Utilization of *Nitrosomonas* sp. and *Nitrobacter* sp. probiotic towards nitrite and nitrate level in nile tilapia (*Oreochromis niloticus*) using aquaponic system

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Abstract. Based on KKP data, nile tilapia production is highly increasing from 2015 – 2018. To generate high production of nile tilapia, intensive culture system is needed. The latter indicated with high density stock and feed amount, which lead to high accumulation of organic waste. Those accumulation of organic waste considered harmful towards fish commodity due to contained ammonia and nitrite which both of them categorized as toxic. The solution for the prior problem is by applying aquaponic system along with the addition of *Nitrosomonas* and *Nitrobacter* probiotic to support nitrification process. Nitrification process described as decreasing process of ammonia and nitrite by oxidizing them into nitrate. The effective dosage of the probiotic has yet to be known, so this study aim to figure out whether the addition of those probiotic with certain dosage can decrease nitrite and increase nitrate. This study uses Rancang Acak Lengkap (RAL) with 5 treatments repeated 4 times. Nitrite and nitrate data analyzed by ANOVA. The result showed the addition of *Nitrosomonas* and *Nitrobacter* probiotic decrease nitrite and increase nitrate with 1,5 mg/L as the most effective dosage. Prior dosage decrease nitrite level into 0,0672 ± 0,0013 mg/L and increase nitrate level into 3,936 ± 0,1175 mg/L.

1. Introduction

Based on KKP data in 2018, nile tilapia production is highly increasing from 1.084.281 ton in 2015 into 1.546.675 ton in 2018 [1]. To generate high production of nile tilapia, intensive culture system is needed. Intensive culture system indicated with high density stock and feed amount, so that possibility of high accumulation of organic waste such as leftover feed and feces [2]. Those accumulation of organic waste considered harmful towards fish commodity due to contained ammonia and nitrite in organic waste which both of them categorized as toxic compound [3]. The solution for the prior problem is by applying aquaponic system.

Aquaponic described as a recirculating aquaculture system combined with hydroponic system. Aquaponic system utilizes organic waste by aquaculture commodity as nutrient for hydroponic system, so that water quality in aquaculture system will be improved, and the hydroponic system will grow [4]. The organic waste contains ammonia as toxic component. Ammonia will be oxidized into Nitrite by *Nitrosomonas* sp., continued by nitrite will be oxidized into nitrate by *Nitrobacter* sp.
Nitrate then absorbed by hydroponic component as nutrient. Both *Nitrosomonas* sp. and *Nitrobacter* sp. are nitrification bacteria, and prior process is called nitrification [5]. The addition of both nitrification bacteria is by applying probiotic.

Previous study showed that the addition of 1 mg/L *Nitrosomonas* and *Nitrobacter* probiotic does increase the biomass of 30 Nile tilapia fingerlings from 21 g to 25 g in 2 weeks [6]. Prior dosage has yet proven the effectivity in water quality especially nitrite and nitrate. Thus, the aim of this study is to figure out whether the addition of *Nitrosomonas* and *Nitrobacter* probiotic with different dosage decrease nitrite and increase nitrate in Nile tilapia culturing using aquaponic system.

2. Materials and methods

2.1. Aquaponic system preparation

The aquaponic system in this study is made of aquarium as the aquaculture component media, gutter as the hydroponic component media, and water pipe as the connector for both media. Aquarium has to be disinfected beforehand with KMNO₄ 0.125 ppm. Then, aquarium filled with 3 – 5 cm sized Nile tilapia fingerlings with stock density of 1 fish/L. Water spinach as the hydroponic component sowed 5 days, then placed on the gutter with rockwool media. The gap between each water spinach is 10 cm. Nile tilapia culturing with aquaponic system held for 29 days. The fingerlings fed by commercial feed (protein 38 – 42%, fat 4 – 6 %, fiber 2 – 3% and water content 9 – 10 %) 3 times a day.

2.2. Probiotic addition

This study uses five different probiotic dosages, each of the dosage repeated four times. Those five different dosage list below:

a. P₀: control (without any addition of probiotic).

b. P₁: 0.5 mg/L

c. P₂: 1 mg/L

d. P₃: 1.5 mg/L

e. P₄: 2 mg/L

The probiotic contained 10⁷ CFU/g *Nitrosomonas* and *Nitrobacter* bacteria. The probiotic also added to the aquaponic system every once a week.

2.3. Observed Parameter

The main observed parameter are nitrite and nitrate, wherein both nitrite and nitrate level measured by spectrophotometry using water sample. Nitrite and nitrate level observed once a week. The other supporting parameter are fish growth data, water spinach growth data and water quality: water temperature, pH, and dissolved oxygen.

2.4. Data Analysis

The main parameter (nitrite and nitrate level) analyzed with One Way ANOVA (Analysis of Variance). If the result shows a significant difference, nitrite and nitrate data analyzed with Tukey HSD as post hoc data. The other supporting parameter written and displayed in table.

3. Results and Discussion

3.1. Results

| Table 1. Nitrite and nitrate level |
|-----------------------------------|
| Treatment | Nitrite (mg/L) | Nitrate (mg/L) |
| P₀        | 0.0806 ± 0.0045 | 2.786 ± 0.2004 |
| P₁        | 0.0755 ± 0.0023 | 3.407 ± 0.4070 |
| P₂        | 0.0779 ± 0.0013 | 3.435 ± 0.1731 |
| P₃        | 0.0672 ± 0.0013 | 3.936 ± 0.1175 |
| P₄        | 0.0644 ± 0.0067 | 3.97 ± 0.6648 |

Note: Different superscript indicate significant difference (P<0.05).
Table 2. Supporting parameter

| Parameter                  | P0          | P1          | P2          | P3          | P4          |
|----------------------------|-------------|-------------|-------------|-------------|-------------|
| DO (mg/L)                  | 4.77 – 4.67 | 3.24 – 3.42 | 3.08 – 3.39 | 2.95 – 3.07 | 2.96 – 3.32 |
| pH                         | 8 – 8.075   | 7.05 – 7.15 | 7.1 – 7.2   | 7.05 – 7.2  | 7.15 – 7.225|
| Water Temperature (°C)     | 27.7 – 29.17| 28.32 – 29.17| 27.72 – 28.8| 28.3 – 29.57| 28.22 – 29.3 |
| Water spinach total leaves | 6           | 8           | 8           | 10          | 12          |
| Water spinach stem length (cm) | 9.12       | 17          | 17.25       | 20.25       | 22.62       |
| Nile tilapia Specific Growth Rate (%/day) | 2.34       | 2.6         | 3.9         | 4.6         | 4.81        |

3.2. Discussion

The result on table 1 shows that the addition of *Nitrosomonas* and *Nitrobacter* does decrease nitrite and increase nitrate significantly (P < 0.05) with the most effective dosage 1.5 mg/L (P3). Those dosage can reduce nitrite into 0.0672 ± 0.0013 mg/L and increase nitrate into 3.936 ± 0.1175 mg/L. On the contrary, highest nitrite level (0.0806 ± 0.0045 mg/L) and lowest nitrate level occurred in P0 (without any addition of probiotic) P4 yaitu 0.0644 ± 0.0067 mg/L. Decreasing nitrite level along with increasing nitrate level is due to nitrification process. Nitrification described as a process wherein nitrification bacteria oxidized ammonia into nitrite, then nitrite into nitrate [7]. The used nitrification bacteria in this study is *Nitrosomonas* and *Nitrobacter* which both are comes from probiotic. Nitrification started with dechipering organic waste such as leftover feed (which contain protein then degradated into amino acid) and feces (which contain urea). Amino acid and urea turn into ammonia by deamination process. Bacteria known as biological agent which can improve water quality by deichpering organic susbtance [8]. Ammonia from organic waste will be oxidized into nitrite by *Nitrosomonas* sp., and nitrite will be oxidized into nitrate by *Nitrobacter* sp. [9].

Overall nitrite level in this study range between 0.0644 ± 0.0067 - 0.0806 ± 0.0045 mg/L whereas those level is well tolerated by nile tilapia. Previous study stated nitrite level in nile tilapia culturing must not surpassed 0.1 mg/L [10]. High level of nitrite can lead fish into hypoxia as the hemoglobin is inhibited by nitrite to bind oxygen [11].

Nitrile level in aquaponic system without probiotic treatment is 2.786 ± 0.204, meanwhile nitrate level in aquaponic system probiotic treatment is slightly higher, ranged between 3.407 ± 0.4070 - 3.97 ± 0.6648 mg/L. Generally, nitrate level in aquaponic system is 3 - 5 mg/L [12], so that we know nitrate level in this study considered as suitable to the previous statement. Low level of nitrate can caused withering in hydroponic system [13], while exaggerated level of nitrate can caused algae blooming. Algae blooming described as water condition with high population of phytoplankton. Algae blooming can lead to low oxygen supply so that possibility of hypoxia fish is high [14].

Overall water temperature, DO, and pH in aquaponic system without probiotic addition (P0) are 27.7 – 29.7°C, 4.77 – 4.67 mg/L, and 8 – 8.075 respectively. Overall water temperature in aquaponic system with probiotic addition relatively is 27.72 – 29.57°C, which has no difference with P0, meanwhile DO and pH level are slightly lower than P0. Average DO and pH level in aquaponic system with probiotic addition are 2.95 – 3.42 mg/L and 7.05 – 7.225. Water temperature, pH, and DO in all aquaponic system of this study considered tolerable for nile tilapia and nitrification bacteria, whereas nile tilapia can tolerate water temperature of 25 – 30°C, DO minimum 2 mg/L, and pH 7 – 8 [15], and nitrification bacteria can tolerate water temperature 5 – 30°C, DO minimum 2 mg/L, and pH 5,8 - 8,5. Lower DO level in aquaponic system with probiotic system is due to high amount of
nitrification bacteria, where nitrification bacteria uses oxygen to oxidize ammonia into nitrate. Similarly, pH level in aquaponic system with probiotic addition is slightly lower due to nitrification bacteria oxidation process, which the process generates acid water condition [16].

4. Conclusion

The addition of *Nitrosomonas* and *Nitrobacter* probiotic significantly decreases nitrite and increase nitrate in nile tilapia culturing using aquaponic system, with 1.5 mg/L as the most effective dosage.

5. References

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