The effect of different stocking densities on ammonia (NH₃) and nitrate (NO₃) concentration on striped snakehead (Channa striata) culture in the bucket

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Abstract. Fish farming in the bucket is a fishery technology that is being developed using an aquaponic system. Striped snakehead fish (Channa striata) is one of the economically important types of fish but the cultivation of striped snakehead fish using an aquaponic system has never been carried out, so it is necessary to know the optimal stocking density of striped snakehead fish to minimize the risk of stress for fish. This study aims to determine the effect of different stocking densities on ammonia (NH₃) and nitrate (NO₃) concentration on striped snakehead (Channa striata) culture in the bucket. This research using 5 treatments and 4 replications, namely P0 1 fish/Liters; P1 2 fish/Liters; P2 3 fish/Liters; P3 4 fish/Liters; and P4 5 fish/Liters. The observed parameters are ammonia (NH₃) and nitrate (NO₃) concentration. The results of this study showed that the value of the ammonia concentration was between 0.089-0.609 mg/L and the nitrate concentration was between 0.931-1.539 mg/L. Based on the results of this study, it can be concluded that treatment of different stocking density affect the concentration of ammonia (NH₃) and nitrate (NO₃) in the cultivation of Snakehead fish (Channa striata) in the bucket.

1. Introduction

Fish farming in the bucket is a fishery technology that is being developed using an aquaponic system. In an aquaponics system, fish is the main product and vegetables are a by-product [1]. Striped snakehead fish (Channa striata) is one of the economically important types of fish. This species has a distinctive taste, thick textured flesh and white in color. In addition, this fish has a high protein content, namely albumin which is needed by the human body in overcoming various diseases [2]. The high price of commercial albumin preparations makes snakehead fish an alternative source of cheap albumin. This is what causes the high level of consumption of this fish. Snakehead fish (Channa striata) has the potential to be cultivated with this system because this fish has an additional respiratory apparatus (labyrinth) that can breathe air directly.
Unfortunately, the cultivation of striped snakehead fish using an aquaponic system has never been carried out, so it is necessary to know the optimal stocking density of striped snakehead fish to minimize the risk of stress for fish. Increasing stocking density until it reaches the maximum carrying capacity will cause fish growth to decrease [3]. Other than that, fish farming activities cannot be separated from the waste generated, especially from leftover feed, feces, and the results of fish metabolism activities [4]. In aquaculture systems, water is a fish rearing medium that must always be considered for its quality because it greatly affects the productivity of aquatic animals, one of which is ammonia. High ammonia concentrations can lead to large reductions in oxygen supply and undesirable changes in aquatic ecosystems [5]. In water, ammonia is naturally liberated and transformed into other forms in the nitrogen cycle. Nitrate is the end product of oxidizing agents associated with nitrogen. The use of ammonia by plants as biological filter media will reduce the concentration of toxins in fish culture media. Nitrates and other nutrients in the water will be absorbed by plants for growth and also serves to clean the air [6].

2. Materials and methods

2.1 Research Location
This research location is in Microbiology and Fish Disease Laboratory of Fisheries and Marine Faculty, Universitas Airlangga.

2.2 Tools and Materials
Tools needed are 80L bucket, plastic cup, wire, pliers, solder, DO meter, thermometer, pH pen, volumetric flask, erlenmeyer, graduated cylinder, volumetric pipette, measuring pipette, beaker glass, spectrophotometer, cuvette, and electronic analytic scale. Materials needed are striped snakehead fish (4-6 cm), water spinach (stem), charcoal, ammonium chloride (NH₄Cl), phenol solution (C₆H₅OH), sodium nitropruside (C₅FeN₆Na₂O) 0.5%, trisodium citrate (C₆H₅Na₃O₇), sodium hypochloride (NaClO) 5%, aquadest, and potassium nitrate (KNO₃).

2.3 Data Analysis Method
This research method is experimental using 5 treatments with 4 replications for each treatment. The treatment given in this study was the difference in stocking density of fish namely P0 1 fish/Liters (60 fish / 60 Liters); P1 2 fish/Liters (120 fish/ 60 Liters); P2 3 fish/Liters (180 fish/ 60 Liters); P3 4 fish/Liters (240 fish/ 60 Liters); and P4 5 fish/Liters (300 fish/ 60 Liters). Water spinach plants are placed in the plastic cup that used as the net pot, this study used 10 plastic cup each bucket. Each bucket filled with 60L water.

3. Result and discussion
Calculation of ammonia, nitrate, water quality (temperature, DO, ph), and plant growth for 28 days using 5 different stocking densities of fish. The results can be seen in table 1, 2, 3, 4 and Figure 1.

Table 1. Ammonia levels during research

| Treatment | AMMONIA (mg/L) | 1st week | 2nd week | 3rd week | 4th week |
|-----------|----------------|----------|----------|----------|----------|
| P0        | 0.089 ± 0.020  | 0.142 ± 0.027 | 0.212 ± 0.034 | 0.336 ± 0.020 |
| P1        | 0.159 ± 0.037  | 0.217 ± 0.036 | 0.292 ± 0.036 | 0.389 ± 0.047 |
| P2        | 0.314 ± 0.044  | 0.357 ± 0.050 | 0.405 ± 0.057 | 0.480 ± 0.044 |
| P3        | 0.379 ± 0.047  | 0.443 ± 0.047 | 0.480 ± 0.041 | 0.405 ± 0.038 |
| P4        | 0.609 ± 0.067  | 0.528 ± 0.036 | 0.491 ± 0.046 | 0.453 ± 0.036 |
Table 2. Nitrate levels during research

| Treatment | 1st week | 2nd week | 3rd week | 4th week |
|-----------|----------|----------|----------|----------|
| P0        | 0.931 ± 0.039 | 0.946 ± 0.001 | 1.025 ± 0.009 | 1.136 ± 0.060 |
| P1        | 1.079 ± 0.004 | 1.190 ± 0.003 | 1.393 ± 0.038 | 1.456 ± 0.033 |
| P2        | 0.980 ± 0.001 | 1.073 ± 0.026 | 1.204 ± 0.004 | 1.278 ± 0.023 |
| P3        | 1.221 ± 0.085 | 1.346 ± 0.142 | 0.966 ± 0.096 | 1.023 ± 0.016 |
| P4        | 1.342 ± 0.013 | 1.538 ± 0.016 | 1.142 ± 0.149 | 0.937 ± 0.095 |

Table 3. Average water quality during research

| Parameter | P0 | P1 | P2 | P3 | P4 | Optimum value | Reference |
|-----------|----|----|----|----|----|---------------|----------|
| Temperature (°C) | 28.1 – 30.2 | 28.1 – 30.3 | 28.2 – 30.3 | 28.3 – 30.3 | 28.3 – 30.2 | 25.5 - 32.7 | [7] |
| DO (mg/L) | 0.99 – 2.19 | 1.00 – 2.08 | 0.44 – 2.02 | 0.30 – 1.81 | 0.30 – 1.74 | 2.0 – 3.7 | [7] |
| pH | 7.4 – 7.8 | 7.3 – 7.9 | 7.6 – 7.9 | 7.5 – 8.0 | 7.5 – 8.2 | 4 - 9 | [7] |

Figure 1. Water spinach growth during research

[8] Aquaponic is a combination of hydroponics (growing plants without soil) and aquaculture (cultivating fish or other aquatic organisms) in an efficient method and can produce plants and fish. Fish manure from the aquaculture section of the system will be broken down by bacteria into dissolved nutrients (such as nitrogen and phosphorus compounds) that will be utilized by plants for growth in the hydroponics section.
Ammonia is highly soluble in water where it exists in a molecular form associated with water and in an ionized form of \( \text{NH}_4^+ \). The extent of ionization depends on temperature and pH that may be toxic to aquatic life [9]. Based on the results of statistical tests that have been carried out on the measurement of ammonia concentration carried out in a 28 days period, it shows that the results are significantly different in each treatment. The measurement of ammonia concentration in the second week showed significant differences in all treatments. In all treatments there was an increase in ammonia concentration, namely at P0 to 0.143 mg/L, P1 to 0.218 mg/L, P2 to 0.357 mg/L, P3 to 0.443 mg/L, while P4 decreased to 0.529 mg/L. In the third week it happened as in the second week. P0 increased to 0.212 mg/L, P1 to 0.293 mg/L, P2 to 0.406 mg/L, P3 to 0.481 mg/L, while P4 decreased to 0.491 mg/L. The value of ammonia concentration in this study was still in the safe range for snakehead fish. According to [10], the ability of snakehead fish tolerance to ammonia concentrations in water at different pH, namely the ammonia concentration > 0.54 mg/L at pH 8 to 1.57 mg/L at pH 10. In addition, [11] explained that the concentration of ammonia that can be toxic to fish farming is at concentrations above 1.5 mg/L.

The cause of the increase in ammonia concentration is the decay of uneaten feed and also a decrease in dissolved oxygen concentration. During the rearing of fish there was an increase in the concentration of ammonia along with the increase in rearing time. This is due to the increasing presence of organic waste, both metabolic waste, fish feces, and feed residue that accumulates in the water [12], while the decrease in ammonia concentration was affected by the presence of plants in the bucket that absorbed nitrate. This is caused by the phytoremediation process carried out by plants [13]. In addition, according to [14], cultivation activities that is not change water, there is a role of bacteria to removing ammonia particles through the nitrification process.

Nitrate is the result of the oxidation of ammonia into nitrite and nitrate by the \textit{nitrobacter} bacteria which takes place under aerobic conditions. Nitrate is an important nutrient for the growth and metabolism of phytoplankton which is an indicator in evaluating the quality and level of fertility of the waters. However, if the concentration is large enough in the waters and exceeds the threshold, a phytoplankton boom will occur which has a negative impact on the biota in the waters[15]. Based on the results of statistical tests that have been carried out on the measurement of nitrate concentrations in a period of 28 days, it shows that the results are significantly different in each treatment. Nitrate concentrations ranged from 0.931 to 1.539 mg/L. Measurement of nitrate concentration during the study showed that the P1 treatment with the last yield of 1,457 mg/L was the highest yield of nitrate while the lowest was found in the P4 treatment of 0.937 mg/L.

The concentration of nitrate that increases in each observation indicates the nitrification process of ammonia by \textit{nitrobacter} bacteria [16]. In addition, nitrate accumulates in the water so that the nitrate concentration increases. The decrease in nitrate concentration is explained in [17] caused by plants that utilize nitrate nutrients for their development and growth.

These results are supported by supporting parameters, such as water quality (temperature, DO, pH) and growth of water spinach. The results of the temperature parameter in this study ranged from 28.1-30.3°C. Based on [7], the temperature that can support the growth of snakehead fish is 25.5°C-32.7°C so that the temperature value during the study can be said to be good in snakehead fish growth. Snakehead fish are tolerant of high water temperatures because they have thick scales and labyrinth organ [18]. However, if the water temperature is too high, it will certainly have an impact on the level of ammonia toxicity. According to [19], the level of toxicity of ammonia depends on the temperature of the water, where the higher the temperature of the water, the more toxic the ammonia in the water. The results of the DO (dissolved oxygen) parameter, DO in this study ranged from 0.30 – 2.19 mg/L. Based on [7], the optimal DO for snakehead fish maintenance is between 2.0-3.7 mg/L. The low DO value in this study was due to the absence of an aerator and also according to [20] that the main cause of the decrease in dissolved oxygen concentration was the presence of organic waste materials that utilized dissolved oxygen during the decomposition process. In addition, the difference in dissolved oxygen content is related to the number of individuals in the treatment.
The higher the population in a container, the higher the need for dissolved oxygen consumption will be [21]. The results of pH parameters, the pH ranged from 7.3–8.2. According to [7], the tolerance pH value for snakehead fish is between 4-9. In addition, according to [22], a good acidity level for fish is 7-8.5. pH of the waters is related to dissolved oxygen where the smaller the dissolved oxygen, the tendency for the pH to be alkaline and vice versa.

Aquaponic cultivation can increase plant productivity and the effectiveness of nutrient absorption. In the growth of water spinach, what needs to be observed is the growth of stems and leaves. According to [23], sufficient nitrogen plays a role in accelerating overall plant growth, especially stems and leaves. Nitrogen plays a role in leaf formation because nitrogen has an important role in the formation of new cells in plants [24]. The results obtained from the measurement of plant length and number of leaves on water spinach increased in all treatments. The most optimal growth observation of water spinach was found in treatment P1 with a stocking density of 120 fish compared to other stocking density treatments. One of the factors supporting the growth of aquatic plants is the water quality of the waters. This is because water quality affects the availability of nutrients for these plants, if the water quality is good then the nutrients for plants are sufficient so that productivity and plant growth are maximized.

4. Conclusion
Treatment of differences stocking density affect the concentration of ammonia (NH₃) and nitrate (NO₃) in the cultivation of Snakehead fish (Channa striata) in the bucket. The value of the ammonia concentration was between 0.089-0.609 mg/L and the nitrate concentration was between 0.931-1.539 mg/L.

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