Effect of fermented feed supplementation in circulated aquaponic system with catfish (Clarias sp.) on growth of lettuce (Lactuca sativa L.)

R Handayani* and A Dinoto

1Research Center for Biology, Indonesian Institute of Sciences, Cibinong Science Center, Cibinong-Bogor 16911, Indonesia

*Email: handayanirini@yahoo.co.uk

Abstract. Aquaponic systems of aquatic animal-plant are a model for conversion of waste into plant substrates and its efficacy is estimated depending on the type of feed given. This study aims to investigate the effect of fermented feed in the circulating aquaponics system with catfish (Clarias sp.) on the growth of lettuce (Lactuca sativa L.). The study was carried out in a circulated serial aquaculture tanks containing catfish. Various inputs of fermented fish feed was applied including low concentration (6% of fish weight) and high concentration (9% of fish weight). The growth of lettuce, water quality, and total microorganisms were measured. As results, maximum length and width of lettuce were higher in supplementation of higher feed concentrations (9%), as well as tendency height and weight of plant clumps plant.

Keywords: fermented feed, aquaponic, catfish, lettuce, plant growth

1. Introduction

Aquaponics is the process of growing aquatic organisms and plants symbiotically. In this system, the effluent of aquaculture undergoes the transformations by microorganisms to be used as a source of nutrients for plant growth, while nutrient absorption from plants remediates water for aquaculture [1]. The main problem in intensive aquaculture is leftover feed, organic matter, and toxic nitrogen compounds. Fish only uses 20-30% of feed nutrients [2].

There are several advantages of aquaponics when compared to conventional land-based cultivation, including not requiring fertilizers and pesticides, very efficient in water use, can be done on non-agricultural land, high productivity, producing two products at once, namely plants and fish, the products produced are categorized organic and free of chemical and biological contamination. The application of aquaponics is also avoid classic problems such as soil compaction, salinization, pollution, disease, and soil fatigue due to intensive use. The types of vegetable plants that can be planted using the aquaponic system, in general, are plants that have a high level of resistance to water such as lettuce and pakcoy [3].

Previous study reported that lettuce plants were able to suppress the increase in the concentration of total ammonia concentration up to 0.399 mg/L in aquariums with an aquaponic system [2]. In aquaponic system, catfish is able to utilize feed with high protein
content, but as much as 65% of the protein will be lost to the environment. Most of the nitrogen is released as ammonia (NH$_3$) through the gills, and only 10% is lost in the form of solid waste [2].

The nutrient content of aquaponic water depends on the nutritional content of fish feed. Feeding containing probiotics in African catfish culture has also improved fish health, fish endurance, feed efficiency and increased fish growth [4]. Based on a literature study on lettuce plants in the aquaponic system and its correlation with catfish feed with functional feed has not been reported. The aim of this study is to find the effect of fermented feed supplementation in recirculated aquaponics system with catfish (Clarias sp.) and correlation to the growth of lettuce (Lactuca sativa L.)

2. Materials and Methods
2.1. Fermented feed supplementation
Fermented feed was obtained from the Industrial Microbiology Laboratory, Research Center for Biology, Indonesian Institute of Science (RCB LIPI).

2.2. Recirculating Aquaculture system
The aquaculture recirculation system in this study consists of three serial tanks with a volume of 100 L per tank, connected by a 3/4 inch pvc pipe with a distance of 15cm each tank. Three tank containing fish, one tank containing bioball for biological filters (bioremediation process), and two additional tank of 20L each for water filter tank and one tank containing submersible pumps for recirculation.

Water from the biofilter tank flows into lettuce growth media in the form of a 4 inches PVC pipe with a length of 250 cm, and 21 planting holes. The distance between the holes is 5 cm with a diameter of 5 cm each. Each hole has a plastic cup as a container for planting lettuce where Rockwool® is used as a growth medium to ensure the seeds survive in the container.

Each fish stocked was 70 specimens with an average length of 10-15 cm and a total weight of 500 gr. They are fed twice a day (8:00 and 3:00 at night). With the percentage of fermented feed 6% and 9% of their total body weight.

The biological plant material used in this study was lettuce (Lactuca sativa L.). For experiments in the aquaponic module at the RCB LIPI Industrial Microbiology Laboratory, planting lettuce seeds after 12 days of seeding.

Throughout the development of nurseries, lettuce was planted under direct solar radiation without the slightest shade.

2.3. Lettuce growth measurement
Measurements of the average plant height (cm), head diameter (cm), number of leaves per plant, carried out on lettuce by feeding fermented fish feed and on commercial fish feed every 7 days for 2 months.

2.4. Water quality measurement
During the study, the water quality was monitored. Dissolved oxygen and water temperature were measured weekly using portable dissolved oxygen meter (JPB-70A®). The concentrations of the total ammonium and nitrate were measured in independent laboratory research of PT SIG.
2.5. Total plate count of aquatic microorganism
The total number of aquatic microorganism was determined by total plate counts. Serial dilution of water sample was conducted in the sterilized 0.85% (w/v) NaCl and the diluted samples were then plated on nutrient agar. After 48h incubation at 37°C the total number of microbes was calculated as cfu/ml.

2.6. Data Analysis
One-way analysis of variance (ANOVA) tests was conducted to determine the significant difference in the growth of the plant with different fed concentration types.

3. Results and Discussion
The results of this study indicated the growth of lettuce in various percentages of fermented feed consumption in catfish. Lettuce leaves grow taller in length and width in 9% fermented feed than in 6% fermented feed (figure 1). Lettuce species grow better in fermented feed with 9% concentrate. Lettuce produces an average wet weight of 31.0 g / plant. In addition, the growth of lettuce leaves reached 14.5 cm / plant (figure 1).

Good water quality must be maintained at all times in a recirculating fish tank to maintain optimal growth lettuce [6]. The most critical water quality parameters to monitor are dissolved oxygen concentrations, temperature, pH, and nitrogen from ammonia, nitrate and nitrite. The biofiltration mechanism in aquaponic systems also removes nitrates quite well and can keep their concentration at much lower levels than this. Other factors that influence the quality of fish tank water include the stocking density of the fish, their growth rate, the rate at which they are fed, the volume of water in the system and environmental conditions for growth lettuce [7].

Figure 1. Lettuce growth in different feed fermented consumption on Catfish
Waste accumulation will increase oxygen demand by bacteria in order to perform organic matter break down, consequently favouring the development of anaerobic patches [8]. While the feed is continuously added in a fish culture system, the wastewater it accumulates in dangerous quantities. Wastewater effluent is rich in nutrients because it comprises mainly feces, uneaten food, and bacterial biomass. Decomposing food creates ammonia. Fish, in metabolic processes of their growth, excrete ammonia at the gills [8].

Table 1. Ammonium levels and water nitrate levels in the catfish-lettuce aquaponic system

|                        | Fermented feed 6% | Fermented feed 9% |
|------------------------|-------------------|-------------------|
| Ammonium level average (mg/L) ± SD | 0,04 ± 0,00 | 0,07 ± 0,00 |
| Nitrate level average (mg/L) ± SD       | 6,92 ± 0,005 | 3,70 ± 0,005 |

Level average of Ammonium and Nitrate in water of tank and correlation with percentage feed fermented concentration 6% and 9% was recorded (table 1). Ammonium level average for 6% fermented feed is 0,04 and 0,07 for 9% fermented feed. Nitrate level average is 6,92 for 6% fermented feed and 3,70 in 9% fermented feed.

The two most common species of nitrifying bacteria are Nitrosomonas sp. and Nitrobacter sp. Nitrosomonas sp. converts ammonia (NH3) to nitrite (NO2) while Nitrobacter sp. use nitrites for their energy source during its conversion to nitrate (NO3). Nitrogen in nitrate form is absorbed and used as a nutrient by plants. It can take a significant amount of time for these bacteria to grow in a system. This might have contributed to lower nitrogen content in the first few weeks [2].

Nitrogen in the form of nitrate and nitrite usually does not present a water quality problem in aquaponic fish tanks as nitrite is quite quickly converted to nitrate and nitrate itself is only seriously toxic to fish at very high levels (300-400 mg/L) [2].

Ammonia in aquaculture ponds generally comes from the process of decomposition of organic materials such as plants, animals and food that are decomposed by microbes and fungi. Ammonia is also derived from fish excretion products (urine and feces). Ammonia in water consists of two forms, namely ammonia NH3 and ammonium NH4 + (Total Ammonia). Ammonia levels are affected by the temperature and pH of ponds [2].

The use of microorganisms as a source of probiotics has been preferred since the early 1990s. It is argued that microbiota living in healthy hosts are most likely to be part of the natural defence system and beneficial in multiple ways [9].

Probiotics are known as living microbial cells that promote the health of their host by improving the balance of the intestinal microbial flora. Recently, it became known in aquaculture that probiotics in diets could help to improve fish growth and the culture environment in which it is reared (biocontrol and bioremediation) [10].

Functional feeds containing gut health promotors deliver with every meal an adequate concentration of natural compounds which can work through multiple mechanisms to reduce the success of parasitic infestations. A wide range of additives with different mode of actions are currently offered including yeast extracts, phytobiotics, probiotics, prebiotics, organic acids and their derivates [10]. Table 2 showed total microorganisms that feeded with 6% fermented feed and 9% fermented feed.

A probiotic application for aquaculture practices can be defined as a live microbial supplement that administered via feed or directly into the rearing water provides a benefit to
The animal by enhancing nutrient utilization, health status, stress response, disease resistance and performance, and this is partly achieved by optimizing the microbial balance within the animals and water environment [10]. The growth of lettuce significantly higher in aquaculture recirculated with fermented fish feeding. According to [11] the positive contribution in grown lettuce occurs in recirculate aquaculture system with microbial treatment.

*Bacillus* spp. is considered a bioremediation agent, concept referring to the use of microorganisms that help to maintain proper pond environment by the secretion of exo-enzymes that break down organic matter [12]. In addition, *Bacillus* spp. may use ammonia nitrogen as nitrogen source for their growth [12].

**Table 2.** Total aquatic microorganisms in the catfish-lettuce aquaponic system

| Feed 6% | Feed 9% |
|---------|---------|
| 83,33 ± 5,25 | 13,33 ± 0,94 |

Fermented feed is feed formulated by adding probiotics. The use of probiotics in aquaculture has been shown to have a beneficial impact on fish health and an increase in economic value on fish farming. At the same time, the use of probiotics also has important environmental benefits. By reducing the risk of disease, needs reduce the risk of disease, the need for drugs and thus the risk of residuals remaining in the environment is reduced [12][13][14] reported the ability of *Lactobacillus* spp. simultaneously removes nitrogen and pathogens from contaminated shrimp ponds. Water probiotics contain several strains of bacteria such as *Bacillus acidophilus, B. subtilis B. lecheniformis, Nitrobacter* sp, *Aerobacter* and *Saccharomyces cerevisiae* [15]. The application of probiotics through water tanks and ponds might also affect fish health by improving some water quality, because they modify the bacterial composition of water and sediments [16].

4. Conclusion

The data clearly indicated the positive effect gained from sterilizing the aquaponic water prior to transferring to the plants which may have contributed some benefits. The maximum length and width of lettuce were higher in supplementation of higher feed concentrations (9%), as well as tendency height and weight of the plant clumps plant.

5. References

[1] Nofiandi Riawan 2015 *Step by Step Membuat Instalasi Akuaponik Portabel 1m² Hingga Memanen* (Jakarta: Agro Media Pustaka)

[2] Hargreaves J A and Tucker C S 2004 *Managing ammonia in fish ponds* (Southern Regional Aquaculture Center) No 4603

[3] Somerville C, M Cohen, E Pantanella, A Stankus and A Lovatelli 2014 *Smallscale Aquaponics Food Production: Integrated Fish and Plant Farming* Rome: FAO

[4] A Dohail, M Abdullah, H Roshada and M Aliyu 2009 Effects of the probiotic, *Lactobacillus* acidophilus, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) *Aquaculture Research* 40(14): 1642-1652

[5] Martins C I M, Eding E H, Verdegem M C J, Heinsbroek L T N, Schneider O, B Lancheton J P, Roque Castel D’orb E and Verreth J A J 2010 New developments in
recirculating aquaculture systems in Europe: A perspective on environmental sustainability *Aquacultural Engineering* **43**(3): 83-93

[6] Erkki-Einar Søberg 2016 *The Growth and Development of Lettuce, Coriander and Swiss chard in a Cold Water Aquaponic System Optimized for Lettuce Production*. (Master’s Thesis), The Faculty of Veterinary Medicine and Biosciences, The Department of Plant Sciences Norwegian University of Life Science

[7] Carvalho R O, Machado M B, Scherer V S, Fuentes G C, Da Luz C A S, Da Luz L G S 2015 Hydroponic lettuce production and minimally processed lettuce. *Aquacultural Engineering International: CIGR Journal* 290-293

[8] Sumeth W, Zhen H, Kartik C, Jae WL, Samir K K 2017 Nitrogen transformations in aquaponic systems: A review *Aquacultural Engineering* **76**: 9-19

[9] Gómez G D and Balcazar J L 2008 A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunology and Medical Microbiology* **52**: 145-154

[10] Lazado C C, Caipang C M A and Estante E G 2015 Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions *Fish and Shellfish Immunology* **45**: 2–12

[11] Zachary J Wielgosz, Tyler S Anderson and Michael B Timmons 2017 Microbial Effects on the Production of Aquaponically Grown Lettuce *Journal of Horticulturae* **3** (46)

[12] Ma G W, Cho Y S, Oh K H 2009 Removal of pathogenic bacteria and nitrogen by *Lactobacillus* spp. JK-8- JK-11 *Aquaculture* **287**: 266-270

[13] Vine N G, Leukes W D and Kaiser H 2006 Probiotics in marine larviculture *FEMS Microbiology Reviews* **30**: 404-427

[14] Hai N V 2015 The use of probiotics in aquaculture *J. of App. Microbiology* **119**: 917–935

[15] Newaj-Fyzul A, Al-Harbi A H and Austin B 2014 Review: Developments in the use of probiotics for disease control in aquaculture *Aquaculture* **431**: 1–11

[16] Michael E T, Amos S O, Hussaini L T 2014 A Review on Probiotics Application in Aquaculture *Fish Aquac J* **5**: 111