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Effectiveness Probiotic of *Nitrosomonas* sp. and *Bacillus sp*. in Improving the Water Quality and Growth of Tilapia Larasati (*Oreochromis niloticus*)

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ABSTRACT

Water quality is the main factor that has a major influence on the growth and survival of Tilapia Larasati (*Oreochromis niloticus*). *Nitrosomonas* sp. and *Bacillus sp*. can optimize water quality through nitrification and denitrification processes and optimize feed utilization. This study aims to determine the effectiveness of using probiotic *Nitrosomonas* sp. and *Bacillus sp*. in *O. niloticus* maintenance media in improving water quality and growth. The method used was CRD with 4 treatments and 3 replications, namely treatment A (without probiotic), B (probiotic 1.0 µL/L), C (probiotic 1.5 µL/L), and D (probiotic 2.0 µL/L). This study used an aquarium measuring 40 x 30 x 40 cm filled with 20 liters of fresh water, and filled with 10 fish weighing 6.97 ± 0.20 g each (density of 1 fish/2 liters). Molasses as feed bacteria and probiotics were given according to the treatment dose. Siphon was accomplished twice a week according to the level of turbidity. Water changes once a week as much as 50 - 60%. The results from the study showed that the use of probiotics had a significant effect ($P<0,05$) in improving water quality and fish growth. The best result observed from treatment C, with the reduce ammonia and nitrite (0.002 and 1.291 mg/L), highest fish growth, specific growth rate, and survival rates (14.63±0.24 g; 2.48±0.01%/day; and 97±0.06%, respectively), and also lowest feed conversion ratio (1.07±0.02). Based on these results, the best dose for improving water quality and growth of *O. niloticus* is 1.5 µL/L.

Keywords: aquaculture, bioflock, organic matter, sludge, waste

ABSTRAK

Kualitas air merupakan faktor utama yang mempunyai pengaruh besar terhadap pertumbuhan dan kelangsungan hidup Ikan Nila Larasati (*Oreochromis niloticus*). *Nitrosomonas* sp. dan *Bacillus sp*. dapat mengoptimalkan kualitas air melalui proses nitrifikasi dan denitrifikasi serta mengoptimalkan pemanfaatan pakan. Penelitian ini bertujuan untuk mengetahui efektivitas penggunaan probiotik *Nitrosomonas* sp. dan *Bacillus sp*. pada media pemeliharaan *O. niloticus* dalam meningkatkan kualitas air dan pertumbuhan. Metode yang digunakan adalah RAL (Rancangan Acak Lengkap) dengan 4 perlakuan dan 3 ulangan yaitu perlakuan A (tanpa probiotik), B (probiotik 1,0 µL/L), C (probiotik 1,5 µL/L), dan D (probiotik 2,0 µL/L). Penelitian ini menggunakan akuarium berukuran 40 x 30 x 40 cm yang diisi air tawar sebanyak 20 liter, dan diisi 10 ikan dengan berat masing-masing 6,97 ± 0,20 g (kepadatan 1 ekor/2 liter). Molase sebagai pakan bakteri dan probiotik diberikan sesuai dosis perlakuan. Siphon dilakukan dua kali seminggu sesuai dengan tingkat kekeruhannya. Penggantian air seminggu sekali sebanyak 50 – 60%. Hasil penelitian menunjukkan bahwa penggunaan probiotik berpengaruh nyata ($P<0,05$) terhadap peningkatan kualitas air dan pertumbuhan ikan. Hasil terbaik diamati pada perlakuan C, dengan amonia dan nitrit terendah (0,002 dan 1,291 mg/L), pertumbuhan ikan, laju pertumbuhan spesifik, dan kelangsungan hidup ikan tertinggi (14,63±0,24 g; 2,48±0, 01%/hari; dan 97±0,06%), serta rasio konversi pakan terendah (1,07±0,02). Berdasarkan hasil tersebut maka dosis terbaik untuk meningkatkan kualitas air dan pertumbuhan *Oreochromis niloticus* adalah 1,5 µL/L.

Kata kunci: akuakultur, bioflok, bahan organik, lumpur, limbah

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1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the main commodities that has great potential in improving freshwater aquaculture in Indonesia because of its relatively high cultivation yields. Based on the data of the Minister of Marine Affairs and Fisheries (2023), production of *O. niloticus* in 2019 to 2021 has increased by up to 33% with a total production amount reaching 141,830 tons or worth IDR 3,2 billion. Seeing this increase, the Minister of Marine Affairs and Fisheries is targeting *O. niloticus* production in 2023 reached 2 million tons. However, in the cultivation of *O. niloticus* there are various obstacles, one of which is declining water quality which can cause the fish to experience stress, disrupt growth, be susceptible to disease and affect their survival (El-Kady et al., 2022). In cultivation businesses, water quality will worsen due to the accumulation of metabolic waste and decomposition of undigested feed and the decay of other organic materials (Padmavathi et al., 2012). One effort to ensure optimal water quality is providing probiotics.

According to the FAO definition (2001), probiotics are live microorganisms that can provide benefits. The role of probiotics in cultivating *O. niloticus* can help increase growth, feed utilization (Hlordzi et al., 2020; El-Kady et al., 2022), fish survival in defending against pathogens (Kuebutornye et al., 2020), and maintain quality water remains optimal (Elsabagh et al., 2018). There are several types of probiotic bacteria that can improve water quality, including Bacillus, *Nitrosomonas*, Nitrosococcus, Nitrobacter, Nitrosospira, and Pseudomonas (Sutthi et al., 2018; Hlordzi et al., 2020; Wang et al., 2020). The use of probiotics in rearing media can be applied to all ages of fish and the absorption of the content is more effective for fish to sustainably absorb (Hlordzi et al., 2020; El-Kady et al., 2022) making it the best method for improving water quality.

The bacteria in probiotics that can be used to improve water quality are *Nitrosomonas* sp. and *Bacillus sp*. The *Nitrosomonas* sp. able to degrade organic materials through the nitrification process, namely breaking down ammonia compounds into nitrites (Sonia et al., 2015). *Bacillus sp*. able to reduce nitrite and nitrate compounds in waters through the denitrification process (Nara et al., 2013; Hlordzi et al., 2020) and can increase feed utilization (Elsabagh et al., 2018; Hlordzi et al., 2020). Probiotic administration *Nitrosomonas* sp. and *Bacillus sp*. have been widely carried out on cultivated cultivars, including vannamei shrimp (Karthik et al., 2015), goldfish (Sonia et al., 2015), and tilapia (Sutthi et al., 2018).

Research on improving water quality through probiotics against *O. niloticus* has been widely carried out. Based on previous studies, probiotics have been given to *O. niloticus* through feed (El-Haroun et al., 2006) and maintenance media (Sutthi et al., 2018; El-Kady et al., 2022; Haraz et al., 2023). However, no research has been conducted that examines the administration of *Nitrosomonas* sp. probiotics and *Bacillus sp*. probiotics against *O. niloticus* maintenance media. Therefore, it is necessary to carry out this research to improve water quality and the growth of *O. niloticus*. This study was conducted with the aim to investigate the effectiveness of probiotics *Nitrosomonas* sp. and *Bacillus sp*. added to the *O. niloticus* cultivation media in order to improve water quality and to increase fish growth.

2. Material and Methods

This research was carried out in January – March 2023 at the Faculty of Fisheries and Marine Sciences, Universitas Diponegoro. The test fish used in this research were Larasati Tilapia seeds (*Oreochromis niloticus*) measuring 7 - 8 cm and weighing 6.97$\pm$0.20g/fish obtained from the Janti Freshwater Fish Hatchery and Cultivation Work Unit. Seed *O. niloticus* first acclimatized in a maintenance tank for 14 days. This research was carried out for 42 days (López-Luna *et al.,* 2014) with regular feeding using the *ad satiation* method (Tran-Duy *et al.,* 2008) twice at 08.00 and 16.00. The density in the aquarium is 1 fish/two liters. The containers used were 12 aquariums measuring 40 x 30 x 40 cm filled with 20 liters of fresh water. Water that has been deposited in the aquarium for 24 hours was given aeration for one night.

After one night, the 1.1 mL of molasses was given according to the calculation results of the Avnimelech (1999). Then, given probiotics according to the treatment dose and left with aeration for 3 days. The probiotics used contain *Nitrosomonas* sp. and *Bacillus sp*. with a bacterial density of 2.0 x 10^3 CFU/mL. Commercial probiotics were added directly to the rearing medium without being mixed with feed (Sonia *et al.,* 2015; Sutthi *et al.,* 2018; El-Kady *et al.,* 2022). Probiotics were given once a week after changing the water by 50 – 60% (Sutthi *et al.,* 2018). During the research, fish growth sampling was carried out every 7 days.

Determination of probiotic dosage was based on Sonia *et al.* (2015) with slightly modification, that the best dose in the Cyprinidae family is 1 g/m^3 or the same as 1.0 µL/L. This research was designed using a Completely Randomized Design (CRD) consisting of 4 treatments and 3 repetitions. The treatments were A (probiotic administration of 0 mL/L); B (probiotic administration of 1.0 µL/L); C (probiotic administration of 1.5 µL/L); and D (probiotic administration of 2.0 µL/L). The water quality parameters observed include DO, pH, temperature, ammonia and nitrite (El-Kady *et al.,* 2022). The DO, pH and temperature were routinely carried out every morning and evening before feeding (Hisano *et al.,* 2021), while ammonia and nitrite were periodically carried out every 14 days (López-Luna *et al.,* 2014).

Bacterial density was calculated using the Total Plate Count (TPC) method so that the final result obtained visually is Colony Forming Unit (CFU) per mL. Water samples were stored in sample bottles before microbiological testing. The test began with dilution of the water sample. A total of 1 mL of water sample was put into a test tube, then 9 mL of sterile NaCl solution was added and then homogenized. The diluted water sample in the tube was incubated for 24 hours at 37°C. After incubation, the sample was seeded on non-selective Trypticase Soy Agar (TSA) media. Seeding was carried out using the spread method with a sterile L glass. Following that, the sample was incubated for 24 hours at 37°C. Bacterial identification and colony counting were carried out after incubation. The calculation formula is based on that used in previous studies (El-Kady et al., 2022; Haraz et al., 2023).

$$\frac{\text{CFU}}{\text{mL}} = \text{Number of colonies} \times \frac{1}{\text{Dilution factor}}$$

Absolute weight gain (W, g), Specific growth rate (SGR, %/day), Feed Conversion Ratio (FCR), and Survival Rate (SR, %) were also calculated as indicator of fish growth. Daily water quality data and bacterial density were analysed descriptively. Meanwhile, others data were analysed using the Analysis of Variance (ANOVA) method at a significance level of 5%. If the analysis results are significantly different (P < 0,05), the Duncan test is continued to determine the difference in the mean value between treatments and the best treatment.

3. Results and Discussion

3.1. Results

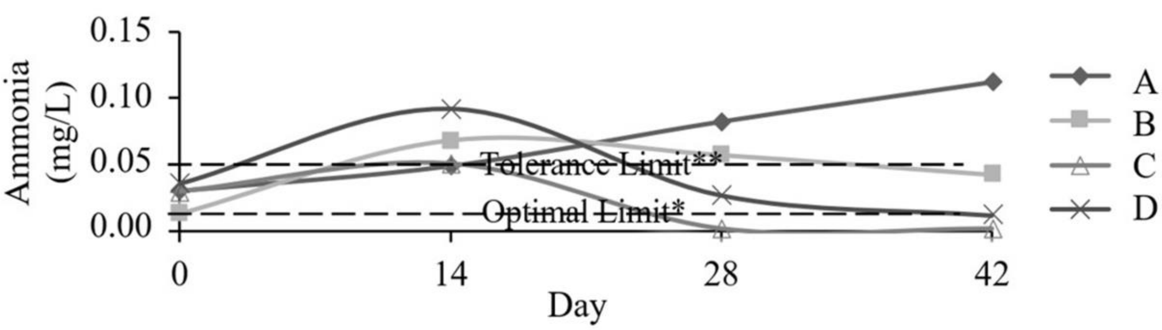
Water quality

a. Degree of Acidity (pH), Temperature, Dissolved Oxygen (DO)

Based on the results, it is known that the pH value in the morning ranges from 6.8 to 8.8 and in the afternoon ranges from 7.2 to 8.9. The temperature value in the morning ranges from 24.6 to 28.1 ^{o}C and in the afternoon is 25.0 to 28.3 ^{o}C. The average DO value in the morning is relatively higher, namely ranging from 5,0 to 7.9 mg/L and in the afternoon ranging from 5.0 to 7.8 mg/L. The results of daily water quality measurements for each treatment are presented in Table 1.

b. Ammonia (NH_3)

Based on the research results, it was



Note: *SNI 7550 (2009); **Yan *et al.* (2021)

Figure 1. Fluctuation of Ammonia Values in *Oreochromis niloticus* Cultivation Media During Study (A: without probiotic, B: probiotic 1.0 µL/L, C: probiotic 1.5 µL/L, and D: probiotic 2.0 µL/L)

found that the ammonia value in the treatment given probiotics decreased on day 28, while the treatment A continued to experience an increase in ammonia. The results of measuring the ammonia value are presented in Figure 1. It is known that the treatment B experienced a decrease in the ammonia value which was not too low, namely 27.51%. When compared with treatments C and D, there was a lower decrease in ammonia values, namely 1225% and 182.87% (Figure 1).

c. Nitrite (NO_2)

The nitrite value in the treatments given probiotics was decreased on day 28, while in the treatment A was continued to experience an increase (Figure 2). Furthermore, it is known that the treatment C has the lowest nitrite value with a decrease in nitrite value of 21.74%. When compared with treatments B and D, the nitrite value decreased by 54.05% and 39.80%.

Bacterial Density

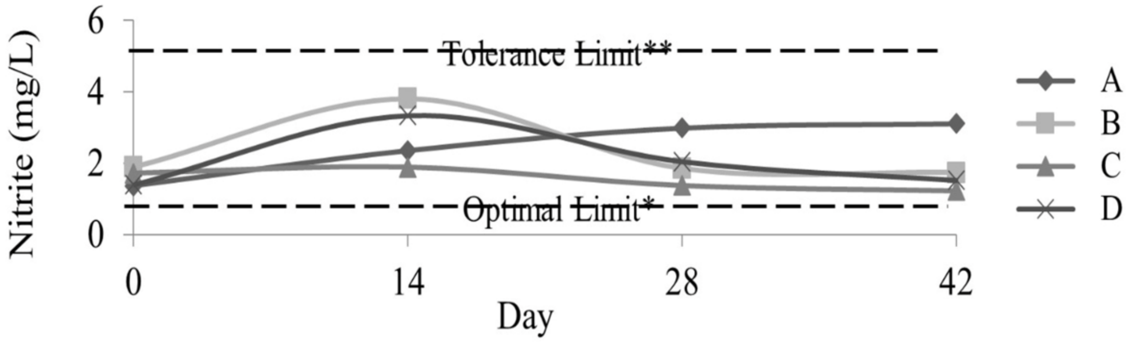
Based on the results, it is known that treatments B, C and D had a significant increase in bacteria until the end of the study (Figure 3). It can be seen that the bacterial population from treatments B, C and D has a relatively higher growth rate compared to treatment A. Treatment D also appears to have the highest increase in bacterial density compared to other treatments.

Table 1. Values of Water Quality in *Oreochromis niloticus* Cultivation Media During Study (A: without probiotic, B: probiotic 1.0 µL/L, C: probiotic 1.5 µL/L, and D: probiotic 2.0 µL/L)

Treatment	Parameter					
	pH		Temperature (^oC)		DO (mg/L)	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
A						
Range	6.8 – 8.8	7.3 – 8.8	25.0 – 28.1	25.4 – 28.2	5.3 – 7.8	5.2 – 7.5
Average±SD	(8.1±0.47)	(8.2±0.41)	(26.5±0.77)	(26.6±0.73)	(6.3±0.62)	(5.9±0.55)
B						
Range	7.3 – 8.8	7.5 – 8.9	24.9 – 28.1	25.3 – 28.3	5.3 – 7.9	5.0 – 7.5
Average±SD	(8.1±0.45)	(8.2±0.41)	(26.5±0.79)	(26.5±0.78)	(6.3±0.63)	(5.9±0.54)
C						
Range	7.2 – 8.7	7.2 – 8.8	24.8 – 28.0	25.0 – 28.3	5.2 – 7.9	5.0 – 7.8
Average±SD	(8.1±0.48)	(8.1±0.48)	(26.4±0.83)	(26.5±0.84)	(6.3±0.66)	(5.8±0.57)
D						
Range	7.3 – 8.8	7.5 – 8.8	24.6 – 28.0	25.2 – 28.2	5.0 – 7.8	5.0 – 7.4
Average±SD	(8.2±0.45)	(8.2±0.42)	(26.4±0.84)	(26.5±0.80)	(6.3±0.65)	(5.8±0.56)
Feasibility value of *O. niloticus*	6.5 – 8.5*		25 – 31*		≥ 3*	
Feasibility value of *Nitrosomonas* sp.	5.8 – 8.5**		5 – 30**		≥4***	
Feasibility value of *Bacillus sp*.	7.8 – 10.5**		25 – 35**		≥ 2****	

Note: *SNI 7550 (2009); **Holt *et al.* (1994); ***Silyn-Roberts and Lewis (2001); ****Hlordzi *et al.* (2020)

Based on the measurement results, this value is suitable for *O. niloticus*, *Nitrosomonas* sp. and *Bacillus sp*. because it is in accordance with the feasibility value.



Note: *Putra *et al.* (2011); **Atwood *et al.* (2005)
Figure 2. Fluctuation of Nitrite Values in *Oreochromis niloticus* Cultivation Media During Study (A: without probiotic, B: probiotic 1.0 µL/L, C: probiotic 1.5 µL/L, and D: probiotic 2.0 µL/L)

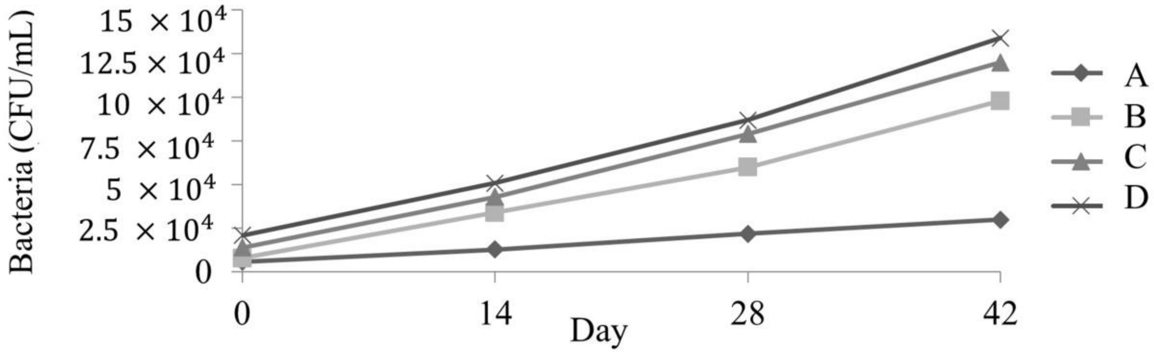


Figure 3. Total Density of Bacteria in *Oreochromis niloticus* Cultivation Media During Study (A: without probiotic, B: probiotic 1.0 µL/L, C: probiotic 1.5 µL/L, and D: probiotic 2.0 µL/L)

Table 2. The Growth of *Oreochromis niloticus* During Study
(A: without probiotic, B: probiotic 1.0 µL/L, C: probiotic 1.5 µL/L, and D: probiotic 2.0 µL/L)

Parameter	Treatment			
	A	B	C	D
Average initial weight (g)	6.73±0.12	7.00±0.07	7.11±0.11	7.08±0.09
Average final weight (g)	14.34±0.09	14.45±0.07	20.61±0.19	15.53±0.18
Weight gain (g)	7.60±0.17[a]	8.21±0.20[b]	14.63±0.24[d]	10.72±0.12[c]
SGR (%/day)	1.80±0.05[a]	1.78±0.05[a]	2.48±0.01[c]	2.19±0.02[b]
FCR	2.15±0.33[c]	2.02±0.02[c]	1.07±0.02[a]	1.48±0.11[b]
SR (%)	67±0.06[a]	67±0.06[a]	97±0.06[b]	70.00±0.00[a]

Note: Values (±SD) with different superscript letters in the same row are significantly different ($P < 0.05$).

Growth

The results of the study showed that giving the probiotic *Nitrosomonas* sp. and *Bacillus sp*. in the rearing media has a significant effect on the growth of *O.niloticus*. The measurement results include absolute weight gain (W) as well as calculations of the SGR, FCR and SR values of *O. niloticus* in each treatment are presented in Table 2. The highest values of weight gain, SGR, and SR, i.e. 14.63±0.24 g; 2.48±0.01%/day; and 97±0.06%, respectively, were observed in treatment C. The lowest value of FCR (1.07±0.02) was also found in treatment C.

3.2 Discussion

Water quality

The pH, temperature and DO values during the research were in optimal conditions as shown in Table 1. The difference in pH values in the morning and evening is due to the processes of photosynthesis and respiration by phytoplankton. The increase in pH value is due to photosynthesis occurring in the waters which produces oxygen, while the low pH value is due to respiration which produces carbon dioxide (Hlordzi *et al.,* 2020). The temperature value in the afternoon is relatively higher due to the influence of the environment, weather, wind and the intensity of incoming light. Optimal temperature can also support bacterial activity through enzyme performance, namely the higher the water temperature, the bacterial metabolic process will increase so that the nitrification process is faster (Hlordzi *et al.,* 2020). The DO value during the research was at the optimal value for the growth of fish and bacteria. DO value ≥ 2 can support the growth of *Nitrosomonas* sp. (Silyn-Roberts and Lewis, 2001) and *Bacillus sp*. (Hlordzi *et al.,* 2020) so that it can break down organic materials effectively.

Ammonia is a toxic compound that can affect fish health. The ammonia value for each treatment at the start of maintenance until the second measurement presented in Table 2 is still above the optimal value, namely < 0.02 mg/L (SNI7550, 2009). However, those values are still within the tolerance range for *O. niloticus*. This is supported by Yan et al. (2021) who stated that ammonia begins to be toxic and can inhibit fish growth at a concentration of 0.05 mg/L. The results showed that treatments B, C and D resulted in a decrease in ammonia values. This result has also been recognized by several previous researchers (Elsabagh *et al.,* 2018; El-Kady *et al.,* 2022).

Nitrite is the result of the decomposition of ammonia by the bacteria *Nitrosomonas* sp. which is toxic to fish (Hlordzi *et al.,* 2020). The nitrite value for each treatment during the research presented in Figure 2 is above the optimal value, namely < 1 mg/L (Putra *et al.,* 2011). This causes the death of fish so that the SR value for *O. niloticus* becomes low. However, not all test fish died. This is because *O. niloticus* can provide high tolerance to nitrite values, namely < 4 mg/L (Atwood *et al.,* 2005). The results showed that B, C and D treatments resulted in a decrease in nitrite values. This result has also been recognized by several previous studies (Elsabagh *et al.,* 2018; El-Kady *et al.,* 2022).

Bacterial Density

The decomposition process of organic material by *Nitrosomonas* sp. and *Bacillus sp*. requires an energy source obtained from ammonia and nitrite found in waters (Hlordzi *et al.,* 2020). Ammonia is a source of nitrogen because it contains proteins, carbohydrates and fats which are used by bacteria as a food source (Saravanan *et al.,* 2021). This is also supported by Validelu *et al.* (2006) that the bacteria *Nitrosomonas* sp. spends 75% of the energy it produces on maintenance processes. In addition, *Bacillus sp*. can produce extracellular enzymes and microbial peptides that control pathogenic bacteria and improve water quality (Xu *et al.,* 2013). The reduced values of ammonia and nitrite in treatments B, C and D were influenced by the density of bacteria found in the water. However, the treatment A experienced a relatively lower increase in bacterial density. Naturally, bacteria can grow by themselves in water. The low number of bacteria in the treatment A was due to not being given probiotics so that relatively few bacteria grew and natural bacteria in the water did not grow (Moriarty, 1997). Therefore, the probiotic *Nitrosomonas* sp. and *Bacillus sp*. can contribute to reducing the value of ammonia and nitrite in waters so that it can improve water quality that is good for fish.

Growth Performance

Growth is the process of increasing the length and weight of an organism which is influenced by the quality of water and food. Based on the research results, it is known that the absolute weight gain (W) value of *O. niloticus* in the treatment C is relatively high, namely 14.63 ± 0.24 g. This is also in accordance with research conducted by Elsabagh *et al.* (2018); El-Kady *et al.* (2022); and Haraz *et al.* (2023) that the appropriate use of probiotics can increase fish growth and productivity because probiotic bacteria can work optimally. Apart from that, giving *Bacillus sp*. in probiotics affect plankton populations in waters and can help optimize growth through increasing the activity of digestive enzymes and the gut microbiota of *O. niloticus* (Elsabagh *et al.,* 2018; Hlordzi *et al.,* 2020). This is in line with this results that the treatment A has relatively low fish growth values. Furthermore, El-Kady *et al.* (2020) mentioned that the low growth of *O. niloticus* can occur due to the high level of undecomposed organic material so that there are relatively few bacteria that support fish growth.

The SGR results on *O. niloticus* can be seen in Table 2. Based on this research, it is known that the best SGR value was in the treatment C (2.48 ± 0.01%/day). These results are better when compared with the SGR value for maintaining *O. niloticus* given *Bacillus sp*. in commercial probiotics, i.e. 1.45±0.04%/day (El-Kady *et al.,* 2022) and which are maintained in a biofloc system and use *Bacillus* subtilis of 1.48±0.05%/day (Haraz *et al.,* 2023). The high SGR value in the study is probably because of the presence of *Nitrosomonas* sp bacteria. which can decompose organic materials well. In addition, *Bacillus sp*. as heterotrophic bacteria which have an important role in the growth of phytoplankton (Hlordzi *et al.,* 2020) and have the ability to improve fish growth performance (Elsabagh *et al.,* 2018). However, the low SGR value in treatment D is thought to be due to excessive activity of probiotic bacteria which can inhibit *Nitrosomonas* sp. and *Bacillus sp*. to work optimally. According to Kuebutornye *et al.* (2020), excessive microbial activity can affect bacterial growth, thereby affecting the efficiency of probiotics. This is because these bacteria use energy and nutrients from the same source so they need to compete for organic substrates that are available as carbon (Mohapatra *et al.,* 2013). Therefore, excess bacteria in water can affect the performance of *Nitrosomonas* sp. and *Bacillus sp*. in maintaining water quality thereby influencing the growth of *O. niloticus*.

The FCR results on *O. niloticus* can be seen in Table 2. It is known that the best results in feed utilization were the treatment C with the lowest FCR value of 1.07. These results are better when compared with the FCR value for maintaining *O. niloticus* given *Bacillus sp*. in commercial probiotics, ie. 1.36±0.04 (El-Kady *et al.,* 2022) and which are maintained in a biofloc system and use *Bacillus* subtilis of 1,54±0.09 (Haraz *et al.,* 2023). This is because the bacteria *Bacillus sp*. probiotics can increase appetite by increasing the activity of fish digestive enzymes resulting in better feed utilization (Hlordzi *et al.,* 2020; Elsabagh *et al.,* 2018) so that less waste is produced. Furthermore, *Bacillus sp*. can stimulate the growth of phytoplankton (Hlordzi *et al.,* 2020) which will be utilized by *O. niloticus* as its natural food. *Bacillus sp* bacteria also plays a role in decomposing organic matter optimally and maintaining water quality so that the fish's appetite does not decrease. However, the high FCR values in treatments A and B suggest that the feed provided was not utilized properly by the fish. Apart from that, it is possible that the high FCR value is due to the low SR value of *O. niloticus* and the high value of ammonia in the water.

The best SR value was observed in the treatment C (97±0.06%). These results are relatively good because they are not much different when compared with the SR value for maintenance of *O. niloticus* given *Bacillus sp*. in commercial probiotics, i.e. 100% (El-Kady *et al.,* 2022). This is probably due to the administration of *Nitrosomonas* sp. and *Bacillus sp*. can change the composition of bacteria in water so that it can help improve water quality to the maximum so that it can increase fish survival. However, giving too much probiotics is giving the negative effect for fish survival. It is known that treatment C is the best treatment compared to treatment D. This is likely due to the ammonia and nitrite values in treatment D waters were much higher compared to C. The higher ammonia and nitrite in D presumably because of too much bacteria. It is possible for competition between bacteria to use energy and nutrients from the same source, namely organic substrates that are available in the form of carbon (Mohapatra *et al.,* 2013). This competition is thought to cause the death of many bacteria, which eventually decompose and increase ammonia and nitrite.

4. Conclusion

The administration of probiotic *Nitrosomonas* sp. and *Bacillus sp*. with a dose of 1.5 µ/L in *O. niloticus* water culture is quite effective in improving water quality and the growth of *O. niloticus*. Further research is needed regarding microbes in waters that inhibit the performance of *Nitrosomonas* sp. and *Bacillus sp*. works optimally in improving water quality.

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