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The effect of stocking density and the application of Nitrobacter as ammonia decomposer in aquaponics system of *Clarias gariepinus* with water spinach (*Ipomoea aquatica*)

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Abstract

This study aimed to determine the effect of *Nitrobacter* bacteria on specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) of African catfish (*Clarias gariepinus*) in aquaponics systems. The sample used was African catfish with a size of 5-7 cm as many as 720 individuals. The research method used was experimental design in the form of Completely Randomized Design (CRD). This study used 5 types of treatments with 4 repetitions. The P0 and P1 contain of 30 fish in each aquarium; 35 fish in P2; 40 fish in P3; and 45 fish in P4. The control group (P0) were treated without *Nitrobacter* administration and the other 4 intervention groups were given *Nitrobacter* 1 ml with a density of 1×10^8 CFU ml^{-1}. The main parameters studied were SGR, SR and FCR of African catfish in different aquarium with different stocking densities. This research also studied the growth of water spinach (*Ipomoea aquatica*) also water quality conditions in aquaponics systems. The administration of *Nitrobacter* in aquaponics systems gave a significant effect on the SGR, SR, and FCR of catfish. The administration of *Nitrobacter* in an aquaponics system can give maximum effect if there are 45 fish stocking densities and 1 ml *Nitrobacter* with a concentration of 1×10^8 CFU ml^{-1}.

Introduction

The world of aquaculture is experiencing a rapid development. One of the supporting programs is the aquaculture promotion in various sectors by the Indonesian government. One sector that is being promoted is the freshwater aquaculture. Increased productivity of freshwater fisheries is a government program in welcoming the Minapolitan program (1). Catfish is a commodity of freshwater fish with a fairly high nutrient content. Production of African catfish (*Clarias* gariepinus) in Indonesia has increased rapidly. In 2004 African catfish production was 55,691 tons, and in 2014 African catfish production reached 543,774 tons with an annual increase of 29.62% (2).

Increasing catfish production required more cultivated land and water use (3). The technology that has been widely applied by farmers in many countries to overcome the problem of limited land is conducting cultivation with an aquaponics system (4). Aside from reducing the land and water use, aquaponics also increases business efficiency through the utilization of nutrients from feed waste and fish metabolism (4,5). Aquaponics constitutes a recirculation system that reuses water which has been used in fish farming with many kinds of filters, such as biological and unbiological filters. One of the plants that can be used is water spinach (*Ipomoea aquatica*). Water spinach has roots which are not strong and easy to maintain. It requires optimum pH of nutrient solution which is influenced by

HNO_3, H_3PO_4, and H_2SO_4 for its growth process (6). These nutrients are obtained by absorbing the existing nutrients through its root. In aquaponics systems, plants absorb nitrates in the waters. The use of water spinach in aquaponics systems is able to reduce nitrogenous fish waste (7).

Fish cultivation with high stocking densities may impair the water quality due to the accumulation of organic matter (8). Factors that cause poor water quality are the remnants of fish feces and feed scraps that are not consumed by fish and are left to pile up and turn into ammonia (NH_3). Ammonia is toxic and dangerous for the survival of fish. The toxic concentration of ammonia in a short period of time ranges from 0.6 to 2.0 mg L^{-1} (9). This condition may cause several health problems, such as gill damage, that will further affect feed utilization and growth rate (10-12). One of the effective effort to maintain water quality in aquaponics systems is by adding *Nitrobacter* bacteria, as these bacteria are suitable materials to improve ammonia decomposition in fish aquarium (12). Therefore, this study was conducted to determine the effect of the administration of *Nitrobacter* bacteria as ammonia decomposer and stocking density on catfish's' growth rate, survival rate, and feed conversion ratio in aquaponics systems.

Material and method

Description of the study sites

This research was an experimental study using Complete Randomized Design (CRD). This research was conducted at the Faculty of Fisheries and Marine, University of Airlangga, Surabaya, Indonesia on January 28th until February 28th 2018. The independent variable in this study was the density of fish. This study gave different stocking density in each group. The high number of fish would influence the water quality because of fishs' metabolic waste. The dependent variables were SGR, SR, and FCR of African catfish. SGR is a growth of fish in a certain time, which is shown by fish weight and lenght. It was influenced by water quality, the amount of fish wastes, and feed. SR is the one of parameters that shows the success of fish cultivation which is influenced by water quality. FCR is a feed effectivity indicator and becomes a parameter that uses for increasing fish food intake. There was a *Nitrobacter* addition as a control variable. Every group was given *Nitrobacter* as a decomposer for ammonia from fish metabolic wastes. These group would be given the same concentration of *Nitrobacter*.

Sample

The materials used were African catfish with a size of 5-7 cm as many as 720 fish and *Nitrobacter* (MSC HIMBIO UNAIR, Indonesia). There were 20 aquaria with (35*25*25) cm long, wide, and height which was filled by local company water about 20 cm height (17.5 L). Fish were fed with commercial feed in the form of HI-PRO VITE 781-1 pellets (PT Central Proteina Prima Tbk, Indonesia) at 6 a.m and 6

p.m. These pellets consist of 31%-33% protein. The average of commercial feed for catfish contain of 28%-32% protein level (13). This meant that feed in this study was very optimal for catfish growth. The water spinach plants were 8-10 cm long. they derived from water spinach seeds that were sown on burnt husk for 2 weeks. They were harvested about 8-10 cm. Furthermore, water spinach was put into a net pot, was made from a plastic cup that has been modified as a way for water entry. Each net pot contained 5-8 water spinach seeds. Burnt husk addition is one way to reduce the use of soil as planting media. After the water spinach were ready, they moved to the trays. There were about 8 water spinach which put in the tray above each aquarium.

Materials

This study used pump set with AT-105 types (Atman) for recirculation. This study also used thermometer Vivid Oval Broader type (Safety), pH meter pen type PH-009 (ATC), DO meter HI9146 (Hanna), digital scale FEJ-5K (Quttro), fish nets 20 cm (PAM), hose 1-meter type (Puso), and ruler 30 cm (Butterfly).

Procedures

This study used five types of treatment with four repetitions in each treatment. The control group got treatment without *Nitrobacter* addition in the aquarium. *Nitrobacter* was planted in a test tube the put in an incubator with a temperature of 30°C about 2-3 days for developing bacteria. Bacterial incubation caused the test tube become turbid. Furthermore, bacteria washed using a centrifuge three times for 15-20 minutes. After the centrifuge appears precipitate, NaCl 0.9% was added so that the bacteria separated from the media. Mc Farland control contained suspended bacteria using NaCl 0.9% solution until turbidity was obtained according to 1 Mc Farland's standard or aquivalent to $1x10^8$ CFU ml^{-1}. Bakteria were then countd using dilution formula $V_1xN_1 = V_2xN_2$. The calculation results showed that the ratio of 1 ml bacterial suspension in liquid form in a microtube is 4:1, so it took 0.25 ml of bacterial suspension and 0.75 ml of NaCl 0.9%. *Nitrobacter* was taken using a micropupette and blur tip and then pured into each aquarium except P0 group. The 4 intervention groups were added 1 ml *Nitrobacter* with a density of $1x10^8$ CFU ml^{-1}. P0 = 30 fish in 15 L freshwater (fish density 2000 m^{-3}) without *Nitrobacter*. P1 = 30 fish in 15 L freshwater (fish density 2000 m^{-3}) with 1 ml Nitrobacter. P2 = 35 fish in 15 L freshwater (fish density 2300 m^{-3}) with 1 ml *Nitrobacter*. P3 = 40 fish in 15 L freshwater (fish density 2600 m^{-3}) with 1 ml *Nitrobacter*. P4 = 45 fish in 15 L freshwater (fish density 3000 m^{-3}) with 1 ml *Nitrobacter*.

The optimal stocking density of catfish was about 400 fish m^{-3} to 2400 fish m^{-3}, so that they can grow well. The pattern of treatment aquarium was randomized to avoid bias.

It considered the bias of the treatment happened naturally; and also avoided impartialy to the one treatment.

The main parameters studied were catfish specific growth rate (SGR), survival rate (SR) and feed conversion ratio (FCR). Whereas, the supporting parameters observed were water spinach plant growth and water quality conditions such as temperature, pH, DO (dissolved oxygen), nitrite and nitrate in aquaponics systems. SGR is calculated based on individual weight gain per day with formula (14):

$$SGR = \frac{\ln W_e \text{ (g)} - \ln W_s \text{ (g)}}{d \text{ (days)}} \times 100$$

where: SGR = specific growth rate (%/day);
We = mean of fish weight at the end of fish rearing (g);
Ws = mean of fish weight at the beginning of fish rearing (g);
d = total rearing days (day).

SR is calculated based on the number of catfish that can survive during observation in units of percent with formula (15):

$$SR = \frac{N_t}{N_0} \times 100$$

Where: SR = survival rate (%);
Nt = amount of fish in the end of fish rearing;
Nt = amount of fish in the beginning of fish rearing.

Whereas, FCR is calculated based on the Buwono's Equation (16):

$$FCR = \frac{F}{(W_t + W_d) - W_0}$$

Where: FCR = Feed Convertion Ratio;
Wt = weight of fish in the end of fish rearing (g);
Wd = weight of dead fish during rearing (g);
W0 = weight of fish in the first day of fish rearing (g);
F = amount of feed (g).

Measurement of supporting parameters, such as water spinach plant growth and water quality, were carried out manually. Measuring water spinach plants was done by using a ruler, from the base of the leaf to the tip of the leaf. On the other hand, pH and DO were observed every day in the morning at 7 a.m. The water temperature was recorded twice a day at 6 a.m and 4 p.m. However, ammonia, nitrite, and nitrate were analyzed once a week.

Statistical analysis

All data were analyzed using ANOVA (Analysis of Variance) test with SPSS Statistics 21 version. If there was a difference, it was followed by Duncan's multiple test with significant level 5% (17).

Results

The results of calculating the SGR, SR and FCR of African catfish feed for 1 month can be seen in Table 1.

Based on table 1, it can be seen that all treatments using *Nitrobacter* on P1, P2, P3, and P4 have higher SGR and SR value than control group. Whereas, the highest value of FCR was in the P0 treatment.

In figure 1, it can be seen that the highest SGR is found in treatment P4 which is equal to 5.0500% and the lowest is found in treatment P0 which is 2.8175%.
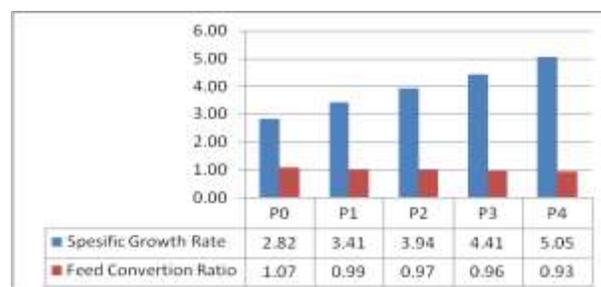

Figure 1: Graph of the average specific growth rate (SGR) and feed conversion ratio (FCR) of African catfish.

Whereas, the highest FCR was found in P0 treatment which was equal to 1.07 and the lowest was found in treatment P4 which was equal to 0.93. In Figure 2 it can be seen that the highest SR was found in treatment P4 which was equal to 92.775% and the lowest was found in treatment P0 which was equal to 41.665%.

Water quality data which includes temperature, pH, DO, ammonia, nitrite and nitrate in maintenance of african catfish can be seen in table 2.

Data on water spinach growth rates, including water spinach length and number of leaves, in the maintenance of African catfish for 1 month can be seen in Table 3.

Table 1: The average SGR, SR, FCR of African catfish

Treatments	Variables		
	SGR ($\pm$SD) (%)	SR ($\pm$SD)(%)	FCR ($\pm$SD)
P0	2.8175 $\pm$ 0.11087[a]	41.665 $\pm$ 8.81791[a]	1.068450 $\pm$ 0.0281757[a]
P1	3.4050 $\pm$ 0.15111[b]	68.330 $\pm$ 4.30418[b]	0.989700 $\pm$ 0.0052814[b]
P2	3.9350 $\pm$ 0.10376[c]	75.710 $\pm$ 1.65122[c]	0.969650 $\pm$ 0.0048707[bc]
P3	4.4125 $\pm$ 0.11871[d]	82.500 $\pm$ 2.04124[c]	0.956950 $\pm$ 0.0114859[c]
P4	5.0500 $\pm$ 0.07303[e]	92.775 $\pm$ 1.11000[d]	0.927825 $\pm$ 0.0115538[d]

Different superscripts show significant or non-significant differences P < 0.05.

Table 2: The water quality average of African catfish maintenance

T	DO (mg L^{-1})		pH		Temperature (ºC)		Ammonia (mg L^{-1})		Nitrite (mg L^{-1})		Nitrate (mg L^{-1})	
	I	F	I	F	I	F	I	F	I	F	I	F
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

Initial calculation - week 0, and final calculation - week 4; T = treatment; I = initial; F = final.
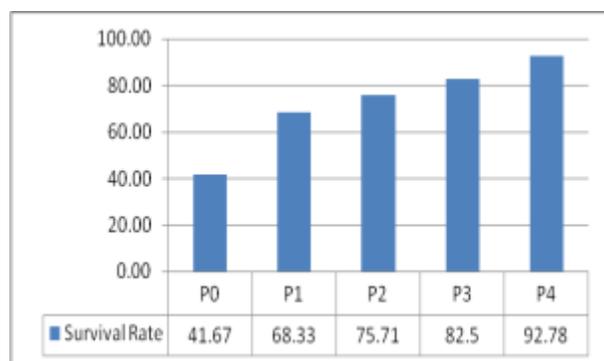

Figure 2: Graph of the average survival rate (SR) of African catfish.

Table 3: Average water spinach growth

Treatments	Water spinach length (cm)		No. of leaves	
	Initial	Final	Initial	Final
P0	10	15.5	2	6
P1	10	21	3	9
P2	10	23	3	9
P3	10	25.5	3	10
P4	10	27.5	3	11

Discussion

Nitrobacter addition in aquaponics systems affected the SGR of African catfish. This study showed each treatment groups (P1, P2, P3, and P4) had specific differences of SGR with control group (P0). The highest SGR was found in treatment P4 (5.05%), while the lowest SGR was found in treatment P0 (2.82%). It was caused by different water quality for each treatment. Residual energy from protein in P0 did not converted into fish weight, but it converted into fish lenght. There are several factors affect to SGR's catfish such as: fish type, genetic, feed, disease resistance, water quality, and stocking density (18). Fish emitted 80-90% ammonia (N-inorganic) through the osmoregulation process, while from feces and urine, it is around 10-20% of total nitrogen (19). P0 group had not got *Nitrobacter* treatment where ammonia concentration was accumulated. The high concentration of ammonia decreased water quality which affected to the catfish's cultivation. This condition triggered stress in fish and reduced feed intake (20). Long term of stress could influence fish's homeostatic also may impaired immune systems (12). Energy sources of African catfish changed into body weight because of the high of density and aquarium size. It was also helped by *Nitrobacter* which decreased the accumulation of ammonia and accelerated to water quality reparation (21). It converted nitrite into nitrate in fish metabolic wastes (12).

The administration of *Nitrobacter* also showed significant effect and significant differences on the catfish's SR ($p < 0.05$). SR as a parameter of fish's sustainability in a cultivation place (22). The highest SR was on P4 (92.77%) and the lowest was found P0 (44.6%). Intervention groups (P1, P2, P3, and P4) had a number of fish mortalities, but the amount was less than control group (P0). P4 had number of stocking at the most which produced large quantities of feces, urine, and residual feed. African catfish excreted ammonia across the gill membranes and a few amounts in urine as a metabolism product (23). Ammonia chemical structure, the length of exposure, pH, and water temperature influenced the ammonia toxicity (23). Ammonia accumulation form its exposure caused intoxication on the central nervous system (24,25). At higher temperature and pH values, ammonia become more toxic to fish, especially african catfish (23). *Nitrobacter* administration could compensate the organic waste in the water and improve water quality.

FCR for all treatments were ranged from 0.93 to 1.07, which the highest FCR obtained in control group where they were only 30 catfish and without any *Nitrobacter* administration. FCR of P0 was about 1.068; it's meant that African catfish needed around 1.7 kg of feed to reach 1 kg fish's total weight. The lowest FCR was obtained by P4 where there were 45 fish with 1 ml *Nitrobacter* administration. It was about 0.93 which meant that it required 0.93 kg of food to produce 1 kg of fish's weight. Recent research explained that FCR below 1.00 affected feed efficiency above 100% (26). Since organic wastes weren't degraded optimally, these wastes caused high concentration of P0 water due to the accumulation of these wastes. The less optimum environmental condition had effect such as inefficiently converted feed (27). This condition could initiate neuromotors impairments (24). Some diseases

caused by poor environmental condition lead worse feeding process than the healthy fish (27). Healthy fish normally consumed feed and converted feed in efficient process (27).

Water quality's parameter was also important to be observed (28). DO level was decreased in intervention groups (P1, P2, P3, and P4) because of *Nitrobacter* administration. Dissolved oxygen is an important parameter because it is needed for the ammonia oxidation process and is a limiting factor for fish survival (7). Low DO concentration could influence the condition of aquatic life (29). The high amount of fish stock in a limited space increased oxygen consumption and metabolic waste product (30). In this study, optimum temperature was 27.2-28.8ºC for the fish growth. Water temperature becomes the major factor to influence the toxicity of ammonia (31). The temperature for catfish to reach optimum growth is 26-30ºC (15,28). Increasing water temperature caused higher toxicity of ammonia (32).

Ammonia is one form of N-organic that is harmful to fish (31). Ammonia values in this study ranged from 0.12 to 0.27 mg L^{-1}. This condition was caused by *Nitrobacter* activity as an autotrophic nitrifying bacterium that converted ammonia into nitrate. On the other hand, pH also has important role to fish cultivation (33). However, in this study, pH value in each group was in fair value which was safe for fish. The optimum pH ranged from 7.5 until 8.5 (28,33) .

The growth of water spinach was observed by measuring the length of water spinach using ruler, also counting the number of leaves. The measurement was conducted at the beginning of study and at the end. During the observation, water spinach growth experienced a significant difference. The lowest growth rate of water spinach was at P0 which only increased about 5.5 cm. Whereas, the best water spinach growth was found in treatment P4.

In the treatment of P4, water spinach growth was 17.5 cm, from 10 cm to 27.5 cm. These results showed that water spinach in P4 had optimal growth. This was due to the help of *Nitrobacter* and organic wastes that changed into nitrogen and oxygen, in the aquarium. This component was absorbed by the water spinach roots. Adequate lighting and appropriate spacing also influences the growth. Plant roots need adequate oxygen for respiration so that the absorption of nutrients can be optimal (34,35).

Conclusions

The administration of *Nitrobacter* in different aquaponics system with renewable solids had effect on the SGR, SR, and FCR of African catfish (*Clarias gariepinus*). The best SGR, SR, FCR values were found in treatment 4 with 45 fish stocking densities and the addition of 1 ml *Nitrobacter*. SGR was 5.05%, SR was 92.77%, and FCR was 0.927. Effective stocking density in the administration of *Nitrobacter* in aquaponics systems was found in P4 treatment with 45 fish stocking densities and 1 ml *Nitrobacter* with a concentration of $1x10^8$ CFU ml^{-1}.

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Conflict of interest

There are no conflicts of interest regarding the publication of this manuscript.

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تأثير كثافة التخزين واستخدام جراثيم النترية كمحلل للأمونيا في نظام الزراعة المائية لأسماك السلور الأفريقي مع السبانخ المائي

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كلية الثروة السمكية والبحرية، جامعة إيرلانجا، جاوة الشرقية، إندونيسيا

الخلاصة

هدفت الدراسة إلى تحديد تأثير جراثيم النترية على معدل النمو الخاص، ومعدل البقاء على قيد الحياة، ونسبة تحويل العلف في سمك السلور الأفريقي في أنظمة الزراعة المائية. كانت العينة المستخدمة هي اسماك السلور الأفريقي بحجم ٥-٧ سم وباستخدام ٧٢٠ سمكة. كانت طرائق البحث المستخدمة معتمدة على التصميم العشوائي الكامل. تكونت تجارب الدراسة من خمسة مجاميع بأربعة تكرارات. احتوت المجموعة الأولى (سيطرة) والمجموعة الثانية على ٣٠ سمكة في كل حوض، و ٣٥ سمكة في المجموعة الثالثة، و ٤٠ سمكة في المجموعة الرابعة، و ٤٥ سمكة في المعاملة الخامسة. لم يتم معالجة مجموعة السيطرة بجراثيم النترية، كما تم إعطاء المجموعات الأربعة البقية ١ مليلتر من بجراثيم النترية بتركيز ١٠×٨ وحدة عد المستعمرات/ مل. كانت المعايير الرئيسية التي تمت دراستها هي معدل النمو الخاص، ومعدل البقاء على قيد الحياة، ونسبة تحويل العلف في سمك السلور الأفريقي في أحواض مختلفة وبكثافة اسماك مختلفة. كما تم دراسة نمو السبانخ المائي فضلاً عن معايير جودة المياه في أنظمة الزراعة المائية. أدى إضافة جراثيم النترية في أنظمة الزراعة المائية إلى تأثير كبير في معدل النمو الخاص ومعدل البقاء على قيد الحياة ونسبة تحويل العلف في أسماك السلور الأفريقي. إن إضافة جراثيم النترية في أنظمة الزراعة المائية يمكن أن يعطي اقصى تأثير إذ كانت الأحواض تحتوي على كثافة تخزين ٤٥ سمكة وبتركيز ١٠×٨ وحدة عد المستعمرات لكل واحد مليلتر من جراثيم النترية.