Dietary supplementation of probiotic bacteria and fermented aquatic fern in commercial feed improves growth performance of Nile tilapia (*Oreochromis niloticus*)

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Abstract. This study evaluated the effects of probiotic *Lactobacillus casei* and fermented *Azolla microphylla* incorporated into commercial feed on the growth performance, feed conversion ratio, and survival rate of Nile tilapia (*Oreochromis niloticus*) fry. The experiment was conducted for 35 days using a completely randomized design with four dietary treatments and five replicates. The treatments consisted of commercial feed as control, commercial feed supplemented with *L. casei* (4 %), commercial feed containing fermented *A. microphylla* flour (30 %), and a combination of *L. casei* (4 %) and fermented *A. microphylla* flour (30 %). Growth performance was evaluated based on specific growth rate (SGR), feed utilization efficiency was assessed using feed conversion ratio (FCR), and fish viability was determined through survival rate (SR). The results showed that dietary treatments significantly affected SGR and FCR ($p < 0.05$), whereas no significant differences were observed in SR among treatments ($p > 0.05$). The combined treatment resulted in the highest SGR (4.26 ± 0.23 % day^{-1}) and the lowest FCR (1.35 ± 0.13), indicating superior growth performance and feed utilization efficiency. Survival rates ranged from 76.0 ± 8.94 % to 80.0 ± 0.00 %, remaining within acceptable levels for tilapia fry culture. In conclusion, the combined application of probiotic *L. casei* and fermented *A. microphylla* in commercial feed effectively enhances growth performance and feed efficiency of Nile tilapia fry without negatively affecting survival.
Keywords: *Azolla microphylla*, *Lactobacillus casei*, feed conversion ratio, fermented feed, probiotic.

Introduction. Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater fish species for worldwide aquaculture due to its rapid growth, high adaptability to diverse culture conditions, and broad consumer acceptance. It is also considered an affordable source of high-quality animal protein, making tilapia a key species in supporting global food security (Vajargah, 2021). Despite its production potential, the sustainability of tilapia aquaculture is constrained by the high cost of commercial feed, which may account for approximately 60–70% of total production costs, along with fluctuations in the prices of major feed ingredients (Sari et al., 2017; Aragão et al., 2022). Conventional protein sources such as fish meal and soybean meal are increasingly expensive and limited in supply, highlighting the urgent need for alternative, locally available, and sustainable feed resources (Hua et al., 2019).

Aquatic ferns such as *Azolla microphylla* have gained increasing attention as alternative protein sources for aquafeeds due to their rapid biomass production, low cultivation cost, and adaptability to low-input systems. *Azolla microphylla* has been reported to contain relatively high crude protein levels compared to many other aquatic plants (Xu et al., 2021). However, the utilization of *Azolla microphylla* in fish diets is often limited by its high crude fibre content, the presence of antinutritional compounds, and imbalanced amino acid profiles, which may reduce nutrient digestibility and negatively

affect fish growth performance (Zulkhasyni & Andriyeni, 2018). Several studies have demonstrated that microbial fermentation, particularly using filamentous fungi such as *Trichoderma harzianum*, can improve the nutritional quality of *Azolla microphylla* by reducing crude fibre and increasing protein availability, resulting in enhanced growth performance and feed efficiency in tilapia (Warasto et al., 2013; Utomo et al., 2011).

In addition to fermented plant-based ingredients, dietary supplementation with probiotics has emerged as an effective and environmentally friendly approach to improving growth performance and feed utilization in aquaculture systems. Probiotics, especially lactic acid bacteria such as *Lactobacillus casei*, have been reported to enhance digestive enzyme activity, suppress pathogenic microorganisms, and improve gut health in fish (Magouz et al., 2020; Torres-Maravilla et al., 2024). *Lactobacillus casei* is known to produce extracellular enzymes, including protease and amylase, which may enhance the digestion of plant-based feed ingredients with relatively high fibre content (Yuriana et al., 2017; Wang et al., 2021). Furthermore, this bacterium exhibits resistance to acidic conditions and bile salts, allowing it to survive and function effectively in the gastrointestinal tract of fish (Aini & Hariani, 2019).

Although the individual effects of fermented plant ingredients and probiotic supplementation on fish growth have been widely reported, studies evaluating the combined application of probiotic *Lactobacillus casei* and fermented *Azolla microphylla* incorporated into commercial tilapia feed remain limited. Therefore, this study aimed to evaluate the effects of dietary supplementation with probiotic *Lactobacillus casei* FNCC 0090 and fermented *Azolla microphylla* on feed conversion ratio, growth performance, and survival rate of Nile tilapia (*Oreochromis niloticus*) fry.

Material and Method

Experimental design and fish rearing. The research was conducted at the Animal Physiology Laboratory, Biology Study Program, Faculty of Engineering and Science, Universitas PGRI Adi Buana Surabaya in July 2025. The experiment was conducted using a completely randomized design (CRD) consisting of four dietary treatments with five replicates each, resulting in a total of 20 experimental units. The number of replicates was determined based on the formula $t(r-1) \geq 15$, where t represents the number of treatments and r the number of replicates.

A total of 100 GIFT Nile tilapia (*Oreochromis niloticus*) fry, aged one month with an initial body weight ranging from 4.0 to 5.5 g per fish, were used in this study. Fish were randomly stocked at a density of five fish per aquarium (53 × 35 × 35 cm), each filled with 55 L of freshwater. The experiment was conducted over a 35-day rearing period. Fish were fed twice daily at 07:00 – 08:00 and 17:00 – 18:00 (WIB) at a feeding rate of 5 % of total biomass per day. Water quality was maintained throughout the experiment by siphoning waste daily and replacing 25% of the water every three days. Water temperature was measured twice daily (morning and afternoon), while pH was monitored at each sampling period to ensure suitable conditions for tilapia growth and survival.

Dietary treatments. The fish were fed with HI-PRO-VITE 781-1, a commercial floating pellet feed manufactured by PT Central Proteina Prima Tbk (CP Prima, Indonesia). According to the manufacturer's specifications, the feed contains a minimum of 31% crude protein, 5% crude fat, a maximum of 5% crude fibre, 12% moisture, and 13% crude ash. The feed was selected because of its high water stability and balanced nutritional composition suitable for the growth of freshwater fish. This commercial feed was the base for all the dietary treatments. The dietary treatments applied in this study were as follows:

- A: 100 % commercial feed (control)
- B: 96 % commercial feed supplemented with 4 % *Lactobacillus casei* FNCC 0090
- C: 70 % commercial feed supplemented with 30 % fermented *Azolla microphylla* flour
- D: 66 % commercial feed supplemented with 4 % *Lactobacillus casei* and 30 % fermented *Azolla microphylla* flour

Preparation of fermented Azolla microphylla. Fresh *Azolla microphylla* was processed into flour and mixed with rice dregs at a ratio of 4:1 (w/w). Water was added at 30 % (v/w), and the mixture was steamed for 30 minutes to reduce microbial contamination. After cooling, *Trichoderma harzianum* inoculum was added at 5 % (w/w) of the total substrate and incubated for 48 hours under aerobic conditions. The fermented product was then dried at 60 °C for 2 hours and ground into flour prior to feed formulation.

Preparation of probiotic-supplemented feed. The probiotic used in this study was *Lactobacillus casei* strain FNCC 0090, prepared following the method described by Aini and Hariani (2019). The bacteria were cultured in de Man, Rogosa, and Sharpe (MRS) broth until reaching a density of approximately 10^8 CFU mL^{-1}.

For treatment B, the probiotic suspension was added at 4 % (v/w) of commercial feed weight and sprayed evenly onto the feed pellets using a spray bottle, followed by thorough homogenization. The feed was then incubated for 30 minutes prior to feeding.

Preparation of combination feed. For treatment C, fermented *Azolla microphylla* flour was added at 30 % (w/w) of commercial feed weight and homogenized thoroughly. For treatment D, a combination of 4 % (v/w) *Lactobacillus casei* probiotic suspension and 30 % (w/w) fermented *Azolla microphylla* flour was mixed with commercial feed until homogeneous. All treated feeds were incubated for 30 minutes before being administered to the fish. All experimental diets were administered twice daily by broadcasting the feed evenly on the water surface.

Measured parameters. The parameters measured during the experiment included specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) of tilapia fry.

Data collection. Fish sampling was conducted weekly over the 35-day experimental period. Fish were weighed collectively using a digital balance to determine growth performance. Survival rate and feed conversion ratio were calculated at the end of the experiment.

Specific growth rate (SGR). Specific growth rate was calculated according to Muchlisin et al. (2016) using the following formula:

$$\text{SGR}\ (\%) = \left(\sqrt[t]{\frac{W_t}{W_0}} - 1 \right) x\ 100$$

where W_t = mean final body weight (g), W_0 = mean initial body weight (g), t = culture period (days).

Survival rate (SR). Survival rate was calculated according to Suminto and Chilmawati (2015):

$$\text{SR}\ (\%) = \frac{N_t}{N_0}\ x\ 100$$

where N_t = number of fish at the end of the experiment, N_0 = number of fish at the beginning of the experiment.

Feed conversion ratio (FCR). Feed conversion ratio was calculated following Lasena et al. (2017) formula:

$$\text{FCR} = \frac{F}{(W_t + W_d) - W_0}$$

where F = total feed consumed (g), W_t = final biomass (g), W_d = biomass of dead fish (g), W_0 = initial biomass (g).

Statistical analysis. All data were analysed using one-way analysis of variance (ANOVA) at a 95 % confidence level using SPSS software. When significant differences were detected ($p < 0.05$), Duncan's multiple range test was applied to determine differences among treatments.

Results and Discussion

Specific growth rate (SGR). The specific growth rate (SGR) of Nile tilapia fry is presented in Figure 1. Analysis of variance showed that dietary treatments significantly affected SGR ($p < 0.05$). The highest SGR was observed in treatment D (4.26 ± 0.23 % day^{-1}), which combined probiotic *Lactobacillus casei* (4 %) and fermented *Azolla microphylla* flour (30 %), whereas the lowest SGR occurred in the control treatment (A), with a value of 2.88 ± 0.35 % day^{-1}. Treatments B (probiotic only) and C (fermented *Azolla microphylla* only) resulted in moderate SGR values and did not differ significantly from each other, indicating that the individual application of probiotics or fermented plant material alone was insufficient to maximize growth performance.

In contrast, the combined treatment (D) produced a synergistic effect, resulting in significantly higher growth. This improvement may be attributed to enhanced nutrient availability and digestibility. Fermentation of *Azolla microphylla* using *Trichoderma harzianum* has been reported to reduce crude fibre content and increase crude protein levels, thereby improving the nutritional quality of plant-based feed ingredients (Hatta et al., 2014; Nuraini et al., 2022). Reduced fibre content facilitates nutrient accessibility and minimizes digestive constraints in herbivorous and omnivorous fish species such as tilapia. In addition, probiotic *L. casei* promotes nutrient digestion by producing extracellular enzymes and modulating intestinal microflora, which enhances nutrient absorption efficiency (Buruiană et al., 2014; Yuriana et al., 2017). These findings are consistent with previous studies reporting that fermented feed ingredients and probiotics can convert complex organic compounds into simpler forms, facilitating digestion and absorption in fish (Afriyanti et al., 2020; Lasena et al., 2017). The higher availability of dietary protein in the combined treatment likely accelerated tissue synthesis and somatic growth, as protein is a key nutrient influencing fish growth performance (Krabi et al., 2015; Iskandar & Elrifadah, 2015).

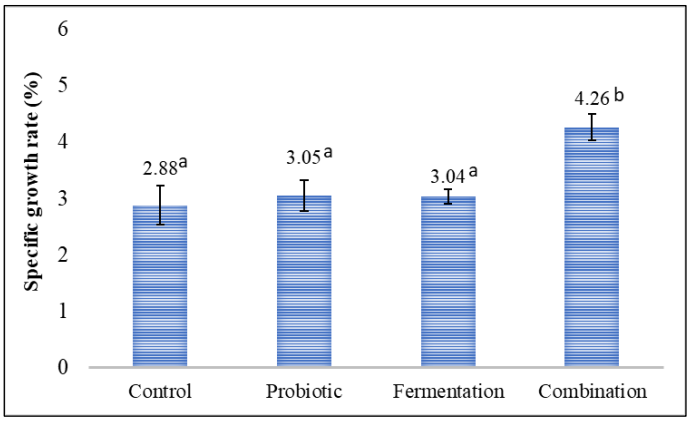


Figure 1. Specific growth rate of Nile tilapia (*Oreochromis niloticus*) fry. Different superscript letters indicate statistically significant differences among treatment means ($p < 0.05$).

Survival rate (SR). The survival rate (SR) of Nile tilapia fry across treatments ranged from 76.0 ± 8.94% to 80.0 ± 0.00% (Figure 2). Statistical analysis showed that dietary supplementation with probiotics, fermented *A. microphylla*, or their combination had no significant effect on SR compared to the control treatment ($p > 0.05$).

Although the control treatment exhibited a slightly higher mean SR value, the differences among treatments were minimal and within acceptable survival ranges for tilapia fry. According to Sari et al. (2017), a survival rate above 50 % is considered satisfactory in freshwater fish culture. Mortality observed during the early cultivation period was most likely associated with handling stress and adaptation to new rearing conditions rather than dietary factors.

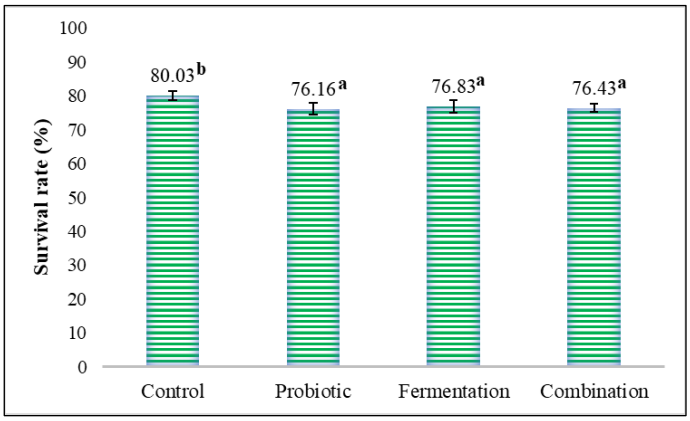


Figure 2. Survival rate of Nile tilapia (*Oreochromis niloticus*) fry. Different superscript letters indicate statistically significant differences among treatment means ($p < 0.05$).

The absence of a significant dietary effect on SR suggests that the inclusion of probiotic *L. casei* and fermented *A. microphylla* did not adversely affect fish health. Similar results were reported by Wilisetyadi et al. (2022), who found that probiotic supplementation enhanced growth performance without significantly affecting survival rate. Although probiotics are known to stabilize intestinal microflora and suppress pathogenic microorganisms (Wang et al., 2021), such benefits may not necessarily translate into improved survival under non-stressful culture conditions.

Feed conversion ratio (FCR). Feed conversion ratio (FCR) is a key indicator of feed utilization efficiency in aquaculture. In the present study, FCR values ranged from 1.35 ± 0.13 to 1.94 ± 0.13 (Figure 3). The lowest (best) FCR was recorded in treatment D, while the highest FCR was observed in treatment C (fermented *A. microphylla* only). Statistical analysis confirmed that the combined treatment significantly improved FCR compared to the other treatments ($p < 0.05$).

The superior FCR obtained in the combined treatment indicates more efficient feed utilization, which can be attributed to the complementary roles of fermented *A. microphylla* and probiotic supplementation. Fermentation by *T. harzianum* enhances protein content and reduces crude fibre, improving substrate availability for digestion (Hatta et al., 2014). Meanwhile, *L. casei* contributes digestive enzymes such as protease and amylase, facilitating the breakdown of nutrients into absorbable forms (Buruiana et al., 2014; Yuriana et al., 2017).

Interestingly, although fermented *A. microphylla* flour alone exhibited relatively high crude protein content, its FCR performance was inferior to that of the combined treatment. This suggests that increased protein content alone is insufficient without adequate microbial activity in the digestive tract to optimize nutrient conversion. The presence of probiotics likely enhanced enzymatic activity and intestinal conditions, resulting in more efficient protein hydrolysis and absorption.

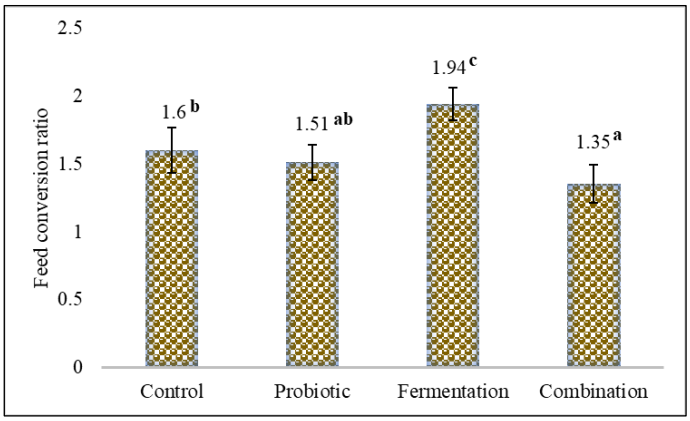


Figure 3. Feed conversion ratio of Nile tilapia (*Oreochromis niloticus*) fry. Different superscript letters indicate statistically significant differences among treatment means ($p < 0.05$).

Overall, the synergistic interaction between fermented plant protein and probiotic bacteria played a critical role in improving feed efficiency, as reflected by the lower FCR values. Similar synergistic effects have been reported in previous studies combining probiotics with fermented feed ingredients in aquaculture diets (Virnanto et al., 2016; Utomo et al., 2011).

Conclusions. The addition of probiotic *Lactobacillus casei*, fermented *Azolla microphylla* flour, and their combination significantly affected the growth performance and feed efficiency of Nile tilapia fry. The combined treatment of *L. casei* (4 %) and fermented *A. microphylla* flour (30 %) resulted in the highest specific growth rate (4.26 ± 0.23 % day^{-1}) and the lowest feed conversion ratio (1.35 ± 0.13), indicating a synergistic effect on nutrient utilization.

In contrast, dietary treatments had no significant effect on survival rate, which remained within acceptable ranges (76 – 80 %). These results suggest that combining probiotic supplementation with fermented plant-based feed ingredients is an effective strategy to improve growth and feed efficiency of Nile tilapia without negatively affecting survival.

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Authors Contributions. Conceptualization: TS. Methodology: PSWK. Formal Analysis: DH. Investigation: MRF. Data Curation: VA. Writing: TS, DH. Original Draft: TS, DH. Writing – Review & Editing: TS, PSWK, DH, MRF, VA. Supervision: PSWK.

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

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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