The Addition of Superior Heterotrophic Bacteria in Increasing The Growth of Saline Tilapia (*Oreochrommis Niloticus*)

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**Abstract.** Aquaculture is one of the important types of economic businesses in Indonesia, this can be seen from the many people’s livelihood depend on aquaculture sector. This study aims to analyse the addition of superior heterotrophic bacteria to the growth of saline tilapia. The method used experimental method with a Completely Randomized Design (CRD) where four levels of treatment are carried out. The treatment given is the addition of feed. T0: Without the addition of heterotrophic bacteria. T1: Addition of heterotrophic bacteria isolates J. T2: Addition of heterotrophic bacteria isolates N. T3: Addition of heterotrophic bacteria combined isolates B, C, D, J, H, N.. The results showed that the highest absolute weight growth rate was found in treatment 3 or P3 of 6.09 g, followed by treatment 2 of 4.48 g, treatment 1 of 3.24 g, and the smallest in treatment 0 of 1.86 g. Graduation rate of fish life during the study was not influenced by the addition of heterotrophic bacterial isolates. The number of heterotrophic bacteria and water quality parameters were not significantly different from the addition of heterotrophic bacterial isolates. The addition of superior heterotrophic bacteria can increase the growth of Saline tilapia.

1. **Introduction.**

Freshwater tilapia can be cultivated in ponds even in the sea through the process of adaptation. Tilapia that successfully adapt to salt water are known as Saline Tilapia. Saline tilapia is able to develop and grow in brackish waters with a salt content> 20 ppt or even in sea waters with salinity up to 32 ppt, through the utilization of euryhaline character possessed by tilapia. The development of tilapia culture in brackishwater and high salinity waters has become a concern in various countries such as Thailand, Vietnam, Jamaica, Egypt, Israel. The tendency of changes in the aquatic environment due to global warming which causes sea levels to rise, ground water becomes saltier and paddy fields in coastal areas flooded with sea water, has led to an increase in the area of brackish or salty land that requires anticipatory solutions to use [1].

The principle of fish health management involves 3 main components, namely: the host (fish), pathogens (germs) and the environment. If the balance of these three components can be maintained then the problem of disease will not arise. Pressing the disease case to zero is indeed impossible, but minimizing the occurrence of the disease depends on the condition of fish health management in each business unit [2]. The fish's immune system is also closely related to water quality. Poor water conditions will result in chronic and depressing fish health, changing biochemical parameters, and suppressing innate and adaptive immune responses of fish. The use of antibiotics against sick fish often has a resistance effect, increases bacterial virulence, causes residue in meat and pollutes the environment. To reduce the dependence of antibiotic use, the use of probiotics as an immunomodulator needs to be developed [3].

Adding probiotics to the feed can also increase body immunity and influence survival [4]. Probiotics, according to [5], are microorganisms that live in fish farming that can prevent disease, increase immunity, help break down food to be simpler, thereby increasing production and reducing economic losses.

Heterotrophic bacteria have antimicrobial potential in inhibiting the growth of fish pathogens [6]. Based on the superior heterotrophic isolates that have been owned by previous studies, it is necessary to study more about the benefits of using heterotrophic bacteria as probiotics in fish feed to improve...
health and increase the rate of fish growth. It is expected that the addition of heterotrophic bacteria in fish feed can provide benefits, especially for saline tilapia farmers. The purpose of this study was to analyze the feeding of fish mixed with heterotrophic bacteria to increase fish growth based on water quality measurements.

2. Methodology.
This study used an experimental method with a Completely Randomized Design (CRD) where four levels of treatment were carried out with three replications. The isolates used were superior isolates from marine microbiology laboratory research collections. Test fish samples were given feed that had been mixed with heterotrophic bacteria until the fish felt full, then the remaining feed was taken to avoid the accumulation of leftover feed in the water base. The density of bacteria used in feed is 10-5 CFU / ml. The treatments given are:
- T0: Without heterotrophic bacteria
- T1: Provision of heterotrophic bacteria isolates J
- T2: Provision of heterotrophic bacteria isolates N
- T3: Provision of heterotrophic bacteria combined isolates B, C, D, J, H, N.
The procedure of this research was carried out based on several stages namely preparation of the container, preparation of test fish, preparation of heterotrophic bacterial isolates, testing of heterotrophic bacterial isolates, and measurement parameters.

2.1 Test Fish Preparation
The fish samples used were 120 tilapia seeds, with 10 fish in each bucket (tank). Tilapia are acclimatized for 24 hours to reduce stress on the fish. After 24 hours the fish are transferred to a bucket of 20 liters of water with a salinity of 5 ‰ and left for 24 hours. The salinity of the water is increased gradually from 5-10-15-17 ‰ per day to familiarize the condition of fish with salinity water.

2.2 Testing for heterotrophic bacterial isolates
Feeding with bacterial isolates in Saline Tilapia was carried out for 30 days at 08.00, 13.00, and 17.00. During the test, water was changed every 2 days to avoid accumulation of leftovers and fish droppings.

2.3. Measurement Parameters
During the study, parameters were measured in terms of life passing rate, growth in absolute weight, heterotrophic bacterial counts, and water quality parameters.

2.4. Survival Rate
Based on the formula [7], to measure the passing rate of life, namely:
\[ SR = \frac{N_t}{N_0} \times 100\% \]
Note: SR = Life graduation rate
Nt = Fish population at the end of the rearing period (tail)
N0 = Fish population at the beginning of the rearing period (tail)

Absolute Weight Growth
Based on the formula [8], to measure growth in absolute weight per individual, namely:
\[ W_m = W_t - W_o \]
Note: Wm = Growth in absolute weight of test sample (g)
Wt = Weight of the test sample at the end of the study (g)
W0 = Weight of the test sample at the beginning of the study (g)

2.5. The number of heterotrophic bacteria
Measurement of the number of heterotrophic bacteria in water samples was used to see the state of bacterial colonies during the study. This measurement is carried out once a week using the Total Plate Agar (TPA) method.

The formula used is:
3. Results and Discussion

3.1 Survival rate
 Survival rate of Saline Tilapia is used to see the number of fish that can live during the study by being given different treatments. Survival rates can be seen in Table 1.

| Treatment | Repetition | T0     | T1     | T2     | T3     |
|-----------|------------|--------|--------|--------|--------|
| R 1       | 80         | 90     | 80     | 80     |        |
| R 2       | 80         | 90     | 90     | 80     |        |
| R 3       | 80         | 80     | 80     | 80     |        |
| Total     | 240        | 260    | 250    | 240    |        |
| Average   | 90,83±8,33 | 95,00±6,38 | 94,16±7,87 | 90,70±7,42 |        |

Based on the data in Table 1, it can be seen that the highest passing rate of life is in treatment 1 or T1 of 95.00%, followed by treatment 2 of 94.16%, treatment 0 of 90.83% and treatment 3 of 90.70%.

In the ANOVA test, P> 0.05 was obtained, which showed that there was no effect of the addition of heterotrophic bacteria during the study to the survival level of saline tilapia. The level of life survival obtained can be influenced by the body condition of the fish itself, as well as from the environmental conditions of the research tank. During the study, the survival rate of tilapia was good because it was still above 80%. According to [9], that a survival rate of ≥ 50% is good, survival of 30-50% is moderate and less than 30% is not good.

Fish experiencing death during the study can be caused by stress factors. According to [10] Fish are experiencing stress because the water quality is not completely the same as the water quality of the fish where the tilapia was raised.

3.2 Absolute Weight Growth
 Absolute weight growth is used to see the weight gain of fish that occurred during the study. Absolute weight growth can be seen in Table 2.

| Treatment | Repetition | T0  | T1  | T2  | T3  |
|-----------|------------|-----|-----|-----|-----|
| R 1       | 2.2        | 4.43| 5.22| 6.2 |
| R 2       | 1.8        | 2.67| 4.54| 5.9 |
| R 3       | 1.6        | 2.62| 3.69| 6.17|
| Total     | 5.6        | 9.72| 13.45| 18.27|
| Average   | 1,86±0,30a | 3,24±1,03b | 4,48±0,76b | 6,09±0,16c |

R = Repetition
T = Treatment
Based on the results of table 2 it can be seen that the highest absolute weight growth rate is in treatment 3 or T3 at 6.09 g, followed by treatment 2 at 4.48 g, treatment 1 at 3.24 g, and the smallest at treatment 0 at 1 at 1, 86 g. The effect of weight gain that occurs can be influenced by the type of heterotrophic bacteria used during the research.

In testing using ANOVA, P value <0.05 which indicates the influence of the addition of heterotrophic bacteria during the research of the growth of absolute weight of saline tilapia. Each treatment showed a significantly different average absolute weight, this can be influenced by the characteristics of heterotrophic bacteria added to fish feed. During the research, fish that were given the addition of heterotrophic bacteria in the feed experienced a heavier weight growth compared to fish that were not given the addition of heterotrophic bacteria. Probiotic bacteria in food which then enter the digestive tract and suppress pathogenic bacteria in the intestines can help the process of absorption of food more quickly. According to [11], that giving microbial diets directly benefits the host animal through increased appetite, increasing microbes in the intestine, synthesizing vitamins and stimulating the immune system.

3.3 The Density of Bacteria
The number of heterotrophic bacteria was used to see the density of bacteria found in each treatment. The number of bacteria obtained can be compared to the effect on the health condition of fish and the weight gain of fish. The bacteria that are grown cannot be seen from any type because no further testing is done to find out whether the given bacteria can grow in the environment where the fish are raised. The number of heterotrophic bacteria obtained during the study can be seen in Table 3.

| Observation day | T0  | T1  | T2  | T3  |
|-----------------|-----|-----|-----|-----|
| 0               | 1.34| 1.32| 1.39| 1.45|
| 7               | 1.40| 1.34| 1.45| 1.69|
| 14              | 1.83| 1.79| 1.96| 2.20|
| 21              | 2.37| 2.26| 2.33| 2.22|

Based on table 3 obtained the number of heterotrophic bacteria in each treatment has a density that is not much different. The highest number of heterotrophic bacteria found on day 0 was 1.32-1.45 x 10^6 / ml on day 21 increasing from 2.26 to 2.37 x 10^6 / ml. Heterotrophic bacteria will utilize organic material in their environment. This is consistent with research [12], that heterotrophic bacteria in waters will utilize organic material as a source of carbon for energy metabolism and growth.

The bacteria used during the study were types of bacteria from the genus Bacillus. According to [13], the genus Bacillus is one of the heterotrophic bacteria whose energy dependence comes from the oxidation or de-assimilation of organic carbon compounds. Bacillus sp. can live well in synthetic medium containing sugar, organic acids, alcohol as a source of carbon and as a source of nitrogen. Morphologically the genus Bacillus is a thick stem with central, subterminal and terminal spores, its movement with flagella. Bacillus sp. Is mostly found in the rhizosphere and possibly its habitat. In these habitats Bacillus grows actively at pH 5.5-8.5

Bacillus type of bacteria given serves to form biofloc and play a role in the nitrification process. The nitrification process is also influenced by the availability of dissolved oxygen to be able to carry out nitrification. According to [14] bacteria capable of forming biofloc include: Bacillus cereus, Bacillus subtilis, Escherichia intermedia, Flavobacterium, Paracolobacterium aerogenoids, Pseudomonas alcaligenes, Sphaerotilla natans, Zoogloe ramigera. Bacteria that can form biofloc such as Bacillus circulans, Bacillus coagulans and Bacillus licheniformis. The bacteria that contribute to this floc have a function in the nutrient cycle in the biofloc system. These bacteria are referred to as functional cycle bacteria, for example Bacillus licheniformis which plays a role in the nitrogen cycle.

3.4 Water Quality Parameters
Water quality parameters are used to look at the perarian conditions where saline tilapia nurseries are maintained. The condition of the waters is endeavored in such a way as to be in accordance with the
conditions needed for the place to live fish. Water quality parameters during the study can be seen in Table 4.

**Table 4. Condition of water quality parameters during the study.**

| Parameter | T0       | T1       | T2       | T3       |
|-----------|----------|----------|----------|----------|
| Temperature | 27-28   | 27-28   | 27-28   | 27-28   |
| pH        | 7.0-8.0 | 7.0-8.0 | 7.0-8.0 | 7.0-8.0 |
| OD (mg/l) | 3.8-4.5 | 3.9-4.1 | 4.0-4.2 | 4.0-4.5 |
| NH3 (ppm) | 0.71-1.18 | 0.98-1.43 | 1.06-1.82 | 1.28-1.72 |

T= treatment  
OD = Oxygen Demand

Based on Table 4 it can be seen that the water temperature during the study was relatively stable, which ranged between 27-28°C. In the measurement of water pH obtained ranges from 7.0 to 8.0 in all research tanks. The pH measurement also shows a relatively stable number between each tank. DO meter measurements obtained levels of dissolved oxygen between 3.8 to 4.5 mg / L. In all research trials there was an increase in the amount of dissolved oxygen during the study. Ammonia measurements obtained in the range between 0.70-1.82 ppm during the study. The ammonia levels can change during the study because it is carried out on the research tank.

Tilapia activity that produces acid from the results of metabolic processes can result in a decrease in water pH, ponds that have not been replaced for a long time will cause a decrease in pH, this is due to an increase in acid production by tilapia that accumulates constantly in the pond and this can cause the toxic power of ammonia and nitrite in tilapia culture will increase sharply [15].

Based on the measurement of the ammonia value, a range of 0.71 to 1.82 ppm was obtained, which is high for maintenance of saline tilapia. According to [15], the presence of ammonia in water can cause reduced oxygen binding capacity by blood grains, this will cause decreased fish appetite. Oxygen and ammonia levels in the waters are inversely proportional, if ammonia increases, oxygen levels become low, good ammonia levels are less than 1 ppm, whereas if ammonia levels are more than 1 ppm then it can be harmful to fish and other aquatic organisms.

4. Conclusion.
The addition of heterotrophic bacteria mixed into the feed can increase the growth of absolute weight. The addition of a superior heterotrophic bacterial consortium is better than one superior bacterial species. Graduation rate of fish life during the study was not influenced by the addition of heterotrophic bacterial isolates. The number of heterotrophic bacteria and water quality parameters were not significantly different from the addition of heterotrophic bacterial isolates.

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