

Use of probiotics in fish feed and clams (*Pilsbryconcha exilis*) as biofilter components of aquaponics system in archipelagic dryland

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Manuscript received: 1 July 2020. Revision accepted: 23 November 2020.

Abstract. Santoso P, Sunadji. 2020. Use of probiotics in fish feed and clams (*Pilsbryconcha exilis*) as biofilter components of aquaponics system in archipelagic dryland. *Trop Drylands* 3: 46-50. Aquaponics is a combination system between aquaculture and hydroponic. It is water-use efficient and suitable to be developed in the archipelagic dryland. This study aimed to determine the effect of adding probiotics in fish feed and the use of clam (*Pilsbryconcha exilis*) as additional components in the aquaponic system on feed conversion ratio, productivity of catfish (*Clarias gariepinus*) and aquatic spinach (*Ipomoea aquatica*), and removal efficiency of organic matter suspended in the aquaculture water. This study was an experimental study using a factorial treatment laid out in a completely randomized design with three replications. The treatments applied were administration of probiotics with concentrations of 2 mL kg⁻¹, 4 mL kg⁻¹, and 6 mL kg⁻¹ in feed to improve feed efficiency in catfish, thus minimizing metabolite discharges, and the use of clam in biofilter systems with a density of 50 clams m⁻², 100 clams m⁻², and 200 clams m⁻² to improve the efficiency of absorption of cultivation effluents in the form of suspended particles. The results of ANOVA showed that the treatment of adding probiotics in the fish feed had a significant effect ($P < 0.05$) on improving feed conversion ratio, increasing the production of catfish, and the removal efficiency, but had no significant effect ($P > 0.05$) on aquatic spinach biomass production. Whereas the use of clams as biofilter significantly ($P < 0.05$) improved feed conversion ratio, increased the production of catfish and aquatic spinach, also efficiently removed organic matter in the aquaponic system. The interaction of these two factors had a significant effect ($P < 0.05$) on feed conversion ratio and aquatic spinach production, however, it had no significant effect ($P > 0.05$) on the production of catfish and the organic matter removal efficiency. Post hoc LSD test showed that the best interaction effect was the treatment of probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻² because it generated a combination of the lowest feed conversion ratio (1.07) and the highest of aquatic spinach production (5.28 kg m⁻²).

Keywords: aquaponic, biofilter, clam, probiotic, production

INTRODUCTION

East Nusa Tenggara is an archipelagic province in Indonesia dominated by dryland areas. This province has a semi-arid climatic type with long dry period and low rainfall. As a consequence, food production through farming, fisheries and livestock rearing needs to be developed to minimize water use. In doing so, it requires appropriate technologies to overcome limited arable land and water limitations, especially in the dry season.

One of the appropriate technologies for food production in water-limited areas with scarce arable land is aquaponic. The aquaponic is an agricultural production system that combines aquaculture and hydroponic simultaneously using same land and water as economically as possible. The ability of aquaponics to efficiently utilize land and water is through the use of waste from fish farming activities by the existing plants (Rakocy et al. 2006). Waste derived from fish farming activities is used as input of organic nutrients to support the growth of plants, such as aquatic spinach. Subsequently, the absorption of fish metabolic waste by aquatic spinach, water quality is improved to be used in fish farming. This mutual benefit makes aquaponics suitable to be developed in areas where

freshwater sources and land are limited such as in densely populated urban settlements.

The selection of commodities plays an important role in the yield of agricultural production. In the context of aquaponics, the types of fish suitable to be maintained are fish that are able to live in sub-optimal water conditions, while the plants usually used are those that have economic value such as aquatic spinach, lettuce, and spinach (Rakocy, et al. 2006). Besides considering both the fish and plant, aquaponic also needs to take into account the optimal conditions for other biotic components of the aquaponic system, namely decomposing bacteria contained in biofilter units. There are two groups of decomposing bacteria in the aquaponic, namely *Nitrosomonas* and *Nitrobacter*. The presence of these bacteria is determined by the charge of solid or dissolved organic matter in water. If excessive organic matter is loaded, bacteria will need more time to break it down. As a result, substances containing ammonia can accumulate in water and endanger the life of fish before it is broken down by bacteria (Losordo et al. 1998).

To minimize organic material in the water, these materials need to be removed periodically from the system. Solid organic matter can be removed from the system mechanically by screening and sedimentation (Losordo et al. 1998). Organic matter in the form of suspended particles

can also be removed by suspension-eating organisms such as bivalves. Therefore, there is an opportunity to add these organisms to the biofilter component. The combination of biofilters in the form of plants and bivalves is expected to increase the filtration capacity of aquaponic system.

With the above considerations, this study explored the combination of increasing feed efficiency through the addition of probiotics with the addition of biofilter components in the form of bivalves/clams. The results of this study are expected to be useful for the development of an aquaponic system that is more effective in conserving water to support the development of freshwater aquaculture, especially catfish in the archipelagic dryland regions.

MATERIALS AND METHODS

Study area

The research was conducted at the Fisheries Field Laboratory, Center of Excellent of Archipelagic Dryland, University of Nusa Cendana. Water quality analysis was carried out at the Laboratory of Fisheries, Faculty of Marine Science and Fisheries, University of Nusa Cendana.

Procedures

This study was laid out in a completely randomized design with a factorial treatment, and three replications. The treatments applied were the combination of addition of probiotics in fish feed with concentrations of 2 mL kg⁻¹, 4 mL kg⁻¹ and 6 mL kg⁻¹, and the use of clam (*Pilsbryconcha exilis*) as biofilter with a density of 50 clams m⁻², 100 clams m⁻², and 200 clams m⁻². The probiotic treatment was expected to increase feed efficiency in catfish (*Clarias gariepinus*) so as to minimize metabolite discharges, whereas the biofilter was used to increase the efficiency of absorption of planting waste in the form of suspended particles.

The aquaponic system consisted of the following components: (i) Fish rearing ponds measuring 3 x 2 x 1 m³. (ii) The place for clam and aquatic spinach (*Ipomoea aquatica*) was made of fiberglass multiplex layered. The size of each aquatic spinach basin was 1 x 0.5 x 0.2 m³. Aquatic spinach rearing tubs were filled with 5-7 mm granular gravel media spread evenly covering the tub surface to a thickness of 5 cm. (iii) Mechanical and biological filters. The mechanical filter used was a swirling sedimentation tank. Mechanical filters treated in this study functioned to separate suspended solids in the water. This filter was connected with biological filters that contain media in the form of bio-ball and net sheet. (iv) Submersible pump. The submersible pump with a capacity of 1,500 L h⁻¹ was used to push water from fish culture tanks to the filter unit. (v) Piping network. Piping used for connecting all system components was made of ½ inch and ¾ inch PVC pipe.

Before use, the entire tub was soaked in water for a month and followed by draining and filling with new water. The density of catfish stocking in all treatment units was 100 fish m⁻². The size of the catfish offspring used had a

length of 5-7 cm and weight of 1.5-2.5 g. Before being placed in the treatment unit, the catfish offspring were acclimatized for a month in a 5-ton concrete tub at the study site. Furthermore, catfish was kept for two months by providing commercial feed, where at the beginning of the study, they were given "starter" fish feed, and after two weeks of age the feed was replaced with "initial" fish feed, and the "final" fish feed was given in the following two weeks and was replaced according to the size of the fish until harvesting. The feeding rate was 5% body weight per day. The weight of the pellet given was corrected every week, according to the growth of the fish. During maintenance, no water replacement was carried out, except for those lost due to evaporation.

After the fish tank was scattered with fish and the water turned greenish due to feeding, the aquatic spinach was ready to be planted. Aquatic spinach seeds were immediately buried in gravel until seeding. Seeds were buried at a distance of 15 cm x 15 cm.

Data analysis

Feed conversion ratio

Feed conversion was calculated according to the formula of Pillay (1990) as follows:

$$FCR = \frac{\text{Total Weight of Feed}}{W_t - W_o}$$

Where:

W_o: total weight of fish at the beginning of the study (kg)

W_t: total weight of fish at the end of the study (kg)

Biomass productivity of catfish and aquatic spinach

The biomass productivity of catfish and aquatic spinach was determined at the end of maintenance. (i) Fish biomass production: Calculated as total catfish biomass per pond area (kg m⁻²). (ii) Aquatic spinach biomass production: Calculated as total aquatic spinach biomass per planting area (kg m⁻²).

Removal efficiency

Removal efficiency/absorption efficiency of solid materials. The efficiency of separating solid material by swirling sedimentation was determined according to the formula of Pfeiffer et al. (2008):

$$RE (\%) = (S_{in} - S_{out}) / S_{in} \times 100$$

Where:

RE : removal efficiency (%):

S_{in} : TSS value of water into filter:

S_{out} : TSS value of water out from filter.

The total suspended solids (TSS) analysis was done using APHA (1995) method. In addition, turbidity, pH, temperature, and ammonia were measured.

Data analysis

Data were analyzed using one-way ANOVA and followed by LSD test. Statistical analysis was carried out using SPSS 24 software.

RESULTS AND DISCUSSION

Feed conversion ratio

The results showed that the feed conversion ratio (FCR) of catfish ranged from 1.02 to 1.39. The treatment means of the administration of probiotics and clams as biofilters are presented in Figure 1.

The obtained feed conversion ratio was then subjected to ANOVA to determine the effect of treatments on the conversion ratio of the catfish feed of the aquaponic system under study. The ANOVA results showed that the addition of probiotics in fish feed and the use of clam in biofilter systems, as well as their interaction, had a significant effect ($P < 0.05$) on the feed conversion ratio. The LSD results showed that the lowest feed conversion ratio occurred in the treatment of probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻², and also the treatment of probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻².

Biomass productivity of catfish and aquatic spinach

The study results revealed that the productivity of catfish biomass ranged between 11.68-14.28 kg m⁻², while the production of aquatic spinach biomass was between 3.51-5.43 kg m⁻². The average productivity of biomass of catfish and aquatic spinach is presented in Figures 2 and 3.

The ANOVA results showed that the addition of probiotics in fish feed had a significant effect ($P < 0.05$) on the productivity of catfish but had no significant effect ($P > 0.05$) on the productivity of aquatic spinach. Whereas the use of clam in biofilter systems significantly affected ($P < 0.05$) both the productivity of catfish and aquatic spinach. The interaction effect of these two factors was not

significant ($P > 0.05$) on the productivity of catfish but was significant ($P < 0.05$) on aquatic spinach. The results of LSD showed that the highest aquatic spinach productivity occurred in the treatment of application of probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻², which did not differ significantly with treatment combinations of probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻², probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻², and also probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻².

Removal efficiency

The results demonstrated that removal efficiency (RE), absorption efficiency of solid materials, ranged from 41.28 to 52.73%. The mean RE values of the treatments are presented in Figure 4.

The results of ANOVA showed that adding probiotics in fish feed and using clam in biofilter systems had a significantly different effect ($P > 0.05$) on the removal efficiency. However, interaction effect of these two factors was not significant ($P > 0.05$) on the removal efficiency.

The results of measurements and analysis of other water quality parameters included turbidity (4.12-6.72 NTU), pH (7.11-8.05), water temperature (25.83-28.07 °C), and ammonia concentration (0.01-0.09 ppm). Water quality parameter values are supporting data in the discussion of the results of this study. These parameters relate to the cycle of organic matter, especially nitrogen in the culture media, so it is important to understand the phenomena that occur and determine the development of the aquaponics system.

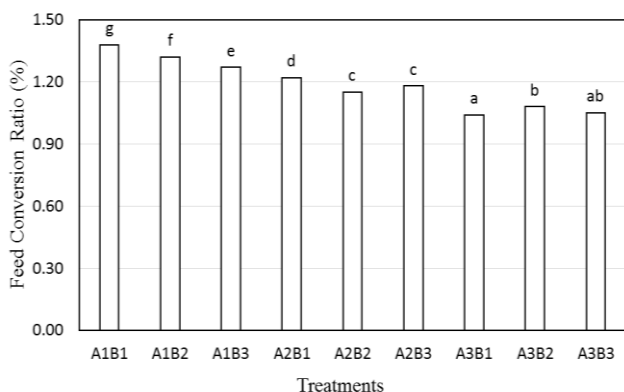


Figure 1. Feed conversion ratio in catfish. Treatment's code: A₁B₁ (probiotic 2 mL kg⁻¹ and biofilter 50 clams m⁻²); A₁B₂ (probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻²); A₁B₃ (probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻²); A₂B₁ (probiotic 4 mL kg⁻¹ and biofilter 50 clams m⁻²); A₂B₂ (probiotic 4 mL kg⁻¹ and biofilter 100 clams m⁻²); A₂B₃ (probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻²); A₃B₁ (probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻²); A₃B₂ (probiotic 6 mL kg⁻¹ and biofilter 100 clams m⁻²); A₃B₃ (probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻²).

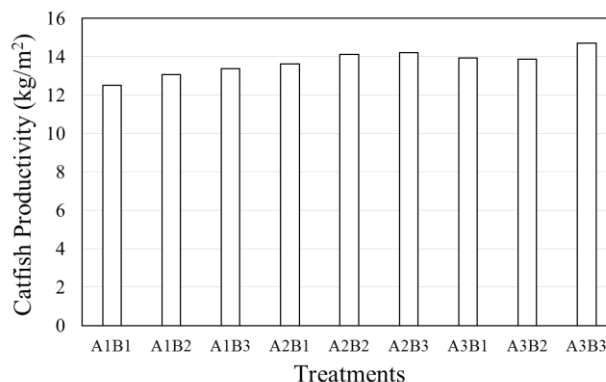


Figure 2. Biomass productivity of catfish. Treatment's code: A₁B₁ (probiotic 2 mL kg⁻¹ and biofilter 50 clams m⁻²); A₁B₂ (probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻²); A₁B₃ (probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻²); A₂B₁ (probiotic 4 mL kg⁻¹ and biofilter 50 clams m⁻²); A₂B₂ (probiotic 4 mL kg⁻¹ and biofilter 100 clams m⁻²); A₂B₃ (probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻²); A₃B₁ (probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻²); A₃B₂ (probiotic 6 mL kg⁻¹ and biofilter 100 clams m⁻²); A₃B₃ (probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻²).

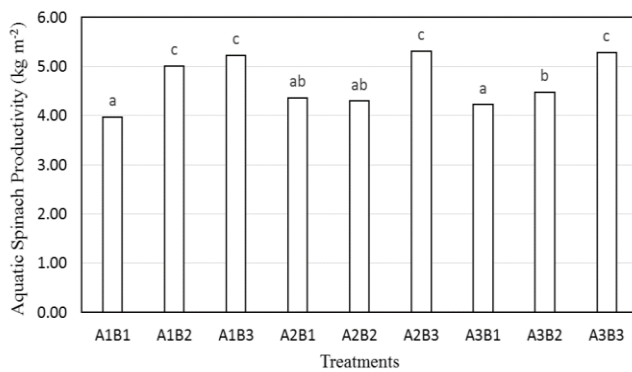


Figure 3. Biomass productivity of aquatic spinach. Treatment's code: A₁B₁ (probiotic 2 mL kg⁻¹ and biofilter 50 clams m⁻²); A₁B₂ (probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻²); A₁B₃ (probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻²); A₂B₁ (probiotic 4 mL kg⁻¹ and biofilter 50 clams m⁻²); A₂B₂ (probiotic 4 mL kg⁻¹ and biofilter 100 clams m⁻²); A₂B₃ (probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻²); A₃B₁ (probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻²); A₃B₂ (probiotic 6 mL kg⁻¹ and biofilter 100 clams m⁻²); A₃B₃ (probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻²)

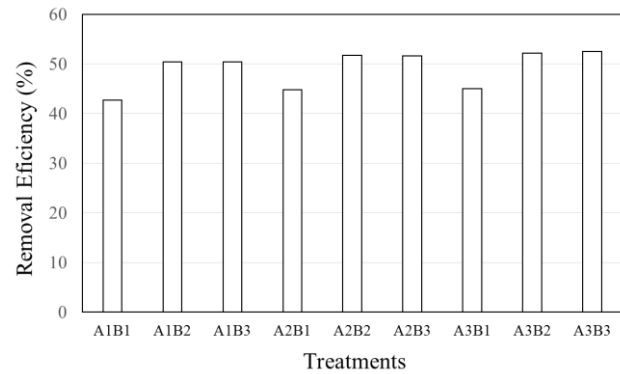


Figure 4. Removal efficiency in aquaculture media water. Treatment's code: A₁B₁ (probiotic 2 mL kg⁻¹ and biofilter 50 clams m⁻²); A₁B₂ (probiotic 2 mL kg⁻¹ and biofilter 100 clams m⁻²); A₁B₃ (probiotic 2 mL kg⁻¹ and biofilter 200 clams m⁻²); A₂B₁ (probiotic 4 mL kg⁻¹ and biofilter 50 clams m⁻²); A₂B₂ (probiotic 4 mL kg⁻¹ and biofilter 100 clams m⁻²); A₂B₃ (probiotic 4 mL kg⁻¹ and biofilter 200 clams m⁻²); A₃B₁ (probiotic 6 mL kg⁻¹ and biofilter 50 clams m⁻²); A₃B₂ (probiotic 6 mL kg⁻¹ and biofilter 100 clams m⁻²); A₃B₃ (probiotic 6 mL kg⁻¹ and biofilter 200 clams m⁻²)

Discussion

Adding probiotics in fish feed had a significant effect on improving feed conversion ratio, increasing the production of catfish, and the removal efficiency, but had no significant effect on aquatic spinach biomass production. The application of probiotics in fish feed during one culture cycle has been proven to reduce the value of feed conversion ratio in catfish (Fackri et al. 2016; Fadholi et al. 2016; Lili et al. 2018). Probiotics mixed in fish food facilitated the absorption process of feed nutrients in the digestive tract and fish's stomach, so that the feed provided was more efficient in stimulating the growth of catfish. Probiotic bacteria used in this study were *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Aspergillus oryzae*, *Rhodopseudomonas*, *Actinomyces*, and *Nitrobacter*. Probiotic bacteria such as *Bacillus* can increase feed digestibility by producing protease enzymes in the digestive tract of fish (Wardika et al., 2014). In addition, the presence of probiotic bacteria also plays a role in improving fish health, because the presence of probiotic bacteria can suppress the population of pathogenic bacteria (Cruz et al. 2012).

Some probiotic bacteria, such as *Nitrobacter* that enter the culture media also play a role in the process of overhauling organic material. This bacterium acts as a decomposer that remodels metabolites and remnants of food waste in aquaculture media into the form of nitrates (NO₃⁻) which can be utilized by vegetables in biofilter components (Tyson et al. 2004). Thus, metabolite discharges and food waste can be used effectively for the growth of vegetables in the biofilter, while the activity of the vegetables themselves can increase the concentration of dissolved oxygen in water. Rakocy (2006) suggested that vegetables need nitrates and phosphates for their growth. Potential of probiotics isolated from Sangkuriang catfish to

improve growth performance and feed efficiency was reported by Manoppo (2018).

The use of clam as biofilter had a significant effect on improving feed conversion ratio, increasing the production of catfish and aquatic spinach, also the removal efficiency in the aquaponic system. Nitrifying bacteria changed fish waste as nutrients that can be utilized by plants. Then, this plant will function as a vegetative filter, which will break down these toxic substances into substances that are not harmful to fish, and supply oxygen (O₂) to the water used to cultivate fish. Thus, this is a cycle of mutual benefit (Tyson et al. 2004). In general, aquaponic uses a recirculation system. That is, reusing water that has been used in fish farming with biological and physical filters in the form of plants and the media. The recirculation used contains maintenance and water treatment compartments. Typically, water treatment systems are composed of decantation compartments, filtration compartments, oxygenation compartments and sterilization compartments.

According to Rakocy (2006), aquatic spinach will grow if the concentration of nitrate (NO₃) and phosphorus (PO₄) compounds is sufficient in the water. The nitrate compound in the aquaponic system is the decomposition of fish excretion and leftover food by bacteria in the water. *Nitrosomonas* and *Nitrobacter* bacteria can work well if there is enough oxygen and not too much organic matter. In the system with whirling sedimentation, solid organic matter is easily trapped in the sedimentation tank so it does not enter directly into the biofilter. This makes the bacteria in the biofilter tankless burdened than the system without swirling sedimentation because it only decomposes dissolved organic matter. As a result, bacteria in a system with swirling sedimentation are able to decompose quickly the organic matter which is dissolved into nitrate. Whereas in a system without swirling sedimentation, a high load of

solid organic matter inhibits the work of bacteria in breaking down organic matter into nitrates. As a result, aquatic spinach production is lower in this system.

Ammonia in the waters comes from fish metabolism that is dissolved in water, fish feces, as well as from fish food that is not consumed and settles at the bottom of the aquaculture pond (Pillay 2004). There are a number of factors that can cause ammonia concentrations to increase, including decaying inedible fish, decreasing levels of dissolved oxygen in ponds; the dissolved oxygen in the range of 1 - 5 ppm will cause slow fish growth while dissolved oxygen less than 1 ppm can be toxic to most fish species (Rully 2011).

The LSD post hoc test showed that the best interaction effect was the treatment combination of probiotic 6 mL kg^{-1} and biofilter 200 clams m^{-2} because it generated a combination of the lowest feed conversion ratio and the highest of aquatic spinach production. The aquaponics system reduces ammonia by absorbing wastewater or by using plant roots so that the absorbed ammonia undergoes an oxidation process with the help of oxygen and bacteria, ammonia is converted into nitrates (Widyastuti 2008). In aquaculture activities without changing the water with afresh one, bacteria have an important role in removing ammonia particles through the process of nitrification (Rully 2011). Ammonia (NH_4^+) is non-toxic, but its non-ionized (NH_3) form is very toxic (Kordi and Tancung 2007). The decay process was first formed ammonia (NH_3) as a result of the overhaul of amino acids by various types of aerobic and anaerobic bacteria. Demolition will produce a free CO_2 gas. If the condition of the water is getting worse, so that O_2 in the water runs out, then the process of dismantling the organic material will slowly be taken up by another well-known bacteria, i.e., Nitrosomonas into nitrite compounds. If the waters contain enough cations, the nitric acid formed can immediately be converted to nitrite salts by Nitrobacter bacteria, the nitrite salts are then further converted into nitrate salts. Nitrite salts are important as a mineral assimilated by green plants to rearrange amino acids in the body to form the protoplasm.

Increasing the effectiveness of the aquaponics system will support the quality of water that is maintained in accordance with the needs of cultivated catfish. The saving of water used in this aquaponics system is very suitable to the condition of the archipelagic dryland which has a limited supply of freshwater. So, the development and improvement of the aquaponics system are expected to be an alternative in the development of freshwater fish farming in the archipelagic dryland areas.

ACKNOWLEDGEMENTS

The authors sincerely thank Alaudin Alayubi, Abram Nggaluama, and Eligius Kono, for their help during observation in the Fisheries Field Laboratory, Integrated Field Laboratory/Center of Excellent of Archipelagic Dryland, University of Nusa Cendana, Kupang, Indonesia.

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