Grouper: Jurnal Ilmiah Perikanan
Vol. 17 No. 2 (2026) pp. 453 - 466
pISSN: 2086-8480 | eISSN: 2716-2702
Journal Homepage: https://grouper.unisla.ac.id/index.php/grouper

Effects of Combined EM4 and Molasses Doses on the Growth Performance of Nile Tilapia (Oreochromis niloticus) Cultured in a Biofloc System

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Submitted: 14 August 2026 Revised: 14 September 2026 Accepted: 28 September 2026

Keywords:
Biofloc; EM4; Molasses; Productivity; Oreochromis Niloticus

ABSTRACT

This study aims to analyze the effect of administering a combination of EM4 and molasses in a biofloc system on the growth of Nile tilapia (Oreochromis niloticus) and to determine the optimal dosage capable of improving aquaculture performance. The study used a Completely Randomized Design (CRD) with four treatments: P0 (control), P1 (15 mL/L), P2 (20 mL/L), and P3 (25 mL/L), each consisting of three replicates. The parameters observed included absolute weight gain, absolute length gain, Specific Growth Rate (SGR), productivity, Survival Rate (SR), Feed Conversion Ratio (FCR), and water quality. The data were analyzed using analysis of variance (ANOVA) after meeting the normality and homogeneity tests, while data that did not meet the parametric assumptions were analyzed using the Kruskal–Wallis test at a 95% confidence level. The results of the study showed that the administration of a combination of EM4 and molasses had a significant effect ($P<0.05$) on absolute weight gain, absolute length, SGR, productivity, and SR, but had no significant effect on FCR ($P>0.05$). Treatment P3 (25 mL/L) yielded the best performance with absolute weight gain 56.33 ± 1.15 g, absolute length of 13.54 ± 0.27 cm, SGR of 4.47 ± 0.14% per day, productivity of 5,633.33 g/m^2, SR of 100%, and the lowest FCR of 2.64 ± 0.40. Furthermore, water quality throughout the study remained within the optimal range for tilapia farming. Thus, the administration of a combination of EM4 and molasses at a dose of 25 mL/L in a biofloc system is recommended to enhance growth and the success of tilapia farming.

INTRODUCTION

Aquaculture is one of the fastest-growing food production sectors in the world and has become a major contributor to the global supply of aquatic animal foods for human consumption. In 2020, aquaculture accounted for 56% of the amount of aquatic animal food production available for human consumption, highlighting its increasing importance in meeting the growing global demand for aquatic foods (FAO, 2022). Nevertheless, efforts to increase aquaculture production still face various challenges, particularly poor water quality, the accumulation of organic waste, high concentrations of nitrogen compounds, and low feed utilization efficiency, which result in a high feed conversion ratio (FCR). These issues not only reduce aquaculture productivity but also increase the risk of eutrophication and water pollution (Anggana *et al.,* 2021; Sahendra *et al.,* 2023). In conventional aquaculture systems, water quality management still relies on periodic water changes, so aquaculture effluent cannot yet be optimally utilized as a nutrient source. This situation prevents fish growth, feed efficiency, and fish survival rates from reaching optimal levels.

This issue is also reflected in the development of Nile tilapia (Oreochromis niloticus) production in North Sumatra Province. Based on production statistics from the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia (KKP), Nile tilapia production in North

Sumatra was recorded at 93,656 metric tons in 2020 and increased to 170,026 metric tons in 2023. The KKP database also recorded 98,041 metric tons in 2024 for Nile tilapia production in North Sumatra. However, because the status of the 2024 figure as a final annual value could not be independently confirmed from the database interface, the 2024 value is presented here as a reported statistical figure rather than as evidence of a confirmed decline in annual production (KKP, 2024).

These fluctuations indicate that the increase in production has not been fully supported by efficient and sustainable aquaculture systems. High production during certain periods does not necessarily reflect increased productivity but may be influenced by intensified aquaculture practices that have not been balanced with optimal environmental quality management (Fradina & Latuconsina, 2022). A similar situation occurred in North Nias Regency, where tilapia (Oreochromis niloticus) production was recorded at only 5 metric tons in 2021 (KKP, 2024). This low production indicates that the adoption of aquaculture technologies capable of improving production efficiency particularly in water quality management and feed utilization remains very limited.

Various studies have developed sustainable aquaculture technologies to address these issues, one of which is through the implementation of the biofloc system. Biofloc technology utilizes the activity of heterotrophic bacteria capable of converting inorganic nitrogen compounds into microbial biomass, thereby allowing aquaculture waste to be reused as a natural feed source for fish. In addition to improving water quality, the biofloc system also reduces ammonia concentrations, decreases the need for water changes, improves feed utilization efficiency, and enhances fish growth and survival rates (Krummenauer *et al.,* 2024; McCusker *et al.,* 2023; Zaidy *et al.,* 2023; Zega *et al.,* 2024). Therefore, the biofloc system is viewed as an environmentally friendly aquaculture approach that can also increase tilapia production, particularly in areas with limited resources and high production costs.

The success of the biofloc system is greatly influenced by the carbon-to-nitrogen (C/N) ratio; therefore, the addition of carbon sources and probiotics is necessary to support the growth of heterotrophic bacteria. Effective Microorganisms 4 (EM4) is a probiotic containing various beneficial microorganisms, while molasses serves as a carbon source that accelerates the formation of microbial flocs. Several studies have reported that the combination of EM4 and molasses can accelerate biofloc formation, increase microbial activity, suppress the growth of pathogenic bacteria, and enhance fish growth (El-Naga *et al.,* 2024; Kavitha & Krishna, 2022). However, these studies generally still focus on the effectiveness of bioflocs or the use of probiotics in general, while information regarding the most effective dosage of the EM4 and molasses combination to improve tilapia aquaculture productivity remains very limited.

Variations in EM4 and molasses dosages have the potential to affect microbial community dynamics, the rate of biofloc formation, water quality stability, feed utilization efficiency, and fish growth. Dosages that are too low can inhibit microbial activity, while dosages that are too high may lead to excessive floc formation, changes in water quality, and an imbalance in the aquaculture ecosystem (Nurfaidah *et al.,* 2024; Vatria *et al.,* 2023; Zaidy *et al.,* 2023; Zega *et al.,* 2025). To date, there have been few studies that comprehensively evaluate the effects of various combined doses of EM4 and molasses on tilapia aquaculture productivity indicators including growth, feed efficiency, survival rate, productivity, and water quality within a single integrated research design.

Based on these conditions, this study proposes an approach involving the application of a biofloc system with four dosage levels of a combination of EM4 and molasses: control (0 mL/m^3), 15 mL/m^3, 20 mL/m^3, and 25 mL/m^3. This approach is designed to evaluate the effect of varying dosages on tilapia aquaculture productivity by measuring growth, feed conversion efficiency, survival rate, productivity, and water quality within a single integrated aquaculture system.

The novelty of this study lies in its comprehensive evaluation of various combination doses of EM4 and molasses on tilapia aquaculture productivity through measurements of absolute growth, absolute length, specific growth rate (SGR), feed conversion ratio (FCR),

survival rate (SR), productivity, and water quality under aquaculture conditions in North Nias Regency. Unlike previous studies, which generally focused only on fish growth or water quality separately, this study integrates biological indicators, feed efficiency, productivity, and environmental quality into a single research framework, thereby providing more comprehensive information regarding the optimal dosage of the EM4 and molasses combination in a biofloc system.

Based on the above description, this study aims to analyze the effect of administering a combination of EM4 and molasses at various doses in a biofloc system on the productivity of tilapia (*Oreochromis niloticus*) aquaculture, as well as to determine the most effective dose for improving growth, feed conversion efficiency, survival rate, productivity, and water quality. The results of this study are expected to provide a scientific contribution to the development of biofloc technology in freshwater fish farming while also serving as practical recommendations for farmers to improve tilapia farming productivity in a sustainable manner.

METHOD

Research Period and Location

This study was conducted from February to March 2026 in Siofabanua Village, Tuhemberua Subdistrict, North Nias Regency, North Sumatra. Water quality analysis was conducted during the maintenance period, while data processing and analysis were carried out at the Laboratory of the Aquatic Resources Study Program, Faculty of Science and Technology, University of Nias.

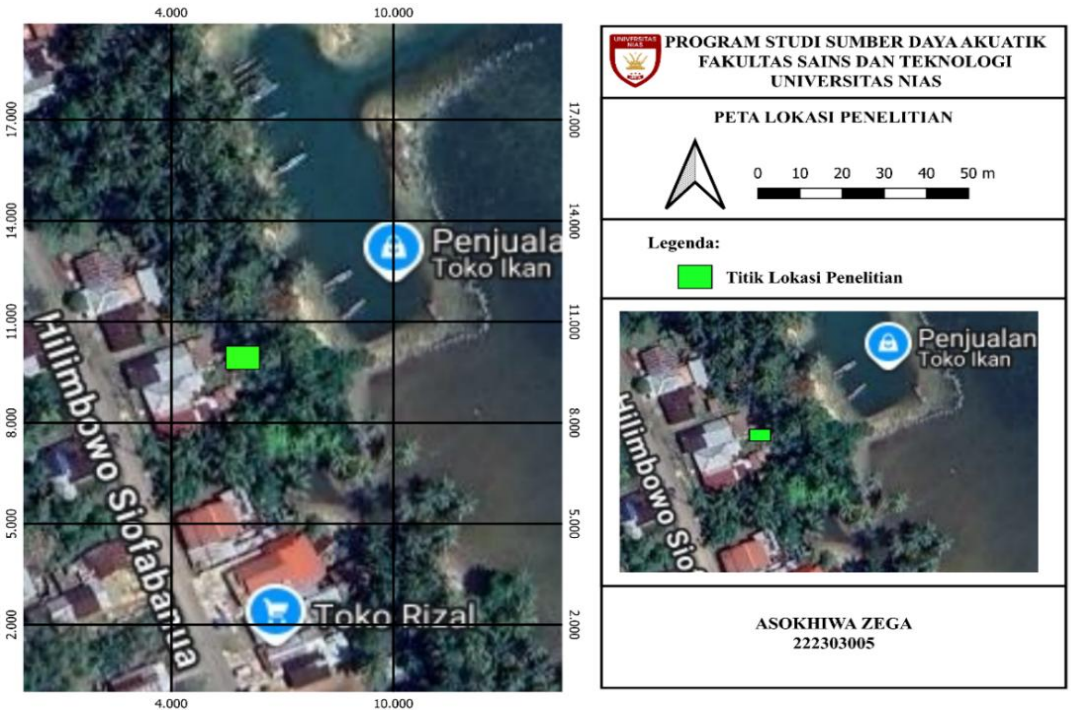


Figure 1. Map of the Study Area, 2026
Source: QGIS (2026)

Research Design

This study employed an experimental method using a Completely Randomized Design (CRD) consisting of four treatments and three replicates, resulting in 12 experimental units. The treatments involved varying doses of a combination of Effective Microorganisms 4 (EM4) and molasses in the biofloc system, as follows:

a) P0 = Control (0 mL/m^3)
b) P1 = EM4–Molasses 15 mL/m^3

c) P2 = EM4–Molasses 20 mL/m^3
d) P3 = EM4–Molasses 25 mL/m^3

Each experimental unit was assigned randomly to minimize the influence of environmental factors outside the treatments.

Preparation of the Culture Medium and Test Fish

The rearing tanks were first cleaned and filled with water to the required culture volume, followed by continuous aeration for 24 h. EM4 and molasses were subsequently added according to the assigned treatment levels to initiate biofloc development. The culture media were maintained under continuous aeration prior to fish stocking. Because specific indicators of biofloc development, such as total suspended solids, settling volume, floc volume, or C/N ratio, were not measured during the preparation stage, biofloc formation was not defined using a quantitative floc-development threshold.

The test fish were Nile tilapia (Oreochromis niloticus) fingerlings with an initial length range of 8–10 cm and an initial body-weight range of 10–12 g/fish. Before stocking, the fingerlings were acclimated to the experimental culture conditions for approximately 30 min. The experiment consisted of four treatments with three replicates, resulting in 12 experimental units. Each experimental unit was stocked with 10 fingerlings; therefore, a total of 120 fish were used in the experiment. Stocking density was expressed based on the actual culture volume of each rearing tank rather than as the number of fish per container.

Fish Rearing

During the study, the fish were reared for 42 days and fed commercial feed amounting to 3% of the total biomass three times daily in the morning, at noon, and in the evening. Aeration was provided for 24 hours to maintain the stability of the biofloc and ensure adequate dissolved oxygen levels. Water quality was monitored periodically, including temperature, pH, dissolved oxygen (DO), and ammonia (NH_3).

Observation Parameters

The parameters observed included absolute weight gain, absolute length gain, specific growth rate (SGR), feed conversion ratio (FCR), survival rate (SR), aquaculture productivity, and water quality.

Absolute Weight Gain

bsolute weight gain was calculated to evaluate the increase in body weight of Nile tilapia during the rearing period. Absolute weight gain was calculated as the difference between the average final body weight and the average initial body weight, using the following formula:

$$G = Wt - W0$$

Notes:
W = Absolute weight gain (g)
Wt = Average final weight (g)
W_0 = Average initial weight (g)

Absolute Length Gain

Absolute length growth is calculated using the formula:

$$Pm = Lt - Lo$$

Definitions:
L = Absolute length growth (cm)
Lt = Average final length (cm)
L_0 = Average initial length (cm)

Specific Growth Rate (SGR)

This parameter reflects the physiological efficiency of fish in utilizing feed and environmental conditions for optimal growth (Zaidy *et al.,* 2022). Mathematically, the specific growth rate can be expressed by the following formula:

$$\text{SGR}(\%) = \frac{In\ \text{Wt} - \text{In Wo}}{t} x100$$

Notes:
SGR = Specific growth rate (% day^{-1})
Wt = Average final weight (g)
W_0 = Average initial weight (g)
t = Rearing period (days)

Survival Rate (SR)

Survival Rate (SR) is an indicator of fish physiological health and the stability of the aquatic ecosystem (Sahendra *et al.,* 2023). The survival rate can be calculated using the following formula:

$$\text{SR}(\%) = \frac{N\text{t}}{N0} x100$$

Notes:
Nt = Number of live fish at the end of the study (individuals)
N_0 = Number of fish at the start of the study (individuals)

Feed Conversion Ratio (FCR)

The Feed Conversion Ratio (FCR) measures the efficiency of converting feed into biomass. A low FCR value indicates high feed efficiency, which is typically influenced by the availability of natural feed in the form of microbial flocs. Mathematically, the FCR value can be calculated using the following formula:

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

Definitions:
F = Amount of feed provided (g)
Wt = Final biomass (g)
W_0 = Initial biomass (g)
D = Biomass of dead fish (g)

Cultivation Productivity

Cultivation productivity is calculated using the following equation:

$$Productivity = \frac{Wt - Wo}{L}$$

Notes:
P = Cultivation productivity (g m^{-2})
Wt = Final biomass (g)
W_0 = Initial biomass (g)
L = Area of the rearing tank (m^2)

Water Quality

The water quality parameters observed included temperature (°C), pH, dissolved oxygen (DO), and ammonia (NH_3). Measurements were conducted periodically throughout the rearing period to monitor water quality and fish growth. Fish growth parameters, including body weight and length, were measured at predetermined sampling intervals using calibrated measuring instruments. Water quality parameters, including temperature, pH, dissolved oxygen (DO), and ammonia (NH_3), were also monitored during the rearing period. All measurements were performed consistently across the experimental tanks using the same measurement procedures and instruments. The instruments were checked and calibrated before use in accordance with the applicable laboratory operating procedures. Water samples for ammonia analysis were collected from each experimental tank at the designated sampling

time and analyzed using the ammonia measurement method applied in the laboratory. Each treatment was observed according to its experimental replication to ensure consistency and comparability of the measurements.

Data Analysis

Data were analyzed using IBM SPSS Statistics version 26. Before the main analysis, all data were tested for normality using the *Shapiro–Wilk* test and for homogeneity of variances using Levene's test. Data that met the assumptions of normality and homogeneity were analyzed using One-Way Analysis of Variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$). If the ANOVA results indicated a significant effect ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was conducted to determine differences between treatments.

RESULTS AND DISCUSSION

Absolute Weight Gain

Absolute weight gain is one of the key parameters used to evaluate the effectiveness of EM4 and molasses dosing in a biofloc system on tilapia growth. The average absolute weight gain of tilapia during the rearing period is presented in Table 1.

Table 1. Absolute Weight Gain of Nile Tilapia (Oreochromis niloticus) Reared Under Different EM4-Molasses Dosages in a Biofloc System

Treatment	EM4-Molasses Dosage (mL L^{-1})	Mean ± SD (cm)
P0	Kontrol	31,37 ± 0,06
P1	15	38,43 ± 1,00
P2	20	45,63 ± 0,55

Source: Research data (2026), adapted from the author's research.

Table 1 shows that absolute weight gain increased as the EM4-molasses dosage increased. Treatment P3 produced the highest weight gain, whereas the control treatment (P0) resulted in the lowest value. Statistical analysis indicated that the treatments had a significant effect on fish growth ($P < 0.05$), demonstrating that different EM4-molasses dosages influenced biomass accumulation during the culture period.

The differences in growth performance among treatments are more clearly illustrated in Figure 2.

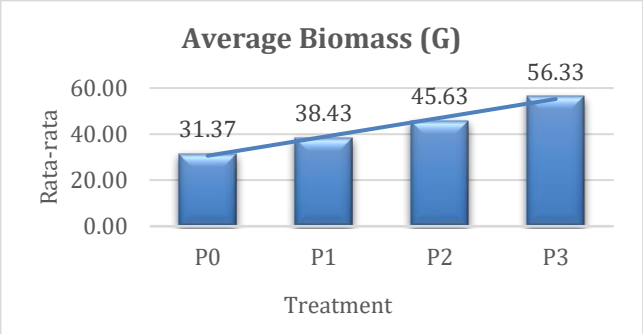


Figure 2. Absolute Weight Gain of Nile Tilapia (*Oreochromis niloticus*) Under Different EM4-Molasses Dosages
Source: Research data (2026), adapted from the author's research.

Figure 2 demonstrates a consistent increase in absolute weight gain from the control treatment to P3, indicating that higher EM4 molasses dosages improved fish growth under the biofloc system.

The highest increase in absolute weight was observed in treatment P3 (25 mL), while the control treatment (P0) yielded the lowest absolute weight. This difference indicates that

increasing the doses of EM4 and molasses had a significant effect on the weight gain of tilapia during the rearing period. These results indicate that increasing the doses of EM4 and molasses can enhance the effectiveness of the biofloc system in supporting tilapia growth. As the dose increased up to treatment P3, the nutrient recycling process within the culture medium became more optimal, allowing more nutrients to be utilized for fish biomass formation. These findings demonstrate that the biofloc system not only functions as a water quality regulator but also as a source of additional nutrients that support fish growth.

This increase in growth may be associated with the combined application of EM4 and molasses, which may support the development and activity of heterotrophic microorganisms in the biofloc system. Molasses can serve as an additional carbon source for microorganisms, while EM4 provides a microbial consortium that may contribute to the decomposition of organic matter and the utilization of nitrogenous compounds originating from fish waste and uneaten feed. These processes may support the formation and development of microbial aggregates (biofloc) within the culture system.

The potential contribution of biofloc to fish growth can be explained by its role as an additional natural food source in biofloc-based aquaculture systems. Microbial aggregates may contain nutrients that can be utilized by cultured fish, potentially improving nutrient utilization and contributing to growth performance. However, in the present study, the C/N ratio, microbial biomass, biofloc composition, protein content, biofloc ingestion, and digestive activity were not directly measured. Therefore, the proposed mechanisms should be considered possible explanations supported by the general principles of biofloc technology rather than direct evidence obtained from the present experiment. The observed improvement in growth performance may thus be related to the combined effects of EM4 and molasses, although the specific microbial and nutritional mechanisms require further investigation.

The results of this study are consistent with research (Putra *et al.,* 2022; Putri *et al.,* 2026; Rattu *et al.,* 2021) indicating that the addition of carbon sources to a biofloc system can increase the activity of heterotrophic bacteria, thereby accelerating biofloc formation and improving nitrogen utilization efficiency. Previous studies have reported that the combined application of probiotics and carbon sources can improve water quality and support fish growth by promoting microbial biomass production, which may serve as a supplementary food source for cultured fish. These findings are consistent with the principles of biofloc technology, in which microbial communities play an important role in recycling organic waste and converting nutrients into microbial biomass. Therefore, the effectiveness of a biofloc-based culture system may be influenced by the development and activity of microbial communities in utilizing organic waste and maintaining water quality. However, the microbial biomass and its nutritional composition were not directly measured in the present study; therefore, these mechanisms are considered possible explanations rather than direct experimental findings.

These findings indicate that the use of EM4 and molasses at a dose of up to 25 mL was the most effective treatment for increasing the absolute weight gain of tilapia under the conditions of this study. Scientifically, these results reinforce the concept that optimizing the microbial community through the adjustment of probiotic doses and carbon sources can improve nutrient cycle efficiency, enhance the quality of the aquaculture medium, and support sustainable fish productivity. These findings also provide a foundation for the development of more efficient and environmentally friendly biofloc-based aquaculture strategies.

Absolute Length Growth

Absolute length growth is an important indicator of linear fish growth and reflects the ability of cultured fish to utilize available nutrients during the rearing period. This parameter was used to evaluate the effect of different EM4-molasses dosages on the body length development of Nile tilapia reared under a biofloc system. The mean absolute length growth of Nile tilapia during the experimental period is presented in Table 2.

Table 2. Mean Absolute Length Growth of Nile Tilapia (*Oreochromis niloticus*) Reared Under Different EM4-Molasses Dosages in a Biofloc System

Treatment	EM4–Molasses Dosage (mL L^{-1})	Mean ± SD (cm)
P0	Kontrol	8,89 ± 0,01
P1	15	10,59 ± 0,31
P2	20	11,72 ± 0,12
P3	25	13,54 ± 0,27

Source: Processed research data (2026).

As shown in Table 2, EM4–molasses supplementation significantly affected the absolute length growth of Nile tilapia ($P < 0.05$). Absolute length increased progressively with increasing EM4–molasses dosage, with treatment P3 producing the greatest length gain, whereas the control treatment (P0) resulted in the lowest growth.

The differences in absolute length growth among treatments are further illustrated in Figure 3.

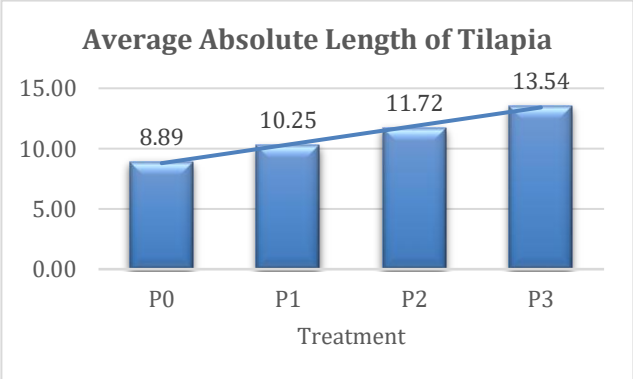


Figure 3. Absolute Length Growth of Nile Tilapia (*Oreochromis niloticus*) Reared Under Different EM4–Molasses Dosages in a Biofloc System
Source: Processed research data (2026).

Figure 3 demonstrates a consistent increase in absolute length growth from the control treatment to P3, indicating that increasing the EM4–molasses dosage promoted a positive linear growth response throughout the culture period. The superior absolute length growth observed in treatment P3 indicates that increasing the EM4–molasses dosage enhanced the effectiveness of the biofloc system in supporting the linear growth of Nile tilapia. This response suggests that the higher dosage created a more favorable microbial environment, allowing fish to utilize available nutrients more efficiently for tissue development and skeletal growth. Consequently, the improvement in fish length reflects not only enhanced nutrient availability but also better physiological performance under biofloc culture conditions.

This growth response is likely associated with the synergistic interaction between EM4 and molasses in optimizing the carbon-to-nitrogen (C/N) ratio within the culture system. Molasses serves as a readily available carbon source that stimulates the proliferation of heterotrophic bacteria, while EM4 supplies beneficial microorganisms capable of converting dissolved inorganic nitrogen, particularly ammonia originating from fish excretion and uneaten feed, into microbial biomass. This process reduces the accumulation of toxic nitrogen compounds while simultaneously producing microbial flocs rich in protein, amino acids, vitamins, and digestive enzymes. The continuous availability of these microbial aggregates as a natural food source improves nutrient utilization efficiency, supports protein synthesis, and promotes the formation of new muscle and skeletal tissues, ultimately resulting in greater absolute length growth.

The present findings are consistent with those reported by Anggana *et al.,* (2021) who demonstrated that the application of biofloc technology combined with probiotics improved

fish growth by enhancing microbial activity and nutrient utilization. Similarly, Ardiansyah *et al.,* (2026) reported that probiotic supplementation in biofloc systems enhanced the growth performance of Nile tilapia by improving water quality and increasing the efficiency of nutrient conversion into fish biomass. In addition, Aini *et al.,* (2025) found that probiotic supplementation promoted better fish growth through improved microbial balance and more efficient metabolic processes. The agreement between the present study and previous reports indicates that the effectiveness of biofloc technology largely depends on maintaining an active heterotrophic microbial community capable of converting organic waste into nutritionally valuable microbial biomass.

Overall, the results demonstrate that the application of 25 mL L^{-1} EM4-**molasses** provided the most favorable conditions for improving the absolute length growth of Nile tilapia under the experimental conditions. These findings reinforce the concept that optimizing probiotic and carbon-source application enhances microbial nutrient recycling, improves culture water quality, and increases fish productivity, thereby supporting the sustainable implementation of biofloc technology in Nile tilapia aquaculture.

Specific Growth Rate (SGR)

Specific growth rate (SGR) was used to evaluate the daily growth response of Nile tilapia under different EM4–molasses dosages in the biofloc system. The mean SGR values obtained during the 42-day culture period are presented in Table 3.

Table 3. Specific Growth Rate of Nile Tilapia (Oreochromis niloticus) Reared Under Different EM4–Molasses Dosages in a Biofloc System

Treatment	EM4–Molasses Dosage (mL L^{-1})	SGR (% day^{-1})
P0	Control	3.61 ± 0.12
P1	15	4.01 ± 0.31
P2	20	4.06 ± 0.13
P3	25	4.47 ± 0.14

Values are presented as mean ± standard deviation (SD).
Source: Processed research data (2026).

The SGR increased progressively with increasing EM4–molasses dosage, with the highest value observed in P3 and the lowest in P0. The analysis of variance confirmed that the treatment significantly affected SGR (F = 10.20; P = 0.004). The pattern of SGR among treatments is illustrated in Figure 4.

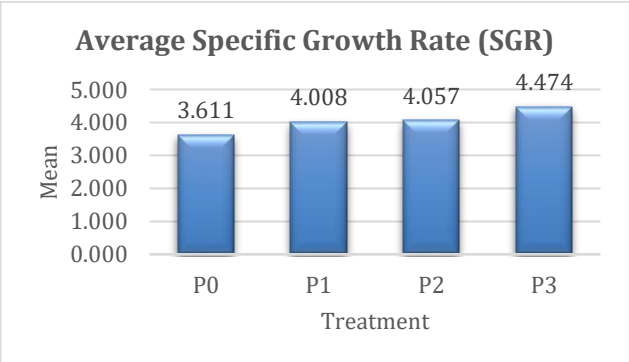


Figure 4. Specific Growth Rate of Nile Tilapia (Oreochromis niloticus) Reared Under Different EM4–Molasses Dosages
Source: Processed research data (2026).

The progressive increase in Specific Growth Rate (SGR) from the control to P3 demonstrates that increasing the EM4–molasses dosage improved the growth efficiency of Nile tilapia under biofloc conditions. This response is attributable to the complementary roles of

molasses as a readily available carbon source and EM4 as a source of beneficial microorganisms, which stimulate heterotrophic bacterial activity, enhance nitrogen assimilation into microbial biomass, and provide additional microbial nutrients for fish. Consistent with Zaidy et al. (2023) and Siregar et al. (2025), these processes improve nutrient recycling and utilization, allowing a greater proportion of assimilated nutrients to support tissue growth. Thus, the 25 mL L^{-1} treatment (P3) provided the most favorable microbial and nutritional conditions and was the most effective dosage for enhancing Nile tilapia growth, directly addressing the objective of determining the optimal EM4–molasses dosage in the biofloc system.

Survival Rate (SR)

Survival rate (SR) was used to evaluate the ability of Nile tilapia to survive throughout the 42-day culture period under different EM4–molasses dosages. The mean survival rate of Nile tilapia is presented in Table 4.

Table 4. Survival Rate of Nile Tilapia (Oreochromis niloticus) Reared Under Different EM4–Molasses Dosages in a Biofloc System

Treatment	EM4–Molasses Dosage (mL L^{-1})	Survival Rate (%)
P0	Control	94.00 ± 0.00
P1	15	97.00 ± 0.00
P2	20	98.67 ± 2.31
P3	25	100.00 ± 0.00

Values are presented as mean ± standard deviation (SD).
Source: Processed research data (2026).

Survival rate increased progressively with increasing EM4–molasses dosage, from 94.00% in the control to 100.00% in P3. The analysis of variance demonstrated that the treatments significantly affected survival rate ($F = 15.06$; $P = 0.001$). The variation in survival rate among treatments is illustrated in Figure 5.

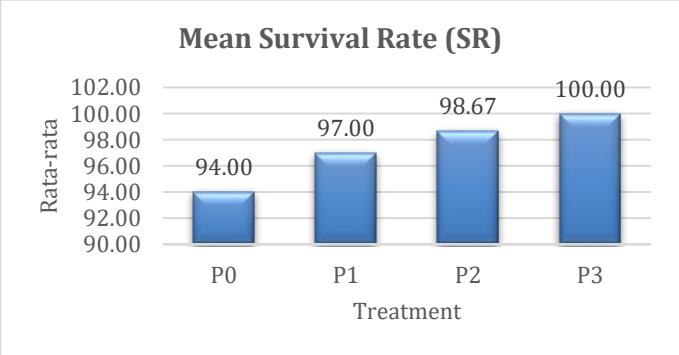


Figure 5. Survival Rate of Nile Tilapia (Oreochromis niloticus) Reared Under Different EM4–Molasses Dosages in a Biofloc System
Source: Processed research data (2026).

The progressive increase in survival rate indicates that EM4–molasses supplementation improved the biological stability of the biofloc system, with the 25 mL L^{-1} treatment (P3) achieving 100% survival, the highest value among the tested treatments. This response is likely associated with enhanced heterotrophic bacterial activity stimulated by molasses and beneficial microorganisms supplied by EM4, which promote nitrogen assimilation into microbial biomass and reduce the accumulation of potentially toxic nitrogenous compounds. The resulting improvement in water conditions can reduce physiological stress and support normal metabolic functions. This finding is consistent with El-Naga et al. (2024) and Dewi and Ulfah (2022), who reported that probiotic application and EM4

supplementation can improve fish survival through better microbial balance and culture conditions. Thus, the 25 mL L^{-1} EM4–molasses treatment was the most effective dosage for maintaining Nile tilapia survival under the experimental conditions, directly supporting the objective of identifying the most effective dosage for improving biofloc-based tilapia production (Hamka *et al.,* 2026).

Feed Conversion Ratio (FCR)

Feed Conversion Ratio (FCR) was used to evaluate feed utilization efficiency in Nile tilapia under different EM4–molasses dosages. The mean FCR values are presented in Table 5.

Table 5. Feed Conversion Ratio of Nile Tilapia (Oreochromis niloticus) Reared Under Different EM4–Molasses Dosages in a Biofloc System

Treatment	EM4–Molasses Dosage (mL L^{-1})	FCR
P0	Control	23.27 ± 4.03
P1	15	22.86 ± 16.02
P2	20	10.10 ± 12.25
P3	25	2.64 ± 0.40

Values are presented as mean ± standard deviation (SD).
Source: Processed research data (2026).

The FCR values showed a descriptive tendency to decrease with increasing EM4–molasses dosage, with the lowest mean value observed in treatment P3 (2.64 ± 0.40). However, the Kruskal–Wallis test showed that the differences in FCR among treatments were not statistically significant (P = 0.076). Thus, although P3 exhibited the lowest numerical FCR value, the observed differences among treatments were insufficient to demonstrate a statistically significant effect of the EM4–molasses dosage on feed conversion. The variation in FCR values among treatments is presented in Figure 6.

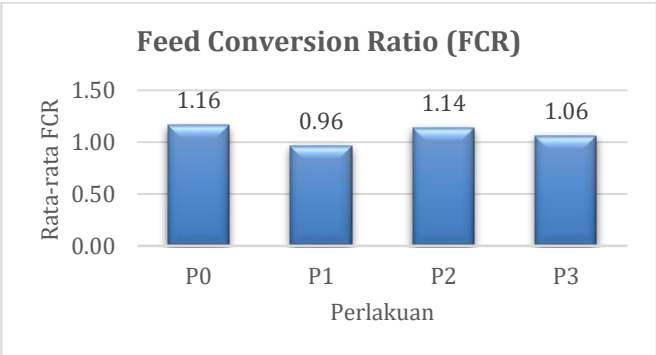


Figure 6. Feed Conversion Ratio of Nile Tilapia (*Oreochromis niloticus*) Reared Under Different EM4–Molasses Dosages in a Biofloc System
Source: Processed research data (2026).

The lower FCR observed at higher EM4–molasses dosages, particularly 2.64 ± 0.40 in P3, suggests improved feed utilization through biofloc-mediated nutrient recycling; however, the difference among treatments was not statistically significant (P = 0.076), indicating that EM4–molasses did not significantly alter feed conversion efficiency under the experimental conditions. The numerical improvement may be related to microbial biomass generated from recycled nutrients, which can provide supplementary nutrition and complement formulated feed, although its effect on FCR depends on feed intake, individual growth variation, and biofloc consumption. Thus, while 25 mL L^{-1} (P3) produced the most favorable numerical FCR, the treatment cannot be considered a statistically proven improvement in feed efficiency, and its main advantage in this study was more clearly reflected in growth and survival performance.

Productivity

Productivity was evaluated to determine the capacity of the biofloc system to convert the available production resources into Nile tilapia biomass. The mean productivity values under different EM4–molasses dosages are presented in Table 6.

Table 6. Productivity of Nile Tilapia (*Oreochromis niloticus*) Reared Under Different EM4–Molasses Dosages in a Biofloc System

Treatment	EM4–Molasses Dosage (mL L^{-1})	Productivity (g m^{-2})
P0	Control	2,895.47 ± 43.81
P1	15	3,701.53 ± 49.73
P2	20	4,489.33 ± 130.93
P3	25	5,633.33 ± 84.71

Values are presented as mean ± standard deviation (SD).
Source: Processed research data (2026)

Productivity increased progressively from 2,895.47 g m^{-2} in P0 to 5,633.33 g m^{-2} in P3, demonstrating that increasing EM4–molasses dosage enhanced biomass production under the biofloc system. This improvement is consistent with the higher weight gain, SGR, and survival observed in P3 and is likely driven by enhanced heterotrophic bacterial activity, nutrient recycling, and microbial biomass formation stimulated by the carbon supplied by molasses and microorganisms from EM4. The resulting biofloc provides additional nutrients while improving the utilization of nitrogenous wastes, thereby supporting more efficient conversion of system nutrients into fish biomass. Consistent with Sahendra et al. (2023) and Ardiansyah et al. (2026), these findings demonstrate that 25 mL L^{-1} (P3) was the most effective EM4–molasses dosage for maximizing Nile tilapia productivity under the experimental conditions, directly addressing the objective of determining the optimal dosage for improving biofloc-based production.

Water Quality Parameters

Water quality was monitored to assess whether the EM4-molasses treatments maintained environmental conditions suitable for Nile tilapia and to clarify the mechanism underlying the observed growth and survival responses. The measured parameters temperature, pH, dissolved oxygen (DO), and ammonia are summarized in Table 7.

Table 7. Water Quality Parameters of Nile Tilapia (*Oreochromis niloticus*) Reared Under Different EM4–Molasses Dosages in a Biofloc System

Parameter	Unit	P0 Control	P1 15 mL L^{-1}	P2 20 mL L^{-1}	P3 25 mL L^{-1}	Optimal Range
Temperature	°C	29.07 ± 0.15	28.71 ± 0.07	28.51 ± 0.05	28.30 ± 0.06	25–32
pH	-	7.80 ± 0.09	8.02 ± 0.06	8.14 ± 0.07	8.19 ± 0.04	6.5–8.5
DO	mg L^{-1}	5.11 ± 0.12	5.75 ± 0.06	6.35 ± 0.03	6.99 ± 0.07	>3
NH_3	mg L^{-1}	1.03 ± 0.13	0.51 ± 0.10	0.38 ± 0.04	0.17 ± 0.00	<0.168

Values are presented as mean ± standard deviation (SD).
Source**:** Processed research data (2026)

Water quality remained suitable for Nile tilapia, with temperature, pH, and DO ranging from 28.30–29.07°C, 7.80–8.19, and 5.11–6.99 mg L^{-1}, respectively. The most notable response was the 83.5% reduction in ammonia, from 1.03 mg L^{-1} in P0 to 0.17 mg L^{-1} in P3, indicating enhanced microbial utilization of nitrogen promoted by EM4–molasses. Molasses supplied carbon for heterotrophic bacterial growth, while EM4 supported microbial activity and nitrogen assimilation into biofloc biomass. This finding agrees with Angriani et al. (2020) and Rinanto et al. (2025), emphasizing the importance of stable water quality for fish physiological performance. Overall, 25 mL L^{-1} EM4–molasses produced the most favorable water-quality

conditions, particularly through improved DO and reduced ammonia, supporting the superior growth and survival observed in P3.

CONCLUSION

The application of EM4–molasses in a biofloc system significantly improved absolute weight gain, absolute length growth, specific growth rate (SGR), productivity, and survival rate of Nile tilapia (Oreochromis niloticus), while its effect on feed conversion ratio (FCR) was not statistically significant despite a descriptive improvement at higher dosages. Among the tested treatments, 25 mL L^{-1} (P3) was the most effective dosage, producing the highest growth, SGR, productivity, and survival, as well as the lowest numerical FCR. These findings indicate that appropriate EM4–molasses supplementation can enhance the overall biological performance of Nile tilapia and support more productive biofloc-based aquaculture.

ACKNOWLEDGEMENT

The author would like to express his sincere gratitude to the Nias Higher Education Foundation and Universitas Nias for providing a full scholarship that enabled him to complete his bachelor's degree in Fisheries.

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