Probiotics as feed additives to improve feed efficiency and growth in catfish (*Clarias gariepinus*) reared in probiotic-enriched media

Subandiyono Subandiyono, Sri Hastuti, Ristiawan A. Nugroho, Diana Chilmawati, Hanungko B. D. Tyastanto

Faculty of Fisheries and Marine Science, Universitas Diponegoro, Tembalang Semarang, Jawa Tengah, Indonesia. Corresponding author: S. Hastuti, hastuti_hastuti@yahoo.com

Abstract. African catfish (*Clarias gariepinus*) aquaculture has increased significantly to support global food security, driven by a rise in feed production. However, super-intensive farming systems face challenges such as environmental degradation, fish health and welfare issues, and increased feed demands. Innovative feed solutions are necessary to address these concerns. Probiotics emerged as a promising option, offering disease prevention and pathogen inhibition in the digestive tract, thereby improving fish health and performance. This study investigated the impact of probiotic-enriched feed (i.e.: *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Bacillus* sp., *Nitrosomonas* sp., *Nitrobacter* sp., and *Saccharomyces cerevisiae*) on the feed efficiency and growth of catfish. A completely randomized design (CRD) was applied with three treatments (i.e. A: 10^5 CFU mL^{-1}, B: 10^6 CFU mL^{-1}, C: 10^7 CFU mL^{-1}) and three replicates. All trial groups of juvenile catfish (8.83±0.09 cm; 5.186±0.152 g) were reared in probiotic-enriched biofloc-based system. The fish were reared in HDPE cages placed in a tarpaulin pond for 42 days. The probiotic-enriched feeds, according to treatments, were administered three times daily using the relative feeding rate method. Feeding trial C gave the best results for total feed consumption (TFC: 1427.18±102.16 g), feed utilization efficiency (FUE: 67.57±3.53%), feed conversion ratio (FCR: 1.41±0.09), protein efficiency ratio (PER: 194±10%), and relative growth rate (RGR: 4.55±0.18% day^{-1}). These findings indicate that probiotics given as feed additives with a dose of 10^7 CFU mL^{-1} can improve feed efficiency and boost growth performance; therefore, this treatment is recommended in catfish aquaculture.

Key Words: African catfish, biofloc system, *Clarias gariepinus*, digestive enzyme activity, fish health, probiotic-enriched feed, sustainable aquaculture.

Introduction. The African catfish (*Clarias gariepinus*), a freshwater species native to Africa, has become a key focus of aquaculture in Indonesia (Hastuti & Subandiyono 2016, 2018; Basuki et al 2020; Kristiana et al 2022). Its ability to thrive in high-density conditions is attributed to specialized respiratory organs known as arborescent organs, which enable survival in suboptimal environments, particularly those with low dissolved oxygen levels (Hastuti & Subandiyono 2016; Sunardi et al 2016; Hastuti et al 2019). Although *C. gariepinus* have adapted well to artificial feeds (Subandiyono & Hastuti 2016), their feed utilization efficiency can still be optimized to further enhance growth rates. Probiotics, including certain photosynthetic bacteria, have demonstrated effectiveness in improving feed efficiency and promoting fish growth (Shabrina et al 2018). One promising approach is the use of probiotics, applied either as feed additives or through the formation of a floc or biofloc in the culture water. Probiotic flocs in the water medium, together with the feed consumed, can be directly utilized by *C. gariepinus* as a supplement, and can also play a role in improving the quality of the water medium itself (Hastuti & Subandiyono 2016, 2018; Salamah & Zulpikar 2020; Rarassari et al 2021).

Probiotics are beneficial bacteria known for their ability to stimulate the immune system (Soto-Dávila et al 2024; Xia et al 2024). In the digestive tracts of fish (Mareta et al 2017; Qi et al 2024) and quail (Lokapirnasari et al 2023), probiotics produce additional enzymes that enhance feed digestion and nutrient absorption (Mareta et al 2017;

Lokapirnasari et al 2023). By incorporating probiotics into feed, the nutritional value is improved, and complex nutrients are broken down into forms more easily absorbed by the body, reducing nutrient waste (Shabrina et al 2018; Sulasi et al 2018). Moreover, probiotics play a critical role in disease prevention by inhibiting the growth of pathogenic bacteria in the digestive tract, as highlighted by Umasugi et al (2018) and Qi et al (2024). These combined functions of probiotics, i.e. enhancing nutrient utilization and preventing disease, ultimately contribute to improved fish growth through increased feed efficiency.

In recent years, *C. gariepinus* aquaculture production has rapidly expanded to meet the growing demand driven by the increasing global population. However, with the projected human population growth by 2050, the aquaculture industry is expected to intensify further, potentially leading to deteriorating water quality and increased stress on aquaculture systems (Proxton 1992; Mohammadi et al 2021). This intensification can compromise the immune system of farmed fish, making them more susceptible to emerging pathogens (Abdel-Latif et al 2020), thereby threatening fish health and welfare. Moreover, the limited space in these systems facilitates the spread of pathogens, resulting in high mortality rates and significant financial losses (El-Son et al 2021; Korzekwa et al 2022). Although antibiotics are commonly used to control bacterial fish diseases, their use has become restricted in many countries due to concerns about harmful effects on human health (Cabello 2006; Limbu et al 2018), the rise of antibiotic-resistant bacteria (Alderman & Hastings 1998; Smith 2008; Chaklader et al 2020), and environmental hazards (Holmström et al 2003; Lulijwa et al 2020). Consequently, there is an urgent need for cost-effective, environmentally safe alternatives. The use of probiotics, prebiotics, synbiotics, and postbiotics in aquaculture has gained significant attention in recent years due to their proven benefits in improving fish and shrimp health (El-Saadony et al 2021; Abdel-Latif et al 2022; Yilmaz et al 2022).

The optimal use of probiotics as a supplement in catfish feed, particularly regarding dosage and application methods, remains an area requiring further research. While the benefits of probiotics are known, the optimal dosage of a specific multi-strain consortium for catfish reared in a system combining probiotic-enriched feed and water remains unclear. This study aimed to investigate the effects on feed intake, efficiency, and growth performance in *C. gariepinus* catfish of a dual approach, incorporating probiotics in feed as well as through a biofloc-based rearing system.

Material and Method

Experimental design. A completely randomized design (CRD) was applied with three treatments and three replicates. The treatments consisted of the use of different concentration levels of probiotics in feed formulation as follows:
- treatment A: feed with probiotics at 10^5 CFU mL^{-1};
- treatment B: feed with probiotics at 10^6 CFU mL^{-1};
- treatment C: feed with probiotics at 10^7 CFU mL^{-1}.

Selection of experimental fish and acclimation. The test subjects in this study were African catfish (*C. gariepinus*) selected for their uniform size, with an average initial weight of 5.186±0.152 g, and reared in June-August 2025. Fish selection was based on criteria such as size consistency, complete body organ development, and overall physical health (Subandiyono & Hastuti 2016; Astuti et al 2017). The selected fish were then acclimated for seven days in the experimental containers to allow them to adapt to the environment and the type of feed to be used (Hartami et al 2015; Hastuti & Subandiyono 2018). Before being placed in the biofloc-based rearing media, a proximate analysis was conducted to assess the nutritional content of the fish.

Preparation of probiotic-based diets. In this study, two types of commercial pellet feed (F-1 and F-2) were used. The feed types differed in size to accommodate the widening of the mouth gape as the fish grew during the rearing process. Feed F-1 was smaller than F-2, but the nutritional quality was similar (Table 1). The probiotics used were a consortium of probiotic bacteria consisting of *Lactobacillus casei, Lactobacillus bulgaricus, Bacillus* sp., *Nitrosomonas* sp., *Nitrobacter* sp., and *Saccharomyces cerevisiae*. This probiotic mix was incorporated into the feed at bacterial concentrations of 10^5, 10^6, and 10^7 CFU mL^{-1} respectively. The probiotic-enriched F-1 feed was administered to the experimental fish during the first to fourth weeks, followed by the probiotic-enriched F-2 feed for the last 2 weeks. The nutritional composition of each of the base pellet feeds and the corresponding probiotic-enriched feeds is presented in Table 1.

Table 1

Nutritional profile of base feeds and probiotic-enriched feeds

Feed type	*Nutritional value of feed (% dry weight basis)*					*Digestible energy (DE)* (kcal g^{-1})*	*E/P ratio** (kcal g^{-1} protein)*
	Protein	*Lipids*	*Crude fiber*	*Nitrogen free extract*	*Ash*		
Base feed F-1	35.62	5.15	6.56	42.18	10.50	271.81	7.63
Base feed F-2	35.66	4.92	4.71	44.96	9.75	277.06	7.77
F-1 with probiotics at 10^5 CFU mL^{-1}	35.45	4.99	3.67	45.49	10.41	278.19	7.85
F-1 with probiotics at 10^6 CFU mL^{-1}	35.55	4.69	7.41	42.00	10.34	267.41	7.52
F-1 with probiotics at 10^7 CFU mL^{-1}	35.31	4.08	6.86	43.27	10.49	264.79	7.50
F-2 with probiotics at 10^5 CFU mL^{-1}	35.17	5.44	7.31	42.27	9.80	272.87	7.76
F-2 with probiotics at 10^6 CFU mL^{-1}	35.12	4.76	7.53	42.60	9.98	267.95	7.63
F-2 with probiotics at 10^7 CFU mL^{-1}	34.79	5.00	6.07	44.36	9.78	273.13	7.85

Note: *Digestible energy for protein 3.5 kcal g^{-1}, nitrogen free extract 2.5 kcal g^{-1}, lipids 8.1 kcal g^{-1} (NRC 1977); **E/P ratio = DE/protein.

Preparation of probiotic media (biofloc). The biofloc system probiotic media was prepared before rearing by mixing 1.5 kg of feed, 125 mL of molasses, 125 g of wheat flour, 1 L of the probiotics consortium (at a concentration of 10^6 CFU mL^{-1}), and water. The probiotic dosage was selected based on research by Simanjuntak et al (2016), who used a similar concentration for rearing media. The mixture was combined in a bucket and left to ferment for two days in a closed container. After fermentation, dolomite lime was added and the mixture was evenly spread across the water surface in the culture pond, followed by aeration for six days to facilitate biofloc formation. This process was only carried out once and was designed to promote the growth of heterotrophic bacteria and the development of biofloc in the rearing medium before the test fish were stocked (Salamah & Zulpikar 2020). Nevertheless, to maintain the stability of the floc-forming bacterial population in the water media during the rearing period, 50 mL of molasses and 50 mL of probiotics were added for each cubic meter of the rearing medium once a week (Rarassari et al 2021).

Fish rearing, husbandry, and feeding trial design. The experimental fish were reared in nine high-density polyethylene (HDPE) cages, each measuring (80 x 60 x 60) cm^3, arranged within a tarpaulin pond of dimensions (4 x 2 x 0.8) m^3. All cages were located in a single tarpaulin pond and received the same probiotic-enriched medium. Five aeration points were provided, namely at the four corners and at the center of the tarpaulin pond. The stocking density was 96 fish per cage, and the rearing period lasted for 42 days. Throughout the rearing process, fish were fed on probiotic-enriched feed using a relative feeding rate of 5% of their biomass during the first and second weeks, 4% during the third and fourth weeks, and 3% during the fifth and sixth weeks. The feed was given three times a day. The relative feeding rate method involved administering feed based on a percentage (3-5%) of the fish biomass weight (Subandiyono & Hastuti 2021). Regular maintenance activities included daily siphoning, weekly water changes, and weekly supplementation of molasses and probiotics (at 10^6 CFU mL^{-1}). Additionally, the water removed during the siphoning process was replaced by fresh clean water from the reservoir.

Samples of 10 experimental fish from each rearing container were weighed every seven days to monitor changes in fish weight, as well as to adjust the amount of feed given. The fish were weighed with digital scales and the results were recorded. Fish survival was calculated at the end of the rearing period. Water quality parameters, including temperature, pH, and dissolved oxygen (DO), were measured daily at 07:00 and 16:00, while ammonia levels were assessed at the beginning and end of the study.

Variables calculated

Total feed consumption. The total feed consumption (TFC) was calculated using the formula from Subandiyono & Hastuti (2021), as follows:

$$TFC = F1 + F2 + ... + Fn$$

where: TFC = total feed consumption (g);
F1 = amount of feed consumed on the first day (g);
F2 = amount of feed consumed on the second day (g);
Fn = amount of feed consumed on the n_{th} day (g).

Feed utilization efficiency. The feed utilization efficiency (FUE) was calculated using the formula from Hastuti et al (2024), as follows:

$$FUE = \frac{(Wt - Wo)}{F} \times 100$$

where: FUE = feed utilization efficiency (%);
Wt = weight of the test fish at the end of the study (g);
Wo = weight of the test fish at the beginning of the study (g);
F = total amount of feed consumed during the study (g).

Feed conversion ratio. The feed conversion ratio (FCR) was calculated using the formula from Subandiyono & Hastuti (2022), as follows:

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
F = total amount of feed consumed during the study (g);
Wt = weight of the test fish at the end of the study (g);
D = number of fish that died during the study (fish);
Wo = weight of the test fish at the beginning of the study (g).

Protein efficiency ratio. According to Sulasi et al (2018), the protein efficiency ratio (PER) was calculated using the following formula:

$$PER = \frac{(Wt - Wo)}{Pi} \times 100$$

where: PER = protein efficiency ratio (%);
Wt = weight of the test fish at the end of the study (g);
Wo = weight of the test fish at the beginning of the study (g);
Pi = total amount of dietary protein consumed during the study (g).

Relative growth rate. The relative growth rate (RGR) quantifies the daily percentage increase in the fish's weight relative to their initial weight. The RGR was determined using the formula provided by Subandiyono & Hastuti (2022) :

$$RGR = \frac{(Wt - Wo)}{(Wo\ x\ t)} \times 100$$

where: RGR = relative growth rate (% day^{-1});
Wt = weight of the test fish at the end of the study (g);
Wo = weight of the test fish at the beginning of the study (g);
t = duration of the rearing period (days).

Survival rate. According to Hastuti et al (2024), the survival rate (SR) represents the percentage of experimental fish that survive to the end the study period. The SR was calculated using the formula:

$$SR = \frac{Nt}{No} \times 100$$

where: SR = survival rate (%);
Nt = number of fish alive at the end of the study (fish);
No = number of fish at the beginning of the study (fish).

Statistical analysis. The biological variables of TFC, FUE, FCR, PER, RGR, and SR were analyzed using SPSS version 26. The analysis involved one-way ANOVA to assess the effect of treatments at the 95% confidence level. If significant differences were detected, Duncan's multiple range test was performed to identify differences in mean values between treatments. Water quality parameters were analyzed descriptively based on the measurement results and compared to standard reference values to assess their suitability for fish aquaculture media.

Results

Biological performance. The results of this study demonstrate that feeding *C. gariepinus* with varying doses of probiotics positively influences the fish growth and feed efficiency. As presented in Table 2, higher probiotic doses significantly increased the fish body weight compared to the 10^5 CFU mL^{-1} dose ($p < 0.05$). Moreover, higher probiotic doses resulted in a lower FCR value, reflecting improved feed efficiency. Probiotics also enhanced the PER value, indicating more effective utilization of dietary protein for growth. Notably, the RGR and SR values were highest at a probiotic dose of 10^7 CFU mL^{-1}, suggesting that this dose not only optimizes growth but also promotes better health and survival in catfish.

Table 2

Biological performances of catfish (*Clarias gariepinus*) fed on probiotic-enriched feed

Biological performance indicator	*Probiotic dose in feed (CFU mL^{-1})*		
	10^5	*10^6*	*10^7*
TFC (g)	1088.65±27.25[a]	1232.82±37.81[b]	1427.18±102.16[c]
FUE (%)	51.66±3.71[a]	57.82±5.40[a]	67.57±3.53[b]
FCR (unit)	1.90±0.12[b]	1.70±0.13[b]	1.41±0.09[a]
PER (%)	166±12[a]	164±15[a]	194±10[b]
RGR (% day^{-1})	2.70±0.18[a]	3.45±0.55[b]	4.55±0.18[c]
SR (%)	97.22±0.60[a]	97.57±1.59[a]	97.57±0.60[a]

Nutritional profiles. The nutritional profiles of *C. gariepinus* fed on probiotic-enriched feed at varying doses revealed notable changes in the fish's body composition (Table 3).

The results indicated that higher probiotic doses led to an increase in body protein content, highlighting more efficient utilization of dietary protein (as supported by FUE variable in Table 2). Additionally, fish receiving higher probiotic doses exhibited a reduction in body fat levels, suggesting enhanced lipid metabolism. The inclusion of probiotics also positively influenced the mineral and moisture content of the fish flesh, contributing to improved overall nutritional quality. These changes in the nutritional profile underscore the role of probiotics in enhancing nutrient utilization and promoting better body composition in catfish.

Table 3

Nutritional profiles of catfish (*Clarias gariepinus*) given probiotic-enriched feed (% dry weight basis)

Catfish (C. gariepinus) group	*Protein*	*Lipids*	*Crude fiber*	*NFE*	*Ash*
Fish stocked (before treatment)	73.63	7.67	2.04	4.32	12.34
Fish fed on probiotic-enriched feed 10^5 CFU mL^{-1}	80.03	11.93	0.54	0.14	7.35
Fish fed on probiotic-enriched feed 10^6 CFU mL^{-1}	79.45	9.93	1.44	1.44	7.74
Fish fed on probiotic-enriched feed 10^7 CFU mL^{-1}	78.25	13.64	0.78	0.29	7.03

Body weight gain. Body weight gain in *C. gariepinus* fed probiotic-enriched feed showed significant improvement with increasing probiotic concentration (Figure 1). Fish receiving higher doses of probiotics consistently exhibited substantially greater weight gain compared to those given lower doses ($p < 0.05$). This indicates that dietary probiotics enhance nutrient absorption and growth efficiency, contributing to improved overall health and development. These findings emphasize the potential of probiotics as a valuable feed additive in aquaculture to boost growth performance and support sustainable fish production.

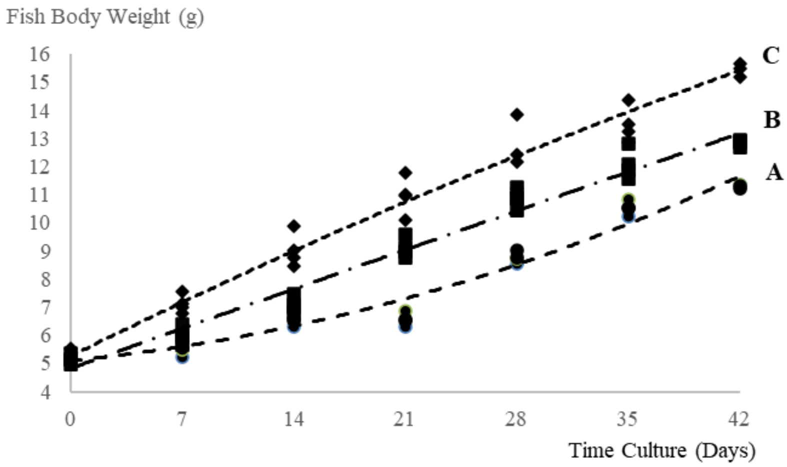

Figure 1. The average individual weight gain of catfish (*Clarias gariepinus*) fed on probiotic-enriched feed (Note: A: fish fed on probiotic-enriched feed at 10^5 CFU mL^{-1}, B: fish fed on probiotic-enriched feed at 10^6 CFU mL^{-1}, C: fish fed on probiotic-enriched feed at 10^7 CFU mL^{-1}).

Water quality parameters. Successful aquaculture relies on maintaining an optimal environment to support fish growth and health (Table 4). Water quality plays a critical role, with several factors directly influencing fish well-being. Water temperature is a key determinant, as it regulates the metabolic rate of fish, impacting their growth and overall physiological processes. During the grow-out phase, parameters such as DO, carbon dioxide, and ammonia are particularly significant. Imbalances in these factors can induce stress in farmed fish, potentially hindering growth and increasing disease susceptibility (Boyd & Tucker 1998). Maintaining stable and appropriate water quality is, therefore, essential for achieving successful aquaculture outcomes.

Table 4

Water quality parameters measured during the experimental fish rearing

Parameters	*Values*	*Recommended ranges**
Temperature (°C)	25.00-31.90	25.00-32.00
Dissolved oxygen (mg L^{-1})	4.01-6.06	> 3.00
pH	6.50-8.30	6.50-8.50
Total ammonia (mg L^{-1})	0.50-1.20	< 1.50

* Boyd & Tucker (1998).

Discussion. Growth and survival are critical factors in farmed fish production. Probiotic complexes have emerged as valuable tools in aquaculture due to their synergistic effects (Jin et al 2024). This study evaluated the impact of a multispecies probiotic formulation, comprising five bacterial strains (*L. casei*, *L. bulgaricus*, *Bacillus* sp., *Nitrosomonas* sp., *Nitrobacter* sp.) and yeast strain (*S. cerevisiae*), on key performance metrics. These included TFC, FUE, FCR, RGR, PER, and SR values in *C. gariepinus*. Feeding *C. gariepinus* a diet supplemented with probiotics for 42 days significantly improved growth performance ($p < 0.05$), as evidenced by increases in the RGR value and reductions in the FCR value (Table 2). The RGR of catfish fed diets enriched with probiotics at concentrations of 10^5, 10^6, and 10^7 CFU mL^{-1} was 2.70±0.18, 3.45±0.55, and 4.55± 0.18 (% day^{-1}), respectively. Meanwhile, the FCR values for the same treatments were 1.90±0.12, 1.70±0.13, and 1.41±0.09 respectively. Probiotic supplementation at a concentration of 10^7 CFU mL^{-1} resulted in a marked reduction in FCR. A similar pattern was observed for FUE, which increased with higher probiotic levels, reaching values of 51.66±3.71, 57.82±5.40, and 67.57±3.53%, respectively (Table 2). The results underscore the potential of dietary probiotics to enhance productivity and sustainability in aquaculture systems.

The highest TFC value in catfish was observed in treatment C (10^7 CFU mL^{-1}), with a value of 1427.18±102.16 g. Treatment B (10^6 CFU mL^{-1}) recorded feed consumption of 1232.82±37.81 g, while treatment A (105 CFU mL^{-1}) had the lowest feed consumption at 1088.65±27.25 g. A similar trend was found in the FUE values (Table 2). The probiotic dose of 10^7 CFU mL^{-1} in the feed for *C. gariepinus* is an effective dose for bacterial colonization in the digestive tract. These findings align with studies by Cai et al (2022) and Abdel-Latif et al (2023). Cai et al (2022) reported that supplementing *Lactococcus lactis* and *Enterococcus faecalis* probiotics in the feed of *Litopenaeus vannamei* at 10^8 CFU g^{-1} for 30 days significantly enhanced growth performance and feed utilization in probiotic-treated groups compared to controls. They further suggested that administering probiotics in *L. vannamei* feed at 10^6 CFU g^{-1} for 30-45 days was more effective in suppressing *Vibrio harveyi* infections than other feeding strategies. Multi-species probiotic feed containing *Bacillus subtilis*, *Lactobacillus plantarum*, *Enterococcus faecium*, and the probiotic yeast *S. cerevisiae* significantly improved growth performance in *Pangasianodon hypophthalmus*; the probiotic-enriched group demonstrated superior final weight, weight gain, and specific growth rate compared to the control group, along with improvements in feed consumption and feed conversion ratio (Abdel-Latif et al 2023). This research provides a strong theoretical basis for the practical application of probiotics in aquaculture.

The incorporation of probiotics into aquaculture feed has emerged as a highly effective approach to enhancing the health and welfare of aquatic animals, including fish. Numerous studies have documented the positive impacts of probiotics on various fish and shrimp species (Denev et al 2009; Abdel-Latif et al 2022; Yilmaz et al 2022). Notably, it is hypothesized that using multispecies probiotic mixtures could offer greater benefits than single-strain probiotics. This enhanced efficacy may result from synergistic interactions among the different probiotic strains (Mohammadi et al 2021; Puvanasundram et al 2021; Wang et al 2019).

The observed increase in growth and FCR in the group of fish fed a probiotic-enriched diet (Table 2) aligns with previous findings in *Pangasianodon* species (Abdel-Latif et al 2023). Similar results were reported by Chowdhury & Roy (2020), who found that dietary supplementation with 0.2% probiotic cocktail-comprising two *Bacillus* species (*B. subtilis* and *B. licheniformis*), two *Aspergillus* species (*A. oryzae* and *A. niger*), and the probiotic yeast *Saccharomyces boulardii* enhanced the growth and survival of *P. hypophthalmus*.

The findings of this study (Table 2) are also consistent with those from numerous prior studies on other finfish species. These include tilapia (*Oreochromis niloticus*) (Ayyat et al 2014), catla (*Catla catla*) (Parthasarathy & Ravi 2011), rohu (*Labeo rohita*) fingerlings (Mohapatra et al 2012), barramundi (*Lates calcarifer*) (Lin et al 2017; Siddik et al 2022), rainbow trout (*Oncorhynchus mykiss*) (Giannenas et al 2015), and Siberian sturgeon (*Acipenser baerii*) seedlings (Hassani et al 2020) when fed diets supplemented with multispecies probiotics.

The improved growth performance of fish observed in this study aligns with enhanced digestive enzyme activity, highlighting the functional benefits of multispecies probiotic supplementation compared to single-strain probiotics (Puvanasundram et al 2021). Several factors may contribute to the growth-promoting effects of multispecies probiotics, including: a) significant improvements in nutrient utilization and absorption (Chowdhury & Roy 2020); b) modulation of intestinal epithelial histoarchitecture by *Lactobacillus* species, which enhances nutrient uptake through increased absorptive surface area (Pirarat et al 2011); c) *Bacillus* and *Saccharomyces* species known for improving nutrient digestibility, particularly proteins, which may explain the growth rate increase in treated fish groups (Hai 2015; Soto-Dávila et al 2024); d) stimulation of digestive enzyme activity, as observed by Abdel-Latif et al (2023), aiding in improved feed digestion (Suzer et al 2008); e) the ability of probiotics to dominate intestinal bacterial flora, preventing pathogenic microorganism colonization through competition for essential nutrients or the production of inhibitory compounds (Ringø & Gatesoupe 1998; Ringø et al 2018); f) *E. faecium*, which can produce bacteriocins or bacteriocin-like substances (BLS) (Sonsa-Ard et al 2015) and enzymes that aid in food particle digestion and nutrient absorption (Tachibana et al 2020); and g) enhanced development of the brush border membrane in fish intestinal cells, leading to improved nutrient utilization (Sáenz de Rodrigáñez et al 2009).

The exact mechanisms by which probiotics enhance fish growth remain not fully understood. Further research is needed to explore this hypothesis in greater depth, particularly by examining gut health aspects such as mucosal barrier function and gut microbiota, and their connections to digestive enzyme activity. We propose that multispecies probiotics may offer synergistic effects, yielding greater overall benefits compared to the use of individual probiotic strains in isolation.

The body composition of *C. gariepinus*, including crude fat, crude protein, and ash content, was significantly influenced by probiotic-supplemented feed (Table 3). In addition to enhancing growth performance and feed utilization, probiotic supplementation also affected the body composition of *C. gariepinus*. The protein content of fish fed probiotic-enriched diets at concentrations of 10^5, 10^6, and 10^7 CFU mL^{-1} increased to 80.03%, 79.45%, and 78.25% (dry weight basis), respectively, compared to the initial protein content of 73.63% (Table 3). This improvement in protein content is likely associated with enhanced digestive enzyme activity, such as protease and amylase, stimulated by probiotic microorganisms, which improves protein digestion and absorption (Abdel-Latif at al 2023). Furthermore, probiotics may positively modulate the intestinal microbiota, leading to improved gut health, increased nutrient availability, and more efficient protein assimilation (Pérez-Sánchez et al 2014; Standen et al 1015; Allameh et al 2016). These mechanisms are consistent with the observed increases in RGR and FUE, as well as the reduction in FCR, indicating that probiotic supplementation enhances overall nutrient utilization and promotes protein deposition in catfish (Hastuti & Subandiyono 2016, 2018). This demonstrates that dietary probiotics can affect the nutritional profile of fish. However, contrasting results have been reported by Boonanuntanasarn et al (2019), who found that feeding microencapsulated *S. cerevisiae* did not alter the body composition of *P. hypophthalmus.*

In this study, dietary probiotics significantly impacted moisture, ash, and crude protein content. However, similar effects were not observed in other finfish species, such as tilapia (*O. niloticus*) (El-Haroun et al 2006), juvenile flounder (*Paralichthys olivaceus*) (Niu et al 2019), rainbow trout (*O. mykiss*) (Ramos et al 2015), and tawes fish (*Puntius gonionotus*) (Allameh et al 2016). Conversely, Mukherjee et al (2019) reported that the combined application of native *Bacillus* strains significantly increased crude protein and crude fat content in rohu fish (*Labeo rohita*). The discrepancies among these studies may be attributed to several factors, including differences in the composition of the probiotics used, variations in fish species, or experimental design variations such as feeding duration and rearing conditions

Dietary probiotics have shown the potential to enhance fish growth, boost antioxidant activity, improve food digestion, modulate the microbial community in the host's digestive tract, and prevent bacterial infections (Soto-Dávila et al 2024). Abdel-Latif et al (2023) conducted a study on *P. hypophthalmus* (initial weight: 9.54±0.50 g), evaluating the effects of feed containing multispecies probiotics. The probiotics included three bacterial strains (*B. subtilis*, *L. plantarum*, and *E. faecium*) and yeast (*S. cerevisiae*) at supplementation levels of 0.0 (control), 0.5, 1.0, and 1.5 g kg^{-1} of feed. The results showed a significant improvement in growth performance in the probiotic-supplemented 10^7 CFU mL^{-1}, 10^6 CFU mL^{-1} compared to the 10^5 CFU mL^{-1} ($p < 0.05$), as evidenced by increased final weight, weight gain, and specific growth rate. Feed intake and feed conversion ratio also improved. However, no differences in body composition were observed among the experimental groups. Notably, digestive enzyme activities (lipase, amylase, and protease) and enzymatic antioxidant activities (CAT, SOD, and GPx) were significantly higher in all probiotic-treated groups compared to the control (Abdel-Latif et al 2023). Additionally, liver malondialdehyde levels, an indicator of oxidative stress, were lower in the probiotic-supplemented groups (Abdel-Latif et al 2023). Abdel-Latif et al (2023) concluded that feed supplementation with multispecies probiotics, combining beneficial bacteria and yeast, enhances the functionality and effectiveness of diets for commercially valuable fish species such as pangasius.

The study by Abdel-Latif et al (2023) demonstrated that increasing the dose of dietary probiotics enhanced intestinal villus width, villus height, and muscle thickness, although only villus width exhibited statistically significant differences. Similar findings have been reported in tilapia (*O. niloticus*) (Standen et al 2015) and Pacific white shrimp (*L. vannamei*) (Xie et al 2019). The increases in villus height and muscle thickness are associated with improved intestinal peristalsis and nutrient digestion (Daniels et al 2010), which likely contribute to enhanced growth performance in fish (Zan et al 2023). These structural improvements in the intestine are believed to drive better FUE, FCR, protein utilization efficiency, and RGR.

Probiotics, in the form of biofloc, play a crucial role in decomposing organic matter in aquacultural rearing systems, significantly reducing sludge and mucus accumulation (Hastuti & Subandiyono 2016, 2018). Their application in rearing systems improves water quality, minimizes odors, and ultimately enhances aquaculture production (Sahu et al 2008). In this study, multispecies probiotics were introduced into pond water, and the experimental conditions were maintained using net enclosures. Water quality monitoring over 42 days revealed that the water conditions remained within acceptable ranges for fish health and growth, as defined by Boyd & Tucker (1998) (Table 4). During the experimental period, water quality parameters remained within acceptable ranges for catfish culture. Water temperature ranged from 25.00 to 31.90°C, DO levels varied between 4.01 and 6.06 mg L^{-1}, and pH values ranged from 6.50 to 8.30. Total ammonia concentrations were recorded between 0.50 and 1.20 mg L^{-1}. The SR of *C. gariepinus* ranged from 97.22 to 97.55% (Table 2), demonstrating the effectiveness of probiotic application in the form of biofloc in maintaining optimal rearing conditions.

Conclusions. This study found that incorporating probiotics into the feed at varying doses significantly influenced nutrient utilization, feed efficiency, and overall fish growth performance. The highest dose tested, with a probiotic concentration of 10^7 CFU mL^{-1} (feeding trial C) provided the best results in terms of nutrient intake, feed efficiency, and growth performance in catfish (*C. gariepinus*), as evidenced by the FCR, PER, and RGR values. Feeding trial C resulted in the most favorable performance, as indicated by higher total feed consumption (TFC: 1427.18±102.16 g), feed utilization efficiency (FUE: 67.57±3.53%), protein efficiency ratio (PER: 194±10%), and relative growth rate (RGR: 4.55±0.18% day^{-1}), along with a lower feed conversion ratio (FCR: 1.41±0.09). Additionally, probiotic-enriched rearing media can contribute to the overall health of fish. These findings highlight the potential of probiotics to improve feed quality and boost catfish production.

These findings suggest that probiotic supplementation at a dosage of 10^7 CFU mL^{-1} may enhance feed efficiency and growth performance in catfish. However, further studies under different culture conditions and longer rearing periods are recommended to confirm the consistency and broader applicability of these effects.

Acknowledgements. The authors gratefully acknowledge the Dean of Fisheries and Marine Science Faculty, Diponegoro University, for his support in implementing this research.

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

References

Abdel-Latif H. M. R., Dawood M. A. O., Menanteau-Ledouble S., El-Matbouli M., 2020 The nature and consequences of co-infections in tilapia: a review. Journal of Fish Diseases 43(6):651-664.

Abdel-Latif H. M. R., Yilmaz E., Dawood M. A. O., Ringø E., Ahmadifar E., Yilmaz S., 2022 Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: a review. Aquaculture 551:737951.

Abdel-Latif H. M. R., Chaklader M. R., Shukry M., Ahmed H. A., Khallaf M. A., 2023 A multispecies probiotic modulates growth, digestive enzymes, immunity, hepatic antioxidant activity, and disease resistance of *Pangasianodon hypophthalmus* fingerlings. Aquaculture 563:738948.

Alderman D. J., Hastings T. S., 1998 Antibiotic use in aquaculture: development of antibiotic resistance - potential for consumer health risks. International Journal of Food Science and Technology 33(2):139-155.

Allameh S. K., Yusoff F. M., Ringø E., Daud H. M., Saad C. R., Ideris A., 2016 Effects of dietary mono- and multiprobiotic strains on growth performance, gut bacteria and body composition of Javanese carp (*Puntius gonionotus*, Bleeker 1850). Aquaculture Nutrition 22(2):367-373.

Astuti A. P. K., Hastuti S., Haditomo A. H. C., 2017 [Effect of curcuma extract in feed as an immunostimulant in tawes fish (*Puntius javanicus*) using bacterial challenge test]. Journal of Aquaculture Management and Technology 6(3):10-19. [in Indonesian]

Ayyat M. S., Labib H. M., Mahmoud H. K., 2014 A probiotic cocktail as a growth promoter in Nile tilapia (*Oreochromis niloticus*). Journal of Applied Aquaculture 26:208-215.

Basuki F., Yuniarti T., Nugroho R. A., Hastuti S., 2020 Preliminary study of various intervals and administration of feed enriched with turmeric and garlic on the growth performance of catfish. E3S Web of Conferences 147:01010.

Boonanuntanasarn S., Ditthab K., Jangprai A., Nakharuthai C., 2019 Effects of microencapsulated *Saccharomyces cerevisiae* on growth, hematological indices, blood chemical, and immune parameters and intestinal morphology in striped catfish, *Pangasianodon hypophthalmus*. Probiotics and Antimicrobial Proteins 11(2): 427-437.

Boyd C. E., Tucker C. S., 1998 Pond aquaculture water quality management. Springer Science Business Media, New York, 700 pp.
Cabello F. C., 2006 Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental Microbiology 8(7):1137-1144.
Cai X., Wen J., Long H., Ren W., Zhang X., Huang A., Xie Z., 2022 The probiotic effects, dose, and duration of lactic acid bacteria on disease resistance in *Litopenaeus vannamei*. Aquaculture Reports 26:101299.
Chaklader M. R., Fotedar R., Howieson J., Siddik M. A. B., Foysal M. J., 2020 The ameliorative effects of various fish protein hydrolysates in poultry by-product meal-based diets on muscle quality, serum biochemistry and immunity in juvenile barramundi, *Lates calcarifer*. Fish and Shellfish Immunology 104:567-578.
Chowdhury M. A., Roy N. C., 2020 Probiotic supplementation for enhanced growth of striped catfish (*Pangasianodon hypophthalmus*) in cages. Aquaculture Reports 18: 100504.
Daniels C. L., Merrifield D. L., Boothroyd D. P., Davies S. J., Factor J. R., Arnold K. E., 2010 Effect of dietary *Bacillus* spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. Aquaculture 304(1):49-57.
Denev S., Staykov Y., Moutafchieva R., Beev G., 2009 Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture. International Aquatic Research 1:1-29.
El-Haroun E. R., Goda A. M. A. S., Chowdhury M. A. K., 2006 Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquaculture Research 37:1473-1480.
El-Son M. A. M., Nofal M. I., Abdel-Latif H. M. R., 2021 Co-infection of *Aeromonas hydrophila* and *Vibrio parahaemolyticus* isolated from diseased farmed striped mullet (*Mugil cephalus*) in Manzala, Egypt - a case report. Aquaculture 530:735738.
El-Saadony M. T., Alagawany M., Patra A. K., Kar I., Tiwari R., Dawood M. A. O., Dhama K., Abdel-Latif H. M. R., 2021 The functionality of probiotics in aquaculture: an overview. Fish and Shellfish Immunology 117:36-52.
Giannenas I., Karamaligas I., Margaroni M., Pappas I., Mayer E., Encarnação P., Karagouni E., 2015 Effect of dietary incorporation of a multi-strain probiotic on growth performance and health status in rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry 41(1):119-128.
Hai N. V., 2015 The use of probiotics in aquaculture. Journal of Applied Microbiology 119(4): 917-935.
Hartami P., Syahputra N., Erlangga, 2015 [Aquaponic technology with different plants on the growth performance of tilapia (*Oreochromis niloticus*).] Jurnal Perikanan Tropis 2(1):72-90. [in Indonesian]
Hassani M. H. S., Jourdehi A. Y., Zelti A. H., Masouleh A. S., Lakani F. B., 2020 Effects of commercial superzist probiotic on growth performance and hematological and immune indices in fingerlings *Acipenser baerii*. Aquaculture International 28:377-387.
Hastuti S., Subandiyono S., 2016 Application of biofloc technology in intensive farming affected production and blood performances of the catfish, *Clarias gariepinus* (Burchell, 1822). Jurnal Teknologi 78(4-2):221-226.
Hastuti S., Subandiyono S., 2018 Hematological parameters of the North African catfish *Clarias gariepinus* farmed using biofloc technology. AACL Bioflux 11(4):1415-1424.
Hastuti S., Subandiyono S., Windarto S., 2019 Blood performance of jaundice catfish *Clarias gariepinus*. AACL Bioflux 12(2):480-489.
Hastuti S., Yuniarti T., Harwanto D., Subandiyono S., 2024 Growth performance of Java barb, *Barbonymus gonionotus*, in relation with water temperature. AACL Bioflux 17(3):949-959.

Holmström K., Gräslund S., Wahlström A., Poungshompoo S., Bengtsson B. E., Kautsky N., 2003 Antibiotic use in shrimp farming and implications for environmental impacts and human health. International Journal of Food Science and Technology 38(3):255-266.
Jin Y., Chen C., Ai C., Song W., Zhao J., 2024 Development of probiotic complex based on the synthetic microbial community and probiotic effects in farming Yunlong grouper. Aquaculture 586:740708.
Korzekwa K., Gomułka P., Nowosad J., Kucharczyk D. J., Targonska K., Czarkowski T. K., Żarski D., Horváth L., Müller T., Kujawa R., Łuczyński M. J., Łuczyńska J., Abdel-Latif H. M. R., Kucharczyk D., 2022 Bacterial survey of skin ulcers in *Anguilla anguilla* L. females during artificial maturation under controlled conditions. Aquaculture Reports 25:101259.
Kristiana I., Karisma A. S., Astiyani W. P., Akbarurrasyid M., Pietoyo A., 2022 [Duckweed (*Lemna sp.*) application on seed feed of pearl catfish (*Clarias gariepinus*)]. Journal of Aquatropica Asia 7(2):78-84. [in Indonesian]
Limbu S. M., Zhou L., Sun S. X., Zhang M. L., Du Z. Y., 2018 Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk. Environment International 115:205-219.
Lin H. L., Shiu Y. L., Chiu C. S., Huang S. L., Liu C. H., 2017 Screening probiotic candidates for a mixture of probiotics to enhance the growth performance, immunity, and disease resistance of Asian seabass, *Lates calcarifer* (Bloch), against *Aeromonas hydrophila*. Fish and Shellfish Immunology 60:474-482.
Lokapirnasari W. P., Al Arif M. A., Maslachah L., Kirana A. L. P., Suryandari A., Yulianto A. B., Sherasiya A., 2023 The potency of *Lactobacillus acidophillus* and *L. lactis* probiotics and *Guazuma ulmifolia* Lam. extract as feed additives with different application times to improve nutrient intake and feed efficiency in *Coturnix coturnix japonica* females. Journal of Animal and Feed Sciences 32(1):59-67.
Lulijwa R., Rupia E. J., Alfaro A. C., 2020 Antibiotic use in aquaculture, policies and regulation, health and environmental risks; a review of the top 15 major producers. Reviews in Aquaculture 12(2):640-663.
Mareta R. E., Subandiyono S., Hastuti S., 2017 [Effect of papain and probiotics in artificial feed on feed efficiency utilization level and growth rate of gourami (*Osphronemus gouramy*)]. Indonesian Journal of Tropical Aquaculture 1(1):21-30. [in Indonesian]
Mohammadi G., Rafiee G., Tavabe K. R., Abdel-Latif H. M. R., Dawood M. A. O., 2021 The enrichment of diet with beneficial bacteria (single- or multi-strain) in biofloc system enhanced the water quality, growth performance, immune responses, and disease resistance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 539:736640.
Mohapatra S., Chakraborty T., Prusty A. K., Das P., Paniprasad K., Mohanta K. N., 2012 Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquaculture Nutrition 18:1-11.
Mukherjee A., Chandra G., Ghosh K., 2019 Single or conjoint application of autochthonous *Bacillus* strains as potential probiotics: effects on growth, feed utilization, immunity and disease resistance in rohu, *Labeo rohita* (Hamilton). Aquaculture 512:734302.
National Research Council (NRC), 1977 Nutrient requirements of warmwater fishes. National Academies Press, 87 pp.
Niu K. M., Khosravi S., Kothari D., Lee W. D., Lim J. M., Lee B. J., Kim K. W., Lim S. G., Lee S. M., Kim S. K., 2019 Effects of dietary multi-strain probiotics supplementation in a low fishmeal diet on growth performance, nutrient utilization, proximate composition, immune parameters, and gut microbiota of juvenile olive flounder (*Paralichthys olivaceus*). Fish and Shellfish Immunology 93:258-268.
Parthasarathy R., Ravi D., 2011 Probiotic bacteria as growth promoter and biocontrol agent against *Aeromonas hydrophila* in *Catla catla* (Hamilton, 1822). Indian Journal of Fisheries 58(3):87-93.

Pérez-Sánchez T., Ruiz-Zarzuela I., de Blas I., Balcázar J. L., 2014 Probiotics in aquaculture: a current assessment. Reviews in Aquaculture 6(3):133-146.
Pirarat N., Pinpimai K., Endo M., Katagiri T., Ponpornpisit A., Chansue N., Maita M., 2011 Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. Research in Veterinary Science 91(3):92-97.
Proxton M. G., 1992 Water quality fluctuations and monitoring in intensive fish culture. In: Aquaculture and the environment: reviews of the International Conference Aquaculture Europe '91, Dublin, Ireland, June 10-12, 1991. De Pauw N., Joyce J. (eds), EAS Special Publication 16, pp. 121-143.
Puvanasundram P., Chong C. M., Sabri S., Yusoff M. S., Karim M., 2021 Multi-strain probiotics: functions, effectiveness and formulations for aquaculture applications. Aquaculture Reports 21:100905.
Qi X., Xue M., Shi K., Wang G., Ling F., 2024 Evaluating *Pseudomonas monteilii* JK-1 as an in-feed probiotic: enhancing growth, immune-antioxidant, disease resistance and modulating gut microflora composition in grass carp (*Ctenopharyngodon idella*). Aquaculture 585:740715.
Ramos M. A., Gonçalves J. F. M., Batista S., Costas B., Pires M. A., Rema P., Ozório R. O. A., 2015 Growth, immune responses and intestinal morphology of rainbow trout (*Oncorhynchus mykiss*) supplemented with commercial probiotics. Fish and Shellfish Immunology 45(1):19-26.
Rarassari M. A., Dwinanti S. H., Absharina F. D., Gevira Z., 2021 [Application of biofloc and probiotics in feed for raising pearl catfish (*Clarias gariepinus*)]. Journal of Fisheries and Marine Research 5(2):329-334. [in Indonesian]
Ringø E., Gatesoupe F. J., 1998 Lactic acid bacteria in fish: a review. Aquaculture 160(3-4):177-203.
Ringø E., Hoseinifar S. H., Ghosh K., Doan H. V., Beck B. R., Song S. K., 2018 Lactic acid bacteria in finfish - an update. Frontiers in Microbiology 9:1818.
Sáenz de Rodrigáñez M. A., Díaz-Rosales P., Chabrillón M., Smidt H., Arijo S., León-Rubio J. M., Alarcón F. J., Balebona M. C., Moriñigo M. A., Cara J. B., Moyano F. J., 2009 Effect of dietary administration of probiotics on growth and intestine functionality of juvenile Senegalese sole (*Solea senegalensis*, Kaup 1858). Aquaculture Nutrition 15(2):177-185.
Sahu M. K., Swarnakumar N. S., Sivakumar K., Thangaradjou T., Kannan L., 2008 Probiotics in aquaculture: importance and future perspectives. Indian Journal of Microbiology 48(3):299-308.
Salamah, Zulpikar, 2020 [Addition of probiotic on commercial feed with different proteins on the performance of catfish (*Clarias* sp.) using biofloc system]. Acta Aquatica: Aquatic Sciences Journal 7(1):21-27. [in Indonesian]
Shabrina D. A., Hastuti S., Subandiyono S., 2018 [The effects of probiotics on blood performance, feed utilization efficiency, protein efficiency ratio, survival rate, and growth of Java barb (*Puntius javanicus*)]. Indonesian Journal of Tropical Aquaculture 2(2):26-35. [in Indonesian]
Siddik M. A. B., Foysal M. J., Fotedar R., Francis D. S., Gupta S. K., 2022 Probiotic yeast *Saccharomyces cerevisiae* coupled with *Lactobacillus casei* modulates physiological performance and promotes gut microbiota in juvenile barramundi, *Lates calcarifer*. Aquaculture 546:737346.
Simanjuntak I. C. B. H., Suminto, Sudaryono A., 2016 [The effect of probiotic bacteria concentration associated in intestinal gut as biofloc on feed utilization efficiency, growth and survival rate of catfish (*Clarias gariepinus*)]. Journal of Aquaculture Management and Technology 5(2):1-8. [in Indonesian]
Smith P., 2008 Antimicrobial resistance in aquaculture. Revue Scientifique et Technique 27:243-264.
Sonsa-Ard N., Rodtong S., Chikindas M. L., Yongsawatdigul J., 2015 Characterization of bacteriocin produced by *Enterococcus faecium* CN-25 isolated from traditionally Thai fermented fish roe. Food Control 54:308-316.

Soto-Dávila M., Webb R. A., Rodríguez-Ramos T., Gillian McDonald G., Dang X., Heath J. W., Reid G., Dixon B., 2024 Effect of dietary supplementation of probiotic on growth, survival, and immune-related biomarkers in chinook salmon (*Oncorhynchus tshawytscha*) challenged with *Vibrio anguillarum*. Aquaculture 583:740582.
Standen B. T., Rodiles A., Peggs D. L., Davies S. J., Santos G. A., Merrifield D. L., 2015 Modulation of the intestinal microbiota and morphology of tilapia, *Oreochromis niloticus*, following the application of a multispecies probiotic. Applied Microbiology and Biotechnology 99(20):8403-8417.
Subandiyono S., Hastuti S., 2016 Trivalent chromium (Cr^{+3}) in dietary carbohydrate and its effect on the growth of commonly cultivated fish. Jurnal Teknologi 78(4-2):233-237.
Subandiyono S., Hastuti S., 2021 [Management application of parent feeding in catfish (*Clarias gariepinus*) hatcheries]. Tigamedia, Semarang, 85 pp. [in Indonesian]
Subandiyono S., Hastuti S., 2022 Growth performances, feed utilization and hematological parameters of the carp (*Cyprinus carpio*), according to the dietary glutamate. AACL Bioflux 15(2):830-839.
Sulasi S., Hastuti S., Subandiyono S., 2018 [The effect of dietary papain and probiotic on feed protein utilization and growth of carp (*Cyprinus carpio*)]. Indonesian Journal of Tropical Aquaculture 2(1):1-10. [in Indonesian]
Sunardi, Syahrizal, Arifin Z., 2016 [Effectiveness of biodecomposers in high-density closed transport of sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang) for aquaculture]. Jurnal Akuakultur Sungai dan Danau 1(1):44-52. [in Indonesian]
Suzer C., Çoban D., Kamaci H. O., Saka S., Firat K., Otgucuoglu Ö., Küçüksari H., 2008 *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata* L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture 280:140-145.
Tachibana L., Telli G. S., de Carla Dias D., Gonçalves G. S., Ishikawa C. M., Cavalcante R. B., Natori M. M., Hamed S. B., Ranzani-Paiva M. J. T., 2020 Effect of feeding strategy of probiotic *Enterococcus faecium* on growth performance, hematologic, biochemical parameters and non-specific immune response of Nile tilapia. Aquaculture Reports 16:100277.
Umasugi A., Tumbol R. A., Kreckhoff R. L., Manoppo H., Pangemanan N. P. L., Ginting E. L., 2018 [The use of probiotic bacteria to prevent *Streptococcus agalactiae* infection on Nile tilapia (*Oreochromis niloticus*)]. Jurnal Budidaya Perairan 6(2):39-44. [in Indonesian]
Wang Y. C., Hu S. Y., Chiu C. S., Liu C. H., 2019 Multiple-strain probiotics appear to be more effective in improving the growth performance and health status of white shrimp, *Litopenaeus vannamei*, than single probiotic strains. Fish and Shellfish Immunology 84:1050-1058.
Xia L., Cheng G., Wang P., Wang X., Dong Z., Mu Q., Yu J., Jiang Z., Xiao J., Feng H., Li X., Kong W., Xu Z., 2024 Screening and identification of probiotics from the intestinal tract of largemouth bass (*Micropterus salmoides*) for use as a feed additive and bacterial infection control. Aquaculture 584:740661.
Xie J., Liu Q., Liao S., Fang H., Yin P., Xie S., Tian L., Liu Y., Niu J., 2019 Effects of dietary mixed probiotics on growth, non-specific immunity, intestinal morphology and microbiota of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Fish and Shellfish Immunology 90:456-465.
Yilmaz S., Yilmaz E., Dawood M. A. O., Ringø E., Ahmadifar E., Abdel-Latif H. M. R., 2022 Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: a review. Aquaculture 547:737514.
Zan Z., Chen K., Wang H., Han Z., Sun J., 2023 Effects of a multistrain probiotic on the growth, immune function and intestinal microbiota of the tongue sole *Cynoglossus semilaevis*. Aquaculture 575:739813.

Received: 28 January 2026. Accepted: 25 February 2026. Published online: 13 April 2026.
Authors:
Subandiyono Subandiyono, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Prof Jacup Rais street, Tembalang Semarang, Central Java, Indonesia, 50275, e-mail: sby.subandiyono@gmail.com
Sri Hastuti, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Prof Jacup Rais street, Tembalang Semarang, Central Java, Indonesia, 50275, e-mail: hastuti_hastuti@yahoo.com
Ristiawan Agung Nugroho, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Prof Jacup Rais street, Tembalang Semarang, Central Java, Indonesia, 50275, e-mail: ristiawan_1976@yahoo.com
Diana Chilmawati, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Prof Jacup Rais street, Tembalang Semarang, Central Java, Indonesia, 50275, e-mail: dianachilmawati@yahoo.com
Hanungko Bayu Dwi Tyastanto, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Prof Jacup Rais street, Tembalang Semarang, Central Java, Indonesia, 50275, e-mail: hanungko@yahoo.com

How to cite this article:
Subandiyono S., Hastuti S., Nugroho R. A., Chilmawati D., Tyastanto H. B. D., 2026 Probiotics as feed additives to improve feed efficiency and growth in catfish (*Clarias gariepinus*) reared in probiotic-enriched media. AACL Bioflux 19(2):641-655.