Effectiveness of *Nitrobacter* on the specific growth rate, survival rate and feed conversion ratio of dumbo catfish *Clarias* sp. with density differences in the aquaponic system

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Abstract. The increased production of catfish has resulted in the addition of cultivated land area and the use of water specifically for this purpose, so technology is needed in catfish culture that promotes high density stocking that can be applied to narrow land and limited water sources; this is the system of aquaponics. With the addition of *Nitrobacter*, it can reduce the increase of ammonia and nitrite compounds in the water so as to produce a high nitrate content that can be absorbed if the roots of the plants are to grow optimally. The purpose of this research was to determine the density of dumbo catfish stocks that are effective for the specific growth rate, survival rate, and feed conversion ratio by putting *Nitrobacter* into an aquaponic system. This was an experimental study using a Completely Randomized RAL Design consisting of 5 treatments and 4 replications with different stocking solids; P0 = 30 fishes/15 L without *Nitrobacter*; whereas P1 = 30 fishes/15 L; P2 = 35 fishes/15 L; P3 = 40 fishes/15 L and P4 = 45 fishes/15 L with *Nitrobacter* 1 x 10⁸ CFU/ml. The data was processed using ANOVA and continued with Duncan’s Multiple Test. The best results obtained were in the stocking solid of 45 fishes/15 L.

1. Introduction
The catfish production of *Clarias* sp. has increased very rapidly in Indonesia. In 2004, catfish production amounted to 55.691 tons in 2014 dumbo catfish production with an average theft of 29.62% per year [1]. Dumbo catfish are a freshwater fish that is widely cultivated in almost all of Indonesia. This is because catfish are a superior commodity, and have a good market prospect. The advantages of catfish, among others, include a fairly high nutritional content, economic value, fast growth and easy maintenance [2].

The increased production of catfish has resulted in the addition of a cultivated land area and water use. The technology that has been widely applied by farmers to overcome the problem of land limitation is to cultivate using the system of aquaponics [3]. The cultivation of fish with high stocking densities causes a decrease in water quality due to the accumulation of organic matter. The factors that cause poor water quality are due to the accumulation of fish stool and the remains of feed that are not consumed by the fish. The rest of the feed and fish feces, if left to accumulate, will turn into ammonia (NH³). Ammonia is toxic in water and is harmful to fish survival.

The high levels of ammonia in the aquaponics system are not enough to give an idea that the amount of present *Nitrobacter* is not able to compensate for the amount of ammonia produced.
Therefore, the provision of *Nitrobacter* into the aquaponic system is expected to improve the quality of water so as to increase the growth of the watercress plants and thus to increase fish production. This study aims to determine the effect of the density of stocking dispersion when related to *Nitrobacter* administration on the growth rate, survival rate, and feed conversion ratio of dumbo catfish in an aquaponics system.

2. Material and method

2.1. Material

We prepared the tools and materials to be used. The weight of the nutrient broth was as much as 1.3 grams in Erlenmeyer. This was dissolved with 100 ml of aquadest. After leaving it overnight, we took 5 ml using a pipette 16 times. The reaction tube that already contained the nutrient broth was covered with cotton and wrap. This was then put in an autoclave for 15 minutes with a temperature of 121°C.

We prepared 16 media basket reactions of nutrient broth and *Nitrobacter*. We selected the *Nitrobacter* using a test tube to avoid contamination. After the *Nitrobacter* was all planted, the test tubes were inserted into an incubator with a temperature of 30°C for 2 - 3 days to allow for the process of bacterial development. After the bacteria was incubated, it became cloudy. Turbidity in the test tube meant that the *Nitrobacter* had developed. After that, we washed bacteria using 3 x centrifuge for 15 - 20 minutes. After the centrifuge process showed there to be sediment, we then added NaCl 0.9% and put it in the vortex to separate the media [4].

The turbidimeter tool was used to hold the vial used as a container for the bacteria. The bacteria present in the reactions in the vortex first remained homogeneous. They were then put in the hole and closed. The numbers of the bacterial densities were shown in the units of McFarland based on the turbidimeter’s position. The McFarland control contained suspended bacteria using NaCl 0.9% as the solution until it was obtained using a standardized conformity of 1 McFarland, or equal to the number of bacteria $1 \times 10^8$ CFU/ml [5].

We conducted poverty results on one suspension, which we then calculated using a dilution formula; $V_1 \times N_1 = V_2 \times N_2$. This was in order to get 1 ml of the teaching material in liquid form in the microtube with the comparison obtained being 4:1, requiring 0.25 ml Bacteria and 0.75 ml of Nacl Physiological 0.9%. This was taken with a micropipette and blue tip. 16 microtubes that had bacteria added to them were then poured into each aquarium, except for aquarium P0. The more microorganisms that are produced in the sample or media, the more that the sample will become cloudy [6].

2.2. Method

The aquaponic system was designed by placing a container into a fish pond. The container plants used a tray tub equipped with inlet and outlet channels. The inlet channel was directly connected to the pump that pumped the pond water into the cultivation plant. The outlet channel drained water from the plant maintenance container to the aquarium. The water flowed using the principle of recirculation, so then the water from the process of the cultivation of the dumbo catfish were used as a source of water in the cultivation process. The provision of *Nitrobacter* was given after the fish entered the aquarium.

The research parameters observed during the study were survival rate, specific growth rate, and the feed conversion ratio of dumbo catfish, and the supporting variables were water quality (ammonia, temperature, pH, DO, nitrite, and nitrate) and water-end spinach kangkung. The water media management was done carefully so then the water quality was controlled. The observation of the pH and DO in the media was done every day, and the temperature was observed every day in the morning and afternoon. The ammonia and nitrate was observed every 7 days. The feed given was a feed that was adapted to the catfish. Artificial feed was given in the form of pellets with a protein content over 40%. The feeding dose was given at 5% of the body weight of the catfish. The feeding was done in the morning and evening. Catfish maintenance in the aquaponic system was done for 30 days.
3. Results and discussion

3.1. Specific growth rate
The results of the statistical analysis showed that the specific growth rate of the dumbo catfish with different solid densities showed very different results ($P < 0.01$) in SGR (Table 1). The best SGR was P4 (45 fishes / 15L) with 5.0500, while the lowest was P0 (control) with 2.8175 (30 fishes / 15L). P1 was 3.4050 higher than the control. P2 was 3.9350 higher than the control. P3 was 4.4125 higher than the control.

| Treatment | Specific Growth Rate  |
|-----------|-----------------------|
| P0        | 2.8175 ± 0.11087      |
| P1        | 3.4050 ± 0.15111      |
| P2        | 3.9350 ± 0.10376      |
| P3        | 4.4125 ± 0.11871      |
| P4        | 5.0500 ± 0.07303      |

Note: P0 (30 fishes, without *Nitrobacter* or Control), P1 (30 fishes, *Nitrobacter*), P2 (35 fishes, *Nitrobacter*), P3 (40 fishes, *Nitrobacter*), P4 (45 fishes, *Nitrobacter*). Different superscripts in the same column indicate a highly significant difference ($P < 0.01$).

3.2. Survival rate
The results of the statistical analysis showed that the survival rate of dumbo catfish with differently spread solids showed very different results ($P < 0.01$) in the context of SR (Table 2). The best SR was in P4 (45 fishes / 15L) with 92.775 while the lowest was in P0 (control) with 41.665 (30 fishes / 15L). P1 was 68.330 higher than the control. P2 was 75.710 higher than the control. P3 was 82.500 higher than the control.

| Treatment | Survival rate (%)     |
|-----------|-----------------------|
| P0        | 41.665 ± 8.81791      |
| P1        | 68.330 ± 4.30418      |
| P2        | 75.710 ± 1.65122      |
| P3        | 82.500 ± 2.04124      |
| P4        | 92.775 ± 1.11000      |

Note: P0 (30 fishes, without *Nitrobacter* or Control), P1 (30 fishes, *Nitrobacter*), P2 (35 fishes, *Nitrobacter*), P3 (40 fishes, *Nitrobacter*) and P4 (45 fishes, *Nitrobacter*). Different superscripts in the same column indicate a highly significant difference ($P < 0.01$).

3.3. Feed conversion ratio
The results of the statistical analysis showed that the ratio of the feed conveyed to the dumbo catfish with different stocking solids showed very different results ($P < 0.01$) in the context of FCR (Table 3). The best FCR was P0 (30 fishes / 15L) with 1.068450 while the lowest was P0 (control) with...
0.927825 (45 fishes / 15L). P1 was 0.989700 higher than the control. P2 was 0.969650 higher than the control. P3 was 0.956950 higher than the control.

Table 3. Feed Conversion Ratio of the dumbo catfish in the aquaponic system with different densities

| Treatment | Feed conversion ratio |
|-----------|-----------------------|
| P0        | 1.068450 ± 0.0281757  |
| P1        | 0.989700 ± 0.0052814  |
| P2        | 0.969650bc ± 0.0048707|
| P3        | 0.956950 ± 0.0114859  |
| P4        | 0.927825d ± 0.0115538 |

Note: P0 (30 fishes, without Nitrobacter or Control), P1 (30 fishes, Nitrobacter), P2 (35 fishes, Nitrobacter), P3 (40 fishes, Nitrobacter) and P4 (45 fishes, Nitrobacter). Different superscripts in the same column indicate a highly significant difference (p <0.01).

3.4. Water quality
The water quality parameters measured during the study were dissolved oxygen, pH, temperature, ammonia, nitrite, and nitrate. The measurements of the water quality parameters were performed in order to monitor the water quality conditions during the maintenance period (Table 4).

Table 4. The water quality parameters in the aquaponics system at different densities

| Treatment | DO  | pH  | Temperature | Ammonia | Nitrite | Nitrate |
|-----------|-----|-----|-------------|---------|---------|---------|
|           | 1   | 4   | 1   | 4   | 1   | 4   | 1   | 4   | 1   | 4   | 1   | 4   |
| P0        | 3.58| 2.17| 8   | 8   | 28.8| 28.4| 0.24| 0.27| 0.04| 0.06| 1.77| 1.59|
| P1        | 3.28| 1.53| 8   | 7   | 28.4| 27.4| 0.23| 0.15| 0.04| 0.03| 1.77| 2.79|
| P2        | 3.18| 2.19| 8   | 7   | 28.3| 27.3| 0.22| 0.14| 0.04| 0.02| 1.78| 2.76|
| P3        | 3.09| 2.27| 8   | 7   | 28.8| 27.5| 0.21| 0.13| 0.04| 0.02| 1.80| 2.87|
| P4        | 3.01| 1.42| 8   | 7   | 28.5| 27.4| 0.19| 0.12| 0.04| 0.02| 1.83| 2.93|

Note: (1) in the first week of the experiment, (4) in the last week of the experiment

3.5. Discussion
It can be seen that the highest specific rate of growth was found in the P4 treatment of 5.0500%, while the lowest specific growth rate was in the P0 treatment of 2.8175%. This was due to the varying quality of the water in each treatment. Fish cultivation wastes that were the result of metabolic activity contain a lot of ammonia [7]. Fish emit 80 - 90% ammonia (N-inorganic) through the osmoregulation process, whereas feces and urine produces about 10 - 20% of total nitrogen. The accumulation of ammonia in the cultivation media was one of the causes of water quality degradation which could result in the failure of fish farming production. The highest specific growth rate was found in the P4 treatment (5.05%). This is because foods with high protein are a source of energy. In the P4 treatment with a high stocking density of 45 head and a narrow scope, the rest of the energy source became heavy growth and was assisted by the presence of Nitrobacter. This could reduce the amount of ammonia released and accelerate the improvement of water quality because Nitrobacter can convert the nitrite into nitrate with nutrients as in the feces of dumbo catfish.
The lowest specific growth rate was found in the P0 treatment of 2.82%/day. This was because the treatment of P0 (control) and its residual energy source from protein to long growth was not heavy. The non-deliverance of *Nitrobacter* on the maintenance media also caused a decrease in water quality as indicated by the high levels of ammonia in treatment P0 compared with treatments P1, P2, P3, and P4. This was in accordance with the previous study [9], which stated that high levels of ammonia can trigger disease, cause stress and decrease the appetite of the fish so then the growth becomes obstructed.

It can be seen that the highest survival rate was found in the P4 treatment which was 92.775%, while the lowest survival rate was in the P0 (control) treatment, which was 41.665%. The fish cultivation activities were inseparable from the waste produced, especially from the remaining feed, feces, and the results of the fish metabolic activities. In the cultivation system of P0 (Control) without the administration of *Nitrobacter*, there was no reduction in the amount of ammonia in the aquarium. The concentrations of the cultivated wastes such as ammonia (NH\textsubscript{3}), nitrite (NO\textsubscript{2}) and CO\textsubscript{2} carbon dioxide increased. High ammonia concentrations lead to physiological disturbance and triggered stress in the fish [8]. Stress in the fish results in a decreased endurance, which makes the fish susceptible to disease and a decreased appetite in the fish can cause death [7].

The highest survival rate was found in the P4 treatment with the stocking of the catfish being 45 fish. This is where the feces, urine, and the rest of the feed caused the ammonia to increase. The fish became stressed and experienced death. Adding *Nitrobacter* was able to lessen the amount of organic waste and it also improved the water quality so then it was suitable for fish life and prevented death. This was in accordance with the previous study [9], stating that water quality also affected the survival rate.

It was seen that the highest specific rate of growth was found in the P0 treatment of 1.07, whereas the lowest specific growth rate was found in the P4 treatment of 0.93. This was allegedly due to the presence of dense stocking differences. The high density of stocking in P4 caused more feces, urine, and feed more than the other treatments. The role of *Nitrobacter* was capable of promoting nitrogen and oxygen in the presence of organic matter. Nitrogen and oxygen are used by the body of the fish. P4 showed the high energy that caused fast growth and a low FCR value.

The high value of the FCR in the treatment without *Nitrobacter* was due to the accumulation of organic material in the culture medium that was not degraded optimally. The organic materials that were not degraded optimally could cause high ammonia levels, so the water quality in the culture medium can decrease. The decline of the water quality in the culture media made the fish become stressed and prompted a loss of appetite [10].

In this study, there was a decrease in the pH value that could be caused by the activity of bacteria and fish in the aquarium. The degree of acidity (pH) was one of the environmental factors that affected the growth and activity of the ammonia-oxidizing bacteria. However, the pH value range in this study was still appropriate for the life of a catfish.

The dissolved oxygen level was decreased in each treatment, especially in P4 with 1.42 mg / l. This is because the dumbo catfish and *Nitrobacter* were given autotrophic bacteria in P1, P2, P3 and P4, which would reduce the dissolved oxygen levels throughout the study. The presence of an increased density of dispersion in a limited container and in the denser conditions of fish stocking meant that the oxygen consumption and accumulation of the metabolic waste was also higher. The dissolved oxygen became an important parameter because it is needed in the ammonia oxidation process and thus became the main limiting factor for fish survival. In this research, the temperature was in the very good range of 27.2 - 28.8°C. The optimum temperature for the catfish was 27 - 30°C [11].

Ammonia is one of the N-organic forms that are harmful to fish. The value of the ammonia in this study ranged from 0.12 - 0.27 mg / l. This was the activity of *Nitrobacter* as a nitrifying autotrophic bacterium that converts ammonia to nitrate. Nitrification was performed by two distinct groups of autotrophic bacteria. *Nitrosomonas* requires ammonium as a source of energy while *Nitrobacter* requires nitrite. During the study, the ammonia yields tended to fall in the treatment of P1, P2, P3 and P4. This was presumably because the administration of *Nitrobacter* in each treatment as a bacterium
altered the nitrification so then it was able to offset the organic material present in the water. Nitrate was the end result of the nitrification process. In the P1, P2, P3 and P4 treatments, the increase was suspected because of the *Nitrobacter* bacteria being capable of converting waste into nitrate. P0 (control) decreased because the P0 treatment was not given *Nitrobacter*.

Based on the results of the measurements conducted at the beginning and end of the study, it showed that the optimal plant growth was in P4. This was due to the help of *Nitrobacter* and the amount of organic in the aquarium that would become nitrogen and oxygen absorbed by the roots of the spinach plants as well as the adequate lighting and the appropriate spacing.

The process of the absorption of the organic matter by plants took place through the roots. The plant roots required enough oxygen for respiration so then the absorption of nutrients could be optimal. The spacing used depended on the type of plant. The plant spacing in accordance with the type of plant will make the plant grow well because of the existence of an adequate growing space. The plant spacing used for kale was 10 cm (Nugroho and Sutrisno, 2008). In addition, the ammonia content that had been converted to nitrate with the help of *Nitrobacter* was also an important factor in optimizing the growth of the kale in the aquaponic system. The nitrate in the plant served as the main fertilizer that was converted into protein.

4. Conclusion

The density of the dumbo catfish stocks that was most effective for the specific growth rate, survival rate, and feed conversion ratio was by giving *Nitrobacter* to an aquaponic system of 45 fishes/15L.

5. References

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Acknowledgments

We would like to thank the staff of the Faculty of Science and Technology and the Faculty of Fisheries and Marine, Universitas Airlangga, for their technical assistance.