

Dietary Supplementation of *Moringa oleifera* Leaf Extract as a Prebiotic for Improved Growth in Nile Tilapia (*Oreochromis niloticus*)

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Abstract: Functional feed additives from plant sources are increasingly explored to improve aquaculture efficiency and sustainability. This study was conducted to assess the influence of dietary *Moringa oleifera* leaf extract supplementation as a natural prebiotic source on the growth performance and feed utilization efficiency in Nile tilapia (*Oreochromis niloticus*). The experiment was carried out for a period of 40 days in a completely randomized design with four dietary treatments of moringa leaf extract levels, controlling (0), 10, 15 and 20 mL/100 g diets, each replicated three times. The performance criteria were survival rate (SR), absolute weight and length gain, specific growth rate (SGR), feed conversion ratio (FCR), feed utilization efficiency (EPP) and water quality. Results indicated that there are no significant effects of *M. oleifera* leaf extract supplementation SR, absolute weight gain, absolute length gain and SGR ($p > 0.05$) however it significantly affects FCR and EPP ($p < 0.05$). The lowest FCR and the highest EPP were observed in fish fed 20 mL/100 g feed, which suggests a better utilization of the feed. All water quality parameters were adequate during the culture period. In conclusion, *M. oleifera* leaf extract has potential as a natural feed additive with prebiotic-like properties to enhance feed efficiency in Nile tilapia, thereby supporting more efficient and sustainable aquaculture practices.

Keywords: Feed conversion ratio, *Moringa oleifera*, Nile tilapia, prebiotic.

Introduction

Nile tilapia (*Oreochromis niloticus*) is among the most important freshwater aquaculture commodities worldwide due to its rapid growth rate, broad environmental tolerance, and high adaptability to diverse culture systems. This species contributes substantially to food security and economic development, particularly in developing countries where fish consumption continues to increase (El-Naggar et al., 2021; Sherif et al., 2021; Mbokane & Moyo, 2023). In Indonesia, Nile tilapia also constitutes a major pillar of national aquaculture production because of its high economic value and relatively accessible farming technology. However, intensification of tilapia production still faces major constraints, particularly those associated

with feed quality, aquatic environmental degradation, and low feed utilization efficiency. In intensive culture systems, feed represents the principal production input, accounting for approximately 50% of total operational costs.

Reliance on commercial feeds based on conventional ingredients, including fishmeal, often fails to provide fully balanced nutrition and may generate economic and environmental pressures (Silva et al., 2021; Charoenphun et al., 2023). Nutrient imbalances between feed composition and the physiological requirements of Nile tilapia can result in suboptimal growth, elevated feed conversion ratios, and increased organic waste release into culture waters. Therefore, innovations in feed formulation are urgently needed, particularly through the use of alternative ingredients and functional additives

with improved sustainability profiles (El-Naggar *et al.*, 2021; Ragozzino-Paulino, 2025; Lumbessy *et al.*, 2024, 2025; Lestari *et al.*, 2025; Nisa *et al.*, 2024; Ramdhani *et al.*, 2024).

The concept of functional feeds is also a fast growing technique in aquaculture nutrition. Functional feeds are formulated for higher performance and they go beyond of fulfilling only the fundamental nutritional needs by increasing growth performance, health condition and resistance to stress and diseases (Mansour *et al.*, 2022; Munni *et al.*, 2023; Sallam *et al.*, 2021). In this respect, prebiotics have attracted more and more interest as they cannot be digested by the host, but specifically promote growth and activity of beneficial microorganisms in the intestine. Prebiotics have also been reported to enhance the gut health, nutrient absorption efficiency and immune response in fish, resulting in improved feed efficiency and growth performance (Kengkittipat *et al.*, 2025; Yostawonkul *et al.*, 2023; He *et al.* 2022; Phinrub *et al.* 2024).

In the current era of sustainable aquaculture, the importance of natural prebiotics derived from plants is gaining popularity as a safe and eco-friendly alternative. Several plant extracts have been reported to act as natural prebiotics, thus improving antioxidant status, intestinal health and disease resistance in Nile tilapia (Elgendy *et al.*, 2021; Elgendy *et al.*, 2022; Almarri *et al.*, 2023). Among these botanical resources, *Moringa oleifera* leaves are especially interesting for their nutritional power and rich bioactive profile.

The leaves of *Moringa oleifera* contain proteins, essential amino acids, vitamins (A,C,E), minerals and bioactive compounds like flavonoids; polyphenols and natural antioxidants (Sajina *et al.*, 2021; Abidin *et al.*, 2021; Li *et al.*, 2024). These characteristics indicate that the leaves of *Moringa* are a candidate as a functional feed additive with prebiotic effects. A number of studies have shown positive effects on antioxidant capacity, immunity response, growth performance and feed utilization efficacy in Nile tilapia fed with dietary inclusion of MLE (Elgendy E.R., 2021; El-Son R.F.A., 2022; Kamble *et al.*, 2024; Ndebele-Murisa, 2024).

However, the reported -results on supplementation of Nile tilapia diets with moringa leaf extract remain erratic with respect

to optimal inclusion level and subsequent physiological responses. A number of research report non-linear dose-response of ZnO, which means increasing of supplemented level is not always associated with improvement in growth performance and feed efficiency (Kumar *et al.*, 2021; Kamble *et al.*, 2024). Furthermore, the mechanisms responsible for AME enhancing properties of moringa leaf extract as a prebiotic in feed utilizations—particularly under high-density culture conditions—are poorly characterized.

This research was designed to investigate the impact of *Moringa oleifera* leaf extract supplementation for enhancing natural prebiotics properties in diets on growth performance and feed utilization efficiency in Nile tilapia (*Oreochromis niloticus*). The results will contribute scientifically to ascertain an effective supplementation dosage, and reinforce the rationale for the use of locally available plant resources as sustainable feed additives in aquaculture.

Material and Methods

Study Period and Location

This experiment was carried out during 40 days, from August to September 2025 at the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Faculty of Agriculture of Universitas Mataram. The laboratory was chosen because it has a good infrastructure for rearing fish and water quality monitoring, along with evaluating growth performances and feed utilization efficiencies.

Experimental Design

Experimental One-way Completely Randomized Design (CRD) was employed which consisted of four dietary treatments with three replicates making a total of 12 experimental units. This approach, inspired by aquaculture nutrition trials where environmental control and replication are key (Kumar *et al.*, 2021; Nottanalan *et al.*, 2021) is in line with the common practice.

Dietary Treatments

The tested treatments were as follows:

- P1: Commercial feed without moringa leaf extract (control)

- P2: Commercial feed + moringa leaf extract at 10 mL/100 g feed
- P3: Commercial feed + moringa leaf extract at 15 mL/100 g feed
- P4: Commercial feed + moringa leaf extract at 20 mL/100 g feed

Experimental Fish and Rearing System

Nile tilapia (*Oreochromis niloticus*) juveniles (4–5 cm in total length) were used as the experimental animals. Fish were stocked in uniform-sized container units at an equal stocking density across all treatments. Aeration and water circulation were maintained to ensure optimal water-quality conditions throughout the rearing period.

Materials and Equipment

Materials included Nile tilapia juveniles, commercial feed, *Moringa oleifera* leaf extract, 70% ethanol, and distilled water. Equipment used comprised aerators, a dissolved oxygen (DO) meter, a pH meter, a thermometer, a blender, an analytical balance, a sieve, a ruler, and a rotary vacuum evaporator.

Preparation of *Moringa oleifera* Leaf Extract

The fresh leaves of *Moringa* were sorted, washed and dried until minimal level of moisture was attained. The powdered samples were macerated with 70% ethanol, then filtered and dried. Extraction through maceration 3×24 h followed by filtration. Remaining solution was concentrated under reduced pressure over rotatory evaporator to get a viscous extract.

Rearing Management and Feeding

Fish were reared for 40 days. Feeding was carried out to apparent satiation twice daily (morning and midday). Body weight and total length were measured every 10 days to monitor growth dynamics.

Observed Parameters

Growth Performance and Feed Utilization: Absolute weight gain (g), Absolute length gain (cm), Specific growth rate (SGR), Feed conversion ratio (FCR), Feed utilization efficiency (FUE) / feed efficiency (EPP). **Survival:** Survival rate (SR). **Water Quality:** Temperature (°C), pH, Dissolved oxygen (DO).

Data Analysis

Distributional assumptions were tested with a normality test on the data. Statistical analysis of normally distributed data was performed by means of one-way ANOVA at the 5% level of significance. Duncan's Multiple Range Test (DMRT) was performed to determine significant differences when detected. Non-parametric data were analyzed descriptively.

Results and Discussion

Survival Rate (SR)

During the 40-day rearing trial, Nile tilapia survival rates ranged from 75% to 82% across treatments. The highest survival was observed in P3 (15 mL/100 g feed) ($82.0 \pm 0.0\%$), followed by P4 ($80.0 \pm 0.1\%$). The lowest survival occurred in P1 (control) ($75.0 \pm 0.0\%$) and was comparable to P2 ($75.0 \pm 0.1\%$). However, one-way ANOVA indicated that survival did not differ significantly among treatments ($p > 0.05$), suggesting that moringa leaf extract supplementation did not statistically improve survival under the present experimental conditions.

From a biological perspective, the observed survival range (75–82%) can be considered acceptable for the experimental period, indicating that dietary moringa leaf extract at the tested doses did not cause adverse effects on tilapia survival. Although a descriptive increase was apparent in P3–P4, this pattern should be interpreted cautiously because it was not supported by statistical significance.

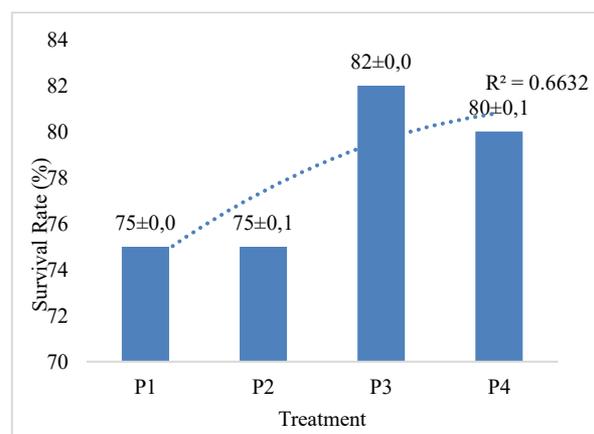


Figure 1. Survival Rate

Absolute Weight Gain

Absolute weight gain of Nile tilapia during the study ranged from 1.4 to 2.3 g. The P1 (control) group showed the lowest absolute weight gain (1.4 ± 0.6 g), whereas the highest value was observed in P4 (20 mL/100 g feed) (2.3 ± 0.2 g). Fish in P3 (15 mL/100 g feed) also exhibited relatively high weight gain (2.2 ± 0.1 g) with low variability among individuals, indicating a more consistent growth response.

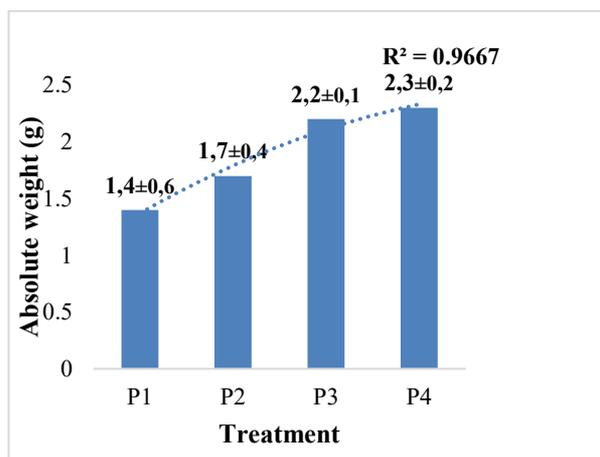


Figure 2. Absolute weight gain (g) of Nile tilapia across treatments

One-way ANOVA indicated that the treatments did not significantly affect absolute weight gain ($p > 0.05$). Nevertheless, a descriptive increasing trend from P1 to P4 suggests that higher doses of moringa extract may be associated with improved growth performance. This pattern may reflect a positive biological response to functional supplementation; however, further studies with larger sample sizes and/or longer feeding periods are recommended to confirm this effect.

Absolute Length Gain

Mean absolute length gain of Nile tilapia ranged from 6.1 to 6.8 cm during the 40-day trial. The lowest length gain was observed in P1 (6.1 ± 0.54 cm), while the highest value was recorded in P4 (6.8 ± 0.51 cm). Treatments P2 (6.7 ± 0.17 cm) and P3 (6.7 ± 0.34 cm) showed comparable responses, indicating similar linear growth performance at intermediate moringa extract doses.

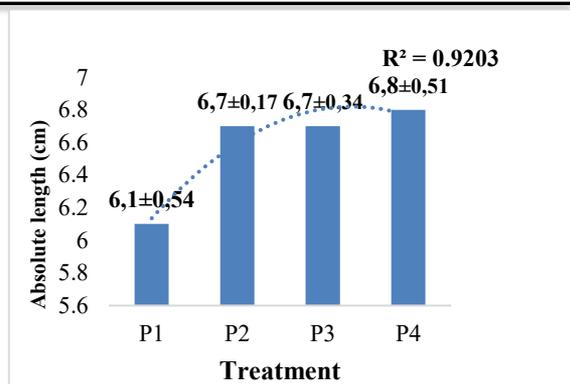


Figure 3. Absolute length gain (cm) of Nile tilapia across treatments

Statistical analysis (one-way ANOVA) indicated that absolute length gain did not differ significantly among treatments ($p > 0.05$). This suggests that dietary moringa leaf extract supplementation up to 20 mL/100 g feed did not significantly affect linear growth of Nile tilapia under the present experimental conditions, although a slight descriptive increasing trend from P1 to P4 was observed.

Specific Growth Rate (SGR)

Specific growth rate (SGR) of Nile tilapia ranged from 3.55 to 5.75% day⁻¹ during the 40-day feeding trial. The lowest SGR was recorded in P1 (control) ($3.55 \pm 1.6\%$ day⁻¹). SGR increased in the moringa-supplemented groups, reaching $4.05 \pm 1.4\%$ day⁻¹ in P2, $5.56 \pm 0.2\%$ day⁻¹ in P3, and the highest value in P4 ($5.75 \pm 0.6\%$ day⁻¹). Overall, a descriptive upward trend was observed from P1 to P4.

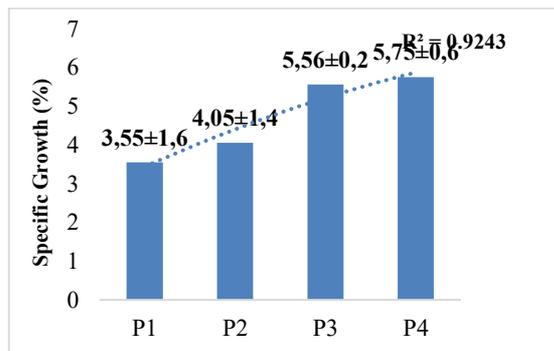


Figure 4. Specific growth rate (SGR, % day⁻¹) of Nile tilapia across treatments

However, one-way ANOVA indicated that SGR did not differ significantly among treatments ($p > 0.05$). Therefore, moringa leaf extract supplementation up to 20 mL/100 g feed

did not produce a statistically significant improvement in SGR under the present conditions. Nonetheless, the consistently higher mean SGR in P3–P4 suggests a potential positive tendency in growth performance, which may be related to improved feed utilization or the bioactive properties of moringa; this trend should be confirmed with further trials (e.g., higher replication, longer duration, and inclusion of FCR/feed intake measurements).

Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) ranged from 1.17 to 1.91 across treatments. The highest FCR (least efficient feed utilization) was observed in P1 (control), whereas the lowest FCR (most efficient) was obtained in P4 (20 mL/100 g feed). One-way ANOVA indicated that moringa leaf extract supplementation significantly affected FCR ($p < 0.05$), suggesting that the dietary treatments influenced feed utilization efficiency during the experimental period.

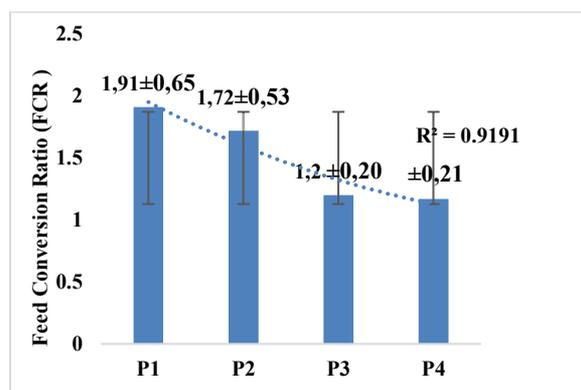


Figure 5. Feed conversion ratio (FCR) of Nile tilapia across treatments

The progressive reduction in FCR with increasing moringa extract doses implies that moringa supplementation improved feed efficiency in Nile tilapia. This improvement may be associated with the bioactive compounds in moringa that support digestion and nutrient assimilation; however, mechanistic confirmation would require supporting evidence (e.g., feed intake, digestibility coefficients, gut histology, or digestive enzyme activity).

Feed Utilization Efficiency (EPP)

Feed utilization efficiency (EPP) ranged from 56.7% to 86.1% across treatments. The lowest EPP was observed in P1 (control),

whereas the highest occurred in P4 (20 mL/100 g feed), indicating improved conversion of feed into biomass with higher moringa extract inclusion.

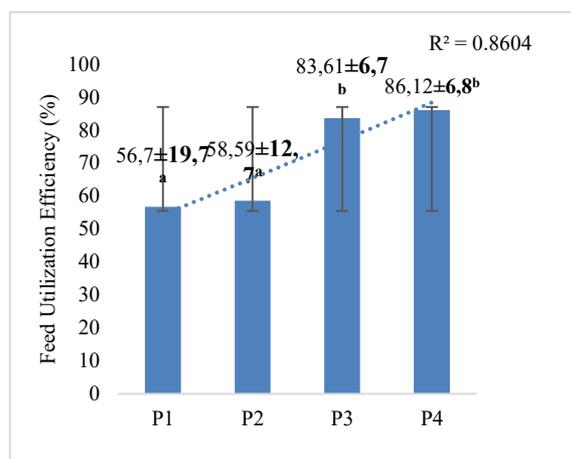


Figure 6. Feed utilization efficiency (EPP, %) of Nile tilapia across treatments (mean ± SD)

One-way ANOVA showed that dietary treatments significantly affected EPP ($p < 0.05$). Post hoc analysis using Duncan's Multiple Range Test (DMRT) revealed that P3 and P4 formed a significantly higher group compared with P1 and P2 ($p < 0.05$), suggesting that medium-to-high moringa leaf extract inclusion levels enhanced feed utilization efficiency in Nile tilapia under the present experimental conditions.

Water Quality During the Experiment

Water quality monitoring indicated that temperature ranged from 26.9 to 28.1°C, pH ranged from 6.42 to 6.45, and dissolved oxygen (DO) ranged from 5.7 to 6.3 mg/L. All measured parameters remained within suitable ranges for Nile tilapia culture and were relatively stable throughout the experimental period.

Table 1. Water quality during the experiment

No.	Parameter	Observed range	Ideal range
1	Temperature (°C)	26.9–28.1	25–30
2	pH	6.42–6.45	6.5–8.0
3	DO (mg/L)	5.7–6.3	5–6

Discussion

Effects of *Moringa oleifera* Leaf Extract Supplementation on Nile Tilapia Survival

Survival of Nile tilapia under rearing conditions (75 to 82%) suggests that all treatments maintained acceptable physiological condition. The lack of a difference between treatments means that moringa leaf extract did not increase stress or toxicity which would influence survival. This result is in agreement with former studies that demonstrated that *Moringa oleifera* leaf extract can be safely used as a dietary additive and could help to improve fish tolerance by inducing innate immune responses (Elgendy *et al.*, 2021; Kamble *et al.*, 2024).

The relatively uniform survival across treatments also reflects the high adaptability of Nile tilapia to variations in diet composition. In addition, the water-quality values were acceptable throughout the course of experiment in both treatments, which was presumably favorable for physiological equilibrium and would have led to decreased environmental limitation regarding survival.

Absolute Weight Gain Response to Moringa Leaf Extract

Absolute weight gain showed a descriptive tendency to increase with higher moringa leaf extract doses, although the differences were not statistically significant. This pattern suggests a positive biological response to moringa supplementation, particularly at medium to high inclusion levels. The result aligns with earlier reports indicating that *Moringa oleifera* can promote fish growth by improving nutritional status and digestive health, although growth responses are often dose-dependent and may not follow a linear relationship (Kumar *et al.*, 2021; El-Son *et al.*, 2022; Kamble *et al.*, 2024). The 15 mL/100 g feed dose produced relatively stable weight gain with low variability, implying a balance between nutrient intake and the fish's metabolic capacity. At higher doses, the weight gain increase was no longer proportional, suggesting that growth responses may approach a physiological saturation point.

Absolute Length Gain and Its Implications

Absolute length gain differed only slightly among treatments and did not show statistical significance. This suggests that linear growth in

Nile tilapia is less responsive to moringa extract supplementation than body-weight gain. Physiologically, length increment is strongly influenced by genetic factors and ontogenetic stage, whereas body-weight gain is generally more sensitive to nutrient availability and feed utilization efficiency. This observation is consistent with studies reporting that plant-based dietary additives more frequently influence body composition and metabolic efficiency than linear morphometric traits (Guan *et al.*, 2024; Angom *et al.*, 2025).

Specific Growth Rate as an Indicator of Metabolic Efficiency

Specific growth rate (SGR) tended to increase with increasing moringa extract doses, although the differences were not statistically significant. This trend indicates that moringa supplementation may improve metabolic efficiency and dietary nutrient utilization. Bioactive compounds in moringa leaves—such as flavonoids, saponins, vitamins, and minerals—may enhance gut health and digestive enzyme activity, thereby facilitating more efficient nutrient assimilation (Sajina *et al.*, 2021; Moghadam *et al.*, 2021).

Improvement in Feed Conversion Ratio as the Key Finding

A significant reduction in feed conversion ratio (FCR) in moringa-supplemented treatments represents the major outcome of this study. The lowest FCR observed at 20 mL/100 g feed indicates that fish required less feed to achieve comparable weight gain. This agrees with previous evidence that *Moringa oleifera* supplementation can significantly improve feed efficiency in Nile tilapia and other aquaculture species (El-Son *et al.*, 2022; Reyes-Becerril *et al.*, 2021; Kamble *et al.*, 2024). Improved FCR is closely associated with enhanced digestive function and intestinal microbial balance. Moringa leaf extract may act as a natural prebiotic by modulating gut microbiota, promoting beneficial bacteria, and suppressing pathogenic microorganisms, thereby improving digestion and nutrient absorption (Kengkittipat *et al.*, 2025; Moghadam *et al.*, 2021).

Feed Utilization Efficiency and Consistency of Findings

The significant increase in feed utilization efficiency (EPP) in **P3 and P4** further supports the functional role of moringa leaf extract as an effective feed additive. High EPP values indicate a greater proportion of consumed feed being converted into fish biomass. The inverse relationship between low FCR and high EPP demonstrates internal consistency, as improvements in feed efficiency were concurrently reflected in both indicators. These findings support the concept that prebiotic effects may be detected first through improved feed utilization efficiency before producing statistically significant increases in absolute growth, as commonly reported in aquaculture nutrition studies (Elgendy *et al.*, 2021; Yostawonkul *et al.*, 2023).

Role of Water Quality in Supporting Experimental Outcomes

Water-quality parameters (temperature, pH, and dissolved oxygen) remained within optimal ranges for Nile tilapia culture. Such stable environmental conditions likely contributed to high survival and allowed fish to utilize feed efficiently. Consequently, the observed differences in growth trends and feed utilization parameters can be more confidently attributed to dietary treatments rather than environmental fluctuations.

Practical Implications and Aquaculture Sustainability

From a practical standpoint, the use of *Moringa oleifera* leaf extract as a natural dietary additive has the potential to enhance Nile tilapia production efficiency by improving feed utilization and reducing overall feed requirements. Additionally, the natural and environmentally friendly properties of moringa support the development of sustainable aquaculture by reducing reliance on synthetic additives and less sustainable conventional feed resources. Overall, these results strengthen the applicability and economic feasibility of moringa leaf extract as a functional feed additive for modern Nile tilapia farming systems.

Conclusion

Dietary supplementation with *Moringa oleifera* leaf extract for 40 days improved feed

utilization efficiency in Nile tilapia, as evidenced by a significant reduction in feed conversion ratio and increased feed utilization efficiency at medium to high supplementation levels. Although absolute growth parameters and specific growth rate did not differ significantly among treatments, the observed upward trends suggest a positive biological response to moringa supplementation. The relatively high survival rate and stable water quality indicate that *Moringa oleifera* leaf extract is safe and does not negatively affect the rearing environment. Overall, *Moringa oleifera* leaf extract shows promise as an eco-friendly natural feed additive to enhance production efficiency in Nile tilapia aquaculture. Further studies are recommended to elucidate the underlying physiological and microbiological mechanisms responsible for the prebiotic-like effects and to determine the optimal dosage under larger-scale farming conditions.

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