The Effect of EM-4 and ST Probiotic Fortification on the Growth of Tilapia (Oreochromis niloticus) in the Biofloc-Aquaponic System

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The Effect of EM-4 and ST Probiotic Fortification on the Growth of Tilapia (*Oreochromis niloticus*) in the Biofloc-Aquaponic System

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Abstract.Tilapia (*Oreochromis niloticus*) is a fish that is in great demand by the public as a source of protein with low cholesterol with a nutritional content of 17.7% protein and 1.3% fat. So it is necessary to innovate to increase production and meet market needs. Probiotics are one of the supporting factors for success in tilapiacultivation. The aims of this study were to determine the effect of probiotic fortification of EM-4 and ST on the growth rate of tilapia in the Biofloc-Aquaponic system. This study used a CompletelyRandomizedDesign (CRD) with three treatments and three replications, namely P0 (without probiotic), P1 (EM-4 probiotic), P2 (ST probiotic). The data obtained were analyzed using the Analysis of Variance ANOVA then continued with the BNT Test. The results showed that the highest growth rate was in treatment P2 with a weight gain of 37.81 g and a length growth of 13 cm, then followed by treatment P1 with a weight gain of 26.66 g and a length growth of 11 cm and the lowest growth found in the P0 treatment with a weight gain of 18.47 g and a length growth of 10 cm. The results showed that the administration of probiotics in the Biofloc-Aquaponic system was significantly different ($p<0.05$). From the results of the study,it can be concluded that probiotic fortification can affect the growth of weight and length of tilapia.in biofloc-aquaponic systems.

Key Words:Probiotics, Growth, Tilapia, Biofloc-Aquaponics.

Introduction.International market demand for tilapia reaches 200,000 tons/year (Wijaya, 2011). Tilapia as a fish commodity has a very important economic value. Tilapia has several advantages, including being easily cultivated and favored by consumers (Christinet al.,2021). Tilapia is also a fish that is in great demand by the public so that market demand increases. So far, Indonesia is known as the largest Tilapia exporting country, which is around 10 million tons/year (Nugroho et al., 2013) therefore the community's need for tilapia continues to increase.

The increase in tilapia production causes an increase in the area of cultivation and water use. The limited land and available water sources need to find a solution so that cultivation activities can continue to develop, one of which is the application of an biofloc-aquaponic system. The existence of biofloc technology is able to utilize nitrogen waste into high protein feed through the growth of heterotrophic bacteria in aquaculture ponds. Microbes that are included in heterotrophic bacteria are from the genera *Mycobacterium*, *Streptomyces*, *Agrobacterium*, *Bacillus* and *Pseudomonas*. Umasugi et al., (2018)suggested that the use of *Bacillus* sp can improve water quality because it can decompose organic matter, suppress the growth of pathogens and balance the microbial community so as to provide a better environment for fish. In addition, the aquaponics system is able to save land and water use and increase business efficiency through the utilization of nutrients from leftover feed and fish metabolism. The aquaponic system is able to remodel ammonia through the process of oxidation to nitrite by bacteria *Nitrosomonas* which is then oxidized under aerobic conditions to nitrate by bacteria *Nitrobacter* (Saptarini, 2010). The main function of this system is to optimize water function and water bioremediation using plants in fish farming systems (Nugroho et al., 2012). This biofloc-aquaponic system has a basic principle that benefits fish and plants from the utilization of nitrogenous waste which becomes nutritious feed for fish and nutrients from leftover feed and fish manure that can be used as fertilizer for plants. So that the combination of biofloc technology and aquaponics system is not only capable of optimizing land and water use, it is also able to optimize nutrients to increase the growth rate of tilapia. The-Eco Friendly Biofloc-Aquaponic system in tilapia rearing is able to minimize the occurrence of aquaculture waste, there by preventing pollution.

The increasing market demand must also be balanced with an increase in the productivity of tilapia aquaculture. One alternative that can be done by tilapia farmers is by giving probiotics. Probiotics are microbial substances that are used as additional nutrients that have the advantage of being able to improve the balance of the microflora of the host's digestive tract. The purpose of giving probiotics is to increase the digestibility of fish by increasing digestive enzymes that can hydrolyze proteins into simpler compounds so that they are more easily absorbed and used as growth deposits. Probiotics can regulate the microbial environment in the intestines, blocking pathogenic microorganisms in the intestines by releasing enzymes that help the process of digestion of food (Istiqomah et al., 2019; Rostika et al., 2020). Several studies have proven that the application of probiotic technology plays a role in improving water quality, increasing biosecurity, increasing productivity. Increasing feed efficiency and decreasing production costs through reducing feed costs (Avnimelech and Kochba, 2009; Salamah, S., & Zulpikar, Z. 2020; Dewi, ERS & Maria U., 2022). One of the factors supporting the success of probiotics in increasing the growth rate is the activity of photosynthetic bacteria, *Lactobacillus* sp., *Actinomycetes* sp., *Streptomycetes* sp., and yeast contained in probiotics. These microorganisms are able to produce digestive enzymes and compounds that can increase the growth of tilapia.

In this study, two types of probiotics are often used in cultivation, namely probiotic EM-4 (Effective Microorganisms) and probiotic ST (Sukses Tani). These two probiotics have each different content to increase the growth rate of fish. Probiotics EM4 in the form of a brown liquid and sweet and sour smell (fresh) which can be used as an addition to optimize the utilization of food substances because the bacteria contained in EM-4 can digest cellulose, starch, sugar, protein, fat (Surung, 2008). In the probiotic EM-4, there are several microorganisms that support the growth of fish such as lactic acid and other fermentative microorganisms. The fermenters in EM-4 probiotics secrete exogenous enzymes such as amylase, lipase, amylase and cellulase. These enzymes degrade complex nutrients that make up feed into simpler nutrients. So that it can facilitate the digestive process of fish and increase the rate of fish growth. ST probiotic is a bioactivator containing *Lactobacillus*, *Actinomycetes*, *Streptomycetes*, and photosynthetic bacteria obtained from the isolation of moist soil in the forest, grass roots, and cow colon (Indriani, 2005). Bacteria *Lactobacillus* can increase the digestibility of fish to feed so that it can stimulate fish growth (Bukhori,, 2020). The ST probiotic also contains bacteria *Nitrobacter* which play an important role in the nitrification process in aquaculture ponds.

The basic principle in this biofloc-aquaponic system is that food residues and fish manure that have the potential to worsen water quality can be used as fertilizer for aquatic plants. There are various types of probiotics that are marketed to support growth, especially in the growth rate of fish weight and length. Therefore, the author will examine the comparison of probiotics of EM4 and ST types fortified in tilapia in an biofloc-aquaponic system. The-Eco Friendly Biofloc-Aquaponic system as an appropriate technology can help farmers to produce tilapia in high quantity and quality

Materials and Method. Thesubjects used in this study were local tilapia with an initial length of 5-7 cm as many as 200 fish/pond. This research was conducted in May – September 2020 at the Biology Education Laboratory Campus 3 Universitas PGRI Semarang and measurements of dissolved oxygen, ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3) carried out at the Chemistry Laboratory, Faculty of Science and Mathematics, Satya Wacana Christian University Salatiga.

This study used a Completely Randomized Design (CRD) with three treatments and three replications, namely P0 treatment without using probiotic treatment (control), P1 using EM4 probiotic treatment and P2 using ST probiotic treatment (10 ml/m3).The variables observed in this study were the observation of the growth rate of tilapia as primary data and the observation of water quality as secondary data. Variables observed for the growth rate of tilapia included the growth rate of fish weight and length. Measurements of weight (WG) and length gain (LG) followed Purbomartono et al., (2022) guidelines by calculating the final weight (Wt) and length (Lt) reduced with the initial weight (Wo) and length (Lo): WG = Wt – Wo; LG = Lt – Lo. WG and LG were the gain in weight (g) and length (cm) of fish after 90 days of rearing.

Water observation variables include DO, levels of ammonia, nitrite and nitrate. Observations were made at the end of the study. The growth rate of tilapia was observed by taking 30 fish samples from each pond.The tools used in this study include; tarpaulin pools, aerators, gutters, water pumps, PVC pipes, plastic buckets, bioballs, pots, coconut fiber, rockwool. The materials used in this study include; paprika seeds, tilapia, fish pellets, probiotic EM4 and probiotic ST.Treatment is carried out during maintenance by giving probiotics and replacing/filling water regularly. Measurements of the growth rate of fish weight and length were carried out every 3 weeks so that there were two calculations in this study. Observation of the growth rate of fish weight and length was measured entirely by sampling 30 fish per pond. Data analysis was used to see the effect of EM-4 and ST probiotic fortification on the growth rate of tilapia in anbiofloc-aquaponic system. The variables analyzed were absolute weight gain, and an increase in absolute length of tilapia. The research data were analyzed by *Analysis of Variance* ANOVA. If $F_{count}>F_{table}$ 0.05, it can be concluded that the probiotic fortification of EM-4 and ST has significant differences in the observed variables. If $F_{count}<F_{table}$ 0.05, it can be concluded that the probiotic fortification of EM-4 and ST did not show significant differences in the observed variables. If the data analysis shows a significant difference, then proceed with the BNT test.

Results and Discussion.

Growth Rate of Tilapia

Weight of Tilapia, Based on the results of the study, the average weight gain of tilapia fry in each treatment for each measurement ranged from 18.47 g to a size of 37.81 g. The average weight gain of Tilapia was highest in the P2 treatment with ST probiotic fortification, which was 37.81 g. Followed by treatment P1 with probiotic fortification EM-4 which is 26.66 g. While the lowest average weight gain was in the P0 treatment without probiotic fortification, which was 18.47 g.

During 92 days of rearing tilapia fry, the highest weight gain was achieved in the treatment of probiotics P2 of 37.81 namely from 2.97 g to 40.78 g, followed by P1 treatment of 26.86, namely from 3.37 g to 30.80 g, and the lowest was in P0 treatment of 18.47, from 3.37 g to 21, 84 g. The results of the rate of weight gain of Tilapia can be seen in Figure 1.

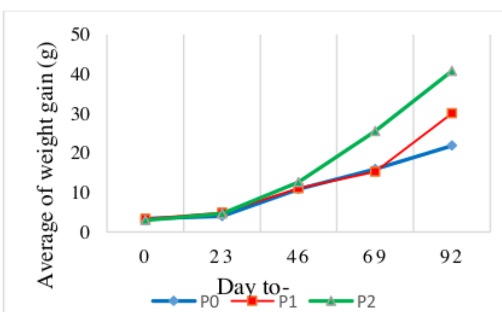

Figure 1. Weight Gain of Tilapia

Theresearch data were analyzed using 3 tests as follows:

Homogeneity Test,The results of the homogeneity test are as follows :

Table 1.

Test of Homogeneity of Weight Tilapia

Levene Statistic	df11	df2	Sig.
.192	2	6	.830

Based on table 2. It can be seen that the results of the homogeneity of variance have a statistical value of sig value of 0.830 > 0.05 at the probability level. So that it can be interpreted that the variance of the three groups of sample data is the same or homogeneous.

ANOVA test, Analysis of Anova variance using SPSS 16 software with the following results:

Table 2.

Results ofVariance Analysis on *One Way Anova* Tilapia Weight

	Sum of Squares	*df*	*Mean Square*	*F*	*Sig.*
Between Groups	565.375	2	282.688	85.038	.000
Within Groups	19.945	6	3.324		
Total	585.321	8			

Based on the analysis of variance (ANOVA) on the weight gain obtained $Fvalue_{calculated}$ of 85.038 while the result of the calculation $Ft._{abel}$ by 5.14 Then F_{count} 85.038 >F_{table} 5.14 which means that between treatments shows a very significant difference. This shows that there is a significant effect between the administration of different types of probiotics (EM4 and ST) on the weight growth rate of tilapia.

BNT Test, Theanalysis was continued with the Smallest Significant Difference Advanced Test (BNT). Analysis of the BNT follow-up test using SPSS 16 software with the following results:

Table 3.

Results of the Advanced Test of BNT Weight of Tilapia

Comparison of Treatments		Differences Mean	Sig
P0 (Control)	P1 (EM-4)	-8,20000[a]	0,002
P1 (EM-4)	P2 (ST)	-11,14000[b]	0,000
P2 (ST)	P0 (Control)	-19,34000[c]	0,000

a, b, c : The difference in letter notation states that there is a significant difference between treatments.

In the further BNT test, it was found that the treatment had a very significant difference in the weight growth of tilapia (P>5%) in each treatment.

Length of Tilapia, Based on the results of the study, the average length of tilapia fry in each treatment for each measurement ranged from 10 cm to 13cm. The average length increase of Tilapia was highest in the P2 treatment with ST probiotic fortification, which was 13 cm. Followed by treatment P1 with probiotic fortification EM-4 which is 11 cm. While the lowest average weight gain in P0 treatment without probiotic fortification was 10 cm.

During 92 days of rearing tilapia fry, the highest length gain was achieved in the treatment with P2 probiotics of 13 cm, from 7 cm to 17 cm, followed by P1 treatment of 11 cm from 6 cm to 15 cm, and the lowest in P0 treatment of 6 cm from 6 cm to 13 cm. The results of the rate of increase in the length of tilapia can be seen in Figure 2.

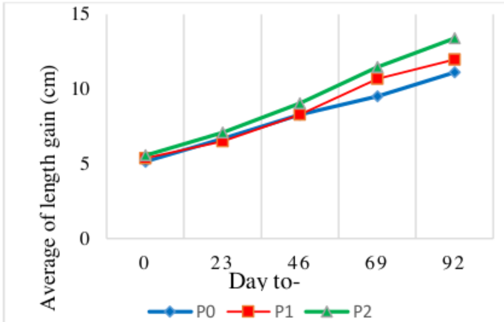

Figure 2. Length of Tilapia

The research data were analyzed using 3 tests as follows:

Homogeneity Test, the results of the homogeneity test are as follows:

Table 4.

Test of Homogeneity of Variances of Tilapia Weight

Levene Statistic	df11	df2	Sig.
1.699	2	6	.260

Based on table 4. It can be seen that the homogeneity of variance has a statistical value of sig 0.260 > 0.05 at the probability level. So it can be interpreted that the variance of the three groups of sample data is the same or homogeneous.

ANOVA test, Analysis of ANOVA variance using SPSS 16 software with the followingresults:

Table 5.

Results ofVariance Analysis on *One Way ANOVA* Tilapia Weight

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	5.798	2	2.899	5.297	.047
Within Groups	3.284	6	.547		
Total	9.082	8			

Based on the analysis of variance (ANOVA) on the weight gain obtained $Fvalue_{calculated}$ of 5.297 while the yield calculation $Ft._{abel}$ by 5.14 Then F_{count} 5.297 >F_{table} 5.14 which means that between treatments shows a very significant difference. This shows that there is a significant effect between the administration of different types of probiotics (EM4 and ST probiotics) on the length growth rate of tilapia.

BNT Test, The analysis was continued with the Smallest Significant Difference Advanced Test (BNT). Analysis of further BNT test using SPSS 16 software with the following results:

Table 6.

BNT Follow-up Test Results Tilapia Fish Weight

Comparison of Treatments		Differences Mean	Significance
P0 (Control)	P1 (EM-4)	-0,69333[a]	0,295
P1 (EM-4)	P2 (ST)	-1,24667[b]	0,085
P2 (ST)	P0 (Control)	1,94000[c]	0,018

a,b,c : The difference in letter notation states that there is a significant differencebetween treat ments

In the further BNT test, it was found that the treatment had a very significant difference in the length growth of tilapia (P>5%) in each treatment.

Growth is an increase in length or weight over time (Supiyanto & Endah Sri Redjeki, 2019). According to Saparinto & Rini (2011) that the growth rate of fish is influenced by two factors, namely internal factors and external factors. Internal factors include; genetics, sex, age, disease and the influence of hormones and external factors are influenced by habitats that are not in accordance with the tolerance ability of the fish's body so that it can cause disturbances in growth. These external factors include; temperature, water oxygen content, salt content, water fertility, and pollution.Based on the results of the research on the growth rate of tilapia weight and length, it was found that the highest weight growth rate was in the P2 treatment (with ST probiotic) with an average weight gain of 37.81 g. Where the average weight of the seeds was 2.97 g after treatment for 92 days increased to 40.78 g. The highest length growth rate was also in the P2 treatment (with ST probiotic) with an average length increase of 7.84 cm. Where at the time of stocking the average weight of the seeds was 5.56 after undergoing maintenance for 92 days increased to 13.40 cm.

This is certainly influenced by the microorganisms in probiotics. In accordance with the statement of Lisna & Insulistyowati (2015) that the bacteria in probiotics is not only able to improve water quality but also improves the digestive tract of fish so that the addition of probiotics can increase fish growth. ST probiotics contain *Lactobacillus* and *Nitrobacter* which in fish rearing media can improve water quality so as to increase fish growth. This is in accordance with Sugih, (2005) statement that bacteria *Lactobacillus* can increase the digestibility of fish to feed so that it can stimulate fish growth. Bacteria are *Lactobacillus* also able to increase the body's immunity to fight infection. According to Arief et al. (2008), *Lactobacillus* sp. is a type of bacteria that can maintain the balance of microbes in the digestive tract, so that it can increase the digestibility of fish and will ultimately provide a better final weight. Bacteria *Nitrobacter* in ST probiotics also play an important role in the nitrification process in ponds. This process occurs in two steps, firstly ammonia is converted to nitrite (NO^{2-}) by several bacterial genera including *Nitrosomonas*. Both nitrites are converted to nitrate (NO^{3}) by groups of bacteria such as *Nitrobacter*. This process occurs in the sediment surface layer of aquaculture ponds, in plants and other structures (Francis-Floyd et al., 1996). The overall nitrification process can be described through the following reaction (Titiresmi & Sopiah, 2006):

$$NH^{4+} + 2O_2 \longrightarrow NO^{3-} + 2H^+ + H_2O$$

Water Quality, In this study, water quality tests were carried out at the end of the study. The water quality in the biofloc-aquaponic system can be seen in table 7.

Table 7.

Pond Water Quality

Water Quality Parameters	Treatment			Standard
	P0 (control)	P1 (EM-4)	P2 (ST)	
DO	6,40	6,00	6,82	5-7 mg/L*
Amonia (NH_3)	0,13	0,23	0,64	< 0,8 mg/L**
Nitrit (NO_2)	0,065	0,024	0,026	0,06 mg/L***
Nitrat (NO_3)	4,1	5,4	5,8	20 mg/L***

(Sumber : *SNI 7550. 2009, Effendi (2003) dan **BBPBAT (2005) *** PP No 82 Tahun 2001)

Based on the results of the water quality test in each pond, it can be seen in table 9. that the levels of DO, ammonia, nitrite, and nitrate in each pond are different. The difference is due to the probiotic fortification that has been carried out. Dissolved Oxygen, ammonia, nitrite, and nitrate levels determine the quality of pond water. Dissolved Oxygen is the amount of oxygen in water that comes from the process of photosynthesis and air diffusion. Dissolved oxygen in the waters is used for respiration processes, degradation of organic and inorganic materials, metabolic processes and exchange of substances which then produce energy for growth and reproduction. The aquaponic biofloc system has a stable DO level when compared to the conventional system. In table 9. where the DO levels in all treatments ranged from 6-6.82 mg/L. In line with the research results of Putra et al. (2011) that the aquaponic system has a stable DO level as a parameter of good water quality for waters.

In table 9. it can be seen that the level of DO is the most stable, namely in the P2 treatment. Sufficient DO levels are also able to facilitate the nitrification process in the pond so that the need for inorganic nitrogen includes Ammonia (NH_3), Nitrite (NO_2), and Nitrate (NO_3) in the pond. Whereas in the P0 treatment without probiotic fortification, the DO level was lower, resulting in the nitrification process being inhibited and the ammonia and nitrite levels increasing. Increased levels of ammonia and nitrite can be lethal to fish in culture.In treatment P1 (with EM-4 probiotic) had a growth rate of weight and length that was not much different from treatment P2, namely weight gain of 26.86 g. Where the average weight of the seeds was 3.37 g and after undergoing maintenance for 92 days increased to 30.80 g. Then for the growth rate of length is 6 cm. Where the average length of the seed is 5 cm and after undergoing maintenance for 92 days it increases to 13 cm.

Tilapia growth in this treatment was not much different from P2 treatment because in EM-4 probiotics there were several microorganisms that support fish growth such as lactic acid and other fermenting microorganisms. Probiotic EM-4 contains a mixed culture of fermenting microorganisms, namely lactic acid bacteria (*Lactobacillus casei*) and yeast (*Saccharomyces cerevisiae*) (Ardita et al., 2015; Rachmawati et al., 2006). These bacteria will secrete enzymes such as proteases and amylase in the digestive tract (Setiawati et al., 2013). The fermenters in EM-4 probiotics secrete exogenous enzymes such as amylase, lipase, amylase and cellulase. These enzymes will degrade complex nutrients that make up feed into simpler nutrients. So as to facilitate the digestive process of fish and increase the growth rate of the fish. This is in accordance with the statement of Rachmawati et al, (2006) that EM4 microorganism culture works in the body of fish through a synergistic action. Lactic acid in probiotic EM-4 also plays a role in inhibiting the growth of pathogenic microorganisms. The bioactive compounds from the spices in the EM-4 probiotic were able to increase the fish's immunity from disease, so that tilapia could survive until the end of this study. The yeast content is also believed to help accelerate the growth of tilapia. Toxic substances that enter with food into the body can be bound by yeast and then these substances are excreted through feces, so that fish can grow better because toxins in the body can dissolve in food that is wasted in feces (Wulandari, 2008).

However, in table 9. it can be seen that the DO lowest level was in the P1 treatment. This causes fish growth to be less than optimal. An imbalance of dissolved oxygen can cause stress in fish because the brain does not get enough oxygen supply, and can lead to death due to lack of oxygen. This is due to oxygen dissolved in the blood that cannot be bound by fish body tissues (Dahril et al., 2017). While the P0 treatment without probiotic fortification had the lowest growth rate in weight and length. Based on the results of the study, the weight gain was 18.47 g. the average weight of the seeds was 3.37 g after undergoing maintenance for 92 days increased to 21.84 g. Then for the increase in length is 6 cm. Where the average length of the seeds was 5 cm after undergoing maintenance for 92 days increased to 13 cm.

This is presumably due to a decrease in water quality as indicated by the low levels of nitrates which can be seen in table 9. The low levels of nitrates are thought to be due to the inhibition of the nitrification process. The inhibition of the nitrification process can be caused by a lack of nitrifying bacteria such as *Nitromonas* and *Nitrobacter* so that the nitrification process is inhibited and causes the growth rate of Tilapia to decrease.The decrease in water quality in the P0 treatment (control) was thought to be because the feed contained excessive protein, excess undigested amino acids resulted in increased N content in the waters resulting in ammonia and nitrate pollution (Dewi et al., 2020). In addition, the control treatment did not add probiotics to the maintenance media so that only a few bacteria capable of oxidizing organic matter and good bacteria that could help improve fish digestion and increase the growth rate were added. This resulted in the hydrolysis process of protein into simpler compounds that was less than optimal and caused less optimal protein absorption so that the growth of tilapia was lower/less than optimal compared to the probiotic fortification treatment.

Ammonia (NH_3) in pond water is obtained from the decomposition of organic matter in the form of metabolic waste and food waste that is not consumed. Ammonia levels in the pond indicate the smoothness of the nitrification process. Where Ammonia will be converted into Nitrite by bacteria Nitrosomonas and Nitrite will be converted by bacteria Nitrobacter in the pond. The high level of Ammonia is caused by the accumulation of organic matter at the bottom of the tank which comes from the rest of the feed and the results of fish metabolism and is not completely decomposed by decomposing bacteria. However, the ammonia level in each treatment was still within the feasible and safe range for tilapia rearing, this is in accordance with the statement of BBPBAT (2005) that ammonia levels in tilapia rearing < 0.8 mg/L.

In table 9 shows that the levels of Nitrite in each pond ranged from 0.024-0.065 mg/L. Low levels of Nitrite indicate declining water quality. The low levels of Nitrite are thought to be due to the small number ofbacteria Nitrosomonas that play a role in converting ammonia to nitrite. So that the nitrification process is inhibited and results in a less than optimal growth rate of fish. Water quality plays an important role for fish growth because good water quality will affect the rate of metabolism and energy assimilation for growth (Putra et al., 2011). From the test results, obtained an increase in the concentration of nitrate in the ST treatment. This increase in nitrate concentration indicates the conversion of ammonia to nitrate through the nitrification process that runs smoothly. In table 9. it can be seen that the levels of nitrate in each treatment ranged from 4.1 to 5.8 mg/L. Where the highest levels of Nitrate in the P2 treatment with ST probiotic fortification. This is presumably due to the presence of bacteria Nitrobacter in ST probiotics which play an important role in the nitrification process. The nitrite contained in the pond will be converted into nitrate by the bacteria Nitrobacter contained in the ST probiotic. High levels of nitrate indicate good water quality in the tilapia pond. So as to increase the growth rate of tilapia optimally.

Conclusions.Based on the results of the study, it can be concluded that the effect of fortification of probiotics EM-4 (Effective Microorganism) and ST (Sukses Tani) significantly affects the growth rate of weight and length of tilapia (*Oreochromis niloticus*) in an biofloc-aquaponic system. The highest growth rate in weight and length with an average weight gain of 37.81 g and an average increase in length of 13 cm was in the P2 treatment (with ST probiotic) which contained *bacteria Lactobacillus* and *Nitrobacter.*

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