of the herbal ingredients as the primary functional additives influencing fish health and growth.

Table 1

Composition for probiotics enrichment

Ingredient	*Volume*	*Measurement unit*
Turmeric	300	g
Ginger	300	g
Aromatic ginger	300	g
Galangal	300	g
Javanese galangal	300	g
Noni	500	g
Pineapple	500	g
Yeast	100	g
Fish meal	2,000	g
Molasses	1,000	mL
Lactobacillus spp.*	325	mL

Note: Stocking density bacteria 1 x 10^{10} CFU.

Feed preparation. The experimental setup utilized a commercially available floating feed specifically formulated for the growth of red tilapia. The protein content of this feed is 30-31%, and it underwent fermentation with *Lactobacillus* spp. Additionally, it was enriched with herbs for a duration of 12 hours. The treatments involved the addition of different doses of *Lactobacillus* spp. probiotic, enriched with herbs per kilogram of feed, as outlined in the following treatments: A - 0 mL, B - 25 mL, C - 50 mL, and D - 75 mL. The incorporation of herb-enriched probiotics into the feed was achieved through top dressing. The feed was gradually introduced and kept in a cool, dry environment within sealed containers before being administered to the experimental fish.

Cultivation preparation. Red tilapia (*Oreochromis niloticus*) were reared in fine-mesh black nylon net cages (2 x 2 x 1.2 m) installed in a 500 m² concrete pond (1.5 m depth). Each cage, serving as an experimental unit, was supported by bamboo/wooden poles, secured with ropes, and fitted with a wooden plank across the units for feeding and monitoring. The open design allowed free water exchange with the pond, ensuring uniform

water quality while minimizing cross-contamination among treatments. This setup facilitated standardized feeding, sampling, and reliable comparisons of growth and survival. Pond water was sourced from a reservoir, stored for several days, and settled before use.

Implementation of research. The research was conducted at the Sungai Gelam Freshwater Aquaculture Development Center, located in Muaro Jambi Regency, Jambi Province, Indonesia. The research commenced with the weighing of the red tilapia to determine the initial weight; subsequently, the red tilapia juveniles were introduced into the net cage at a density of 100 juveniles per net cage. The feed acclimation process was meticulously organized, beginning with a two-week combination of commercial feed and the experimental diet, progressively elevating the ratio of the test feed until it constituted the entirety of the diet. The gradual transition is crucial as it alleviates stress and fosters acceptance of the new feed, particularly in juvenile fish (Chang et al 2006). The feed was administered at a rate of up to 5% of biomass weight per day using the fixed feeding rate method. The feeding frequency occurred twice daily, at 08:00 PM and 04:00 PM. The investigation spanned 60 days from the middle of July to the end of September 2023. Biomass sampling was conducted only at the beginning and at the end of the trial by weighing the total biomass in each net cage. The mean body weight per fish was calculated from the total biomass divided by the number of surviving fish, while survival was determined by counting the total number of fish in each net cage.

Proximate analysis. Proximate analysis (AOAC 2012) was conducted to assess the protein, lipid, ash, and fiber content of the samples.

Water quality. Water quality parameters, such as temperature, pH, and dissolved oxygen, were assessed twice daily, while feasibility and ammonia (NH_3) evaluations were performed bi-weekly. The evaluation of water quality was carried out following the APHA (2012) methodology.

Parameters observed. This study focused on several key parameters, including feed usage efficiency (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and survival rate (SR). The following formulas are used to measure the parameters for growth performance and feed utilization (National Research Council 2011):

$$\text{Feed usage efficiency (EFU)} = 100 \times \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Diet weight consumed (g)}}$$

$$\text{Specific growth rate (SGR)} = 100 \times \frac{\text{Ln(initial weight in g)} - \text{Ln(final weight in g)}}{\text{Time (days)}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Diet consumed (g)}}{\text{Weight increase (g)}}$$

$$\text{Protein efficiency ratio (PER)} = 100 \times \frac{\text{Weight gain (g)}}{\text{Protein consumed (g)}}$$

$$\text{Survival rate (SR)} = 100 \times \frac{\text{Final number of fish}}{\text{Initial number of fish}}$$

Statistical analysis. The data evaluation employed one-way analysis of variance. In cases where the results demonstrated a highly significant effect ($p < 0.01$) or a significant effect ($p < 0.05$), the Duncan Multiple Area Test was performed. Statistical analyses were conducted utilizing SPSS Version 27. Descriptive analyses were employed to compare the measurement results of water condition and proximate analysis in the study to the required conditions outlined in the literature.

Results. The findings from the observation of parameters are detailed in Table 2. According to Table 2, the supplementation of feed with probiotics enriched with herbs had a significant impact on feed usage efficiency (EFU), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR), while it did not have a considerable effect on protein efficiency ratio (PER).

Table 2

Data on parameter observations during the study

Parameters	*Treatments*			
	A	*B*	*C*	*D*
Initial weight (g)	25.21 ± 0.20^{a}	25.17 ± 0.44^{a}	25.17 ± 0.47^{a}	25.13 ± 0.93^{a}
Final weight (g)	70.28 ± 0.71^{a}	79.40 ± 2.85^{b}	76.10 ± 0.76^{ab}	76.66 ± 5.78^{ab}
EFU (%)	74.10 ± 1.33^{a}	83.84 ± 1.02^{b}	84.07 ± 2.28^{b}	84.56 ± 2.66^{b}
SGR (% day^{-1})	1.44 ± 0.01^{a}	1.62 ± 0.05^{b}	1.56 ± 0.02^{b}	1.57 ± 0.06^{b}
FCR	2.22 ± 0.05^{a}	1.85 ± 0.09^{b}	1.87 ± 0.06^{b}	1.83 ± 0.09^{b}
PER (%)	2.26 ± 0.26^{a}	2.40 ± 0.64^{a}	2.47 ± 0.43^{a}	2.41 ± 0.42^{a}
SR (%)	91.33 ± 0.67^{a}	90.00 ± 0.00^{ab}	91.33 ± 2.31^{ab}	93.00 ±1.00^{b}

Note: Mean values with different superscript letters indicate a significant effect ($p < 0.05$).

The proximate analysis presented in Table 3 indicated that the highest protein content was observed in treatment D (30 mL kg^{-1} diet). Consequently, the supplementation of feed with probiotics enriched with herbs can enhance fish growth.

Table 3

Proximate analysis of feeds

Parameters	*Treatments*			
	A	*B*	*C*	*D*
Protein (%)	28.85	29.25	29.25	29.92
Lipid (%)	6.68	6.19	6.19	7.41
Fiber (%)	12.88	12.20	12.20	12.49
Ash (%)	13.77	13.84	13.84	14.42
NFE (%)	37.82	38.52	38.52	35.76
Energy (kcal)*	378.76	379.33	379.33	383.14
Ratio of E/P**	13.13	12.97	12.97	12.81

Note: *calculated based on digestible energy according to Watanabe (1988); 1 g of protein contains 5.6 kcal g^{-1}, 1 g of nitrogen-free extract contains 4.1 kcal g^{-1}, and 1 g of lipid contains 9.4 kcal g^{-1}; **according to Steffens (1989), the E/P value for optimal growth of fish ranges from 8-12 kcal g^{-1}.

The findings from the polynomial orthogonal test and the regression analysis of herb-enriched probiotics in feed concerning EFU, SGR, FCR, PER, and SR are illustrated in Figures 1-5. Figures 1-5 indicate that the relationship between different doses of the herb-enriched probiotic in feed and EFU, SGR, FCR, PER, ECR, and SR exhibits a quadratic nature, with determination coefficients ranging from 72% to 96%.

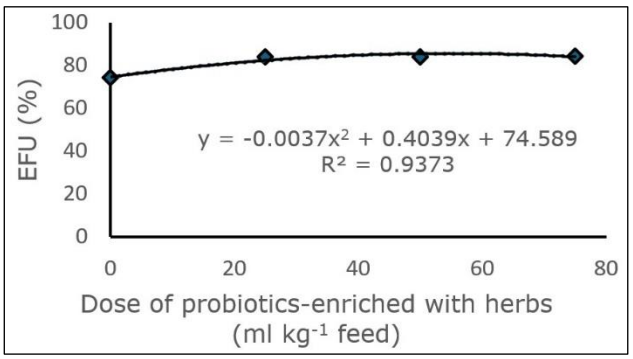

Figure 1. The relationship between probiotic-enriched herbs in feed and EFU in red tilapia.

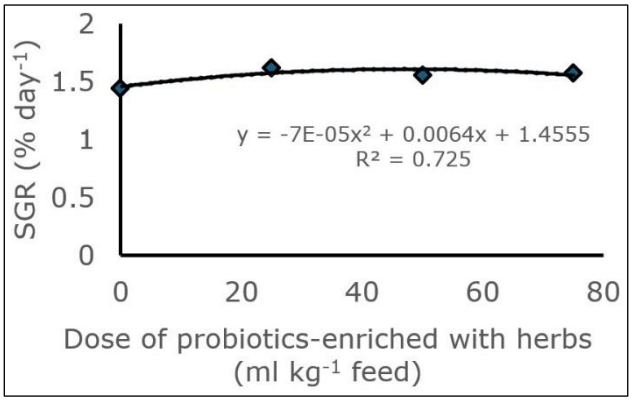

Figure 2. The relationship between probiotic-enriched herbs in feed and SGR in red tilapia.

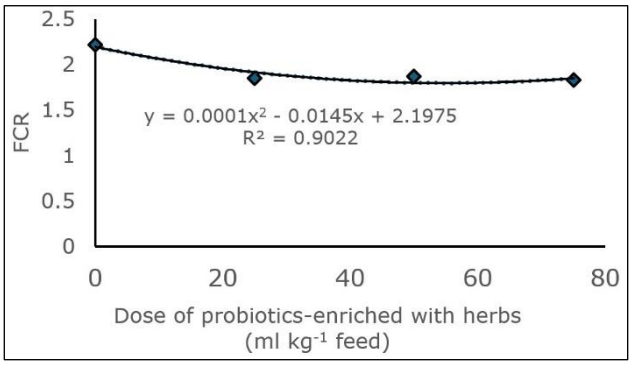

Figure 3. The relationship between probiotic-enriched herbs in feed and FCR in red tilapia.

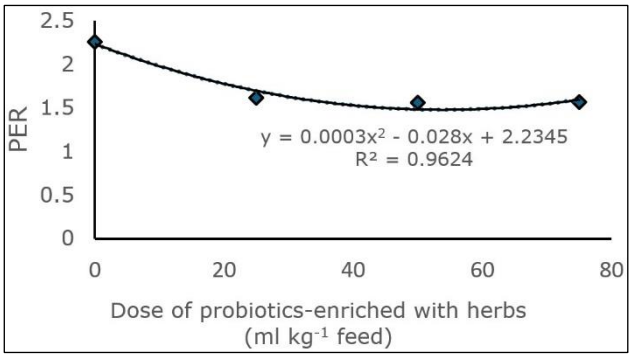

Figure 4. The relationship between probiotic-enriched with herbs in feed and PER in red tilapia.

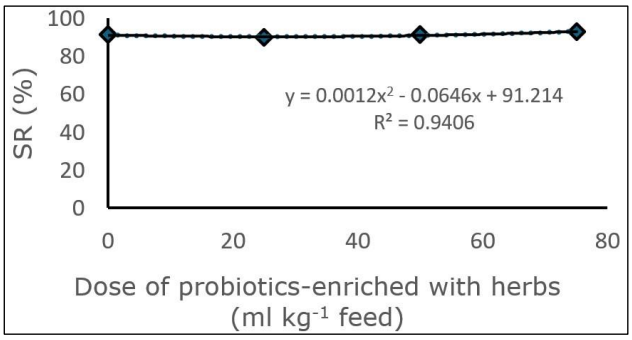

Figure 5. The relationship between probiotic-enriched with herbs in feed and SR in red tilapia.

The water parameters observed during the study (Table 4) met the established criteria outlined in the literature. The findings suggested that the water quality parameters were deemed suitable for the cultivation of juvenile red tilapia.

Table 4

Water condition parameters

Pond	*Parameters*			
	Temperature (°C)	*pH*	*DO (mg L^{-1})*	*NH_3 (mg L^{-1})*
Inlet	26.3 - 29.0	6.18 - 7.21	2.29 - 2.49	0.012 - 0.045
Middle	27.3 - 30.1	6.79 - 7.17	2.98 - 3.09	0.017 - 0.020
Outlet	27.4 - 29.1	6.79 - 7.54	2.02 - 3.01	0.016 - 0.022
Feasibility	14 - 38*	6.50 - 8.50	>2*	<0.1*

Note: *Boyd (2003).

Discussion. The red tilapia (*Oreochromis niloticus*) that received a probiotic diet enriched with herbs (25-75 mL kg^{-1} feed) in treatments B, C, and D exhibited higher feed usage efficiency (EFU) values (83.84-84.56%) in comparison to the treatment without the supplemented probiotic enriched with herbs, as seen in treatment A (74.10%). The highest EFU value recorded was 84.56% in red tilapia that received dietary supplementation of probiotic-enriched with herbs at a rate of 75 mL kg^{-1} feed (treatment D). This was closely followed by treatment C at 84.07%, treatment B at 83.84%, and treatment A at 74.10%. The elevated EFU value of red tilapia receiving a probiotic diet enriched with herbs at a dosage of 75 mL kg^{-1} feed suggests that this amount may effectively promote the growth rate of red tilapia, potentially through enhancements in gut health and nutrient absorption (Azevedo et al 2015).

Table 2 shows that red tilapia fed a probiotic-enriched with herbs diet (25-75 mL kg^{-1} feed) had a higher specific growth rate (SGR) (1.56-1.62% day^{-1}) than those fed a control diet (0 ml kg^{-1}). Figure 2 shows the optimal probiotic-herb supplement dose. The dose is 25 ml kg^{-1} feed with 1.62% SGR day^{-1}. The R^2 value in Figure 2 was 72.5%. Dietary supplementation with probiotic-enriched herbs affected 72.50% of SGR and 27.50%. Red tilapia fed B (25 ml kg^{-1} feed) had the highest SGR value, which was thought to increase protein digestibility due to digestive tract enzyme activity, maximizing feed usage efficiency and fish growth. The treatment with 25 mL kg^{-1} feed had the highest SGR value, indicating that it may enhance protein digestibility by increasing digestive tract enzyme activity and feed usage efficiency (Azevedo et al 2015; Garcia et al 2016). According to Azevedo et al (2015), probiotics increase digestive enzyme production, improving fish nutrient absorption and growth.

The addition of probiotics to water significantly boosts feed conversion ratio (FCR) values and enhances feed efficiency in tilapia aquaculture. Probiotics improve water quality by reducing harmful substances such as ammonia and nitrite, which directly affects the health and growth of tilapia fish, as shown by enhanced feed efficiency and lower FCR values (Idris et al 2023). The incorporation of probiotics has shown to improve survival rates and facilitate increased growth rates, as evidenced in Table 2. Increased doses of probiotics lead to a decrease in feed conversion ratio and an improvement in feed efficiency. Prakasita and Wahyuni (2020) suggest that combining herbs and probiotics can be a viable substitute for antibiotic growth promoters (AGP) in animal feed. The synergy between herbs and probiotics, as illustrated by Prakasita and Wahyuni (2020), shows that herbs such as ginger and turmeric markedly promote the growth of probiotics in a notably effective and efficient way. Amenyogbe (2023) underscored the significance of probiotics in fostering sustainable and environmentally friendly aquaculture, pointing out their contribution to reducing antibiotic usage and improving fish health. The incorporation of probiotics and herbal mixtures improves feed efficiency and strengthens the immune systems of fish, leading to healthier growth and greater longevity in optimal aquaculture settings.

The FCR values varied between 1.83 and 1.87, with the minimum FCR recorded at 75 mL kg^{-1} of probiotic-enriched herb supplementation, in contrast to the FCR of 2.22

observed in the absence of supplementation (0 ml kg^{-1} diet). A lower FCR signifies improved feed efficiency, indicating that fish are more effectively converting feed into body mass. The enhancement in feed conversion ratio with the supplementation of herb-enriched probiotics indicates that such probiotics may improve feed utilization, likely through better digestion and nutrient absorption. The results concerning the improvement of FCR via herbal-enriched probiotic supplementation indicate that this method may enhance feed utilization by optimizing the digestion and absorption of nutrients. According to Samara et al (2023) the use of probiotics may enhance the growth of betta fish (*Betta splendens*) by optimizing digestive enzymes, leading to improved digestion and nutrient absorption. Furthermore, Samara et al (2023) highlighted that probiotic comprising diverse bacterial strains, including *Lactobacillus acidophilus*, *Bacillus subtilis*, *Rhodopseudomonas* spp., *Aspergillus oryzae*, and *Saccharomyces cerevisiae*, have the potential to improve feed quality by elevating the levels of proteases within the digestive system. This has the potential to enhance growth and diminish the presence of potentially harmful bacteria within the fish's digestive system. Figure 3 illustrates the optimal dosage of the herb-enriched probiotics diet. The dosage is 75 mL per kg of feed, resulting in a feed conversion ratio of 1.83. The R^2 value presented in Figure 3 was 90.22%. It indicates that dietary supplementation with probiotics enriched with herbs had an influence on 90.22% of FCR.

Red tilapia that received probiotics-enriched herb supplementation (25-76 mL kg^{-1} feed) exhibited higher protein efficiency ratio (PER) values (2.40-2.47) compared to those that did not receive any supplementation (0 g kg^{-1} feed), which had a PER of 2.26. Red tilapia that received probiotics-enriched with herbs supplementation at a rate of 50 mL kg^{-1} feed (treatment C) exhibited the highest PER value of 2.47, while the lowest PER value of 2.26 was observed in treatment A (0 mL kg^{-1} feed). The highest PER value observed in treatment C (50 mL kg^{-1} of feed) was considered effective in enhancing digestibility and optimizing feed utilization efficiency, leading to an increase in the protein efficiency ratio. The increased rates of probiotic inclusion led to improved protein efficiency ratios, suggesting that the pre-digestion of feed by probiotics facilitated better protein absorption and utilization (Haque et al 2021). Ndobe et al (2021) detail how probiotics facilitate the breakdown of complex compounds, enhancing the digestibility of feed via fermentation. While the differences between treatments did not reach statistical significance, the observed pattern indicated a possible enhancement in growth and feed conversion ratio (FCR) following two days of feed fermentation with probiotics. The observed increase in PER in this study, particularly in treatment C (50 mL kg-1 feed), likely results from a comparable mechanism.

The addition of probiotics to the feed facilitated the breakdown of complex proteins into simpler forms, enhancing the digestibility and efficiency of protein utilization in red tilapia. Ndobe et al (2021) detailed how probiotics can promote fish growth through the enhancement of digestive enzyme activity, resulting in improved digestion and nutrient uptake and found that fermenting over three days did not yield positive results. Increased probiotic dosages do not necessarily guarantee improved outcomes. An optimal point for probiotic supplementation may exist, where the benefits reach their peak before beginning to decline. Figure 4 illustrates the optimal dose of the probiotics-enriched with herbs diet. The dosage is 50 ml per kg of feed, exhibiting a PER value of 2.47. The treatment with 50 mL kg^{-1} feed exhibited the highest PER value, indicating that this dosage significantly improves protein digestibility and optimizes feed utilization efficiency (Azevedo et al 2015). The observed trend of elevated PER with greater probiotic inclusion rates corresponds with the observations made by (Ran et al 2012), who indicated that probiotics could improve protein absorption and utilization by decomposing complex compounds into simpler forms. The R^2 value presented in Figure 4 is 96.24%. It indicates that up to 96.24% of PER was affected by the dietary supplementation of probiotics enriched with herbs.

The dietary supplementation of probiotics enriched with herbs had a significant effect ($p < 0.05$) on the survival rate (SR) of red tilapia fingerlings (Table 2). The SR consistently stayed elevated across all treatments, varying between 93.33% and 96.67%. This suggests that the diets enriched with probiotics did not negatively impact fish health and may have even enhanced disease resistance, as indicated by earlier research (Hossain et al 2022; Ringø et al 2022). Previous studies indicate that probiotics can enhance the

immune response in fish, which contributes to improved survival rates under optimal aquaculture conditions (Pérez-Sánchez et al 2013; Selim & Reda 2015). Figure 6 illustrates the optimal dose of the probiotics enriched with herbs diet. The dosage is 75 ml per kg of feed, with an SR value of 93%. The R^2 value presented in Figure 6 is 94.06%. It indicates that up to 94.06% of SR was affected by the dietary supplementation of probiotics enhanced with herbs.

The significance of probiotics in improving water quality is evident, as they have demonstrated the ability to decrease harmful substances like ammonia and nitrite in aquaculture systems (Yu et al 2019). The enhancement in water quality is directly linked to improved growth and feed efficiency in tilapia, demonstrated by the noted reduction in FCR values with increased probiotic doses. The combined effects of herbs and probiotics, as highlighted by (Ferguson et al 2010), reinforce the idea that these mixtures can act as viable substitutes for antibiotic growth promoters (AGPs) in aquaculture. The herbs, especially those recognized for their immunostimulant properties, can boost the efficacy of probiotics, resulting in better growth and health outcomes in fish. Abdel-Tawwab and Abbass (2016) conducted a study on the effects of turmeric powder on common carp (*Cyprinus carpio*), revealing noteworthy enhancements in growth performance and innate immunity. The results demonstrated that turmeric supplementation enhanced the nonspecific immune system, offering sustained protection against pathogenic infections like *Aeromonas hydrophila*. The findings align with the results of the present study, indicating that diets enriched with probiotics and supplemented with herbs have positively influenced the growth performance and feed utilization efficiency of red tilapia, presumably by enhancing immune responses and gut health.

Similarly, Harikrishnan et al (2011) showed that a diet enhanced with herbal and probiotic elements positively influenced the hematological and immune status of *Oplegnathus fasciatus* in the face of *Edwardsiella tarda* infections. The protective effects noted in their study align with the findings of the current investigation, where the application of probiotics and herbs not only improved growth metrics but also led to increased survival rates, indicating enhanced disease resistance in red tilapia. The study conducted by Dowidar et al (2018) corroborates the findings of the current study, indicating that probiotics enhanced growth performance and disease resistance in Nile tilapia when faced with *Aeromonas hydrophila* challenges. The repeated observation of improved growth and survival rates in multiple studies highlights the effectiveness of incorporating probiotics and herbal supplements into aquaculture diets.

In a separate investigation, Abdel-Tawwab and Abbass (2016) investigated the incorporation of green tea (*Camellia sinensis*) into the diets of Nile tilapia, revealing a notable improvement in phagocytic activity and a decrease in mortality rates after exposure to *Aeromonas hydrophila* infection. The findings of this study correspond with previous results, suggesting that diets enriched with probiotics and supplemented with herbs could strengthen the immune system and increase resistance to pathogens, ultimately promoting better fish health and growth performance. The results presented by Zare et al (2021) provide additional support for the conclusions of the current study, indicating that incorporating nettle (*Urtica dioica*) and tarragon (*Artemisia dracunculus*) into the diets of rainbow trout (*Oncorhynchus mykiss*) led to improved growth and enhanced blood parameters, such as red and white blood cell counts. This indicates that herbal supplements may have a beneficial impact on the physiological health of fish, aligning with the noted enhancements in growth performance and feed efficiency in red tilapia that were provided by probiotic-enriched herbal diets.

Conclusions. The findings indicated that the ideal dosage for maximizing growth performance was determined to be 25 mL kg^{-1} feed, yielding a feed usage efficiency (EFU) value of 83.84%, a feed conversion ratio (FCR) of 1.85, a specific growth rate (SGR) of 1.62% day^{-1}, and a survival rate (SR) of 93%. The investigation underscores the notable advantages of integrating probiotic-enriched diets, enhanced with herbs, into the feeding strategy for red tilapia. The identified optimal dosages for improving growth performance, feed efficiency, and survival rates highlight the promise of probiotics as a sustainable alternative to conventional antibiotic growth promoters in aquaculture. The results indicate

that incorporating probiotics and herbs may enhance gut health, nutrient absorption, and overall fish welfare, thus supporting more sustainable aquaculture practices.

Acknowledgements. The authors take this opportunity to thank to the Freshwater Aquaculture Development Center, Sungai Gelam, Jambi, Indonesia for supporting and helping with the study.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Abdel-Tawwab M., Abbass F. E., 2016 Turmeric powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: growth performance, innate immunity, and challenge against pathogenic *Aeromonas hydrophila* infection. Journal of the World Aquaculture Society 48(2):303–312.

Ahmed S., El-Murr A., Elhakim Y. A., Metwally M. M., Gharib A. A., Amer S. A., Younis E. M., Abdel-Warith A.-W. A., Davies S. J., Khalil E., 2023 Comparative study on ginger powder and ginger extract nanoparticles: effects on growth, immune–antioxidant status, tissue histoarchitecture, and resistance to *Aeromonas hydrophila* and *Pseudomonas putida* infection in *Oreochromis niloticus*. Fishes 8(5):259. https://doi.org/10.3390/fishes8050259

Ali M. A. M., Khuraiba H. M., Elsayed N. E. G., Sharawy Z. Z., 2020 The effect of different stocking densities of marine shrimp larvae *Litopeneaus vannamei* on water quality using biofloc technology. Egyptian Journal of Nutrition and Feeds 23(1):183–195. https://doi.org/10.21608/ejnf.2020.95845

Amenyogbe E., 2023 Application of probiotics for sustainable and environment-friendly aquaculture management - a review. Cogent Food & Agriculture 9:2226425. 23 pp. https://doi.org/10.1080/23311932.2023.2226425

Azevedo R. V. D., Filho J. C. F., Cardoso L. D., Mattos D. D. C., Júnior M. V. V., Andrade D. R. D., 2015 Economic evaluation of prebiotics, probiotics and symbiotics in juvenile Nile tilapia. Revista Ciência Agronômica 46(1):72–79.

Boyd C. E., 2003 Guidelines for aquaculture effluent management at the farm-level. Aquaculture 226(1-4):101-112.

Chang Q., Liang M., Wang J. L., Chen S. Q., Zhang X. M., Liu X. D., 2006 Influence of larval co-feeding with live and inert diets on weaning the tongue sole *Cynoglossus semilaevis*. Aquaculture Nutrition 12(2):135–139.

Dowidar M., El Azeem S. A., Khater A. M., Somayah M. A., Metwally S. A., 2018 Improvement of growth performance, immunity and disease resistance in Nile tilapia, *Oreochromis niloticus*, by using dietary probiotics supplementation. Journal of Animal Science and Veterinary Medicine 3(2):35–46.

Ferguson R. M. W., Merrifield D. L., Harper G. M., Rawling M. D., Mustafa S., Picchietti S., Balcázar J. L., Davies S. J., 2010 The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). Journal of Applied Microbiology 109(3):851–862.

Garcia F., Romera D. M., Sousa N. S., Paiva-Ramos I., Onaka E. M., 2016 The potential of periphyton-based cage culture of Nile tilapia in a Brazilian reservoir. Aquaculture 464:229–235.

Haque M. M., Hasan N. A., Eltholth M. M., Saha P., Mely S. S., Rahman T., Murray F. J., 2021 Assessing the impacts of in-feed probiotic on the growth performance and health condition of pangasius (*Pangasianodon hypophthalmus*) in a farm trial. Aquaculture Reports 20. https://doi.org/10.1016/j.aqrep.2021.100699

Harikrishnan R., Kim M.-C., Kim J.-S., Balasundaram C., Heo M., 2011 Protective effect of herbal and probiotics enriched diet on haematological and immunity status of *Oplegnathus fasciatus* (Temminck & Amp; Schlegel) against *Edwardsiella tarda*. Fish & Shellfish Immunology 30(3):886–893.

Idris A. P. S., Baga I., Wahidah S., Patang, 2023 The effect giving of probiotics in water on feed efficiency and water quality for aquaculture tilapia (*Oreochromis niloticus*).

The International Journal of Science & Technology 11(4). https://doi.org/10.24940/theijst/2023/v11/i4/st2304-009
Muahiddah N., 2023 Use of pineapple (*Ananas comosus*) as an immunostimulant in aquaculture (review). Jurnal Biologi Tropis, 23(4):658–663.
Ndobe S., Rosyida E., Palallo Z. R., 2021 Fermentation of probiotic-enriched commercial feed to improve tilapia (*Oreochromis niloticus*) growout performance. IOP Conference Series: Earth and Environmental Science 674(1). https://doi.org/10.1088/1755-1315/674/1/012056.
Pérez-Sánchez T., Ruiz-Zarzuela I., Blas I. D., Balcázar J. L., 2013 Probiotics in aquaculture: a current assessment. Reviews in Aquaculture 6(3):133–146.
Prakasita V. C., Wahyuni A. E. T. H., 2020 In vitro study: Combination of herbs and probiotics as an alternative antibiotic growth promoters. Scholars Journal of Agriculture and Veterinary Sciences 7(8):198-201.
Rabelo-Ruiz M., Peralta-Sánchez J. M., Martín-Platero A. M., Ruiz A. J., Agraso M. D. M., Bermúdez L., Ariza J. J., Baños A., Valdivia E., Martínez-Bueno M., 2023 Allium-derived compound propyl propane thiosulfonate (PTSO) reduces vibrio populations and increases body weight of European seabass (*Dicentrarchus labrax*) juveniles. Antibiotics 12(1):134. https://doi.org/10.3390/antibiotics12010134.
Ran C., Carrias A., Williams M. A., Capps N. K., Dan B. C. T., Newton J. C., Kloepper J. W., Ooi E. L., Browdy C. L., Terhune J. S., Liles M. R., 2012 Identification of *Bacillus* strains for biological control of catfish pathogens. Plos One 7(9):e45793. https://doi.org/10.1371/journal.pone.0045793.
Ringø E., Li X., Doan H. V, Ghosh K., 2022 Interesting probiotic bacteria other than the more widely used lactic acid bacteria and bacilli in finfish. Frontiers in Marine Science 9. https://doi.org/10.3389/fmars.2022.848037.
Samara S. H., Saputra F. E., Fidyandini H. P., Hudaidah S., 2023 The effect of probiotics to betta fish (*Betta splendens* Regan, 1910) aquaculture performance. IOP Conference Series: Earth and Environmental Science 1273(1). https://doi.org/10.1088/1755-1315/1273/1/012033.
Samidjan I., Rachmawati D., Riyadi P. H., 2022 Development of polyculture engineering technology on milkfish and mud crab farming. Pertanika Journal of Tropical Agricultural Science 45(2):377-389.
Selim K., Reda R. M., 2015 Improvement of immunity and disease resistance in the Nile tilapia, *Oreochromis niloticus*, by dietary supplementation with *Bacillus amyloliquefaciens*. Fish & Shellfish Immunology 44(2):496–503.
Singh M., Borah D., Dutta M., Gogoi S., Saikia C., Sonowal S., Manhai S. K., 2022 A review on immunostimulatory and antioxidant potential of herbs, *Curcuma longa* L., *Camellia sinensis* L., *Zingiber officinale* and *Allium sativum* Linn. in fish health: a sustainable approach for a healthy aquaculture. Ecology Environment and Conservation 28(3):1431–1445.
Siregar R. S., 2023 Ginger as an animal feed additive: an overview. The Journal of Animal and Plant Sciences 34(1):31–49.
Sutthi N., Thaimuangphol W., 2020 Effects of yeast (*Saccharomyces cerevisiae*) on growth performances, body composition and blood chemistry of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) under different salinity conditions. Iranian Journal of Fisheries Sciences 19(3):1428–1446.
Yones A.-M. M., Hussein M. S., Ali M. W., Abdel-Azem A.-A. M., 2019 Effect of dietary Lacto cel-con probiotic on growth performance and hematology indices of fingerlings mono-sex Nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Aquatic Biology and Fisheries 23(2):227-239.
Yu L., Qiao N., Li T., Yu R., Zhai Q., Tian F., Zhang H., Chen W., 2019 Dietary supplementation with probiotics regulates gut microbiota structure and function in Nile tilapia exposed to aluminum. PeerJ 7:e6963. https://doi.org/10.7717/peerj.6963
*** American Public Health Association (APHA), 2012 Standard methods for the examination of water and wastewater, 22ed.; American Public Health Association, Water Environment Federation; Washington DC, USA. 541 pp.

*** Association of Official Analytical Chemists (AOAC), 2012 Official methods of analysis of AOAC international, 17th ed. Arlington. 25–42 pp.
*** National Research Council, 2011 Nutrient requirements of fish and shrimp. The National Academies Press, Washington DC. 392 pp.

Received: 06 November 2024. Accepted: 03 March 2025. Published online: 09 October 2025.
Authors:
Ediwarman Ediwarman, Research Center for Freshwater Aquaculture, National Research and Innovation Agency, KST Soekarno, Jl. Raya Bogor KM 46, Cibinong, Nanggewer Mekar, Bogor, West Java, 16911, Indonesia, e-mail: ediwarman@brin.go.id
Titin Kurniasih, Research Center for Freshwater Aquaculture, National Research and Innovation Agency, KST Soekarno, Jl. Raya Bogor KM 46, Cibinong, Nanggewer Mekar, Bogor, West Java, 16911, Indonesia, e-mail: titin.kurniasih@brin.go.id
Novita Panigoro, Research Center for Freshwater Aquaculture, National Research and Innovation Agency, KST Soekarno, Jl. Raya Bogor KM 46, Cibinong, Nanggewer Mekar, Bogor, West Java, 16911, Indonesia, e-mail: novita.panigoro@brin.go.id
Ena Sutisna, Research Center for Freshwater Aquaculture, National Research and Innovation Agency, KST Soekarno, Jl. Raya Bogor KM 46, Cibinong, Nanggewer Mekar, Bogor, West Java, 16911, Indonesia, e-mail: ena.sutisna@brin.go.id
Adang Saputra, Research Center for Freshwater Aquaculture, National Research and Innovation Agency, KST Soekarno, Jl. Raya Bogor KM 46, Cibinong, Nanggewer Mekar, Bogor, West Java, 16911, Indonesia, e-mail: adang_pusrisdkp@yahoo.com
Paino, Center of Freshwater Aquaculture Development, Sungai Gelam, Jambi, Indonesia, e-mail: paino78@kkp.go.id
Yuliana Fitria, Department of Fisheries Resources Utilization, Jakarta Technical University of Fisheries, Jakarta, Indonesia, e-mail: yulifitria239@gmail.com
Eko Harianto, Universitas Batanghari Jl. Slamet Riyadi Broni Jambi 36122, Indonesia, e-mail: eko.harianto@unbari.ac.id
Donwill Panggabean, Fisheries Management Study Program, Postgraduate School, Indonesia Open University, Jalan Cabe Raya, Kota Tangerang Selatan 15437, Indonesia, e-mail: donwill@ecampus.ut.id
Kasful Anwar, Fisheries Management Study Program, Postgraduate School, Indonesia Open University, Jalan Cabe Raya, Kota Tangerang Selatan 15437, Indonesia, e-mail: kasful@ecampus.ut.ac.id
Brata Pantjara, Research Center for Freshwater Aquaculture, National Research and Innovation Agency, KST Soekarno, Jl. Raya Bogor KM 46, Cibinong, Nanggewer Mekar, Bogor, West Java, 16911, Indonesia, e-mail: brata.pantjara@brin.go.id
Moh Mustakim, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Mulawarman University, Jalan Gunung Tabur No. 1, Gunung Kelua Campus, Samarinda City, Kalimantan Timur, 75129, Indonesia, e-mail: mustakim.unmul@gmail.com
Waryat, Research Center for Agroindustry, National Research and Innovation Agency, Jalan Raya Bogor KM. 47 Cibinong, Nanggewer Mekar, Bogor, West Java, 16912, Indonesia, e-mail: wary003@brin.go.id
Saptana, Research Center for Cooperative, Corporation, and People's Economy, National Research and Innovation Agency – Jakarta- 12710, Indonesia, e-mail: saptana@brin.go.id

How to cite this article:
Ediwarman E., Kurniasih T., Panigoro N., Sutisna E., Saputra A., Paino, Fitria Y., Harianto E., Panggabean D., Anwar K., Pantjara B., Mustakim M., Waryat, Saptana, 2025 Enhancing growth performance and feed efficiency in red tilapia (*Oreochromis niloticus*) by predigesting artificial feed with probiotic bacteria and herbal additives. AACL Bioflux 18(5):2263-2274.