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Balancing of nutrient uptake by water spinach (*Ipomoea aquatica*) and mustard green (*Brassica juncea*) with nutrient production by African catfish (*Clarias gariepinus*) in scaling aquaponic recirculation system

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ABSTRACT

From both engineering and economic perspectives, goals of an aquaponic recirculation system are keeping a healthy environment for fish and plant, by eliminating toxic metabolites and growth-inhibiting substances. The type and quantity of waste excretions produced by the cultured organisms are also the important considerations, especially in designing the component system. Therefore, to be effective at nutrient removal, aquaponic systems should be sized correctly to balance fish output and nutrient uptake by plants. In this study, the plant component was isolated from the fish rearing operation so that nutrient removal could be evaluated independently. Two leafy green vegetables, i.e. water spinach (*Ipomoea aquatica*) and mustard green (*Brassica juncea*) were selected to evaluate the effectiveness of plant nutrient uptake to balance nutrient production from fish culture. Results indicated that nitrogen utilization efficiencies of water spinach and mustard green were 66.5 and 59.9%, respectively. In addition, water spinach-based aquaponics had better water quality than that of mustard green-based aquaponics, primarily due to its higher root surface area. The growth performance of African catfish showed the feed conversion ratio was in the range 1.18–1.33. The results obtained from this study indicated that both crops have considerable impacts on nutrient removal.

Keywords: Aquaponics recirculation system; Feed conversion ratio; Mustard green; Nitrogen utilization efficiencies; Water spinach

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1. Introduction

Effluents from aquaculture operations pose environmental and may contribute to eutrophication of aquatic environments through direct addition of reactive organic matter [1,2]. Aquaponics recirculation system (ARS), which is an integrated system that links recirculating aquaculture with hydroponic production can potentially be a good alternative solution to fish culture as it ensures a good controlled culture conditions by providing a better control of the water quality and prevention of fish disease [3,4]. A well-managed aquaponics could improve nutrient retention efficiency, reduce water usage and nutrients discharge to the environment, and improve profitability by simultaneously producing two cash crops [5–7]. It is generally believed that aquaponics, with concomitant nutrients recovery, will become one of the widely accepted methods of sustainable food production in the near future. These can potentially lead to higher survival and faster growth of the fish and thus enhancing the production of this fish. The renewed interest in recirculation systems are also due to their perceived advantages, including: greatly reduced land and water consumption [8], better control of water quality and waste management [9], better hygiene and disease management [10], and become a key solution for large-scale ecologically sustainable fish production [11]. Through waste recycling, integrated of aquaculture and hydroponics systems can be used to treat aquaculture effluent, spread financial risk through diversification, increase farm productivity through efficient resource utilization, and beneficial use for fresh food production [12–14]. In addition to normal nutrient absorption from plant root, foliar application of some micro and macro-nutrients were also reported to significantly alleviate nutrient deficiencies in the leaves of tomatoes grown on aquaponics [15–19].

Nitrogen is an essential element, with high contents in proteins and nucleic acids. Nitrogen is associated with protein, which is the major source of nitrogen for fish cultivation, representing 50–70% of fish production costs [20]. In aquaculture system, only about 25% of the nitrogen input is harvested through fish biomass, and over 70% is excreted into the surrounding environment in the form of ammonia [21]. In ARS, the biological nutrient wastes excreted by fish (e.g. ammonia) and those generated from the microbial breakdown of fish feed (nitrite, nitrate) are absorbed by plants as nutrients for growth, and thus this method allows the removal of undesirable nutrient wastes from the water by plants and the water can then be reused for fish culture. These could potentially lead to faster growth and higher production both of the fish and plants. The use of ARS techniques has been reported to be highly efficient as it utilizes the generated fish waste as nutrients for the plants and thus providing a symbiotic environment for producing fish and plants in a closed system [14]. Hence, the performance and efficiency of components used in this system can be evaluated through analysis of the nitrogen conversion to fish biomass [22] and to sustain high rates of growth and high stocking densities [23]. When the system is in balance, high production of fish and plant crops at high stocking densities can be obtained without the use of chemical fertilizers, herbicides, or pesticides [24].

To be most effective, ARS must be sized correctly with the optimum balance between nutrient production from fish culture and nutrient uptake by the plant component. Too large of plant growing area could improve water quality, but will lead to slower plant growth rates and reduced production of plants crops. Insufficient plant growing area will result in an accumulation of nutrients in ARS or the excessive release of nutrients inflow through the systems. Waste excretion by fish is directly correlated with the quantity and quality of feed given. This is supported with the study of Lam et al. [25] which report that Marble goby (*Oxyeleotris marmorata*) fed with live tilapia (*Oreochromis niloticus*) exhibit lower waste excretion rate as compared to those fed with minced scads (*Decapterus russellii*). Frequent removal of solid wastes minimizes the generation of dissolved nutrients. However, the need for viable methods for protection of plants grown in ARS is currently under development as there has not been a fish-safe insecticide or fungicide developed for use in aquaponics [26] and there is also a need to design the aquaponics system to maintain the balance of nutrient production and uptake in order to ensure effective nutrient removal [27].

Therefore, balancing plant uptake with nutrient fish production over time could provide crucial information for the design and the optimization of recirculation, feeding strategies as well as water and effluent treatment technologies. It also helps to identify and quantify the role of each process unit in the production system to suggest changes in operating practices, and to predict the impact of these changes on system water quality. Under equilibrium conditions, the inputs in terms of biomass to the system must be balanced with the outputs that have been removed from the system.

In this study, the hydroponic component was isolated from the fish culturing operation so that nutrient removal could be evaluated independently. Fish production, plant growth, and water quality were measured and their dependence on plant species were examined. Furthermore, nutrient removal ability of plants over entire cropping period was examined. African catfish, water spinach, and mustard green were chosen as the consumable and native species in Malaysia.

2. Materials and methods

2.1. Experimental setup

The experiments were conducted in the greenhouse at Universiti Malaysia Terengganu to provide uniform conditions such as temperature, light, pH, and aeration rate throughout the growth phase. Two aquaponic trough were operated side by side for nearly 12 weeks, each consists of three fiber glass rearing tanks, three hydroponics troughs, sump, and water holding tank were used to conduct the study. The schematic diagram of the ARS system based on Endut et al. [6] is shown in Fig. 1.

Pipelines made of polyvinyl chloride were installed to connect each component in the system for the purpose of water recirculation. Water level in each culture tank was kept at 0.80 m deep to maintain the water volume at 960 L. Black net cloth was installed over the culture tank to prevent excessive sun exposure to the fish tank. Water lost through evaporation, transpiration, and sludge removal was replenished with water in the pre-aeration tank. Water drained out and flowed from the culture tank was sprinkled over the vegetables in the grow bed and outflow trickled down to the sump for denitrification process. The water was then pumped vertically to the sand filtration tanks for particulate removal. After exiting the sand filter, the water went directly to water storage tank and was continuously flow under gravitational force to fish culture tank through the water spreader bar. The components were installed such that the water flowed by gravity, by placing components at appropriate elevation relative to one another. Nutrients present in aquaculture effluent were absorbed by plants in grow bed and the water was recirculated continuously between fish tank and grow bed in aquaponics and no water exchange was conducted during the study period except for replenishing evapotranspiration losses. During this study, the hydraulic loading rate (HLR) of the ARS was maintained to be identical as possible by adjusting the gate valve, which is 1.28 m/d. The HLR was selected based on the aquaponic system optimization on the previous study [6].

2.2. Experimental operation

The tank was stocked with high density (30 kg/m^3) of African catfish (*Clarias gariepinus*) fingerlings with an initial body weight in the range of 30–40 g, which was obtained from a local catfish producer. The amount of fish was in the range of 750–850 individuals for high density stocking. The fish were hand fed with 3.2 mm commercial floating pellet (Cargill Company) manually in the range of 2–4% of fish body weight/d. Feeding rates began with 4.0% body weight/d and gradually decreased to 2% body weight/d toward the end of experiment. With this regime, fish were expected to reach a market size of 220–250 g in three

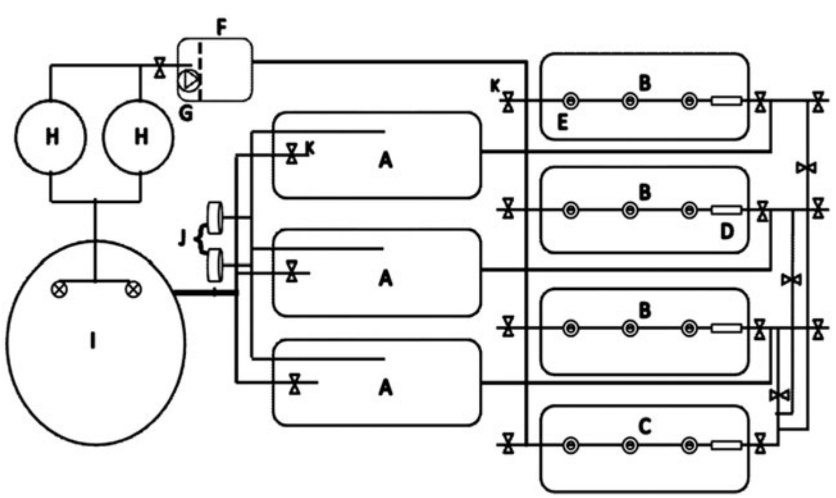

Fig. 1. Schematic diagram of aquaponic system: (A) culture tank, (B) hydroponic trough, planted bed, (C) hydroponic through, control bed, (D) filter, (E) sprinkler, (F) sump, (G) pump, (H) rapid sand filter, (I) water storage tank, (J) air blower, and (K) valves.

months. Feed rates were adjusted weekly based on an estimated growth rate. No water discharge or displacement took place except for replacing water lost through evaporation, transpiration, and sludge removal of less than 5%. Air pump was used to provide sufficient oxygen for fish growth by aerating the tank water, and dissolved oxygen (DO) concentrations were maintained above 5 mg/L. The tanks were covered with by plastic net (20 mm aperture) to hinder the fish jumping out of the tanks.

Rectangle fiber glass tank with an effective volume of 800 L was used as the grow bed. Two different plant species, water spinach (*Ipomoea aquatica*) and mustard green (*Brassica juncea*), both the leafy plants, were used simultaneously in different aquaponics. Experiments were carried out in triplicates. The plant seeds were germinated according to method in Ako and Baker [28]. After two (2) weeks, healthy plant seedlings were transplanted to the grow bed at their optimum planting density as per the seed supplier's guidelines. The plant density of water spinach and mustard green were 30 and 20 plants/m^2, respectively, which was equivalent to a fish feeding rate of 15–42 g/m^2 plant growing area. Nitrogen mass balance in ARS was analyzed according to the method described by Endut et al. [4].

2.3. Water sampling and analytical methods

Water samples were taken from each culture tank, influent and effluent of the 3-m-length-hydroponics and control trough, sump, water holding tank and inflow of culture tank, once a week for chemical analyses and were analyzed immediately for TAN, NO_2^--N and NO_3^--N concentrations, using HACH DR4000 spectrophotometer according to Nessler, diazotization, and cadmium reduction methods, respectively. DO concentrations, temperature, and pH were measured *in situ* using DO meter YSI 55A and pH cyber scan waterproof, respectively. The total suspended solids concentration was determined according to the Standard Methods [29]. Daily fish feed consumption was recorded; fish and plant biomass increase were accurately weighed using analytical balance. At the end of the experiment, samples of fish, plants and fish feed were dried, and their total nitrogen contents were determined by using Vapodest 30s.

The design of experiment was based on Randomized Complete Block Design. Data were presented as a mean of three replicate experiments. Since it is essential to compare whether different forms of nitrogen are statistically significant, the results were statistically analyzed using SPSS v21.0 for Windows software (SPSS v21.0 IBM Corporation, Somers, NY, USA). All the data were presented as mean ± standard deviation throughout the text and possible differences in performance were tested with one-way analysis of variance followed by a Duncan multiple comparison test at $p < 0.05$.

3. Results and discussion

3.1. Aquaponics performance

During the initial stages of the study, a few mortalities occurred. These were then replaced by similar sized of fish. The overall survival rate was about 94% and mortality would be due to the natural death and to the manipulations during the weekly samplings. The plant seedlings in all growing troughs grew rapidly and fairly uniform and appeared healthy with green in color. Both of the plants in all replicates grew quickly and seemed healthy, with no signs of any nutrient deficiency syndromes or toxic effect during the growth period. In terms of ration, one batch of mustard green and two batches of water spinach were harvested due to the short growth of water spinach. More fish feed consumption and higher fish biomass increase were obtained in water spinach-based aquaponics, mainly because of better water quality is this system. The feed conversion ratio (FCR) of water spinach and mustard green-based aquaponics were 1.13 and 1.29, respectively, both within the range of conventional aquaculture system (i.e. 1–3) [30]. Both aquaponics successfully achieved simultaneous production of two cash crops, i.e. fish and vegetable. Performance of ARS is tabulated in Table 1.

Higher water replenishment of mustard green-based aquaponics was resulted from its larger leaf surface exposed to air. In ARS daily exchange of 5–10% with fresh water is part of the operation routine, although some ARS have been operated successfully without water exchange in recent years [31].

3.2. Effect of plant on nutrient removal

Significant decreased in TAN, nitrite-N, nitrate-N, and orthophosphate was consistently observed and the pattern of changes was similar for both treatment systems. As shown in Fig. 2, the effluent concentrations were generally increased with time during the seed germination period (days 3–9) due to the release of dissolved and suspended organic matter from the developing seeds from its cotyledons and was dependent on the quantity and the type of seeds [32]. However, toward the end of growth period, the nutrient effluent concentrations gradually reduced. Reduction in these quantified water quality parameters was well

Table 1
Performance of ARS

Parameters	Water spinach-based aquaponics	Mustard green-based aquaponics
Fish feed consumption[a] (g)	41,250	40,770
Fish biomass increase[a] (g)	36,500	31,600
Plant yield[a] (g/m^2)	10,000	7,400
Specific growth rate[b] (%/d)	2.28	2.18
Daily growth rate[c] (g/fish/d)	2.24	1.91
Feed conversion ratio[d]	1.13	1.29

Note: Values given are mean from triplicate data (n = 3).
[a]Fish feed consumption is the dry weight, while fish biomass increase and plant yield is the wet weight.
[b]Specific growth rate (SGR) = (ln final weight (g) − In initial weight (g)) × 100 d^{-1}.
[c]Daily growth rate (DGR) = (Final weight (g) − initial weight (g))/culture period (d).
[d]FCR = total weight of dry feed give/total wet weight gain.

demonstrated in water spinach than mustard green troughs. Water spinach-based aquaponics reduced the TAN concentration by 88.76% from 0.85 to 0.09 mg/l while mustard green reduced the concentration by 78.21% to 0.18 mg/L.

Several mechanisms exist for the removal of TAN from the aquaculture wastewater. Forms of inorganic nitrogen that were associated with particulate matter may be removed from waste streams by sedimentation and filtration/interception by the root mats of plants. Ionized ammonium (NH_4^+) was one of the major sources of inorganic nitrogen taken up by the roots of higher plants [33]. It may be assimilated by microorganisms and converted back into organic matter or may be removed from waste streams through the process of nitrification.

The concentration of nitrite-N concentration in influent and effluent water measured throughout the 12-week-experiment is depicted in Fig. 1(B). The influent concentration of NO_2-N gradually decreased from 0.1120 to 0.0122 mg/L for week 4 and from 0.1560 mg/L to 0.0076 for week 12 in water spinach troughs. The same decrease was observed in mustard green trough system where the concentration was reduced from 0.1120 to 0.0224 mg/L and from 0.1560 to 0.0192 mg/L, for week 4 and week 12, respectively. The reduction of the nitrite might have resulted from the nitrification process. The concentration of NO_2-N in both planted troughs effluent was in the range of 0.02–0.17 mg/L. At the end of the growth period, water spinach and mustard green-based aquaponics effectively removed 92.51 and 86.67% NO_2-N, respectively. In this study, the continuous aeration by air stones in the system compartments was facilitated the nitrification process. Although NO_2^--N was considerably less toxic than TAN, it may be more important than ammonia toxicity in intensive ARS because it tends to accumulate in the recirculated water as a result of incomplete bacterial oxidation [34].

Changes in NO_3-N concentrations over the 12-weeks' period in both treatment systems are shown in Fig. 1(C). The concentration of NO_3-N in wastewater decreased in both plant-based aquaponics, but the decrease was more rapid for water spinach than for the mustard green. The removal percentages were 90.04 and 86.87% for the water spinach and mustard green-based aquaponics, respectively.

Under natural conditions, major sources of nitrogen for plants are ammonium and nitrate-N [35]. Nitrate-N is the preferred form of inorganic nitrogen taken up by the roots of higher plants [33]. It may also be assimilated by microorganisms in the water column or by biofilms associated with the root mats of plants [36]. In agreement of the previous findings, therefore it can be assumed that the decreases in nitrate concentration in this study are due to plant absorption, assimilation of water microorganisms, and association of biofilms with root mats of vegetables. The average NO_3-N concentrations in the final effluents from the planted system were in the range of 0.3–0.9 mg/L. Although nutrient concentrations in the influent increased with culture time, the highest levels accumulated in the water spinach system are 0.857, 0.156, 3.20 mg/L for TAN, nitrite and nitrate, respectively were well below the levels of 3.0–6.7, 0.4–1.5, and 50 mg/L, for TAN, nitrite and nitrate, respectively which, considered toxic to African catfish [37].

The mechanisms responsible for the removal of NO_3-N were probably assimilated by microorganisms in the water column or by biofilms associated with the root mats of plants. Nitrification or oxidation of ammonia to nitrate as an oxygen demanding process

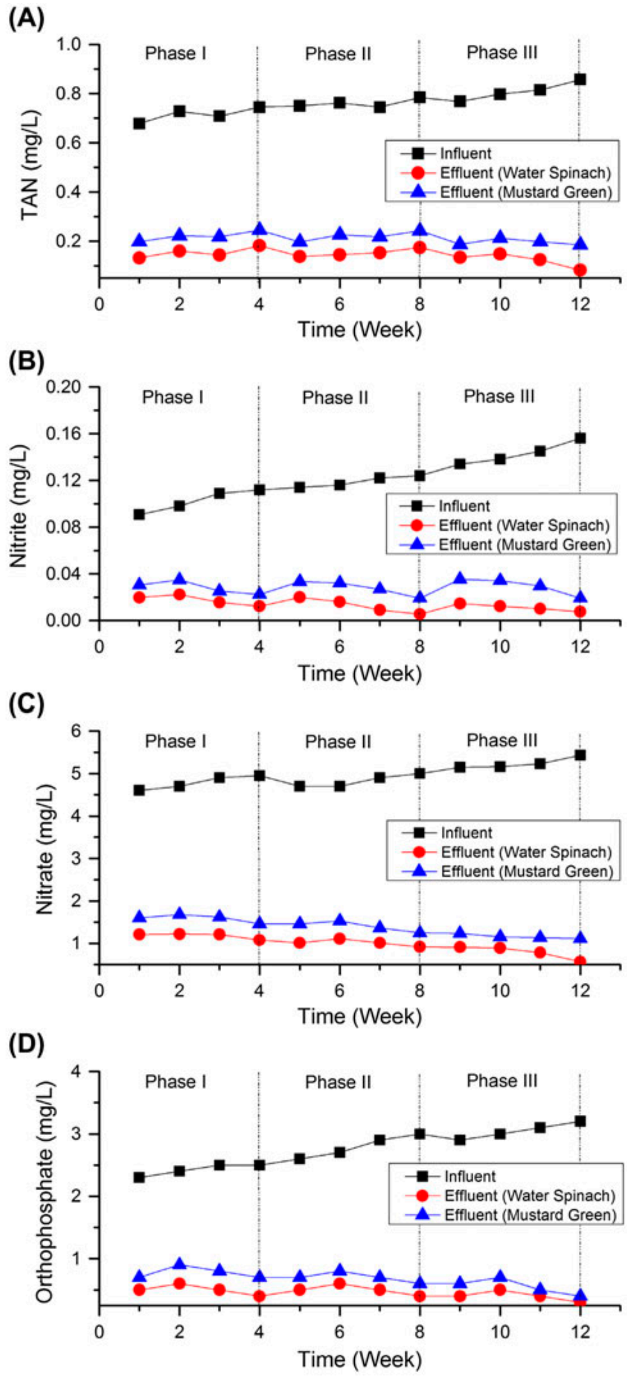

Fig. 2. Changes nutrient concentrations of (A) TAN, (B) nitrite-N, (C) nitrate-N, and (D) orthophosphate during the experiment in both aquaponic treatment systems.

occurred in two steps involving microbial species, e.g. Nitrosomonas and Nitrobacterm [38]. The accumulation of nitrate within the ARS provides further evidence of nitrification activity. Denitrification of nitrate may have been limited in this system, as demonstrated by the accumulation of effluent nitrate. This may be due to an inadequate retention time of only 2.3 h for complete nitrate removal to occur, or the amount of available carbon as a substrate may be insufficient to support denitrifying bacteria.

The measured concentration of orthophosphate (OP) in the ARS over the 12 weeks experimental period is depicted in Fig. 1(D). The pattern of changes in orthophosphate concentration was similar with nitrate concentrations for both treatment systems. The orthophosphate concentration in effluent decreased with time during the growth period and was dependent on type of plants. The concentration of the orthophosphate in the final effluent was low due to the crop root mates were fully developed and the amount of orthophosphate absorbed by the roots increased. At the end of the growth period, orthophosphate reductions of 88.99 and 78.72% were achieved in the troughs containing water spinach and mustard green, respectively. During a 12-weeks period, the orthophosphate concentration in the effluent was reduced from 5.432 to 0.568 mg/L and 5.432 to 1.112 mg/L using water spinach and mustard green, respectively.

Several mechanisms are responsible for the removal of OP from wastewater. Forms of phosphorus that are associated with particulate matter may be removed from wastewater by sedimentation or by filtration by the root mats of plants. Soluble and insoluble forms of organic phosphorus are not biologically available until they have been converted into soluble, inorganic forms. Organically bound phosphorus is converted into inorganic phosphates by microbial oxidation. In aquatic, plant-based treatment systems, microbial communities responsible for this oxidation process are associated with litter, sediments, and the root mats of plants. Ebeling et al. [39] showed that the majority of the phosphorus discharged from intensive aquaculture systems (50–85%) is contained in the filterable or settleable solids fraction. Phosphorus is often the limiting nutrient in natural ecosystems, and excessive algae blooms can occur if discharge concentrations exceed the absorption capacity of the receiving water body.

3.3. *Efficiency of treatment system*

Fig. 3 shows the average percent removal by both of treatment systems. The values are the average of three measurements.

Statistically, there were significant differences in percent removal between water spinach and mustard green based aquaponics for TAN ($p < 0.05$), NO_2-N ($p < 0.05$), NO_3-N ($p < 0.05$), and orthophosphate

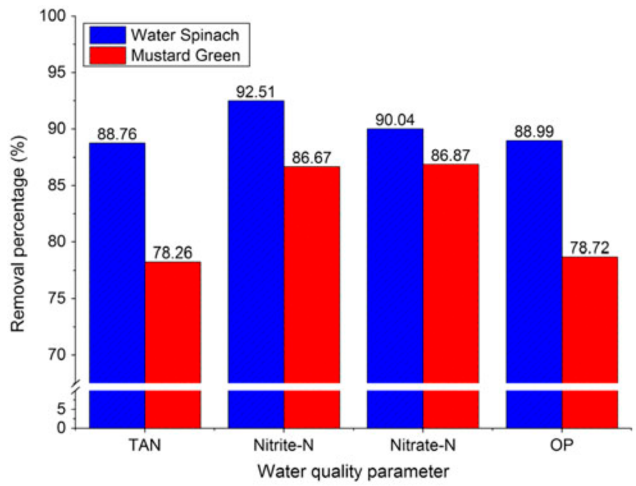

Fig. 3. Removal of TAN, nitrite-N, nitrate-N, and phosphate by water spinach and mustard green.

($p < 0.05$). ARS essentially comprises self-contained artificially engineered wetland ecosystems. It utilizes particular combinations of plants, gravels, bacteria, substrates, and hydraulic flow systems to optimize the physical, chemical, and microbiological processes naturally present in the root zone. Biological nutrient removal is possible due to the special characteristics of hydroponic plants, such as water spinach, which transfer substantial amounts of atmospheric oxygen through their root systems. Additionally, roots, through their growth provide an attachment surface for microbial communities, which is the main function of plants in hydroponics system. These are needed for the effective breakdown of many types of compounds, such as the oxidation of ammonia to nitrate—the first step in the biological breakdown of this compound.

3.4. Nitrogen transformations in aquaponics

Nitrogen compounds such as TAN, nitrite, and nitrate including orthophosphate were monitored throughout the aquaponic system trials. Fig. 2 shows the variation of nitrogen and phosphate compounds concentrations in 12-week-study period. The study period was divided into three phases, where each batch represents a batch of water spinach and mustard green. Water quality parameters mainly regarding nitrogen composition (TAN, Nitrite-N and Nitrate-N) and phosphate composition (Orthophosphate) were analyzed right after fish stocking. In mustard green-based aquaponic system, the highest TAN and nitrite-N concentrations were detected in week 4 and week 9, respectively. This was explained due to the slow growing characteristic of nitrifying bacteria [5]. Typically, nitrogen was introduced to the aquaponic system daily via protein contained in fish feed and was excreted to aquaculture water in the form of ammonia after being metabolized by the fish. The presented ammonia subsequently could be oxidized into nitrite-N and nitrate-N through nitrification. At the initial phase of the aquaponic startup, nitrifying bacteria was insufficient to remove all of the TAN produced by fish metabolism hence accumulation of TAN was observed.

In all the phases of aquaponic trials, TAN concentrations between water spinach- and mustard green-based aquaponic system differs significantly. Water spinach-based aquaponics shows significantly lower concentration of TAN as compared to mustard green. Mustard green possessed fibrous root which traps significant amount of nitrifying bacteria as compared to anchored root of water spinach, thus caused the loss of the bacteria when the plant was removed after each harvest to transplant new seedlings. Another research supported that most nitrifying bacteria were attached on plant root surface [5]. This explains the repeated increase and decrease of TAN concentrations observed in both phase II and III after each root removal. Throughout the study period, NO_2^- concentrations were observed to be under 0.04 mg N/L. This is due to the anaerobic bacteria which were responsible for the rate limiting step of nitrification [40].

Both aquaponics systems were set-up with similar fish stocking density with zero water exchange throughout the study period. In Fig. 2, it was observed that the nitrate-N concentration in the RAS increased linearly and quickly reached its highest concentration of 0.9 and 0.6 mg/L, respectively, for water spinach- and mustard green-based aquaponic system in two weeks. Nitrate-N concentration, then reduced as plant propagated, a similar trend was observed in Phase I and III as the fluctuation was attributed to the growth of water spinach and mustard green. Thus, this simulated the important role of plant in regulating nitrate-N concentration in aquaculture tank. At the beginning of each phase, the newly sowed plant had low nitrate-N absorption ability where the nitrate-N formation rate of nitrifying bacteria exceed the NO_3^- uptake rate of plants. As plant propagated in size, its nitrate-N uptake rate increased, reducing the nitrate-N concentration present in the aquaculture tank. As compared to mustard green, water spinach had more plant biomass resulting in a higher plant uptake rate and lower observed nitrate-N concentration in the aquaculture tank.

In overall, the nitrogen and phosphate composition, concentrations observed in aquaculture tank was significantly lower in water spinach-based aquaponics as compared to mustard green. Thus, better water

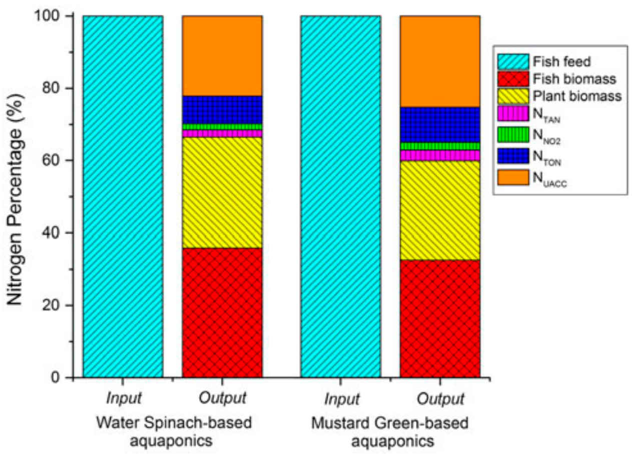

Fig. 4. Input and output nitrogen mass composition in both aquaponic system.

quality parameters in water spinach-based aquaponics was observed to contribute to better fish performance. This explained the observed higher fish feed consumption and lower FCR obtained in water spinach-based aquaponics.

The nitrogen mass balance analysis of water spinach- and mustard green-based aquaponics throughout the 12-week study period was conducted and presented in Fig. 4. Fish feed was the major nitrogen source in both aquaponics amounting almost overall of nitrogen input. Freshwater replenishment was reported to contribute at no more than 0.5% of nitrogen input in aquaponic system [5]. It was found that 35.94 and 32.52% of nitrogen input were recovered as fish biomass in water spinach- and mustard green-based aquaponics, respectively. As discussed before, the different of FCR between the two aquaponic system contributed to the difference in fish biomass elucidated in Table 1. Nitrogen input retained by water spinach and mustard green biomass were, respectively, 30.56 and 27.36%. In overall, the NUE of water spinach- and mustard green-based aquaponics were 66.5 and 59.9%, respectively. This NUE values are higher as compared to previously reported aquaponics system with an average NUE of 40% [5,21,41]. Thus, this supported the commercialization of this aquaponic system on a global scale due to the minimum requirement of crop production of 50% on the recovery of fertilizer N [42].

4. Conclusion

Water spinach and mustard green have the ability to reduce the pollution potential of aquaculture wastewater. The differences in the structure and recruitment of roots by the two plants depicted important consequences for the deprivation of wastewater components and uptake of nutrients. This study indicates that both crops do not affect fish growth performances in terms of SGR, DGR, FCR, and fish biomass. Higher removal efficiency and nitrogen utilization efficiency were obtained in water spinach-based aquaponics due to higher root surface area. Fish effluent can complement or even substitute for organic fertilizers of vegetables production. The ARS showed that it is not only a successful method for biomass production as food crops, but also a useful system to recycle aquaculture wastewater. Selected fish-to-plant stocking density in this research supported the commercialization of the aquaponic system for specific fish (African catfish, *C. gariepinus*) and plants (Water Spinach, *Ipomoea* aquatic and Mustard Green, *B. juncea*). It is interesting to note that results obtained from this study indicate that using crop vegetables can be one of the ways to mitigate the toxicity effect of ammonia to plants and other living organisms.

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