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Influence of Satiation Feeding on the Water Quality and the Growth, Hematological Parameters, and Blood Chemistry of Nile Tilapia *Oreochromis niloticus* Reared in Nutrient Film Technique Aquaponic System

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ABSTRACT:
One of the most significant inputs in an aquaponic system is fish feed. Here, two satiation feeding (SF) levels (60% & 50%) have been tested. It aims to determine its effect on the water quality and the growth, hematological indices, and blood chemistry of Nile tilapia Oreochromis niloticus reared in nutrient film technique aquaponic system (NFTAS) containing upland kangkong Ipomea reptans within 1 month period and compared it in the recirculating system (RS). Results showed that the SF levels have an insignificant effect on the water quality between the systems mainly due to the non-optimal pH level that influenced the nitrification efficiency and the inefficient uptake of nutrients by the plants in NFTAS. Consequently, the growth, hematological parameters, and blood chemistry of the fish reared in NFTAS have not improved compared with those in RS. However, the fish and the plant growth performance were better at 60% SF in both systems. Thus, 60% is suggested SF level when 200 individuals Nile tilapia O. niloticus tilapia fingerling (2.46 g, average initial weight) are reared within 31 days in a healthy and balanced NFTAS with 50 individuals upland kangkong I. reptans.

Keywords: *Satiation feeding, O. niloticus, performance indicators, hematological parameters, blood chemistry*

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INTRODUCTION

Aquaponics is a soilless agriculture method that synergistic aquaculture and hydroponics (Love et al., 2015). In an aquaponics system, waste organic matters from the aquaculture system, which can become toxic to animals, are converted by microbes into soluble nutrients for the plants. Simultaneously, the hydroponics system has already treated the water and recirculates back to the aquaculture system with cleansed and safe water for the animals (Liang & Chien, 2013).

Tilapia is the most used fish in aquaponics systems (Rakocy, Masser, & Losordo, 2006) for its high availability, fast-growing stress and disease resistance, and easy adaptation to the indoor environment (Hussain, 2004). Water spinach is one of the most cultivated plant species in the aquaponic system. A study demonstrated that the tilapia-water spinach raft aquaponics was extremely effective in fish waste treatment and water conserved (Liang & Chien, 2013)

Water quality is an important factor in all soilless production systems, but it is especially tricky in aquaponics systems that must balance the needs of fish, plants, and bacteria. For example, the ideal pH ranges for the three groups are different, so a compromise of a system-wide pH of 7 is maintained (Rakocy, Masser, & Losordo, 2006). Water temperature, dissolved oxygen, ammonia, and nitrite are other important water quality parameters that should be monitored and balanced between the fish, plants, and bacteria. Therefore, the key environmental factor for maximizing aquaponic production is water quality parameters, directly impacting fish welfare/health issues and plant requirements. Nutrient uptake in aquaponic systems should be maximized for the healthy production of the plant biomass without sacrificing the best welfare conditions for the fish in water quality (Yildiz et al., 2017). The nutrient film technique (NFT) in the aquaponic system provides high oxygen to the plant roots, facilitating high yield. However, NFT is only suitable for tiny vegetable species because their grow beds cannot support a high quantity of roots due to the potential blockage of recirculating flow (Engle, 2015). Thus, efficient solid removal is critical for NFT to prevent clogging in the grow bed channel (Wongkiew et al., 2017).

A feeding regime for fish should be designed to allow for optimal growth and nutrient levels within the water without causing a build-up of toxic waste products. Fish feeding can be of two types: satiation feeding and weight feeding. Satiation feeding is to provide food to the fish till they stop eating. The feeding by weight method is understanding the approximate weight of the fish and providing a percentage per day. When tilapia is used in aquaponic system studies, feeding by weight is usually applied (Eissa et al., 2015). In the context of growth and health about a fish, knowledge, and research related to physical parameters of water and hematological parameters can serve as the best health indicators (Fazio, 2019). It also helps diagnose any serious pathological condition, as suggested by Adham et al. (2002).

Studies by some authors have reported an improvement in water quality and the health status of Nile tilapia in the aquaponic system (Eissa et al., 2015). However, the use of SF in the aquaponic system has not been explored. Thus, this study evaluates the influence of satiation feeding on the water quality and the growth, hematological parameters, and blood chemistry of Nile tilapia reared in NFTAS.

MATERIALS AND METHOD

The study was conducted in the Multi-Species Hatchery, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, the Philippines, for 31 days.

Experimental fish and plant

Nile tilapia fingerlings were obtained from the SEAFDEC/AQD, Tigbauan Main Station Iloilo, Philippines. The fish were transported to the study site early in the morning using oxygenated plastic bags and acclimated in 6 units 200 L capacity fiberglass tanks provided with aerations 7 days before the experiment. Feeding was done twice a day to apparent satiation using a Tateh commercial diet. In addition, fish waste was siphoned, and a water change of around 25 percent was done every day to maintain good water quality. Plant seeds were purchased from the local agricultural supply, sowed in a seed tray, and grown within 7 days using a SNAP solution as fertilizer.

Experimental treatments and set-up

Two experiments (60% and 50% SF) were conducted to determine the influence of SF on the water quality and the growth, hematological parameters, and blood chemistry of Nile tilapia reared in NFTAS. Each experiment consisted of two treatments with three replicates and was conducted using an indoor RS (control) and NFTAS (fish and plant).

RS (Treatment 1) is made up of a rearing tank (200 L capacity, fiberglass), a biofilter for nitrification, water transport materials such as PVC pipe, fitting, valves, powered by a 6W submersible pump (Resun B-400) with a plastic water hose. Each tank was provided with a single air stone to provide aeration. The biofilter comprises a 10 L plastic container containing crushed rocks and a biological filter mat with a washable filter mat on the top to collect solid waste.

To complete the NFTAS grows bed (Treatment 2), the same design of the RS was installed with two units 4" orange PVC pipe with a length of 3.33 ft provided with 5 holes each, which hold the net pots (3"). Single daylight led bulb (Firefly basic series, 13W, Non-dimmable) was installed 25 cm above the plants in the grow bed. A 24-hours illumination was maintained throughout the study. The natural photoperiod for the rearing fish was kept in the experiments by providing a black sack between the tanks and grow bed.

Feeding

SF levels were determined on the experiment's 1st, 8th, 16th, and 24th day at 0800h. The total amount of feed consumed by feeding fish at feeding is 100% satiation. Fish were fed using Tateh tilapia Pre Starter surfer feeds following the SF out of the whole meal consumed. Feeding the computed amount to the fish began at 1600h after determining SF level and thrice a day (0800h, 1200h & 1600h) before the next schedule.

Water management

The filter mat in the biofilter system was cleaned to remove solid waste and fish feces every 3 and 2 days in the first half and second half of the experiments. In addition, 1 ml of live nitrifying bacteria (API quick start) was added after cleaning the filter mat within the first half of the investigation. No water exchange and siphoning of fish feces were done. However, clean water was added to maintain the volume of the rearing tanks due to water loss caused by evaporation and during washing of the filter mat.

Water quality

Water quality monitoring was conducted weekly using an API test kit for pH, ammonia, nitrite, and nitrate. A digital water quality tester (Model number: EZ-9908 multifunction) was used for total dissolved solids (TDS), electrical current (EC), and temperature. Dissolved Oxygen (DO) was monitored using a water test kit (TBS®) following the standard protocol.

Estimation of response parameters

After the experiments, the fish and plant's growth and survival were assessed. Then, all the remaining fish in each tank and the plants in the aquaponics system were weighed, measured, and counted. For the experimental fish growth and survival, the following formula's were used as follows:

Weight gain, WG (g) = FABW – IABW

Where:

FABW = Final average body weight (g) and IABW = Initial average body weight (g)

Specific Growth Rate (SGR, %/day) =

$$100*(\ln FABW - \ln IABW) / \Delta t^{-1}$$

Where:

Δt = Rearing period

Feed Conversion Ratio (FCR) =

$$\text{Feed given (g)} / \text{WG (g)}$$

Survival rate (%) =

$$100*(\text{final count} / \text{initial count})$$

For the experimental plant's growth and survival, the following formula's were used as follows:

Weight gain, WG (g) = FAW – IAW

Where:
FAW = Final average weight (g) and
IAW = Initial average weight (g)

Height gain, HG (inch) = FAH – IAH
Where:
FAH = Final average height (inch) and
IAH = Initial average weight (inch)
Survival rate (%) =
100*(final count / initial count)

Blood sampling for hematological parameters and blood chemistry analysis

After the experiment, blood sampling was done for hematological parameters (CBC / platelet) and blood chemistry (Glucose & SGPT) analysis. First, fish samples were anesthetized in a pail with ice (4°C) for less than 2 minutes to avoid struggling during blood extraction. Fish total length was measured and weighed. Then, blood samples were extracted from each fish using a 1ml syringe (25 G x 5/8"). For hemoglobin, hematocrit, red blood cells (RBCs), and platelet count, the extracted blood was transferred immediately to 0.5 ml EDTA.K2 capillary blood collection tube (Guangzhou Improve Medical Instruments Co., Ltd.). Samples were analyzed using Hematology Analyzer Coulter Ac•T diff (Beckman Coulter). For Serum glutamic pyruvic transaminase (SGPT), extracted blood was transferred to a 1.5ml microcentrifuge tube (Eppendorf). Samples were immediately centrifuged in a low-temperature microcentrifuge (4°C) for 10 minutes at 3,000 rpm to separate the serum component of the blood. Serum samples were analyzed using a clinical chemistry analyzer (ILAB 300 Plus).

Blood glucose was determined in-situ using a Blood Glucose Meter (Accu-Chek® Active, Roche Diabetes Care GmbH, 68305 Mannheim, Germany). First, fish samples caudal peduncles were trimmed and allowed the blood to ooze. Then, a drop of blood was placed in the Blood Glucose Meter strip for glucose level reading.

Statistical analysis

Data were subjected to an Independent sample T-test to determine significant differences in feeding, growth parameters, survival, water quality, hematological parameters, and blood chemistry. The SPSS version 20 software was used to conduct the statistical analysis.

RESULTS

In the present study, two separate systems were organized viz: RS and NFTAS. Water quality parameters in both systems were sampled weekly. Nile tilapia- upland kangkong was setup, and the fish were satiate fed for the present investigation. The SF fed groups were further classified as SF 60% and SF 50%. Both systems periodically evaluated all the important water parameters, such as pH, ammonia, nitrite, nitrate, TDS, EC, temperature, and DO during the rearing period. Apart from this, the fish growth, survival, hematological parameters (CBC / platelet), blood chemistry (Glucose & SGPT), and plant growth and survival were evaluated after the experiment.

Water quality parameters examined in the systems are presented in Figures 1 and 2. At 60% SF (Fig. 1), a significant difference ($p \leq 0.05$) was found on nitrite and nitrate on the second week, where the highest level was observed in NFTAS compared to RS. On the other hand, at 50% SF (Fig. 2), a significant difference ($p \leq 0.05$) was found only on EC and TDS levels in the fourth week, where the highest level was observed in NFTAS compared in RS. Performance indicators of the fish reared in the systems are presented in Figure 3. The fish raised between the systems showed an insignificant difference ($p > 0.05$) at 60% SF. Significantly higher ($p \leq 0.05$) fish survival was obtained in RS than in NFTAS under 50% SF. However, an insignificant difference ($p > 0.05$) was observed with other performance indicators (FL, WG, SGR & FCR). On the other hand, the growth and survival of the plant reared in NFTAS are presented in Figure 4. Results indicated that better growth and survival of the plants could be achieved at 60% than 50% SF. Hematological parameters and blood chemistry of fish reared in the systems are presented in figure 5. Results showed an insignificant difference ($p > 0.05$) on fish hematological parameters and blood chemistry between the systems. However, it can be noted the test results value increases as SF decreases in both systems.

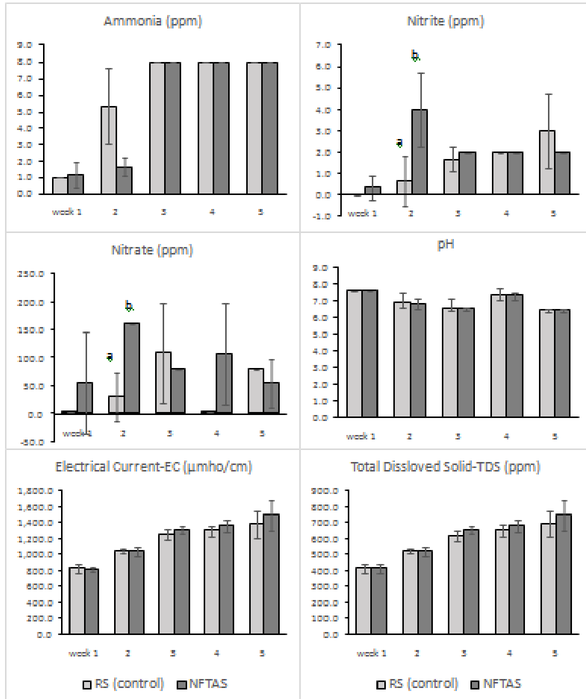

Figure 1: Water quality parameters (mean ± SD) at 60% SF. Different letter on each bar indicates significant differences ($p \leq 0.05$) (Applied to other figures).

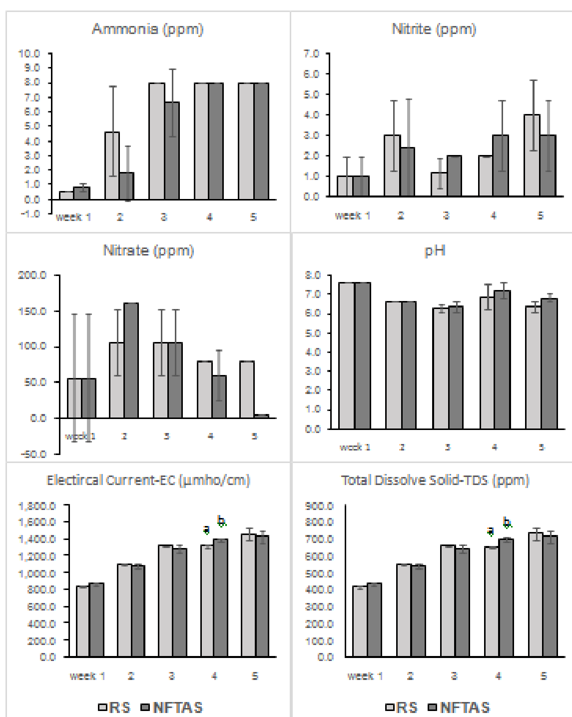

Figure 2: Water quality parameters (mean ± SD) at 50% SF.

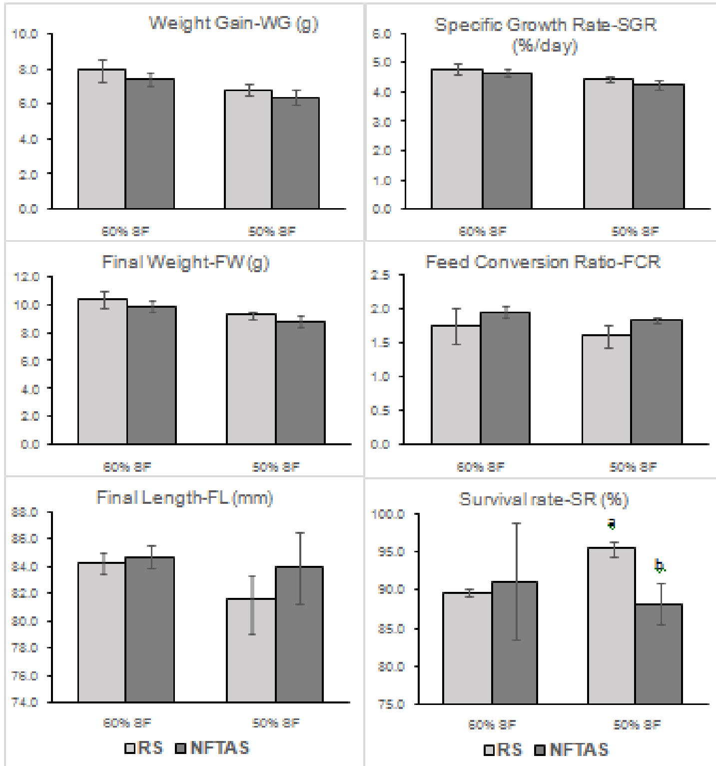

Figure 3: Performance indicators (mean ± SD) of Nile tilapia in the systems.

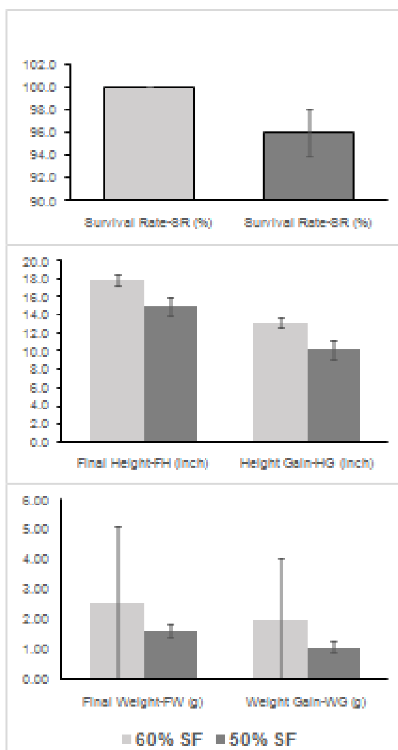

Figure 4: Performance indicators (mean ± SD) of upland kangkong in NFTAS.

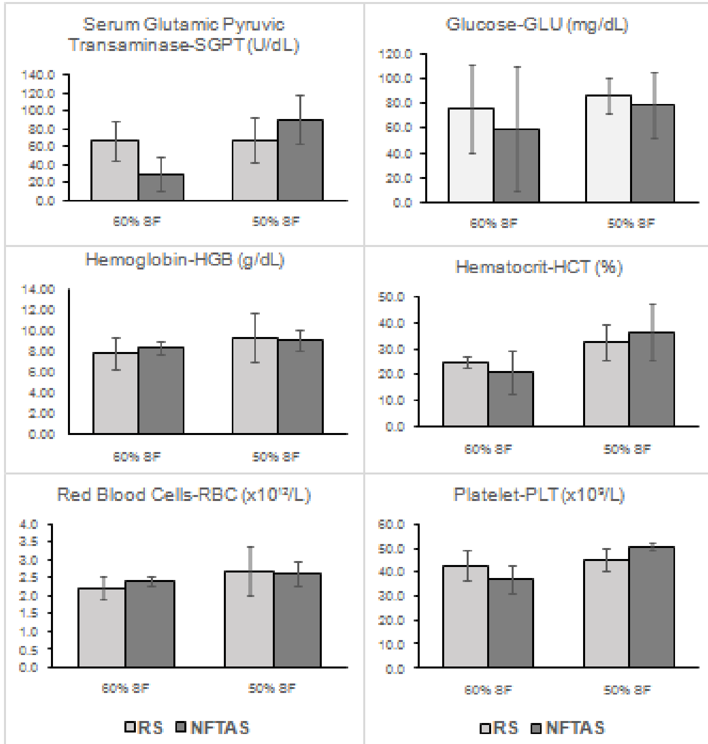

Figure 5: Hematological parameters and blood chemistry (mean ± SD) of Nile tilapia in the systems.

DISCUSSION

In the aquaponics system, feed is the primary source of nutrients eventually tied up as the biomass of animals, plants, and microbes or stayed free in water (Liang & Chien, 2013). As a result, a sudden change in feeding rate might cause significant changes in water quality; thus, regular monitoring of those crucial water quality indicators is required (Yildiz et al., 2017). According to Sallenave (2016), optimal water quality values are required for productive and well-performing tilapia aquaponics systems. In the present studies, it can be noted that the monitored daily temperature ranged from 26.7-29.9^{o}C, and pH values of 6.33-7.60 in NFTAS are slightly lower and slightly higher than the optimal range (27-29^{o}C). For ammonia, TAN values did not exceed 0.24 ppm (Florida Department of Environmental Protection Chemistry Laboratory Methods Manual, 2001), which is within the optimal value (<1 ppm). For nitrite, values (1-4 ppm) in NFTAS exceed the optimal value (<1 ppm) except during the 1st week (0.33 ppm) sampling in 60% SF NFTAS. For nitrate, values exceed about 10 ppm (56.00-160.00 ppm) than the highest optimal value (5-150 ppm) in both SF experiments.

One challenge to developing aquaponic technology is converting the toxic ammonium produced by the fish into nitrate via bacteria in a biofilter to provide nitrogen to the plants (Junge et al., 2017). API quick start was effective and allowed the primary nitrogen cycle in the systems. Although the pH and temperature maintained the ammonia into a non-toxic (NH4+) form, the pH affects the nitrifying bacteria's efficiency for nitrification as observed in the nitrite levels. The pH values in NFTAS and RS range from 6.33-7.60 and 6.46-7.60, respectively. According to Villaverde (1997), nitrification efficiency increased linearly by 13% per pH unit in the pH levels ranging from 5.0 to 9.0, with ammonium oxidizer activity peaking at 8.2. Overall nitrification pH of approximately 7.8 has also been observed by Antoniou et al. (1990).

The capacity of a nutrient solution to conduct an electrical current (TDS) is two ways to quantify dissolved nutrients (EC) (Liang & Chien, 2013). TDS remains 200-400 ppm or EC 0.3-0.6 mmho/cm will produce good plant production in an aquaponic system (Rakocy, Masser, & Losordo 2006). In this study, results showed that TDS is higher than the considered range. However, according to Ibrahim and Ramzy (2013), the permissible limit for TDS should be ≤ 500. Phytotoxicity can develop if dissolved nutrients consistently increase and approach 2000 ppm TDS or 3.5 mmho cm-1 EC. Here, maximum values of TDS in NFTAS only exceed 700 ppm with EC values of around 0.8-1.5 mmho/cm. Liang and Chien (2013) reported an EC of 700 mmho cm^{-1}. Therefore, the level of dissolved nutrients was suitable for plant growth, and only 1/5 of the concerned EC, 3.5 mmho/cm, showed that the aquaponics operated considerably satisfactorily.

Growth trials are tests that measure growth rates, and FCR is calculated and can be used to evaluate fish performance under specific conditions (Larsen et al., 2012). FCR obtained in the experiments reflects that the SF at 60% (RS – 1.76; NFTAS – 1.95) or 50% (RS – 1.60; NFTAS – 1.83) are effective feeding strategies for the fish in the systems. A 1.4–2.4 FCR is generally obtained in intensive tilapia farming (Fry et al., 2018). An FCR value of 1.7 and 1.8 of fish (Nile and Red tilapia) reared in the UVI aquaponic system has been reported (Rakocy, Masser, & Losordo, 2006). The experiments also revealed that the SF level affected the performance indicators supporting the findings of Alejos et al. (2019). The Nile tilapia growth performance slightly improved as the satiation increased.

The survival of the fish in the experiments has also been affected due to the level of nitrite. Nitrite levels of 5 ppm are hazardous to fish and should be kept at or below 1 ppm (Sallenave, 2016). Mortality was observed in the early days (1-7 days), but the fish could adapt to the environment after that. Tilapia is known for tolerance to poor water quality and easy adaptation to an indoor environment (Hussain, 2004). The pH (6.26-6.86) during the experiments, especially after the 1st week of sampling, is lower than the optimal tilapia. However, the fish species tolerate large fluctuations in pH value with a tolerance between pH 3.7 and 11 but achieve the best growth performance between pH 7.0 and 9.0 (McAndrew et al., 2000). Tilapia prefers 27–29°C for maximum growth (Sallenave, 2016), slightly higher than the minimum and maximum value. However, some authors suggested that the optimal growing temperature of tilapia in a controlled environment is approximately 27–30°C (Azaza, Dhraïef, & Kraïem, 2008).

The fish feed and by-products produced in the fish component of aquaponic systems deliver the needed majority of nutrients to the plant's growth (Sallenave, 2016). However, to achieve the proper balance between fish nutrient production and plant uptake in each system, the optimal ratio between fish and plants must be determined (Goddek et al., 2015). For NFTAS, the optimum feeding rate ratio is roughly 25% of the ratio used for the raft system (Rakocy 2007) or feeding value between 15-25 g/day. The average feeding value in this study per day is around 21.41-40.67 g at 60% SF and 19.52-49.69 g at 50% SF in NFTAS. Better growth and survival of the plants could be achieved at 60% SF. Therefore, the feeding level is higher compared with the suggested feeding ratio. Endut et al. (2010) also reported a higher value in the aquaponic recirculation system.

The growth and survival of the plants rely on the available nutrients and the water quality present in the aquaponic system, and nitrate is the primary nitrogen source for plant growth (Graber & Junge, 2014). The dissolved nutrients such as TDS and EC (Liang and Chien, 2013); water quality such as temperature and pH enhance the uptake of nutrients by plants (Goddek et al., 2015). This study's nitrate levels are within the optimal range for plants except on the 2nd week of sampling, exceeding 10 ppm. The TDS levels are within the permissible nutrients and did not exceed 700 ppm, too far from the phytotoxicity level of 2000 ppm for plants. The pH levels (60% SF - 6.46-7.60; 50% SF - 6.33-7.60) also exceed the optimal value between 6 and 6.5 to enhance the uptake of nutrients

(Goddek et al. 2015). Likewise, the daily monitored temperature range of 26.7-29.9°C is higher than the best temperature (21-23°C) needs of vegetables to grow (Sallenave, 2016).

It can be observed that the plants did not significantly reduce the levels of nitrate and TDS in NFTAS compared to RS. However, considerably lower TDS levels have been monitored from the 2nd, 3rd, and 5th week of sampling under 50% SF. One possible reason is a faster nitrogen cycle because of the other surfaces (hydroponic beds) of nitrifying bacteria for nitrification, and the solubilization rates of solid feces from organic material to ionic minerals (Seawright et al., 1998) are present in the NFTAS.

It can be observed that the level of nitrate on the 1st and 2nd week of sampling and the level of TDS and EC in the 1st week of sampling in NFTAS are higher compared to RS. First, according to Cripps and Bergheim (2001), solid wastes are only partially solubilized as they are mechanically filtered out daily under current practices in RS. These filtered wastes can be thoroughly mineralized outside and reintroduced into hydroponic beds (Goddek et al., 2015). Second, the plants' capacity to uptake nutrients has been affected due to non-optimal pH levels. The best temperature range for plant growth has not also been met. Third, NFTAS is less stable as less water, lower nutrient uptake because of smaller root-water contact area, and lower yields (Goddek et al., 2015). As a result, the plant's overall growth performance is poor despite providing continuous illumination. However, the growth and survival of the plants can be improved. The WG of the plants is almost twice at 60% SF than those at 50% SF. Although the nitrate and the dissolved nutrients at 60% SF are not considerably higher than in 50% SF based on sampling periods, other micro and macronutrients that are not measured may be higher at higher SF, which have a beneficial effect on the plants. At 50% SF, the survival of the plants has been reduced by 3%.

Fish welfare is a crucial aspect of successful commercial aquaculture operations, including aquaponics systems. It is not unique from aquaculture in terms of water quality metrics, which are important in hazard identification for various aquaculture operations risk assessments (Yildiz et al., 2017). The effect of SF level on the water quality showed an insignificant influence in the system. It reflects the insignificant difference in the fish's hematological indices and blood chemistry reared between RS and NFTAS, indicating that the health conditions of the fish raised under NFTAS have not improved. Water quality parameters (DO, temperature, ammonia, and pH) measured in the systems fall within the ideal range for fish culture. Although the nitrite level in all sampling periods exceeds the optimal limit, including the nitrate level in the 2nd week of sampling, it does not reach the toxic level for the fish. In the study Osman et al. (2021), water quality was improved in ASTAF-PRO aquaponic system; however, the nitrite level was lower than the permissible limit. As a result, nearly all the hematological indices in the blood of monosex *O. niloticus* reared in the aquaponic system, and those raised in pond systems show insignificant differences. The fish was fed twice a day at 5% body weight.

CONCLUSIONS

The SF levels have an insignificant effect on the water quality between the systems mainly due to the non-optimal pH level that influenced the efficiency of nitrifying bacteria for nitrification and the inefficient uptake of nutrients by the plants in NFTAS. Consequently, the growth, hematological parameters, and blood chemistry of the fish reared in NFTAS have not improved compared with those in RS. However, the growth performance indicators of the fish and plant were better at 60% SF. Thus, 60% SF is suggested when 200 individuals Nile tilapia *O. niloticus* tilapia fingerling (2.46 g, initial weight) are reared within 31 days in a healthy and balanced NFTAS with 50 individuals upland kangkong *I. reptans*.

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Conflicts of Interest:
None at all

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