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Optimizing Tilapia Growth through a Comparative Evaluation of EM4 and Homemade Probiotics in Pellets

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Abstract

One alternative approach to facilitate the utilization of feed by tilapia is the addition of probiotics in feed. In this study, we compare the effects of commercial probiotic EM4 and homemade probiotics on tilapia growth. This research was conducted in PT. Sahaja Berkah Abadi at wonosalam, jawatimur, indonesia. The study was designed as a completely randomized design with 3 treatments and 4 replications, encompassing control group (C), commercial probiotics (G1), and homemade probiotics (G2). The commercial probiotics (G1) contained *Rhodopseudomonas* sp., *Lactobacillus* sp., *Streptomyces* sp., and *Actinomycetes* sp. while the homemade probiotic (G2) contained starter bacteria *Lactobacillus casei* and herbal plant such as ginger, Curcuma, and turmeric. Parameters observed during the study included weight gain (WG), specific growth rate (SGR), and survival rate (SR). The results revealed that both G1 and G2 performed better than the control group. The weight gain for the control, G1, and G2 groups were 2.38±0.03 g, 3.09± 0.01 g, and 3.13 ± 0.03 g, respectively. Similarly, the specific growth rates for the control, G1, and G2 groups were 4.25±0.03%, 5.52± 0.02%, and 5.59 ± 0.03%, respectively, while the survival rate remained at 100% across all groups. The water quality in all treatments during maintenance met the standard requirements for tilapia, with temperatures ranging from 25.5 to 26.7 °C and pH levels between 6.7 and 6.9. These findings suggest that the probiotics incorporated in G2 present a cost-effective alternative to those in G1, offering a promising option for tilapia cultivation.

INTRODUCTION

Aquaculture plays a vital role in providing sustainable livelihood opportunities and food security for the increasing world population. Among diversity in farmed aquatic species, tilapia (*Oreochromis niloticus*) stands out as a widely cultured consumable fish in Indonesia. Tilapia is an important

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freshwater aquaculture species characterized by fast growth, strong environmental adaptability, ease of breeding, and disease resistance (Zhang, 2021). Based on Asianto *et al*. (2021), tilapia production in 2020 reached about 2.5 million tonnes.

Feed is a crucial element in aquaculture, influencing the growth and overall health of cultured organisms (Zlaugotne *et al*., 2022). To enhance fish weight effectively, it is necessary to add supplements to the feed. One approach to improve feed digestibility is by introducing probiotics (El-Saadony *et al*., 2021). Probiotics, as supplementary foods, consist of live microorganism cells with beneficial effects on the host (Soliman *et al*., 2019). This strategy holds significance for improving the efficiency of aquaculture practices and contributes to the overall productivity and sustainability of fish farming.

Various types of commercial probiotics are readily available, among them EM4 stands out as a well-known probiotic in the field of aquaculture (Nurjanah *et al*., 2023; Sinaga and Mukti, 2022). EM4 is composed of a mixture of bacteria, including *Rhodopseudomonas*, *Lactobacillus*, and *Actinomycetes* (Wilisetyadi *et al*., 2022). The accessibility of commercial probiotics has encouraged individuals in Wonosalam, East Java, Indonesia, to engage in the homemade production of probiotics. This shift is driven by the relatively high cost of commercial probiotics, which poses a financial challenge for the local population with limited income. Crafting homemade probiotics involves the use of simple tools and locally available herbal plants, such as ginger, Curcuma, and turmeric, which have been extensively researched for their benefits in aquaculture (Mariappan *et al*., 2023). Notably, despite the widespread use of both commercial probiotics (EM4) and homemade probiotics in the region, their concurrent effectiveness in tilapia cultivation remains untested. This study aims to understand the effectiveness of commercial probiotics of EM4 and homemade probiotics in predigestion feed for tilapia cultivation. The results will inform sustainable practices in aquaculture with low input costs.

METHODOLOGY

Ethical Approval

No animals were harmed or improperly treated during this research. The test animals in this study were appropriately handled, considering factors such as optimal environmental conditions, proper water quality, and the availability of suitable feed.

Place and Time

This research was conducted from December 2023 to January 2024 at PT. Sahaja Berkah Abadi, located in Wonosalam, Jawa Timur, Indonesia.

Research Materials

The tilapia fish were sourced from a local fish farmer in Jombang, located in the province of East Java, Indonesia. The research material utilized in this study consisted of tilapia with an average weight of 0.15 ± 0.00 grams. The experimental tanks measured 100x50x40 cm, with a water volume of 120 liters. Various equipment was used including scales (T-Scale, Taiwan), thermometer (Eutech, USA), and pH meters (Bluelab, New Zealand). The feed utilized in this study consisted of commercial pelletized granules from the Phokphand Group (PT. Central Protein Prima, Indonesia), and the nutritional composition of the feed is detailed in Table 1.

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Table 1. Nutritional composition.

Nutritional composition	Nutritional content (%)
Protein	35
Fat	2
Fiber	3
Ash	17
Water content	12

The chosen commercial probiotic was EM4 (PT. Sinta Prima Feedmill, Indonesia), containing *Rhodopseudomonas* sp., *Lactobacillus* sp., *Streptomyces* sp., and *Actinomycetes* sp. Homemade probiotics used Yakult (PT. Yakult Indonesia Persada, Indonesia), containing *Lactobacillus*, and various herbal plants such as ginger, Curcuma, and turmeric. Before use, the feed underwent a 3-day predigestion process with probiotics. Details regarding the materials used in the preparation of predigestion feed are outlined in Table 2.

Table 2. Composition of ingredients.

G1 (Commercial probiotic)		G2 (Homemade probiotic)	
Material	Amount	Material	Amount
EM 4	15 ml / kg	Yakult	2 bottle/kg
		Ginger	20 g/kg
		Tumeric	20 g/kg
		Curcuma	20 g/kg

Research Design

This study employed a Completely Randomized Design (CRD) with three treatments and four replications. Each treatment unit was randomly assigned and homogeneously conditioned. The treatments included the Control group (C), involving tilapia culture using normal feed; Commercial probiotics (G1), involving tilapia culture using predigestion feed with a commercial probiotic (EM4); and Homemade Probiotics (G2), involving tilapia culture using predigestion feed with a homemade probiotic.

Work Procedure

Predigestion Feed Process

The process of preparing both commercial and homemade probiotics started with gathering the necessary materials, as outlined in Table 2, followed by the fermentation process. The nutritional composition of the pelleted feed is further outlined in Table 1. For commercial probiotics, Sinaga and Mukti (2022) recommend an optimal dosage of 15 ml per kilogram of feed for EM4 in tilapia cultivation. Similarly, the same dosage of 15 ml per kilogram is utilized for homemade probiotics. These materials were combined with commercial pellets and placed inside a container for fermentation. The fermentation process occurred within the container for three days, stored in indirect sunlight. Subsequently, the fermented mixture was dried by exposure to air without direct sunlight until the feed reached a completely dry state.

Feeding Experiment

In total, 120 tilapia (*O. niloticus*) fingerlings, with an average body weight (BW) of 0.15 ± 0.00 g, were randomly allocated into three groups (C, G1, and G2) and cultured in 120-L tanks equipped with an independent recirculation system. Each group comprised 10 fish, and the experiments were conducted in triplicate. This stocking density was chosen to maintain optimal conditions for fish growth and behavior, while mitigating the accumulation of metabolic waste,

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minimizing competition for food and oxygen, and reducing social stress (Dauda *et al*., 2019). Keeping the stocking density consistent across tanks allows for reliable comparisons between different groups in our experiment. The control group (C) was fed a basal pellet diet, while the experimental diets included basal diets with the addition of commercial probiotics (G1) and homemade probiotics (G2). Throughout the feeding trial, fish were weighed once every two weeks, and the diet quantity was adjusted accordingly. Fish were fed twice daily at 09:00 and 17:00, with the daily feeding rate set at 5% of BW. The feeding rate of 5% of body weight per day is commonly used for fish because it provides a balance between optimal growth and feed conversion, while also avoiding overfeeding and pollution (Assan *et al*., 2021). Tank maintenance included daily water siphoning. After 8 weeks of cultivation, the weight gain, specific growth rate, and survival rate of the fish were analyzed.

Parameters

The weights of all tilapia from each aquarium were assessed at both the initial and final samplings to evaluate growth performance. Key metrics including weight gain (WG), specific growth rate (SGR), and survival rate were determined using the formulas outlined in Tan *et al*. (2019).

$$\text{Weight gain} = \text{Final weight} - \text{initial weight}$$

$$\text{SGR} = \frac{\text{Weight gain}}{\text{days}} \text{x}100\%$$

$$\text{Survival rate}(\%) = \frac{\text{final fish number}}{\text{initial fish number}} \text{x}100\%$$

Water Quality Measurement

Throughout the research activities, water quality parameters such as temperature and pH were regularly monitored every morning during routine maintenance.

Data Analysis

The software employed in this experiment is Sigma Plot 12.5, utilizing One-Way ANOVA to obtain scores for growth performance parameters such as weight gain, specific growth rate, feed efficiency, and survival rate. To delineate differences in the data, the Duncan test is applied to provide accurate values and facilitate comprehensive analysis.

RESULTS AND DISCUSSIONS

Weight Gain

The weight gain of tilapia was assessed at various observation times, specifically on days 1, 14, 28, 42, and 56. The measurements revealed a consistent increase in individual weight at each observation time, as illustrated in Figure 1.

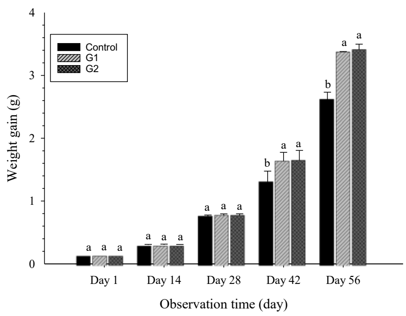

Figure 1. The weight gain of tilapia over 56 days. Different superscripts represent significant differences. The treatments included the control group (C), the commercial probiotic group (G1), and the homemade probiotic group (G2).

The ANOVA test results indicated no significant differences in the weight gain of individual tilapia on the 1st day of observation (P>0.05). Subsequent

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measurements on days 1, 14, 28, 42, and 56 also did not show significant differences in weight gain from days 1 to 14 (P>0.05). However, by days 42 and 56, a clear and significant difference emerged (P < 0.05), indicating higher weight gain in G1 and G2 compared to the control group (C). The weight gain values for the control, G1, and G2 groups were 2.38 ± 0.03 g, 3.09± 0.01 g, and 3.13 ± 0.03 g, respectively.

The uniformity observed in the weight gain of individual tilapia on the 1st day of observation, as indicated by the non-significant differences between treatments (P>0.05), is a crucial baseline for the subsequent growth performance experiments. Consistency in the initial sample is crucial for drawing meaningful conclusions about the impact of treatments on overall tilapia growth (Thorarensen *et al*., 2015). Throughout the observation period on days 7, 14, and 28, there were no significant differences in weight gain, suggesting that the treatments, including both commercial probiotics (G1) and homemade probiotics (G2), did not manifest distinct effects during these early stages. However, visual observation on day 28 showed a slight increase in body weight gain in G1 and G2 compared to the control group (C), but the statistical analysis did not support a significant difference. The turning point in the experiment came on days 42 and 56, where a clear and significant difference (P <0.05) appeared. Both G1 and G2 exhibited higher weight gains compared to the control group, indicating that the treatments started to manifest their effects on tilapia growth at this stage of the experiment.

Lactobacillus species have gained recognition as probiotics in numerous fish studies due to their diverse beneficial effects on host organisms. These effects include stimulating growth performance, gastrointestinal development, digestive function, mucosal tolerance, immune response, and enhanced disease resistance (Chen, 2019). The enzymatic capabilities of *Lactobacillus*, including the secretion of proteases, amylases, and lipases, facilitate the breakdown of complex organic compounds such as proteins, carbohydrates, and fats (Mathur *et al*., 2020). These enzymes act specifically on complex macronutrients, simplifying them into forms readily absorbed by the host.

Moreover, *Lactobacillus* contributes to the fermentation of dietary components, resulting in the production of short-chain fatty acids (SCFAs) and other metabolites (Moradi *et al*., 2020), which promote a stable environment conducive to nutrient absorption. In the current studies, *Lactobacillus* serves as a probiotic starter in both experimental groups, G1 and G2, where it plays a crucial role in enhancing weight gain in tilapia. The secretion of various enzymes by *Lactobacillus*, such as proteases, amylases, and lipases, facilitates the breakdown of complex organic compounds into simpler forms that can be readily assimilated by the host. Moreover, *Lactobacillus* contributes to the fermentation of dietary components, leading to the production of SCFAs and other metabolites, which further optimize nutrient absorption and utilization. This enhanced nutrient assimilation in G1 and G2 likely contributed to the observed higher weight gain compared to the control group.

Specific Growth Rate

The specific growth rate (SGR) is a crucial metric in aquaculture and fisheries management as it provides a quantitative measure of the relative growth of organisms over a specific period (Crane *et al*., 2020). The results of observations of the specific growth rate of tilapia can be seen in Figure 2.

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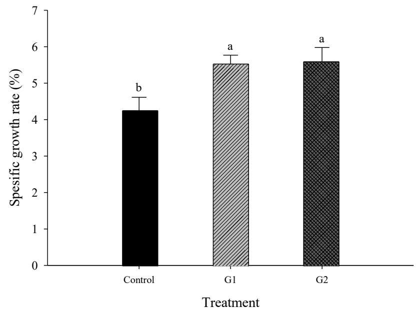

Figure 2. The specific growth rate of tilapia over 56 days. Different superscripts represent significant differences. The treatments included the control group (C), the commercial probiotic group (G1), and the homemade probiotic group (G2).

The ANOVA test revealed significant differences in specific growth rates among the treatments, with subsequent Duncan's test showing no significant difference between G1 and G2. However, both G1 and G2 exhibited significantly higher specific growth rates compared to the control group. The specific growth rates for the control, G1, and G2 groups were 4.25±0.03%, 5.52± 0.02%, and 5.59 ± 0.03%, respectively.

Assan *et al.* (2022) suggest that adding probiotics to fish feed improves digestion and helps fish absorb nutrients more efficiently. Dawood *et al.* (2020) found that using a balanced feed additive with lactic acid bacteria enhances growth and health in tilapia. Wilisetyadi *et al.* (2022) also highlight the positive effects of EM4 probiotics on tilapia growth. In the current study the composition differences between G1 and G2 in terms of their probiotic content provide an interesting perspective on the observed results. G1, consisting of starter probiotics from EM4 such as *Rhodopseudomonas* sp., *Lactobacillus* sp., and *Actinomycetes* sp., represents a commercially available formulation focusing on bacterial strains. On the other hand, G2 while containing *Lactobacillus* sp. as the starter probiotic, incorporates additional herbal plants such as ginger, Curcuma, and turmeric.

The absence of significant differences in the growth performance between G1 and G2 may be attributed to the potential compensatory effects of the herbal plants in G2. Herbal plants like ginger, curcuma, and turmeric are recognized for their multifaceted benefits, including anti-inflammatory properties and enhancement of digestive processes (Tadese *et al.*, 2022). These bioactive compounds present in the G2 probiotic mix could have exerted positive influences on tilapia growth, offsetting the fewer bacterial strains present compared to G1. The interaction between bacterial and herbal elements highlights how probiotic mixtures can be complex and have combined effects in aquaculture. This shows that probiotics can work together with herbal ingredients to improve fish growth in aquaculture.

It is important to highlight the potential benefits of ginger, Curcuma, and turmeric in G2. These herbal ingredients are known for their multifaceted roles in improving feed palatability, stimulating appetite, and facilitating better nutrient absorption in tilapia (Tadese *et al.*, 2022; Mariappan *et al.*, 2023). For example, ginger has been shown to possess anti-inflammatory properties (Ballester *et al.*, 2022; Ma *et al.*, 2021; Fazelan *et al.*, 2020), while curcuma and turmeric exhibit antioxidant effects (Alagawany *et al.*, 2021; Fagnon *et al.*, 2020; Akaberi *et al.*, 2021). These properties can contribute to overall fish health and growth. Additionally, the bioactive compounds present in these herbal plants, such as curcumin in turmeric, may directly impact metabolic processes in fish, further

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enhancing growth rates. Thus, combining bacterial and herbal components in G2 may have led to a synergistic effect, supporting the observed similarities in growth rates between G1 and G2. This underscores the importance of comprehensively understanding the interactions between different components in probiotic formulations for optimizing aquaculture outcomes.

Survival Rate

The survival rate is a vital parameter in aquaculture and fisheries management, offering insight into the overall health and resilience of the fish population within a specific timeframe (Brosset *et al*., 2020). The results regarding the survival rate of tilapia are depicted in Figure 3.

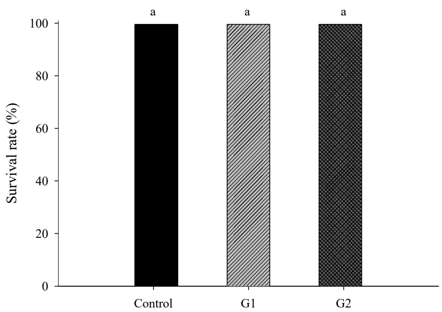

Figure 3. The survival rate of tilapia over 56 days. Different superscripts represent significant differences. The treatments included the control group (C), the commercial probiotic group (G1), and the homemade probiotic group (G2).

The consistent 100% survival rate observed across all experimental groups underscores the robustness of the controlled culturing conditions employed in this study. Importantly, the introduction of probiotics in groups G1 and G2 did not yield any negative effects on overall tilapia survival. This lack of significant differences in survival rates between the control group and the treatment groups indicates the safety and compatibility of the probiotics utilized in G1 and G2 with the tilapia culture environment. These results assure the harmlessness of the probiotics and their suitability for incorporation into aquaculture practices, ensuring the welfare and viability of the cultured tilapia. These findings validate the pivotal criterion that probiotics must be safe for use and should not impart any harmful effects (Amenyogbe, 2023).

Water Quality

Water quality is a fundamental aspect of aquaculture and fisheries management, playing a significant role in the health and well-being of aquatic organisms (Boyd and Tucker, 2012). The results of water quality parameters observed during the study are illustrated in Table 3.

Table 3. Water quality in the tilapia culture tank over 56 days.

Parameters	Treatment			Optimum
	Control	G1	G2	(Abd El-Hack *et al*., 2022)
Temperature (°C)	25 – 26.7	25 – 26.7	25 – 26.7	25 – 27 °C
pH	6.7 – 6.9	6.7 – 6.9	6.7 – 6.9	6.7 – 6.9

Throughout the 60-day tilapia culture period, the observed temperature ranged from 25°C to 26.7°C. This aligns with the optimal temperature range for tilapia growth, which typically falls between 25°C to 27°C. This consistency

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suggests that the environmental conditions within the pond were favorable for the growth and development of tilapia. Similarly, the pH levels recorded during the culture period ranged from 6.7 to 6.9, remaining within the optimal pH range of 6.5 to 9.0 for tilapia growth (Abd El-Hack *et al*., 2022). These findings indicate that the culture maintained suitable water quality parameters conducive to the healthy growth and survival of tilapia throughout the study.

The results of this experiment demonstrate that the probiotics present in G1 and G2 effectively enhance feed quality, resulting in higher growth rates and specific growth rates compared to the control treatment. Importantly, the study reveals that the probiotics group does not have any negative effects on the host. Additionally, despite the considerably lower cost and easy accessibility of ingredients for making G2 probiotics in the Wonosalam area, statistical analysis indicates that G2 is comparably effective to the commercial probiotics found in G1. In conclusion, the findings suggest that the probiotics incorporated in G2 offer a cost-effective alternative to those in G1, making them a promising option for tilapia cultivation.

CONCLUSION

In conclusion, the experiment demonstrated that both commercial probiotics (G1) and homemade probiotics (G2) effectively improved the growth performance of tilapia compared to the control group. While G1 contained a combination of bacterial strains, G2 incorporated additional herbal plants such as ginger, Curcuma, and turmeric. The absence of significant differences in growth performance between G1 and G2 suggests potential compensatory effects of the herbal plants in G2. These findings underscore the complex interactions between bacterial and herbal components in probiotic formulations, emphasizing the importance of comprehensive understanding for optimizing aquaculture outcomes. Furthermore, both G1 and G2 probiotics were found to be safe and compatible with tilapia culture, as evidenced by consistent survival rates across all experimental groups. The study also confirmed that the environmental conditions within the pond remained favorable for tilapia growth throughout the culture period. Overall, the results highlight the promising potential of incorporating cost-effective homemade probiotics like G2 into tilapia cultivation practices, providing a sustainable alternative to commercial probiotics like G1.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

AUTHOR CONTRIBUTION

The experiment was conceived and designed by Izzudin. Izzudin conducted all experimental procedures, collected the data, and performed the statistical analysis. Izzudin drafted the manuscript, which was critically revised and approved by Woro Hastuti Satyantini for final submission.

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