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Plant Growth and Nitrogen Flow in an Aquaponics System Integrating Lettuce Hydroponics and Loach Aquaculture

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ABSTRACT

There is an urgent need to develop production technology that uses water and other material resources effectively to create a stable food supply. Aquaponics combining hydroponics and aquaculture is expected as an efficient system for producing crops and animal proteins sustaining the reuse of water and balance of nutrient elements between both cultures, using dissolved elements in fish excrement for plant growth. To evaluate the possibility of aquaponics combining lettuce hydroponics and loach aquaculture, we analyzed the growth performances of lettuce and loach and the balance of nutrient elements, especially for nitrogen between input and output of the whole system and nitrogen flow in the system. As a result, lettuce grew in aquaponics with half strength standard solution as well as hydroponics with a standard solution by the optimal combination of the number of plants and fish. In nitrogen balance, almost 70% of nitrogen from the feed was used by growing lettuce and about 25% of nitrogen from the feed was accumulated in loach.

keyword: aquaculture, hydroponics, sustainability

INTRODUCTION

Due to the recent growth in the global population, concerns have arisen over the stable supply of food and water resources. Therefore, there is an urgent need to develop production technology that uses water resources effectively to create a stable food supply (Yamada, 2015). This study examines aquaponics, a biological production system combining hydroponics and fish farming that has been attracting attention in recent years as a resource-recycling production system.

Aquaponics can make effective use of water and nutrients (Wongkiew *et al*., 2017). Sharing water between hydroponics and aquaculture systems allows plants to utilize the fertilizer components derived from fish excrement for growth. Therefore, food can be produced while reusing nutrients and converting waste into high-value resources.

To date, the main focus in the field of aquaponics has been the availability, dissolved concentration, and accumulation of nitrogen compounds (Goddek *et al*., 2016). This is because plant growth depends on nitrogen supply (Xu *et al*., 2012). Since aquaponics is a biological production system that combines hydroponics and fish farming, a thorough understanding of the nitrogen balance is required to increase the production of the system.

Most studies dealing with aquaponics have used tilapia or catfish as the fish to be cultured (e.g., Nuwansi *et al.,* 2016; Graber and Junge, 2009; Naznin et al.; Islam et al., 2022) because they are edible and have a high growth rate. However, some parts of these fish are not edible, such as the head, internal organs, and bones, which is a drawback. In contrast, loach has an edible portion of 100% and high nutritional value (Japan Ministry of Education, Culture, Sports, Science, and Technology). It is also a traditional Japanese ingredient. In addition, loaches are resistant to dissolved oxygen deficiency because they can breathe using their intestines. Therefore, loach was adopted as the fish to be cultivated in this study. Many studies have been conducted on aquaponics cultivation with different types of plants. For this study, lettuce was adopted as the plant to be cultivated, as it is common in plant factory cultivation.

The nitrogen balance has been the main focus of aquaponics research (e.g., Hu et al. 2015) As a biological production system combining hydroponics and aquaculture, a thorough understanding of nitrogen balance is necessary to increase system productivity. In this study, we also evaluated the usefulness of components derived from fish excreta as fertilizers for plant growth in aquaponics, using a combination of loach farming and lettuce hydroponics. In particular, we evaluated the flow of nitrogen within the system, as it is an essential element for plants and is abundant in fish excreta.

MATERIALS AND METHODS

Lettuce (*Lactuca sativa L. 'crunch'*) and loach *(Misgurnus anguillicaudatus*) were cultivated and bred for 21 days. Dechlorinated tap water (12 L) and loach (initial weight 2.9 g/fish) were placed in a cultivation container, and 21-day-old lettuce seedlings (fresh weight 2.8 ± 0.2 g/plant) were planted in the holes at 5 cm intervals in a grid pattern (Fig. 1). The environmental conditions for growing lettuce were set at a temperature of 25 °C, relative humidity of 70%, a CO_2 concentration of 1000 μmol mol^{-1}, a photosynthetic effective photon flux density of 200 μmol m^{-2} s^{-1}, and a light period of 16 h d^{-1}. A white fluorescent lamp (FHF32EXD HX-S NEC Corporation) was used as the light source. Continuous aeration (3.5 L min^{-1}) was provided to the nutrient solution. Loaches were fed a commercially available goldfish feed (Kyorin Co., Ltd.) once a day, 5 times a week, equivalent to 1% of their body weight (about 0.03 g / fish).

Test Group

In this study, a commercial fertilizer solution (OAT, Ohtsuka Co., Ltd.) at 1/2 the standard prescription concentration was used as a standard nutrient solution. Separate test plots were

established with the standard nutrient solution, 0.5-fold nutrient solution, and 0.5-fold nutrient solution with 20 loaches, respectively. Furthermore, the amount of dissolved nitrogen discharged from 20 loaches was quantified by establishing a test plot in which only loaches were bred in dechlorinated tap water.

Nitrogen Flow

The nitrogen content (g) of each element in the system was calculated using the following procedure.

The amount of nitrogen contained in the nutrient solution of a test plot ("Nutrient Solution"; Fig. 2) was calculated from the nutrient solution composition specified by the manufacturer. The amount of nitrogen contained in the feed provided to the fish ("Feed"; Fig. 2) was calculated by multiplying the nitrogen content (g g^{-1}) contained in the loach feed by the total feed amount (g). There was no leftover food. The amount of nitrogen contained in the excretion from loaches ("Excretion from loaches"; Fig. 2) is the amount of nitrogen derived from loach emissions calculated from the total amount (g) of dissolved nitrogen components discharged by 20 loaches in 21 days in the test plot where only loaches were bred. The amount of nitrogen remaining in the cultivation container at the end of the experiment ("Residual solution"; Fig. 2) was calculated from the amount of dissolved nitrogen component (g) at the end of the experiment. The amount of nitrogen accumulated in the lettuce grown during the experiment ("Lettuce"; Fig. 2), calculated using the nitrogen content of lettuce at the end of the experiment (g g-dry^{-1}) and the increase in the total dry weight of lettuce during the experiment (g-dry). The amount of nitrogen accumulated in the loach body during the experimental period ("Loaches"; Fig. 2) was calculated from the nitrogen content in the loach body (g g-dry^{-1}) and the increase in the loach dry matter during the experimental period (g-dry).

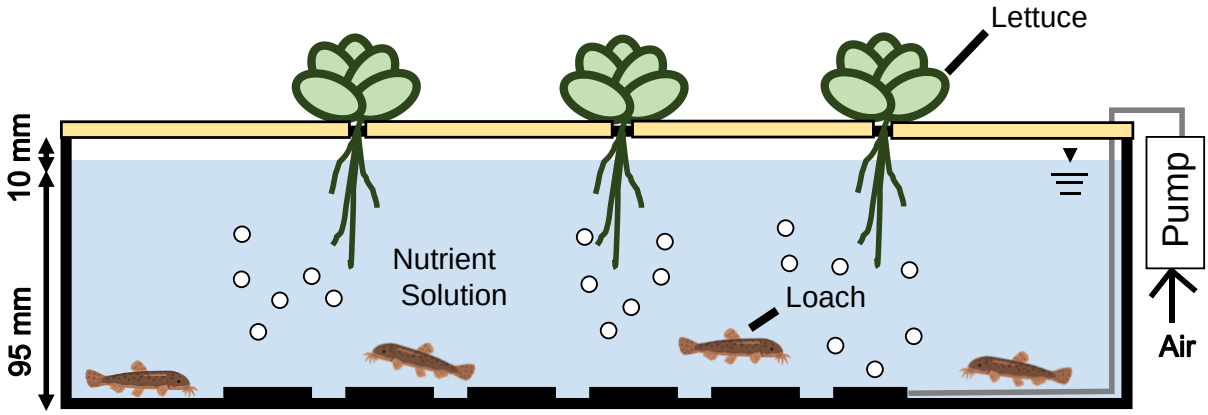


Fig. 2: Schematic diagram of the experimental aquaponics combining lettuce hydroponics and loach aquaculture.

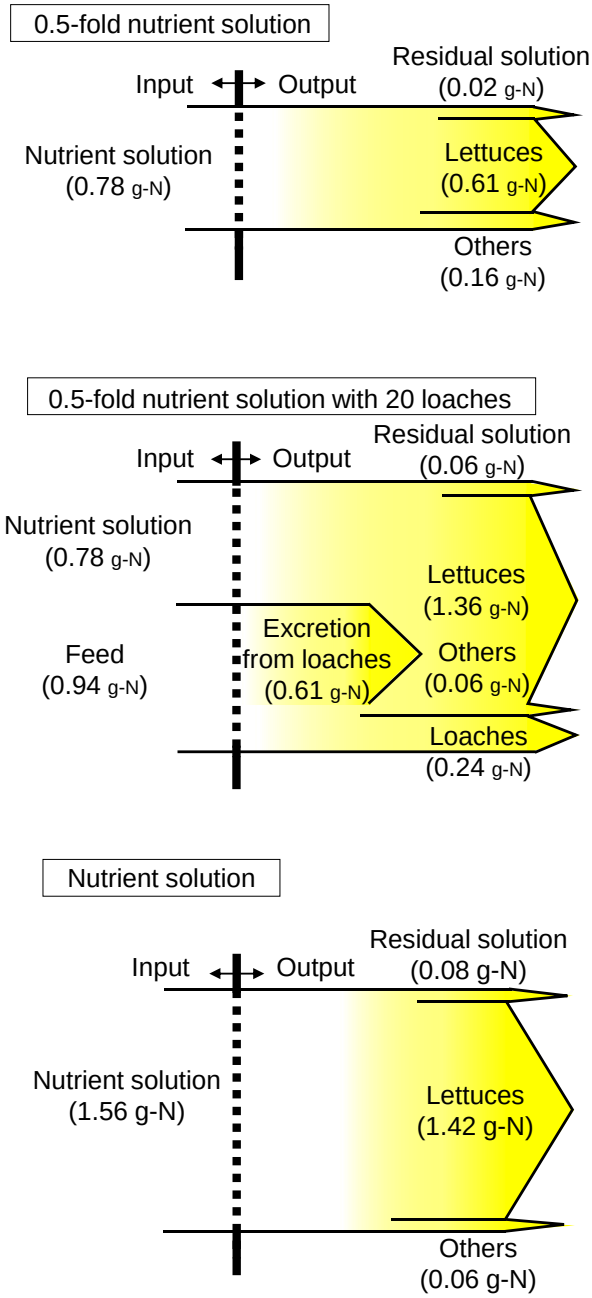


Fig. 2: Schematic diagram of nitrogen flow in each treatment. Numerical values in parentheses indicate amounts of nitrogen (g N) supplied and accumulated in the system during the 21-day experiment.

RESULTS

Lettuce Growth

Table 1 shows the lettuce weight and nitrogen content at the end of the experiment. The total dry matter weight of lettuce was 1.6 times higher in the "0.5-fold nutrient solution with 20 loaches" group than in the "0.5-fold nutrient solution" group, indicating that the excreted components of loach contribute to plant growth. There was no significant difference in the total

dry weight of lettuce between the "0.5-fold nutrient solution with 20 loaches" group and the "standard nutrient solution" group. There was no significant difference in the dry weight of the roots in the test section. Lettuce from the "0.5-fold nutrient solution" group had a lower nitrogen content than lettuce from the other two test groups, and no significant difference was observed between the "standard nutrient solution" group and the "0.5-fold nutrient solution with 20 loaches" group.

Table 1. Growth and nitrogen contents of lettuces in different treatments.

Nutrient solution	No. of lettuces	No. of loaches	Dry weight (g/plant)			Fresh weight of lettuce shoot (g/plant)	Nitrogen content	
			Total	Shoot	Root		(g g^{-1} DW)	(g/plant)
× 0.5	6	-	3.31 ± 0.20 b	2.50 ± 0.16 b	0.81 ± 0.06 a	53 ± 4 b	0.031 b	0.105 b
× 0.5	6	20	5.26 ± 0.24 a	4.47 ± 0.21 a	0.79 ± 0.04 a	97 ± 4 a	0.046 a	0.244 a
× 1	5	-	5.69 ± 0.29 a	4.95 ± 0.27 a	0.74 ± 0.04 a	116 ± 6 a	0.050 a	0.286 a

Mean ± standard error (n = 5-6). Different alphabets indicate a significant difference (Tukey-Kramer test, P<0.05) for each parameter.

Loach Growth

During the 21 days of rearing, loaches were fed approximately 0.45 g of feed per individual and the average loach weight increased from 2.9 g / fish to 3.3 g / fish. The weight gain of the loach was equivalent to 89% of the food provided.

Nutrient Solution Component Composition

The composition of the nutrient solution was compared between the "0.5-fold nutrient solution" group and the "0.5-fold nutrient solution with 20 loaches" group. The breeding of 20 loaches resulted in a solution comprising approximately 80% N, Ca, and K. Increased by 200% and 8% (Table 2).

Table 2. The amount of nutrient elements supplied in each treatment during the 21-day experiment.

Nutrient solution	No. of lettuce	No. of loaches	Macronutrients (mg)					Micronutrients (mg)					
			Na	Ca	K	Mg	P	B	Cu	Fe	Mn	Mo	Zn
× 0.5	6	0	780	492	1008	108	156	1.39	0.10	8.10	3.48	0.10	0.28
× 0.5	6	20	1388	1475	1085	280	245	2.93	0.20	8.13	3.65	0.26	0.36
× 1	5	0	1560	984	2016	216	312	2.78	0.19	16.20	6.96	0.19	0.55
tap water	0	20	608	983	77	172	89	1.54	0.10	0.03	0.17	0.16	0.08

In other words, loach excreta have a different composition from that of the standard nutrient solution.

Nitrogen Flow

In the "0.5-fold nutrient solution with 20 loaches" group, almost all the nitrogen supplied by the loach excreta was used for lettuce growth (Fig. 2). Furthermore, in this test group, the product of the total dry weight of lettuce and the nitrogen content in the lettuce (Table 1) did not differ from that in the "standard nutrient solution" group. This indicates that loach excreta could be used to supplement chemical fertilizer. The amount of nitrogen in the loach excreta and the amount of nitrogen accumulated in the loach were approximately 65% (0.61 g N) and 26% (0.24 g N) of the feed nitrogen content (0.94 g N), respectively.

DISCUSSION

In the "0.5-fold nutrient solution with 20 loaches" group, the lettuce dry weight increased by 59% compared to that of the "0.5-fold nutrient solution" group, but the amount of N supplied to the nutrient solution also increased by about 80%. Since plant growth depends on the amount of nitrogen supplied (Xu *et al*., 2012), we infer that the growth of lettuce in the "0.5-fold nutrient solution" group was suppressed by nitrogen deficiency. However, potassium is also an essential macronutrient, and the potassium content of the nutrient solution increased by only approximately 8%, suggesting that the supply of K in both groups was sufficient. From the above, loach excrement has a different nutritional composition from the standard nutrient solution. Consequently, loach excrement alone will result in insufficient plants due to a shortage of potassium. However, the test results show that aquaponics can reduce the amount of chemical fertilizer required. In all test plots, the dissolved nitrogen component (Residual solution in Fig. 2) at the end of the experiment was less than 5% of the supply, and nitrogen was fully utilized in the entire system. Since excessive nitrogen supply reduces utilization efficiency, further investigations are required into how an increased nitrogen supply in an aquaponics system could affect the nitrogen utilization efficiency of the system.

Almost 70% of nitrogen from the feed was used by growing lettuce and about 25% of nitrogen from the feed was accumulated in loach. This fact indicates that in the case of aquaculture alone, much of the N source supplied as feed is discharged into the environment, suggesting the importance of recovery by plants and water purification in aquaponics.

A wide variety of plant species can be used in aquaponics, so it can be developed according to the needs of producers. For example, Naznin et al. (2015) reported the hydroponic culture of garlic plants combined with tilapia fish culture as a new combination in aquaponics to investigate its potential and medicinal productivity. Garlic is one of the common vegetables used as a spice and a medical herb in the world. The medicinal activities of garlic are attributed to ajoene which is a sulfur-rich compound and derived from heated garlic through the conversion of alliin and allicin. The possibility of hydroponic garlic culture integrated with fish culture was evaluated to increase food production in a limited space. Three treatments including garlic culture only (G), garlic culture with tilapia fish culture (GT) and tilapia fish culture only (T) were applied and growth of plants and fish and water quality were monitored. Dry weights of bulbs, leaves and roots were 1.4, 1.6 and 1.6 times, respectively, greater in GT than in G. The medicinal component `Ajoene` contents in newly developed bulbs, leaves, and roots were 2.6, 2.2, and 2.4 times, respectively, greater in GT than in G. The specific growth rate of fish in GT were 1.3 times greater than in T. It was confirmed that the fish supplied additional nutrients including nitrogen compounds to garlic plants and plants kept the water quality suitable for fish growth. Garlic and fish can be grown well in the integrated culture system with mutual benefit. It was confirmed that the combined culture of garlic with fish promoted food production in a limited space. Islam et al. (2022) reported an aquaponic system with hydroponic culture of sweet potato in a tilapia fish culture pond. In the experiment, styrofoam boxes with holes on the bottom side containing nylon net containers filled with hydroballs were used to culture sweet potato plants on the water surface. The result showed that sweet potatoes can be produced on the water surface of a fish culture pond effectively to enhance food production in a limited space. Aquaponics with loach fish allows for fish farming in plant culture

beds just like this experiment. Loaches, which are relatively small fish, can be kept even in shallow water depths of 5 to 10 cm, and suffer almost no feeding damage or no mechanical stress to plant roots. Therefore, there are many options for plant species to be combined as well as the potential for minimizing water usage.

In conclusion, lettuce grew equally even if a part of the supplied chemical fertilizer was replaced with the excretory component of loach in this study. Therefore, loach excreta can be used to partially supplement chemical fertilizer. Aquaponics has the potential to reduce the environmental load of aquaculture, through the simultaneous production of vegetables and fish, the reduction of chemical fertilizers in lettuce hydroponics, and the purification of aquaculture water. Future studies should examine not only nitrogen but also other fertilizer elements to model the optimum combination of loach, lettuce, and feed supply.

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