Effects of supplementary nutrient in an aquaponic system for production of ornamental red tilapia (*Oreochromis* sp.) and lettuce (*Lactuca sativa* var *longifolia*)

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**Abstract**

Effects of supplementary nutrient in the production of red tilapia (*Oreochromis* sp) and Lettuce (*Lactuca sativa* var *longifolia*) evaluated in a representative water recirculating aquaculture system. The nutrient solution supplemented was 25% level of (L25) nutrient solution as medium used for aquaponic production of lettuce in the NFT system (based on cooper’s formula). Thus, a completely randomized experimental design conducted with two treatments in triplicates (PL25 and PL0). Six black rectangular tanks (114 x 86 x 100cm) used as fish culture tanks and each one equipped with three hydroponic troughs. Each tank filled with 640 L of water and aerated continuously with two circular air stones (3 L min⁻¹) during the experiment. The system was efficiently able to remove high rate of total ammonia -nitrogen (TAN) excreted by fish during the experiment. The fish attained marketable size (200g) during a 110 –day period. Nutrient supply had not significant effects (*p* >0.05) on growth of fish during experimental period. The yields (Biomass/tank) of fish in treatments PL25 and PL0 were 9.97 and 9.26 kg / tank, respectively. Three times cultivation and harvest of lettuce carried out during the experimental period. At the first harvest, the yield (mean wet weight) of lettuce showed significant (*p*<0.05) differences between treatments, 1437g and 85 g in treatments PL25 and PL0, respectively. In the second and third lettuce harvests, the yield of lettuce did not show any significant differences (*p* >0.05) and averaged 2112 and 1419 (Second harvest) and 1173 and 807 (Third harvest) for treatments PL25 and PL0, respectively. It was recorded that red tilapia could tolerate 25% of nutrient solution, used for aquaponic production of lettuce, and introduction of nutrient solution to the culture system is necessary to get higher yield of lettuce at initiation of culture system.

**Keywords:** Nutrient solution, Aquaponic system, Lettuce, Red tilapia, Nitrification, De-nitrification.

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Introduction

Water re-circulating systems initiated based on knowledge of fish requirements, physical and biological factors involving in the production of fish in the culture system. The potential of all physical and biological aspects and system performance in the removal of accumulated N-compounds, determine the capacity of each culture system. Total ammonia nitrogen (TAN), produced by fish, is the main pollutant which must be removed from the culture system then fecal matter and remained food that all being derived due to feeding of the fish (Rafiee and Saad, 2005). During three last decades, various biological methods utilized to treat water for reuse in intensified fish culture system (Spotte, 1970; Rackocy, 1989; Rafiee and Saad, 2005; Roosta and Hamidpour, 2011). In general, ammonia used by bacteria and it converts to nitrite, then to nitrate (Nitrification). In another process, nitrate converted under anaerobic condition to free nitrogen (N2) that the process called De-nitrification. In a recirculating aquaculture system, by using bacterial bio-filters, the toxicity of ammonia removes but whenever the low water exchange rates and high feeding rates is aimed, for higher production of fish, the accumulation of organic matter and consequent mineralized nutrients productions would be greater than nutrient removal by bacterial and physical filters. Therefore, these matters have high potential to influence the fish production and environmental quality problems adversely. Another way for preventing the accumulative frequency of nutrients throughout a fish culture system is to link the plant for assimilating the major components produced in intensified fish culture systems, i.e., ammonia, nitrite, nitrate, then, other nutrients (macro and micro-elements) which are responsible for eutrophication of aquatic environments. In this respect, many research initiated in both warm (Rakocy, 1994; Recovery research system) and moderate climates (Lewis et al., 1978; Pierce, 1980; Sutton et al., 1982; Rackocy, 2000; Seawright, 1993) to utilize waste-water as solution nutrients for vegetable growth. As alkalinity declines in recirculating system as bicarbonate is consumed and acid is formed during nitrification, liming agents [CaCO$_3$, CaMg(CO$_3$)$_2$, CaO, Ca(OH)$_2$] are added to maintain pH at 7.5 for integrated plant-fish culture (Rackocy and Hargreave, 1993). Nutrient accumulation increases the conductivity of culture water as well. As a result, however, nutrient accumulation is one of the critical point but to maintain the condition at steady state and desirable for fish and plant is more important. Because, it cannot be easy and the main goal, since dynamic of nutrients in integrated system are complex and some variables subjected to mineral manipulation. Therefore, the ability to control the nutrient composition of fish and plant in culture water is limited, especially at the initiation of culture
system while small amount of feed added to the system. Some studies indicated that from fish culture system, insufficient quantities of essential plant nutrients obtained (MCMurtry et al., 1997; Parker et al., 1990; Rackocy et al., 1993; Cerozi and Fitzsimmons, 2017). Thus, balancing the profile of nutrient in water at the tolerating ratios or levels for fish, plant and bacteria and achieving high production of fish and plant is so important. In this regards, the aim of this study was to investigate the effect of input-nutrient on fish and vegetable growth in a recirculating aquaculture system in order to using it for ornamental fish culture.

Materials and methods

System and experimental design

This study was conducted at the Aquatic Resources Technology Laboratory, Institute of Bioscience, Universiti Putra Malaysia (UPM) under a tropical condition (water temperature 27-30°C). A completely randomized experimental design was conducted with two treatments in triplicates, a. the experimental integrated system with three lettuce troughs as hydroponic compartment (P) without nutrient supply (PL0), b. with 25% of cooper’s formula (PL25) for hydroponic culture of lettuce (Cooper, 1987). The experimental unit was a black rectangular tank (114 × 86 × 100cm) and three troughs (110 × 28 ×5 cm) were utilized and fixed above each fish- rearing tank (20cm above the rearing tank). An aquarium pump (Aquatic pump model 1500, 16.5 Watt) was positioned inside each rearing tank at the corner for re-cycling the water through the system. Water pumped from rearing tank to L-shaped pipe and under the pressure of pump led to three hydroponics troughs and returned to rearing tank again (Fig. 1).

Figure 1: Schematic diagram of the system: integrated fish and plant co-culture with use of L-shaped pipe.
Water supply
Two days before acclimating the fish, each fish tank filled with 640 L of tap water and aerated continuously with two circular air stones (3 L min⁻¹). Water supply had following characteristics: age tap water: pH=8.1; EC= 0.24 mhos; N=1.1 mg L⁻¹; Mg=1.71 mg L⁻¹; P=0.00031 mg L⁻¹; Fe=0.0001 mg L⁻¹; K=2.4 mg L⁻¹; Mn =0.003 mg L⁻¹; Zn=0.01 mg L⁻¹; Ca=23.2 mg L⁻¹.

The evapotranspiration and leaking loss of water compensated with tap water during the experiment. Prior to initiation the experiment, the fish acclimatized the system for one week. The EC of water in treatments with nutrient supply adjusted at 0.65 ± 0.02 mmhos cm⁻¹, using supplementary nutrient. Two stock solutions were prepared, using below mineral salts:

Stock solution A: Ca (NO3) 2, 3009 g; Fe -EDTA, 23g in 15 liter of tap water

Stock solution B: KNO3, 1700g; MgSO4, 1539g; H2KPO4, 789g; MnSO4, 18.3g; HBO3, 5.1g; ZnSO4, 1.3g in 15 liter of tap water. 800 ml from each stock solution taken and added in each experimental tank to get 25% of nutrient solution, based on hydroponic production of lettuce in NFT system.

L-shaped pipe utilized in all systems as a settling chamber of fecal mat (5 inches diameter, 0.6-meter horizontal length and 1.5-meter vertical length). Each pipe positioned in parallel beside the rearing tank. A valve conducted to the end of horizontal part of the pipe for possible cleaning.

Feed and feeding
The fish fed a floating pellet diet, containing 32% Protein (min), 6% Fat (max), 6% Fiber (max) and 11% moisture (max) that ordered from Car-gill Company, for grower of red tilapia.

Production of lettuce seedlings
Two weeks prior to experiment commencement, for production of lettuce seedling, seeds of lettuce sowed in some sheets of sponge (40×60 cm) already cut to small pieces (3 ×3cm). The seeds irrigated daily until germinating, each newly seedling transferred to a small perforated plastic cups and transferred to hydroponics troughs.

Protocol
The experiment run on March 5, and terminated on 25 August (110 Days). At the day of experiment commencement, the 45 cups, each comprising a one-week aged seedling of lettuce, placed into the holes of polystyrene sheets that already fixed inside all NFT troughs. Three times planting and harvesting of lettuce were carried out during the experiment (averagely, with a five-week period). Each rearing tank stocked with 75 red tilapia juveniles with the average and standard length of 5.62±3.75 g and 5.73 ± 1.41 cm, respectively. The fish fed twice a day ad libitum at 09.00 h and at 15.00h.
Water quality monitoring
Dissolved oxygen (DO) and water temperature (T) in fish tanks measured twice a week by YSI Model 57. Electrical conductivity (EC) was determined twice a week using EC meter model HANA instrument conductivity meter HI 8033. The pH was determined using Orion model 410A pH meter. Total ammonia (NH$_3$+NH$_4^+$) and Nitrite-Nitrogen (NO$_2^-$) were measured (after dilution of the samples). Nitrate (NO$_3^-$) was measured weekly (AHPA, 1980).

Data analysis
Data of variables subjected to paired-comparison t-test analysis, using the software of SPSS (version 10.0).

Results
Fish production
The fish growth was not significantly different ($p>0.05$) between treatments at the end of experimental period. The average biomass of fish was $9.97\pm2.41$ and $9.26\pm1.34$ kg in treatment PL$_{25}$ and PL$_0$, respectively at the end of experimental period (Table 1; Fig. 2). The rate of survival in treatment PL$_{25}$ was significantly lower ($p<0.05$) than treatments PL$_0$ (Recorded plus rates of jumping) (Table 1). The SFG and FCR rates were significantly different ($p<0.05$) between treatments at the end of experimental period.

Table 1: Mean (mean ± S.D.) of fish biomass (FB), individual fish weight (IFT), specific growth rate (SGR), Feed Conversion Ratio (FCR), survival (Sur) between treatments at the end of experimental period.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FB (kg)</th>
<th>IFT (g)</th>
<th>Sur (%)</th>
<th>SGR (g)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL$_{25}$</td>
<td>9.97±2.41</td>
<td>185±23</td>
<td>73±25.43</td>
<td>1.61±0.20</td>
<td>1.25±0.15</td>
</tr>
<tr>
<td>PL$_0$</td>
<td>9.26±1.34</td>
<td>211±14</td>
<td>58±08.57</td>
<td>1.84±0.12</td>
<td>0.88±0.07</td>
</tr>
</tbody>
</table>

The same subscript letters in a column are not significantly different at the 0.05 levels.

Figure 2: Changes in red tilapia growth between treatments during the experimental period.

Vegetable production
The lettuce seedling were cultivated and harvested three times during the experimental period. In the first time, the seedlings replaced through the hydroponic troughs five days after running the systems and harvested thirty days later. The growth rate of seedlings in treatments with supplementary nutrient solution (PL$_{25}$)
was significantly higher (p<0.05) than treatment without nutrient solution (PL₀), 1437±339 and 85±94, respectively. In second and third harvest of lettuce, the yield of lettuce did not show any significant differences (p> 0.05) between treatments. These rates in treatments PL₂₅ and PL₀ were averagely 2112±697 and 1685±192 respectively for first and 1173±402, and 807±180 g, respectively for third harvest (Fig. 3).

![Figure 3](image)

**Figure 3**: Mean yields of lettuce gained from three times planting and harvesting during the experimental period (veg. w. 1, 2, 3 = biomass of lettuce in first, second and third harvests in different treatments).

Nitrate (NO₃⁻)

With using nutrient solution in treatment PL₂₅ at the commencement of experiment, concentration of nitrate increased up to 75 mg L⁻¹. After on smart depletion of nitrate occurred in treatments PL₂₅. The nitrate concentration ranged 0.023 to 6.39 mg L⁻¹ at the end of experiment (Fig. 5).

![Figure 5](image)

**Figure 5**: The Changes of Nitrite-N in different treatments during the experimental period.

**Water quality parameters**

**Total ammonia (TA)**

TA continuously decreased and one week prior to termination of the experiment got to the 0.32 mg L⁻¹.

Nitrite (NO₂⁻): The concentration of nitrite did not show any significant differences (p>0.05) between treatments. After two weeks, the concentration of nitrite increased up to 3 mg L⁻¹ in both treatments. Within the last two weeks, an increase in nitrite concentrations recorded in both treatments and reached to 4 mg L⁻¹ (Fig. 4).

**Dissolved oxygen (DO), Temperature (T), pH and EC**

DO did not show considerable depletion in rearing tanks and at the bottom of L shaped pipe. DO ranged 6 - 8.5 mg L⁻¹ in rearing fish tanks. Water
temperature was between 27-30ºC during the experimental period. The EC rates in different time intervals (in rearing tanks) showed significant differences (p<0.05) by 9th weeks, afterward did not show any significant differences (p>0.05) between treatments. The EC of water constantly increased in both treatments and reached up to 0.86 and 0.85 mmhos cm\(^{-1}\) in PL 25, PL0, respectively (Fig. 6).

At the start of experiment, pH were adjusted at 7.8 (mixed aged tap and well water, prior to adding nutrient). When nutrient added to treatments PL25. It came down and reached to below 7. The sharp depletion of pH recorded in all treatments during the experiment and continuously showed a decrease. After 5 weeks pH reached to below six in both treatments, after that slowly increased and got to 6.5 in 8th weeks. Afterward had fluctuation and ranged from 5.4 to 7.1 by the end of the experiment (Fig. 7).

**Discussion**

In this study, with use of simple feasibilities, a new design for production of fish in integrated water re-circulating fish culture system with use of supplementary nutrient introduced. The system demonstrated that could successfully remove increasingly accumulation of N-compounds through experimental period. The result showed that seven weeks after starting the experiment, systems were able to hold the concentration of total ammonia-N below the 48h LC\(_{50}\) 2.5 mg L\(^{-1}\) reported for *Sarothrodon aurea* (Redner and Stilkney, 1979). The concentration of nitrite also averaged 3 mg L\(^{-1}\) (Fig. 4) at the end of experiment that it was less than the threshold already reported in catfish, 96h mean tolerance of 7.5 mg L\(^{-1}\) (Sutton and Lewis, 1982). The concentration of nitrate in treatments with nutrient supply was 74 mg L\(^{-1}\) at the start of experiment, afterward, smart depletion of nitrate occurred in particular in treatments PL25. Nitrate depletion probably was due to activities of plants and bacteria in assimilation of nitrate. Existence of nitrite in rearing

![Figure 6: Average changes of EC in different treatments during the experimental period.](image)

![Figure 7: The pH changes in different treatments during the experimental period.](image)
tanks also emphasized that the nitrification and de-nitrification activities occurred by bacteria during the experiment; However, at the start of experiment there was not any concentration of ammonia and nitrite in water supply. At the first of the experiment, lack of nutrient was evidence in treatments without nutrient supply because of unsuitable growth of vegetable. When water EC approached to the level of 0.33 mmhos (after 40 days), moderately growth of lettuce occurred in comparison with the nutrient supply system, meaning that standard Ec for introduction of vegetable in integrated system could be 0.33 mmhos, however profile of nutrient must be considered for standardizing the Ec. Concentration of nutrient in water did not reach the levels commonly utilized in commercial production of vegetable in hydroponic system, large amount of feed suggested approximately 8-10 kg/m$^3$ must be used to get suitable medium for culture of most vegetable in hydroponic system (Rackocy et al., 1993). It was resulted that excretion of fish and biodegradation of organic to inorganic matter just provide moderate nutrients required for vegetable (lettuce) culture, emphasizing the effect of nutrient solution either on growth of plant or further removal of N-compounds. Foliar use of nutrient demonstrated as one of practical method to improve nutrient deficiency in hydroponic part of integrated system (Roosta and Hamidpour, 2011). Such a system even emphasis on permanent production of leafy vegetables for local and civil regions due to balancing the nutrient in different aquaponics design (Kyaw and Andrew, 2017). The growth of fish was not significantly different between treatments, it accentuated that supplementary nutrient had not significantly negative effect on growth of fish. The fish reached to marketable size (200 g) within 110 days. In a natural condition at 23°C, tilapia (Sarothrodon niloticus) reaches to 200 g weight within six months (Naegel, 1977). However, it has been reported that tilapia shows maturity after six months of age, but during this experiment some fish started displaying mating behaviors and color changes when attained 100 g weight (after two months) and showed territorial operations and attacked to each other, this condition can be related to welfare condition of fish in the culture system. One of the most important points of this experiment was sharp depletion of pH, from about 8 below 6 during the first four weeks of experiment and below seven for next period, indicating reactions of De-nitrification by bacteria. Depletion of pH had also observed already by other experimenter (Naegal, 1977; Rakocy et al., 1995; Rakocy, 1989). Acidic conditions not only have an important role in neutralizing the toxicity of high concentration of ammonia in rearing tank but also affecting solubility of some elements such as Fe (ferrous), Zn (Zinc) and
Phosphorus (P) which can be more available to plant.

Depletion of N-compounds in all treatments accentuated that the lettuce and L-shaped pipe chamber efficiently operated as de-nitrification parts. Low concentrations of ammonia, nitrite and nitrate and floating of fecal mat above the vertical part of the pipe indicated the ability of horizontal part of L-shaped pipe in de-nitrification (Seawright, 1993). Since, de-nitrification occurs under anaerobic condition, high accumulation of waste fish inside the horizontal compartment of the L-shaped pipe probably prepared anaerobic condition for activity of de-nitrificant bacteria. In fact, It can be said that this part of system operated similar to both nitrification and de-nitrification bio-filters. These compartments separately utilized by some scientists for production of fish and vegetable throughout water re-circulating system (Naegal, 1977; Sutton, 1982; Lewis, 1978; Pierce, 1980; Rakocy, 1984; Wren, 1984; Zweig, 1986; McMurtry et al., 1990; Rafiee et al., 2005).

Comparing the result of this experiment with other works, showed that the efficiency of present system (PL25 and PL0) for high production of fish and vegetable and maintenance the water quality parameters at optimum ranges. If the land area surrounded for production of fish and vegetable considered, the usage of less space (1 m³ area) and high production of fish and vegetable within 15 weeks dominated in this study. Considering the fish yield of 31.5 kg/m², for cat fish (Lewis et al., 1978), 9.20 kg/m² for Nile tilapia (Watten and Budch, 1984) and 13.61-16.41 kg/m² for present system. The present system comprised vegetable plant and nutrient use, PL25, showed capable for production of fish and vegetable in urban and local regions in tropical condition, especially for ornamental plant and fish culture. Determining the roles of biological and physical compartments separately in assimilation of nutrient required for future studies. Since, nutrient supply affects de-nitrification and nitrification bacteria (Austin and Austin, 1989). The impact of nutrient on activity of bacteria and biodegradation of organic to inorganic matter also require further studies.

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