Effect of heterotrophic bacteria on the growth of tilapia (Oreochromis niloticus) cultivated in brackish water

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Abstract. Tilapia is a commercial fish cultivated in both fresh water and seawater frequently attacked by pathogenic bacteria. Some bacteria have become probiotics and help overcome this problem. This study aimed to examined the effect of heterotrophic bacteria administration on survival rate and absolute weight growth of the fish, heterotrophic bacterial population growth and the water quality. The study used a factorial experimental design a x b, where a factor was the method of administering heterotrophic bacteria and b factor was the species of isolate used. Factor a consists of an introduction to fish via feed (a1) and via water test media (a2). Factor b consisted of b1 (diet without heterotrophic bacterial treatment), b2 (diet with heterotrophic bacterial isolate J), b3 (diet with heterotrophic bacterial isolate N), b4 (diet with heterotrophic bacterial combined isolates B, C, D, J (Vagococcus fluvialis), H, N; Bacillus cereus). The fishes were reared in a 60 l container. Administering via a feed of heterotrophic bacteria showed no significant effect to survival rate of tilapia. While administering via water showed a significant influence on the survival rate. Both administration methods caused a better absolute growth rate. However, administration of the bacteria did not affect the heterotrophic bacterial populations in the water.

Keywords: brackish water; heterotrophic bacteria; probiotic tilapia

1. Introduction

Tilapia can be cultivated in freshwater water and through a series of adaptations is also commonly cultivated in brackish water [1]. The fish grow and breed in brackish waters in salinity of > 20 ppt or even in sea waters with salinity up to 32 ppt through the utilization of its euryhaline character. The development of tilapia culture in brackish waters has become a concern in various countries [2] such as Egypt, Vietnam, Jamaica, and Thailand [3].

Current aquaculture activities are vulnerable to disease threats. The emergence of disease due to disease agents may come from the environment or due to engineering actions on intensive cultivation it self. 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 infected fish often has a resistance effect, increases bacterial virulence, causes residue in the flesh, and pollutes the environment. To reduce the dependence of antibiotic use, the use of probiotics as an immunomodulator needs to be developed [4].

The administration of probiotics to fish diet can also increase the body's immunity and influence survival [5]. According to other authors [6] probiotic contains microbial cells that have been prepared,
which is beneficial for the health and life of its host. Others [7] have succeeded in isolating heterotrophic bacteria which can be potential as probiotics for tilapia diet cultivated in brackish water. Based on the superior isolates that have been revealed by previous studies, it is necessary to study the benefits of using heterotrophic bacteria as probiotics to improve health and increase the growth rate of fish. This study aimed to examine the effect of heterotrophic bacteria on tilapia survival rate, absolute weight growth, heterotrophic bacterial population growth, and water quality.

2. Methodology

2.1. Time and place
The fishes (size 5-8 cm), collected from a hatchery, and the research was carried out from July to December 2019 in Marine Microbiology Laboratory, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences.

2.2. Experimental design
The study used a factorial experimental design a x b, where factor a is a method of administering heterotrophic bacteria and factor b is the type of isolate used. Factor a consists of an introduction to fish via feed (a1 or AVF) and via water test media (a2 or AVW). Factor b consisted of b1 (diet without heterotrophic bacterial treatment), b2 (diet with heterotrophic bacterial isolate J), b3 (diet with heterotrophic bacterial isolate N), b4 (diet with heterotrophic bacterial combined isolates B, C, D, J, H, N).

2.3. Preparation of container
The rearing container for the fish was a 60 lb basin of 12 units. The basins were first washed to remove pathogenic microorganisms, then filled with potassium permanganate solution and allowed to stand for 24 hours to sterilize. The container was then rinsed with clean water to remove leftover feed and fish feces and then filled with 40 l of brackish water.

2.4. Acclimatisation of fish
The fishes were placed in a container with up to 10 fishes per container. Tilapia are first acclimatized for 24 hours to reduce stress, then transferred to a bucket of 20 liters of water with a 5 ‰ salinity for 24 hours. The salinity of the water was gradually increased every day from salinity 5 - 10 - 15 - 17 ‰ to familiarize the fish with salinity water to the salinity of 17 ‰.

2.5. Preparation of heterotrophic bacterial isolates
Heterotrophic bacterial isolates were provided by Marine Microbiology Laboratory with code isolates B, C, D, J (Vagococcus fluvialis), H, and N (Bacillus cereus). Rejuvenated isolates in nutrient media were taken 1 loop and grown into nutrient broth media for 24 hours. The isolates were then transferred into a centrifugation tube and centrifuged for 10 minutes to separate the bacteria from the nutrient media broth (supernatant). The supernatant was removed and filled with 10 ml phosphate buffer saline then vortexed. The same process was carried out up to 3 times to obtain bacterial suspension with a density of 10^5 cell/ml.

2.6. Administering the isolates to the fish
Administering heterotrophic bacteria to fish was carried out in 2 ways, namely via food (a1; AVF) and via water (a2; AVW). For AVF, 15 ml of bacterial suspension or 2.5 ml per isolate for experiment unit b4 (combined) was placed into a measuring cup. A volume of 250 ml of distilled water and 2.25 ml of NaCl were added to obtain a volume of a solution of 267.25 ml. The solution was then removed into a spray bottle, then sprayed into a container containing 250 g of fish feed, and allowed to stand until dry, and ready to use. For the experimental unit a2, a heterotrophic bacterial suspension of 10 ml was then mixed into the cultivating media.
2.7. Fish examination

The fishes were fed 3 times a day at 08.00, 13.00, and 17.00. The fish were fed by using a commercial pellet of PF 800 produced by PT. CP Prima, Medan. During the experiment, the containers were cleaned every 2 days to avoid an accumulation of leftovers and fish feces. Water quality was examined every week for 21 days. The measured variables included survival rate, absolute growth rate, and growth of heterotrophic bacteria. The growth of heterotrophic bacteria in water samples was checked every week using the total plate count agar method. Water quality parameters measured were temperature, pH, dissolved oxygen, and ammonia levels. All data were tabulated and analyzed with analysis of variance.

3. Result and discussion

3.1. Survival rate

In the AVF treatment (a1; administering the heterotrophic bacteria via feed) the highest survival rate of fish was found at a1b2 of 95.00%, followed by a1b3 of 94.16%, a1b1 of 90.83%, and a1b4 of 90.70%. But the ANOVA test results showed no significant effect of the administration of heterotrophic bacteria to the survival rate administration of tilapia. While the AVW treatment (a2; administering the heterotrophic bacteria via water) the highest survival rate was 96.77% (a2b3), followed by a2b2 (90%), a2b1 (86.67%), and a2b4 (66.67%). ANOVA test results showed the administration of heterotrophic bacteria to the water influenced the survival rate of the fish. More detailed survival rate data are presented in table 1.

| Table 1. Survival rate (%) of tilapia. |
|--------------------------------------|
| Factor a | b1 | b2 | b3 | b4 |
| a1        | 90.83 | 95.00 | 94.16 | 90.70 |
| a2        | 86.67 | 90.00 | 96.77 | 66.67 |

3.2. Absolute growth rate

Absolute growth rate is used to see the weight gain of fish during the study. In the AVF treatment (a1) the highest growth occurred a1b4 experimental unit (6.09 g), followed by a1b3 (4.48 g), a1b2 (3.24 g), and a1b1 (1.86 g). The statistical analysis showed the influence of the administration of heterotrophic bacteria on the absolute growth rate of test fish. While in AVW (a2), the absolute weight ranges from 1.30-3.03 g. The highest growth was in the unit experiment of a2b3 (3.03 g), then followed by a2b2 (2.05 g), a2b1 (1.80 g), and a2b4 (1.30 g). The statistical analysis showed the influence of the administration of heterotrophic bacteria on the absolute growth rate of test fish. More detailed data are presented in table 2.

| Table 2. Absolute growth rate (g) of tilapia. |
|-----------------------------------------------|
| Factor a | b1 | b2 | b3 | b4 |
| a1        | 1.86 | 3.24 | 4.48 | 6.09 |
| a2        | 1.80 | 2.05 | 3.03 | 1.30 |

3.3. Growth of heterotrophic bacteria

The highest heterotrophic bacterial growth in AVF (a1) treatment on the last day of sampling (21 days) was in the experimental unit of a1b1 at 2,371 cells/ml, followed by a1b3 at 2,327 cells/ml, a1b3 at 2,257 cells/ml and a1b4 at 2,224 cells/ml (table 3). ANOVA test released that the value of P was > 0.05 which means heterotrophic bacterial isolates added to fish feed did not affect the number of heterotrophic
bacteria in the overall test media water. While the AVW (a2) treatment, it was noted that the highest total bacterial colony in water was in the a2b2 experimental unit (2,271 cell/ml), followed by the a2b3 treatment of 2,214 cell/ml, a2b4 of 2,059, and finally in a2b1 leading to heterotrophic bacteria number of 1,937 cells/ml (table 4).

Table 3. Growth of heterotrophic bacteria (cell/ml) in a1 (AVF) experimental unit.

| Experimental unit | Day of experiment |
|-------------------|-------------------|
|                   | 0                 | 7     | 14    | 21    |
| a1b1              | 1.346             | 1.399 | 1.832 | 2.371 |
| a1b2              | 1.322             | 1.335 | 1.789 | 2.257 |
| a1b3              | 1.385             | 1.445 | 1.959 | 2.327 |
| a1b4              | 1.453             | 1.692 | 2.201 | 2.224 |

Table 4. Growth of heterotrophic bacteria (cell/ml) in a2 (AVW) experimental unit.

| Experimental unit | Day of experiment |
|-------------------|-------------------|
|                   | 0                 | 7     | 14    | 21    |
| a2b1              | 1,663             | 1,849 | 2,376 | 1,937 |
| a2b2              | 1,973             | 2,099 | 2,669 | 2,271 |
| a2b3              | 1,971             | 2,197 | 2,412 | 2,214 |
| a2b4              | 1,419             | 1,754 | 2,297 | 2,059 |

3.4. Water quality

Water temperature during the study was relatively stable, which ranged between 27-28°C. The pH range of water was obtained from 7.0-8.0. Dissolved oxygen levels were between 3.8 to 4.5 mg/l. Ammonia ranged from 0.70-1.82 ppm during the study. While in the AVW treatment it was seen that the water quality during the study was classified as good for aquaculture activities. The temperature in all experimental units ranged from 26.5-28 °C, pH ranged from 7.6-7.9, dissolved oxygen ranged from 1.1 to 2.3 mg / L and ammonia ranged from 0.61-1.70 ppm (table 6).

Table 5. Water quality on AVF (a1) treatment.

| Experimental unit | Parameter | Temperature (°C) | pH     | DO (mg/L) | NH3 (ppm) |
|-------------------|-----------|------------------|--------|-----------|-----------|
| a1b1              |           | 28               | 7.0-8.0| 3.8-4.5   | 0.71-1.18 |
| a1b2              |           | 28               | 7.0-8.0| 3.9-4.1   | 0.98-1.43 |
| a1b3              |           | 27-28            | 7.0-8.0| 4.0-4.2   | 1.06-1.82 |
| a1b4              |           | 28               | 7.0-8.0| 4.0-4.5   | 1.28-1.72 |

Table 6. Water quality on AVW (a2) treatment.

| Experimental unit | Parameter | Temperature (°C) | pH     | DO (mg/L) | NH3 (ppm) |
|-------------------|-----------|------------------|--------|-----------|-----------|
| a2b1              |           | 27-28.2          | 7.6-7.8| 2.75-5    | 0.74-1.58 |
| a2b2              |           | 26-28            | 7.6-7.9| 3-5.5     | 0.73-1.63 |
| a2b3              |           | 26.5-28          | 7.7-7.9| 3-5.75    | 0.61-1.62 |
| a2b4              |           | 26.8-27.5        | 7.5-7.8| 3-5       | 0.81-1.70 |
3.5. Discussion
The survival rate of tilapia in the two methods of administering heterotrophic bacteria (AVF and AVW) was higher compared to the control experimental unit. However, all unit experiment still showed relatively good condition (above 80%). According to some workers [8], the survival rate of ≥50% is still good. ANOVA showed that the value of P > 0.05 which means there is no effect of the administration of heterotrophic bacteria on fish survival. The death of some fish is rather to water quality matter, stress factors, or the inability of the fish to adapt to the given environment. According to some researchers [9], fish may experience stress due to water quality differences between new and old environments where tilapia was raised.

Heterotrophic bacteria can increase the survival rate of fish by improving water quality, killing pathogenic bacteria, and improving fish health through digestion. Heterotrophic bacteria used in this study are *Bacillus*, a probiotic bacteria which usually play a role in improving the health and survival rate of some fishes administered through fish digestion tract. The use of *Bacillus* sp has proven to increase and maintain the survival rate of aquatic organisms. Probiotic bacteria in aquaculture plays a role in maintaining the survival rate and enhancing the immune system by changing the bacterial community [10].

The administration of heterotrophic bacteria via diet and water increased the absolute growth rate of fish compare to fish in control in experimental units. This is thought to be a positive effect of heterotrophic bacteria (*Bacillus cereus* and *Vagococcus fluvialis*). The statistical analysis mentioned that P <0.05, meaning that heterotrophic bacteria influenced the growth rate of tilapia. Multiple species or probiotic strains are more efficient than a single species due to the synergistic effect of probiotics [11].

Probiotic bacteria in the digestive tract of fish will compete with pathogenic bacteria in competing for space and food sources. The bacteria also produce enzymes that can break down the long-chain and complex compound materials so that the feed can be more easily digested by fish. Direct microbial diets such as *Lactobacillus* sp provide benefits for host animals by increasing appetite, increasing microbes in the intestine, synthesizing vitamins, and stimulating the immune system [4].

Other authors [12] mentioned that heterotrophic bacteria are found to be better used as antipathogens against *Vibrio alginolitycus* than *A. hydrophila* and *Pseudomonas* sp. *Bacillus* has enzymes that have been produced on an industrial scale, including alanine and formic enzymes, α-amylase, isoamylase, β-amylase, glucoamylase, chitinase, and cholesterol oxidase. *Bacillus* also have enzymes that help digestion of fish by breaking down the complex compounds to simpler so that it can be easily digested by fish [13]. In addition to producing cellulase enzymes, the bacterial isolate of *Bacillus* also produced lipase, amylase, and protease enzymes [14].

The administration of heterotrophic bacterial isolates did not affect the number of bacteria present in the test media water. The bacteria used in this study were *Bacillus cereus* and *Vagococcus fluvialis*. Water quality parameters (temperature, pH, dissolved oxygen, and ammonia levels) during the study showed that the water was optimum and support the growth and survival of tilapia. This is consistent with previous tilapia researchers [15-17].

4. Conclusion and suggestion

4.1. Conclusion
AVF (administering via feed) treatment showed no significant effect of the administration of heterotrophic bacteria on the survival rate of tilapia. While AVW treatment (administering via water) showed a significant influence of heterotrophic bacteria on the survival rate. Statistical analysis showed that administration of heterotrophic bacteria affected the absolute growth rate of the fish. ANOVA test states that administration of heterotrophic bacterial both AVF and AVW treatments does not affect the number of heterotrophic bacterial populations in the water.
4.2. Suggestion
It is suggested heterotrophic bacterial isolates of *Bacillus* and *Vagococcus fluvialis* are also challenged with other fish species.

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