The effect of EM-4 and ST probiotic fortification on the growth of tilapia (*Oreochromis niloticus*) in the biofloc-aquaponic system

[1]Endah Rita Sulistya Dewi, [1]Fitri Rahmawati, [1]Maria Ulfah

[1] Biology Education Department, Faculty of Mathematics Science and Information Technology Education, Universitas PGRI Semarang, Semarang, Central Jawa, Indonesia. Corresponding author: Endah Rita Sulistya Dewi, endahrita@yahoo.co.id

Abstract. Tilapia (*Oreochromis niloticus*) is a fish that is highly demanded by the public as a source of protein with low cholesterol. It has a nutritional content of 17.7% protein and 1.3% fat. Innovative production methods must be found to increase production and meet market needs. Probiotics are one of the supporting factors for success in tilapia cultivation. This study aimed to determine the effect of probiotic fortification of EM-4 (Effective Microorganism) and ST (Sukses Tani) on the growth of tilapia in the biofloc-aquaponic system. This study used a Completely Randomized Design (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 and the Fisher's LSD (Least Significant Different) method. The results showed that the administration of probiotics in the biofloc-aquaponic system generated significant differences in weight and length gain ($p<0.05$). The highest growth was obtained in P2 treatment, with a weight gain of 37.81 g and a length growth of 13 cm, followed by P1 treatment, with a weight gain of 26.66 g and a length growth of 11 cm, while the lowest growth was obtained in the P0 treatment with a weight gain of 18.47 g and a length growth of 10 cm. It was concluded that probiotic fortification could improve tilapia growth in biofloc-aquaponic systems.
Key Words: aquaponics, probiotics, fortification, tilapia, biofloc.

Introduction. International market demand for tilapia reaches 200,000 tons/year (Wijaya 2011). Tilapia has high economic value. Tilapia has several advantages, including easiness of cultivation and consumer' preferences (Christin et al 2021). Tilapia is also highly demanded on the international market. Indonesia is the largest tilapia exporting country, exporting around 10 million tons/year (Nugroho et al 2013).

The increase in tilapia production causes an increase in the cultivation area and water use. The limited land and available water sources impose to find a solution so that cultivation activities can continue to develop. One solution is the application of a biofloc-aquaponic system. The biofloc technology transforms 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 to provide a better environment for fish. In addition, the aquaponics system can save land and water use and increase business efficiency through the utilization of nutrients from left over feed and fish metabolism. The aquaponic system can 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 (Ristiawan 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. The combination of biofloc technology and aquaponics system can optimize land and water use and optimizes nutrients to increase tilapia growth. The-eco friendly biofloc-aquaponic system in tilapia rearing minimizes aquaculture waste, thereby preventing pollution.

The increasing market demand must be balanced with an increase in the productivity of tilapia aquaculture. One alternative is to give probiotics to fish. 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 capacity 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 applying 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 & Kochba 2009; Salamah & Zulpikar 2020; Dewi & Ulfah 2022). One of the factors supporting the success of probiotics in increasing the growth is the activity of photosynthetic bacteria, *Lactobacillus* sp., *Actinomycetes* sp., *Streptomycetes* sp., and yeast contained in probiotics. These microorganisms can produce digestive enzymes and compounds that can increase the growth of tilapia.

In this study, probiotic EM-4 (Effective Microorganisms) and probiotic ST (Sukses Tani) are used. They are two types of probiotics that are often used in cultivation. These two probiotics each have different content to increase fish growth. Probiotics EM-4 in the form of a brown liquid and sweet and sour smell (fresh) can be used as an addition to optimize the utilization of food substances because the bacteria in EM-4 can digest cellulose, starch, sugar, protein, and fat (Surung 2008). Several microorganisms support the growth of fish in EM-4 probiotic, 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. Consequently, EM-4 can facilitate the digestive process of fish and increase 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 *Nitrobacter* bacteria 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 fish growth, especially in weight and length. Therefore, this study will comparatively examine the effect of EM-4 and ST probiotics on tilapia in a biofloc-aquaponic system. The-eco friendly biofloc-aquaponic system as an appropriate technology that can help farmers to produce tilapia in high quantity and quality.

Material and Method. The subjects used in this study were local tilapia with an initial length of 5-7 cm. A number of 200 fish were reared in three ponds. Each pond had 2650 liters. This research was conducted between May and September 2020 at the Biology Education Laboratory Campus 3 Universitas PGRI Semarang. Measurements of dissolved oxygen, ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) were 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 EM-4 probiotic treatment and P2 using ST probiotic treatment (10 ml/m^3). The variables observed in this study were weight gain and length of tilapia and water quality indicators. Measurements of weight gain (WG) and length gain (LG) followed Purbomartono et al (2022) guidelines as follows: WG = Wt – Wo; LG = Lt – Lo, where Wt = final weight; Lt = final length; Wo = initial weight; Lo = initial length; WG = weight gain; LG = length gain after 90 days (3 month) of rearing.

Water observation variables included dissolved oxygen (DO), ammonia, nitrite, and nitrate levels. Observations were made at the end of the study. Tilapia growth 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, and rockwool. The materials used in this study include; paprika seeds, tilapia, fish pellets, probiotic EM-4, and probiotic ST. Treatment was carried out during maintenance by giving probiotics and replacing/filling water regularly. Measurements of the fish weight and length growth were carried out twich during the study. Observation of the growth of fish weight and length was measured by sampling 30 fish per pond.

ANOVA was use to analyze the research data (wehight gain and length gain) If $F_{count}>F_{table}$ 0.05, it can be concluded that the probiotic fortification of EM-4 and ST generated 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. A Fisher's LSD (Least Significant Different) method is run if the data analysis shows a significant difference. The aim of Fisher's LSD method is to compare two average values.

Results and Discussion

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. During 92 days of rearing tilapia fry, the highest weight gain was of 37.81 g, achieved in the treatment of ST probiotics, in P2, namely from 2.97 g to 40.78 g. This was followed by P1 treatment, with EM-4, with a 26.86 g increase from 3.37 g to 30.80 g. The lowest gain was of 18.47g, in P0 treatment (no probiotic), from 3.37 g to 21, 84 g. The results of the weight gain of tilapia can be seen in Figure 1.

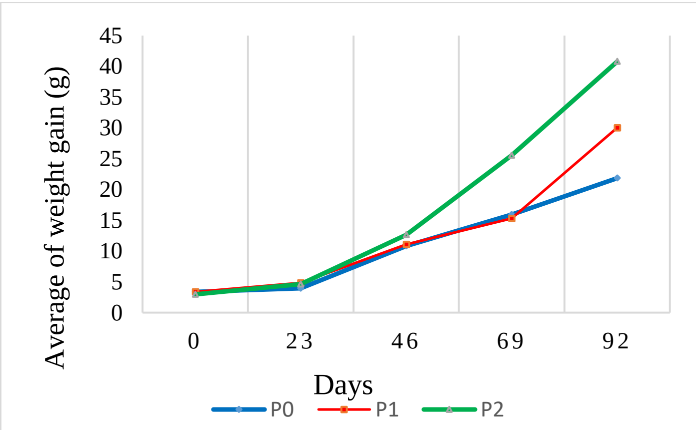

Figure 1. Weight gain of tilapia during the study.

The research data were analyzed using three tests as follows. The first one was the homogeneity test and its results are presented in Table 1. The sig value (0.830, Table 1) is higher than 0.05. This means there is no statistically significant difference between tested variables.

Table 1.

Homogeneity test for the weight gain of tilapia

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

The second test on the weight gain was one-way ANOVA. This generated the results presented in Table 2. 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 there is a very significant difference between treatments. This shows that there is a significant effect between the administration of different types of probiotics (no treatment, EM-4 and ST) on the weight gain of tilapia.

Table 2.

Results of one-way ANOVA regarding tilapia weight gain

	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			

The third test was Fisher's LSD (Least Significant Different) Method. This generated the results presented in Table 3. The Fisher's LSD (Least Significant Different) method showed that the treatment had a statistically significant difference in the weight growth of tilapia ($p<0.05$) in each treatment.

Table 3.

Results of the Advanced Fisher's LSD (Least Significant Different) method of the 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

Note: Different superscript letters indicate a significant difference between treatments.

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 13 cm. During 92 days of tilapia fry rearing, the highest length gain was achieved in the P2 treatment, (with ST probiotic fortification), with an average of 13 cm (measurements ranging from 7 cm to 17 cm). This was followed by P1 treatment, with probiotic fortification EM-4, with an average of 11 cm (measurements ranging from 6 cm to 15 cm). Finally, the lowest increase was in P0 treatment, without probiotic fortification, with an average of 6 cm (measurements raging from 6 cm to 13 cm). The results of the of increase in the length of tilapia can be seen in Figure 2.

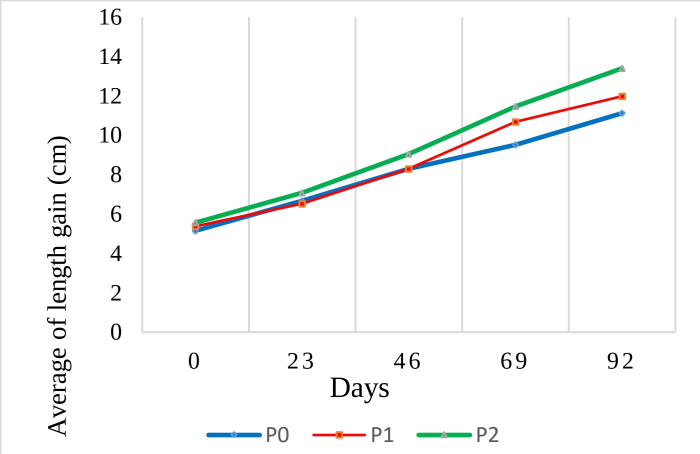

Figure 2. Length of tilapia during the study.

The research data were analyzed using 3 tests as follows. The results of the homogeneity test are indicated in Table 4. The homogeneity of variance has a statistical value of sig 0.260 > 0.05, indicating that the variance of the three groups of sample data is the same or homogeneous.

Table 4.

Test of Homogeneity for Variances of tilapia weight gain

Levene Statistic	*df11*	*df2*	*Sig.*
1.699	2	6	.260

The results of ANOVA test on the weight gain are included in Table 5. They generated $Fvalue_{calculated}$ of 5.297 while the yield calculation $Ft._{abel}$ by 5.14. Then F_{count} 5.297 >F_{table} 5.14 which means that there is a statistically significant difference between treatments. In other words, there is a significant difference between the effect of the administration of different types of probiotics (EM-4 and ST probiotics) on the length growth of tilapia. ST probiotics generated a bigger increase in weight gain.

Table 5.

Results of One Way ANOVA on tilapia weight gain

	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			

The results of Fisher's LSD (Least Significant Different) test are presented in Table 6. According to it, the P2 treatment generated a statistically significant difference in the length growth of tilapia (p<5%).

Table 6.

Fisher's LSD Results tilapia Fish Weight gain

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

Note: Different superscript letters indicate a significant difference between treatments.

Growth is an increase in length or weight gain over time (Suprianto et al 2019). According to Saparinto & Susiana (2011), the fish growth is influenced by two factors, namely internal factors and external factors. Internal factors include genetics, sex, age, disease, and the influence of hormones; external factors include habitat variables 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 pH, and pollution. The results of this study show that the highest weight gain was in the P2 treatment (with ST probiotic) with an average weight gain of 37.81 g. The average weight gain of the seeds was 2.97 g and after treatment for 90 days, it increased to 40.78 g. The highest length growth was also in the P2 treatment (with ST probiotic) with an average length increase of 7.84 cm. The average length of the seeds was 5.56 cm at the beginning and it increased to 13.40 cm after undergoing maintenance for 90 days.

This is certainly influenced by the microorganisms in probiotics. According to Lisna & Insulistyowati (2015), the bacteria in probiotics can improve water quality and 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 and increase fish growth. This is in accordance with Sugih (2005) who mention that bacteria *Lactobacillus* can increase the digestibility of fish to feed so that it can stimulate fish growth. *Lactobacillus* bacteria are 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 weight gain. *Nitrobacter* bacteria 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 & Pouder 2018). 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 is described 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**
Nitrite (NO_2)	0.065	0.024	0.026	0.06 mg/L***
Nitrate (NO_3)	4.1	5.4	5.8	20 mg/L***

Source: * Badan Standariasi Nasional Indonesia (2009), Effendi (2003), ** BBPBAT (2005), *** State Secretariat (2001)

The water quality test results (Table 7) show 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. DO, ammonia, nitrite, and nitrate levels determine pond water quality. DO is the amount of oxygen in the water that comes from photosynthesis and air diffusion. DO in the waters is used for respiration, degradation of organic and inorganic materials, metabolic processes, and exchange of substances, which produce energy for growth and reproduction. The aquaponic biofloc system has a stable DO level when compared to the conventional system. In table 7 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), the aquaponic system has a stable DO level as a parameter of good water quality for waters.

Table 7 shows that the level of DO was not much different between treatments. 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. Treatment P1 (with EM-4 probiotic) had a growth of weight and length that was not much different from treatment P2, namely weight gain of 26.86 g. The average weight of the seeds was 3.37 g and after undergoing maintenance for 90 days increased to 30.23 g. The length growth was 6 cm. The average length of the seed was 5 cm and after undergoing maintenance for 90 days it increases to 11 cm.

Tilapia growth in P1 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, facilitate the digestive process of fish, and increase fish growth. This is in accordance with the statement of Rachmawati et al (2006) that EM-4 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 to 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 eliminated in feces (Wulandari 2008).

However, in Table 7 it can be seen that the lowest level of DO was in the P1 treatment. This causes fish growth to be less than optimal. An imbalance of DO can cause stress in fish because the brain does not get enough oxygen supply and can lead to death. This is because oxygen is dissolved in the blood that cannot be bound by fish body tissues (Dahril et al 2017). The P0 treatment without probiotic fortification had the lowest weight and length gain. Based on the study's results, the weight gain was 18.47 g. Thus, the average initial weight gain of the seeds was 3.37 g, and after undergoing maintenance for 90 days, it increased to 21.84 g. The length increase was 6 cm. The average initial length of the seeds was 5 cm, and after undergoing maintenance for 90 days increased to 11 cm.

This is presumably due to a decrease in water quality as indicated by the low levels of nitrates (Table 7). 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 slow down the growth of tilapia. The decrease in water quality in the P0 treatment (control) was thought to be caused by the feed that 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 have probiotics, so only a few bacteria could oxidize organic matter, and there were few good bacteria that could help improve fish digestion and increase the growth. 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 the one seen in the probiotic fortification treatments.

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. Ammonia is converted into nitrite by *Nitrosomonas* bacteria and nitrite is converted by *Nitrobacter* bacteria in the pond. The high level of ammonia is caused by the accumulation of organic matter at the bottom of the pond 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 recommendations of BBPBAT (2005) that indicate that ammonia levels in tilapia rearing must be < 0.8 mg L^{-1}.

The levels of nitrite in each pond ranged from 0.024-0.065 mg L^{-1} (Table 7). Low levels of nitrite indicate declining water quality. The low levels of nitrite are thought to be due to the small number of *Nitrosomonas* bacteria that play a role in converting ammonia into nitrite. The nitrification process is inhibited and results in a less than optimal fish growth. Water quality plays an important role for fish growth because good water quality affects the rate of metabolism and energy assimilation for growth (Putra et al 2011). Results show an increase in nitrate concentration in the ST treatment was obtained. This increase in nitrate concentration indicates the conversion of ammonia to nitrate through the nitrification process that runs smoothly. In table 7. it can be seen that the levels of nitrate in each treatment ranged from 4.1 to 5.8 mg L^{-1}. The highest level of nitrate was in the P2 treatment (with ST probiotic fortification). This is presumably due to the presence of *Nitrobacter* bacteria in ST probiotics which play an important role in the nitrification process. The nitrite in the pond is converted into nitrate by the Nitrobacter bacteria in the ST probiotic. High levels of nitrate indicate good water quality in the tilapia pond. This increases the growth of tilapia.

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

Conflict of interests. The authors declare that there is no conflict of interests

References

Ardita, N., Budiharjo, A., Sari, S. L. A., 2015 growth and conversion ratio of tilapia feed (*Oreochromis niloticus*) with the addition of probiotics. Biotechnology 12:16-21. [In Indonesian]

Arief, M., Mufidah, Kusriningrum, 2008 Effect of probiotic addition on artificial feed on growth and feed conversion ratio of tilapia gift (*Oreochromis niloticus*). Jurnal Ilmiah Perikanan & Kelautan 3: 46-51. [In Indonesian]

Avnimelech, Y., Kochba, M., 2009. Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using ^{15}N tracing. Aquaculture 287(1-2):163-168.

Badan Standariasi Nasional Indonesia, 2009 SNI No 7550:2009. Tilapia (*Oreochrois niloticus*) production class enlargement in calm water ponds. National Standardization Agency. Indonesia. [In Indonesian]

BBPBAT. 2005. Catfish hatchery instructions (*Clarias sp.*). Sukabumi. [In Indonesian]

Bukhori, A., Sartini, S., Rahmiati, R., 2020 Isolation of lactic acid bacteria (BAL) from the digestive tract of tilapia (*Oreochromis niloticus*) and its ability to inhibit *Staphylococcus aureus* and *Shigella sp*. UMA Journal of Biological Sciences 2: 23-31. [In Indonesian]

Christin, Y., Restu, I. W., Kartika, G. R. A., 2021 Growth rate of tilapia (*Oreochromis niloticus*) in three different recirculation systems. Current Trends in Aquatic Science IV(2):122-127. [In Indonesian]

Dahril I., Tang U. M., Putra.I, 2017. The effect of different salinity on the growth and livelihood of red tilapia fry (*Oreochromis sp*.). Berkala Perikanan Terubuk 45(2):67-75. [In Indonesian]

Dewi, E. R. S, Nugroho, A. S., Ulfah M., 2020 water quality in the maintenance of *Oreochromis niloticus* through environmentally friendly biofloc technology. The 7th

International Conference on DV-Xα Method 2-4 September 2019, Semarang, Indonesia. IOP Conference Series: Materials Science and Engineering 835:1-5.
Dewi, E. R. S., Ulfah M., 2022 Biofloc performance in environmentally friendly biofloc-aquaponic systems. BIOMA: Jurnal Ilmiah Biologi 11:121-134. [In Indonesian]
Effendi, M. I., 2003 Fisheries Biology. Yayasan Pustaka Nusantara. Yogyakarta. [In Indonesian]
Francis-Floyd R., Pouder D., 2018. Use of Formalin to Control Fish Parasites. Institute of Food and Agricultural Sciences, University of Florida. Available at https://edis.ifas.ufl.edu/pdf%5Carchived%5CVM%5CVM061%5CVM061-10568036.pdf
Indriani, Y. H. 2005. Making Compost Quickly. Penebar Swadaya, Jakarta. [In Indonesian]
Istiqomah I., Isnansetyo A., Atitus I. N., Rohman A. F., 2019 Isolation on cellulolytic bacterium *Staphylococcus* sp. JC20 from the intestine of octopus (*Octopus* sp.) for fish probiotic candidate. Jurnal Perikanan Universitas Gadjah Mada 21:93-98. [In Indonesian]
Lisna, Insulistyowati, 2015. The Potential of Microbes Probiotik_FM in Improving Pond Water Quality and The Growth Rate of Dumbo Catfish Fry (*Clarias gariepinus*). Jurnal Penelitian Universitas Jambi Seri Sains 17:16-25 [In Indonesian]
Nugroho A., Arini E., Elfitasari T., 2013 Effect of different densities on the smoothness of life and growth of tilapia (*Oreochromis niloticus*) on the recirculation system with charcoal filters. Journal of Aquaculture Management and Technology 2: 94-100. [In Indonesian]
Purbomartono C., Hapsari A. N., Susanto, Samadan G. M., 2022 Ginger (*Zingiber officinal*e Rosc.) flour diet in gourami (*Osphronemus gourami*) hatchery with biofloc system. AACL Bioflux 15(2):585-592.
Putra I., Setiyanto D. D., Wahyjuningrum D., 2011 Growth and survival of tilapia (*oreochromis niloticus*) in the recirculation system. Jurnal Perikanan dan Kelautan 16:56-63. [In Indonesian]
Rachmawati D., Pinandoyo, P., Purwanti A. D., 2006 Addition of halquinol in artificial feed to increase growth of baung fish fry (*Mystus nemurus C. V*). Jurnal Perikanan 8:92-100. [In Indonesian]
Ristiawan A. N., Lilik T. P., Diana C., Alfabetian H. C. H., 2012 Application of aquaponic technology in freshwater fish farming for optimization of production capacity. Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology 8:46-51. [In Indonesian]
Rostika R., Azhima M. F., Ihsan Y. N., Andriani Y., Suryadi I. B. B., Dewanti L. P. 2020 The use of solid probiotics in feed to growth and survival rate of *Cyprinus carpio.* AACL Bioflux 13:199–206.
Salamah S., Zulpikar Z., 2020 Giving probiotics to commercial feed with different proteins to catfish performance (*Clarias sp*.) using a biofloc system. Acta Aquatika: Aquatic Sciences Journal 7:21-27. [In Indonesian]
Saparinto C., Susiana R., 2011. Tips for success in Tilapia Farming. Andi Offset Publishers. Yogyakarta. [In Indonesian]
Saptarini P., 2010 Effectiveness of aquaponics technology with ground kale (*Ipomea reptans*) against ammonia reduction in goldfish enlargement. Thesis. IPB. Bogor. [In Indonesian]
Setiawati, J. E., Tarsim, Adiputra Y. T., Hudaidah S., 2013. Effect of probiotic addition on feed with different doses, in the face of growth, dilution, feed efficiency and protein retention of catfish (*Pangasius hypophthalmus*). e-Jurnal Rekayasa dan Teknologi Budidaya Perairan 1:151-162. [In Indonesian]
Sugih, F. H., 2005. Effect of Probiotic Addition in Commercial Feed on Gourami Fry Growth. Faculty of Fisheries and Marine Sciences. Thesis. Universitas Padjajaran. Jatinangor. [In Indonesian]
State Secretariat, 2021 PP RI No. 82, 2001. Water Quality Management and Water Pollution Control (Statute Book of the Republic of Indonesia of 2001 Number 153, Supplement to the Statute Book of the Republic of Indonesia Number 4161). Jakarta. [In Indonesian]

Suprianto S., Redjeki E. S., Dadiono M. S., 2019 Optimization of probiotic doses on the growth and survival rate of Nile tilapia (*Oreochromis niloticus*) in biofloc system. Journal of Aquaculture and Fish Health 8(2):80-85. [In Indonesian]
Surung M. Y., 2008 Effect of EM4 (Effective Microorganism-4) dose in drinking water on the body weight of free-range chickens. Jurnal Agrisistem 4:109-113. [In Indonesian]
Titiresmi, Sopiah, N., 2006 Biofilter technology for the treatment of ammonia waste. Jurnal Teknologi Lingkungan 7:173-179. [In Indonesian]
Umasugi A., Tumbol R. A., Kreckhoff R. L., Manoppo H., Pangemanan N. P. L., Ginting, E. L., 2018 The use of probiotic bacteria for the prevention of bacterial infection of *Streptococcus agalactiae* in tilapia, *Oreochromis niloticus.* e-Journal Budidaya Perairan 6:39–44. [In Indonesian]
Wijaya A., 2011 Effect of probiotic bacteria (*Bacillus sp*.) on maintenance media on survival of tilapia fry (*Oreochromis niloticus*) infected with *Streptococcus agalactiae*. Thesis. FIK UNPAD. Jatinagor. [In Indonesian]
Wulandari, R., 2008 Effect of yeast addition in giving red lamtoro (*Acacia villosa*) on rat liver histopathology. Thesis. IPB. Bogor. [In Indonesian]

Received: 8 November 2021. Accepted 25 August 2022. Publised online: 28 Augst 2022.
Authors:
Endah Rita Sulistya Dewi, Biology Education Department, Faculty of Mathematics Science and Information Technology Education, Universitas PGRI Semarang, Semarang, Central Jawa, Indonesia, email: endahrita@yahoo.co.id
Fitri Rahmawati,Biology Education Department, Faculty of Mathematics Science and Information Technology Education, Universitas PGRI Semarang, Semarang, Central Jawa, Indonesia. email: fitri.rhm06@gmail.com
Maria Ulfah, Biology Education Department, Faculty of Mathematics Science and Information Technology Education, Universitas PGRI Semarang, Semarang, Central Jawa, Indonesia, email: mariaulfah@upgris.ac.id

How to cite this article
Dewi E. R. S., Rahmawati F., Ulfah M., 2022 The effect of EM-4 and ST probiotic fortification on the growth of tilapia (*Oreochromis niloticus*) in the biofloc-aquaponic system. AACL Bioflux 15(4):2171-2180.