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Effect of Stocking Density on Sustainable Growth Performance and Water Quality of Nile Tilapia-Spinach in NFT Aquaponic System

Mohammed S. Al-Zahrani [1], Hesham A. Hassanien [2,3,*], Fawaz W. Alsaade [4] and Heider A. M. Wahsheh [5]

[1] Department of Computer Networks and Communications, College of Computer Science and Information Technology, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia; malzahrani@kfu.edu.sa
[2] Department of Animal and Fish Production, Agricultural and Food Sciences College, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia
[3] Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt
[4] Department of Computer Science, College of Computer Science and Information Technology, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia
[5] Department of Information Systems, College of Computer Science and Information Technology, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia
* Correspondence: helsanwey@kfu.edu.sa

Abstract: In Saudi Arabia, there is a scarcity of water used for agriculture and human consumption. Therefore, the aquaponic technique needs to be considered. Aquaponics is a modern, environmentally friendly agricultural technology that combines hydroponics and aquaculture into one system. However, the key to a successful aquaponic system is optimizing the stocking density for the target species. This study estimates the effect of three stocking densities—3 kg/m^{-3}, 6 kg/m^{-3}, and 9 kg/m^{-3}—in five replicates on the growth performance and water quality of fingerling Nile tilapia (*Oreochomis niloticus*), as well as the yield of spinach (*Spinacia oleracea*) grown in the nutrient film technique (NFT) aquaponic system. As for the planting density, 36 spinach plants are planted per m^{-2} for each replicate. The experiment is set up for 8 weeks. The findings reveal that the average final body weight, weight gain, specific growth rate, and survival rate of Nile tilapia were significantly higher in the 3 kg/m^{-3} treatment. It is evident that the total yield of spinach increased as the stocking density increased ($p < 0.05$). Most of the water quality measurements are significant, pH values range from 6.74 to 7.47, dissolved oxygen is 4.33 to 6.35, ammonia is 0.13 to 0.17 mg/L^{-1}, nitrite is 0.045 to 0.089, and nitrate is 2.44 to 3.35. Therefore, to maximize the productivity of spinach and tilapia while achieving the maximum benefit from fresh water, it can be recommended to use the stocking density of Nile tilapia fingerlings in the range of 6 kg/m^{-3} in the aquaponic system.

Keywords: Nile tilapia; stocking density; spinach; aquaponic

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1. Introduction

A major issue for arid and semi-arid countries including Saudi Arabia is the scarcity of water for agriculture and human consumption. Due to the low rainfall and harsh climate, the availability of fresh water is severely restricted in these places [1]. Compared to China and the United States, Saudi Arabia imports more fresh food per capita. Therefore, the aquaculture system may complement traditional agriculture and help achieve the goals and policies set by the government to ensure the availability of food and water security [2]. Importing agricultural products is not a sustainable option, and local food production becomes an important challenge. Tilapia culture is being done at high densities. To cover the growing market demand and production costs, producers have been forced to upgrade their technological capabilities or implement new culturing solutions. This is due to the growing consumer demand for aquaculture products, as well as to rising land- and water-related costs and environmental restrictions [3]. Hence, aquaculture in Saudi Arabia is becoming more crucial for guaranteeing food security and long-term economic prosperity. One of the industries producing food the fastest in the world is aquaculture. Global aquaculture production increased by 5.3% yearly between 2010 and 2018. Production climbed from 57.7 million tons in 2010 to 82.1 million tons in 2018 [4]. In 2030, the world is expected to consume 186 million tons of fish, with aquaculture expected to supply up to 50.2% of that need. Currently, Saudi Arabia produces 72,000 tons of aquaculture, and according to Saudi Vision 2030, it aims to reach 400,000 tons [5].

Aquaponics is a cutting-edge, environmentally friendly agricultural technique that combines hydroponics with aquaculture in a single production system to produce fish and plants [6,7]. Fish are fed in aquaponic systems, and the nutrients in their waste are used to feed the plants. The system depends on bacteria working properly since they are essential to the effective transformation of fish waste into nutrients for the plants [8]. Hydroponics, aquaculture, and the upkeep of microorganisms and nutrients are all necessary for an aquaponics system to be effective. Integrated agri-aquaculture systems (IAAS) are thought to include aquaponics as a subset [9]. There are factors affecting the success of the aquaponics system, such as the plant culture system and the fish tank, the filtration system, aeration and water flow, and the types of both the fish and the cultivated plants. The nutrient film technique (NFT) is frequently run as a closed system and is thought to be the most scalable. With this technique, plant roots are suspended in a tube or trough with a slope of 1% to 2%, and nutrients are fed to the plants continuously through the tube or trough from the upper to lower end by gravity [10]. NFT aquaponics systems are benefits by being easily established, having low construction costs and low-waste systems, and providing oxygen to plant roots.

Aquaponics has been utilized effectively with more than 150 different plant types. The most popular species grown in aquaponic systems include lettuce, tomato, basil, eggplant, pepper, and spinach [11,12]. A perennial green vegetable that is high in vitamins, minerals, protein, and omega-3 fatty acids is spinach (*Spinacia oleracea*) [13]. Globally, systems for hydroponic, aquaponic, and traditional agriculture are used to grow spinach. Spinach has become a widely favored green vegetable for aquaponics systems because of its quick growth rate with minimal nutritional requirements, favorable market acceptability, and exceptional adaptation to a wide range of environmental circumstances [14].

Due to its extensive usage in aquaculture, the Nile tilapia (*Oreochomis niloticus*) has been called the fish of the millennium [15]. Nile tilapia is very effective at converting feed and has a wide range of environmental adaptability. It also grows quickly. These qualities distinguish this species as being the best for aquaculture production. Several parameters influence the quantity of nutrients generated in a fish culture system; the most important of them is the amount of feed provided to the fish in accordance with the density of fish stocked in the system. [12]. The growth rate, fish size, and overall productivity of the farm are affected by the stocking density [16]. Refs. [17,18] both claim that stocking density affects the health of farmed fish. The best use of water, pond water quality, sedimentation rate, growth performance, water productivity, and fish mortality are all impacted by stocking density [15,16,19].

It is crucial to establish the ideal stocking density for any aquaponic system to maximize the productivity of the fish and the vegetables without affecting the system's water quality or its financial returns. This study aimed to estimate the effect of rearing Nile tilapia (*Oreochromis niloticus*) fingerlings in three stocking densities on growth of fish, yield of spinach (*Spinacia oleracea*), and water quality in an NFT aquaponic system.

2. Materials and Methods

2.1. Experiment Design and Aquaponic Setup

The experiment was carried out for 56 days in the nutrient film technique (NFT) aquaponic system situated inside a glass greenhouse that was 50% shaded at King Faisal University, Al-Hasa, Saudi Arabia (26°23′18.56″ N, 50°11′16.01″ E). The experiment conducted a completely randomized design with three treatments, five replicates each, comprising three different stocking densities of Nile tilapia as follows: 3 kg/m^{-3}, 6 kg/m^{-3}, and 9 kg/m^{-3}. The experimental design consists of 15 similar aquaponic units used in this trial, each unit consisting of a recirculating aquaculture system (circular fish tank of capacity 500 L (water volume 400 L), a mechanical filter (250 L), a bio-filter (500 L)), and a hydroponic unit (NFT parallel plastic channels) (Figure 1). Each fish tank contains a submersible water pump (flow rate 1500 L/m) that drives the water to the mechanical filter and then to the biological filter with a pump of 5 m^3/hour and then to the hydroponic units (NFT) before it flows back into the tilapia fingerling tank by gravity. The water that was passing through the bio-filter for nitrification came back degassed and oxygenated. The fish tank's volume was changed twice per hour by regulating the water flow. To keep the fish's oxygen supply constant, an air blower was also used to aerate every tank. The hydroponic system, which was comprised of five parallel plastic channel troughs measuring 10 m in length, relied on the nutrient film technique (NFT) to provide water and nutrients to the roots through a thin film.

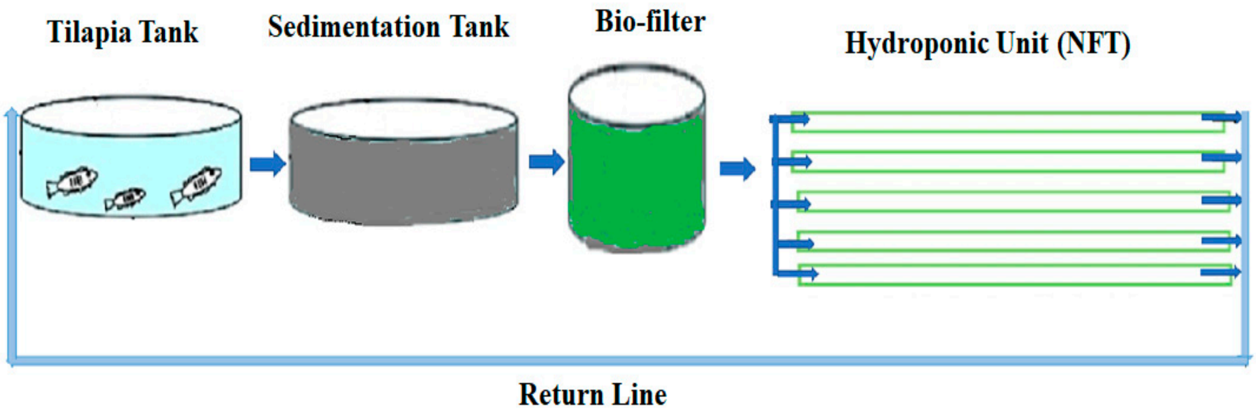

Figure 1. Schematic diagram of an aquaponic unit.

2.2. Spinach and Nile Tilapia Fingerlings

The spinach, *Spinacia oleracea*, seeds were obtained from a commercial market. Prior to transplantation, the spinach seeds were sowed and allowed to develop in a seed tray for 20 days to reach the same size as the seedlings upon planting. Daily watering was given to the seedlings until they began to germinate. The germinated spinach was then transferred to the plastic containers after three weeks. The third and fourth true-leaf stages of the seedlings (21 days old) were moved to the NFT HP with a planting density of 36 plants m^{-2} and a spacing of 15 $\times$ 15 cm^2.

All-male sex-reversed Nile tilapia (*O. niloticus*) fingerlings were from the King Faisal University hatchery and were transported to the greenhouse through the air in sealed polyethene bags. The fingerlings had an average size of 15.21 $\pm$ 1.23 g (36 days old). In circular tanks with a 500 L capacity and constant aeration, Nile tilapia were stocked for acclimation within a week; every day, fecal matter and residual feed siphoning and water exchange were carried out. Fingerlings were fed commercial feed containing 35% protein and 4% crude fat at a rate of 5% of fish body weight twice daily throughout the experimental period.

2.3. Monitoring of Nile Tilapia-Spinach

All fish from each tank were counted and weighed individually at the end of the experiment (56 days) to evaluate weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate, calculated as per the following standard formulas:

Weight gain (g) = final weight (g) − initial weight (g).

$$\text{Specific growth rate} = (\ln \text{final weight (g)} - \ln \text{initial weight (g)})/\text{time (No. of days)} \times 100.$$

$$\text{Feed conversion ratio} = \text{feed intake ((dry weight) (g)/body weight gain ((wet weight) (g)}.$$

$$\text{Survival rate \%} = \text{total number of harvested fish/total number of initial stock} \times 100.$$

The first spinach was harvested on day 26, and the time period between the two harvests was 10 days. Therefore, the days of harvest during the experimental period were days 36, 46, and 56. The spinach was cut manually when it was 6 inches long using scissors. The spinach was weighed (in grams) after it was harvested. With each harvest of spinach, 15 leaves per treatment were used to estimate the content of chlorophyll (SPAD index) using a portable chlorophyll meter (SPAD-502, Minolta, Japan).

2.4. Water Quality Parameters

Throughout the experiment, no water exchange was carried out in the aquaponic units except for refilling evapotranspiration. Water was monitored each day in the fish-rearing tank and the hydroponic NFT before feeding the fish at 8 am and 3 pm for the following, temperature (T; °C), dissolved oxygen (DO; $mg \cdot L^{-1}$), pH, electric conductivity (EC; mS cm^{-1}), and total dissolved solids (TDS; g L^{-1}), using a YSI 556 multiparameter meter (YSI Inc., USA), an EcoSense pH10A Pen Tester, and Hach test kits. Water samples from the fish tanks and spinach NFT were taken once a week for analysis of ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), orthophosphate (PO_4-P), and alkalinity ($CaCO_3$ mg L^{-1}), according to the manufacturer's recommended methodologies using an optical photometer YSI 9500 (YSI Incorporated, Yellow Springs, OH, USA) (±1% precision) (YSI, I. 2014). To determine variations in water quality between the hydropic units and nutrient extractor rate, water quality parameters and nutrients were assessed in the tilapia tanks and spinach hydroponic systems. According to plant absorption and other biogeochemical processes, the rate of nutrient loss was computed as follows.

$$\text{Rate of nutrient loss} = \frac{\text{Nutrient levels in a hydroponic system} - \text{Nutrient levels in fish tank}}{\text{Nutrient levels in fish tank}} \times 100$$

2.5. Statistical Data Analysis

All output data were examined for normality and homogeneity of variances by the one-sample Kolmogorov–Smirnoff test and the Levene's test. Using the Statistical Package for the Social Sciences, Version 25 (SPSS, Chicago, IL, USA), a one-way ANOVA study was performed to evaluate growth performance and water quality parameters. Tukey's test was used to compare the pairwise means, and the nominal level for significance was settled at $p < 0.05$. All the observed data are mentioned as mean ± standard error (S.E.).

3. Results

3.1. Performance of Tilapia-Spinach

Table 1 shows the growth performance of Nile tilapia fingerlings stocked at different levels, viz., 3 kg/m^{-3}, 6 kg/m^{-3}, and 9 kg/m^{-3}. The accuracy of randomization among the experimental treatments is demonstrated by statistically identical values ($p > 0.05$) of mean initial body weight between treatments. The tilapia growth performance was significantly affected by stocking density. The mean final body weight was 41.14 ± 1.20 g, 31.94 ± 2.10 g, and 23.84 ± 1.97 g for fingerlings stocked at 3 kg/m^{-3}, 6 kg/m^{-3}, and 9 kg/m^{-3}, respectively. Fingerlings stocked at 3 kg/m^{-3} at the end of the experiment weighed about 29% heavier than fish stocked at 6 kg/m^{-3} and 72% heavier than fish stocked at 9 kg/m^{-3}. Meanwhile, fish that were stocked at a density of 6 kg/m^{-3} weighed around 34% heavier than fish that were stocked at a density of 9 kg/m^{-3}. Weight gain and specific growth rate (% day^{-1}) showed the same pattern.

Table 1. Growth performance of Nile tilapia produced according to the stocking density in the aquaponic system (mean ± standard error; n = 5) *.

Parameters	*O. niloticus* Stock Density		
	3 kg/m^{-3}	6 kg/m^{-3}	9 kg/m^{-3}
Initial body weight (g)	15.3 ± 0. 37	15.65 ± 1.52	14.94 ± 1.51
Final body weight (g)	41.14 ± 1.20 ^{a}	31.94 ± 2.10 ^{b}	23.84 ± 1.97 ^{c}
Body weight gain (g)	26.11 ± 1.55 ^{a}	18.28 ± 1.73 ^{b}	12.90 ± 1.44 ^{c}
Specific growth rate (% day^{-1})	1.79 ± 0.11 ^{a}	1.38 ± 0.18 ^{b}	1.11 ± 0.14 ^{c}
Feed conversion ratio	1.46 ± 0.09 ^{c}	1.69 ± 0.11 ^{b}	1.88 ± 0.19 ^{a}
Survival rate (%)	92.40 ± 1.39 ^{a}	86.44 ± 1.18 ^{b}	84.91 ± 1.96 ^{b}

* Mean value 5 replicates ± S.E.; Mean values sharing the same letters are insignificantly different (p < 0.05).

Generally, SGR reduced as fingerlings grew over time, with the greatest decline being observed in fingerlings stocked at 9 kg/m^{-3}. Fish stocked at 3 kg/m^{-3} grew faster than fish placed at 9 kg/m^{-3} and fish stocked at 6 kg/m^{-3}, according to the analysis of the rate of change. All treatments had significantly different FCR values (p < 0.05). The lowest FCR was found in 3 kg/m^{-3} (1.46 ± 0.09) after consuming on average 829± 61 g of feed followed by 6 kg/m^{-3} (1.69 ± 0.11) after consuming on average 1434 ± 137.1 g of feed, and the highest in 9 kg/m^{-3} (1.88 ± 0.19) after consuming on average 1734 ± 137.1 g of feed. Over the period of the experiment, differences were observed in the fish survival rate across the three treatments (Table 1). Moreover, the mean survival rate was significantly highest at a stocking density of 3 kg/m^{-3} (92.40 ± 1.39%), followed by 6 kg/m^{-3} (86.44 ± 1.18%), which was similar to fingerlings stocked at 9 kg/m^{-3} (84.91 ± 1.96%).

Four times during the trial, spinach was harvested: on days 26, 36, 46, and 56 following transplantation into the experimental setting (Table 2). The total yield of spinach was 5.84 ± 0.63, 6.52 ± 0.57, and 9.37 ± 0.38 kg/m^{-2} for stocking densities of fingerlings 3, 6, and 9 kg/m^{-3}, respectively. When compared to fish stocked at 3 kg/m^{-3}, 6 kg/m^{-3}, and 9 kg/m^{-3}, the yield of spinach was significantly higher in fish stocked at 9 kg/m^{-3} during the four harvests (p < 0.05). To assess the photosynthetic activity of spinach in different stock densities of tilapia fingerlings, the chlorophyll content of leaves was examined (Table 3). During the experiment, the chlorophyll content (SPAD value) of the first harvest was insignificant (p > 0.05) among treatments. In contrast, it was found that the yield of spinach for the next three harvests was significantly higher (p < 0.05) at the level of stocking tilapia with a density of 9 kg/m^{-3} compared to other treatments.

Table 2. Yield of spinach at different stocking density with *O. niloticus* in aquaponic system (mean ± standard error; n = 5) *.

Fish Stocking Density	Mean Marketable Yield ± S.E. (kg/m^{-2})				Total Harvest
	1st Harvest (26-day)	2nd Harvest (36-day)	3rd Harvest (46-day)	4th Harvest (56-day)	
3 kg/m^{-3}	1.62 ± 0.19 ^{c}	1.59 ± 0.31 ^{b}	1.75 ± 0.34 ^{a}	0.88 ± 0.08 ^{c}	5.84 ± 0.63 ^{ab}
6 kg/m^{-3}	2.11 ± 0.17 ^{b}	1.79 ± 0.34 ^{b}	1.53 ± 0.26 ^{b}	1.09 ± 0.18 ^{b}	6.52 ± 0.57 ^{b}
9 kg/m^{-3}	2.58 ± 0.09 ^{a}	2.39 ± 0.21 ^{a}	2.47 ± 0.15 ^{a}	1.93 ± 0.13 ^{a}	9.37 ± 0.38 ^{a}

* Mean value of 5 replicates ± S.E.; in each column sharing the same letters are insignificantly different (p < 0.05).

Table 3. Total chlorophyll content (SPAD) in leaves of spinach in different stocking density of Nile tilapia in an aquaponic system (mean ± standard error; *n* = 5) *.

Fish Stocking Density	1st Harvest (26-Day)	2nd Harvest (36-Day)	3rd Harvest (46-Day)	4th Harvest (56-Day)
3 kg/m^{-3}	47.11 ± 2.27^{ns}	27.39 ± 3.33^{b}	32.11 ± 3.14^{b}	22.82 ± 3.37^{b}
6 kg/m^{-3}	49.91 ± 3.31^{ns}	29.76 ± 2.95^{b}	30.59 ± 4.23^{b}	25.77 ± 3.19^{b}
9 kg/m^{-3}	52.21 ± 4.23^{ns}	46.39 ± 5.33^{a}	45.31 ± 4.74^{a}	41.61 ± 5.20^{a}

* Mean value of 5 replicates ± S.E.; in each column sharing the same letters are insignificantly different ($p < 0.05$). ns = not significant.

3.2. Water Quality Management

Table 4 shows the water quality measurements examined during the experiment. The average water temperatures of the aquaponic system ranged from 25.63 ± 1.8 to 25.88 ± 1.3 °C and 24.37 ± 1.5 to 24.65 ± 1.1 for fish tank and NFT hydroponic, respectively, during the entire experimental period, not showing statistically significant differences (Figure 2a). Different stocking densities of tilapia had different effects on DO, pH, alkalinity, ammonia, nitrate, nitrite, and phosphate levels (because of the amount of feed intake per density). The pH in 3 kg/m^{-3}, 6 kg/m^{-3}, and 9 kg/m^{-3} varied significantly ($p < 0.05$). At the middle stocking density of 6 kg/m^{-3}, pH was driven closer to neutral and ranged from pH 7.15 to 7.24 (Figure 2b). Moreover, it was similarly found that the level of dissolved oxygen was significantly higher at the 3 kg/m^{-3} stocking density compared to other treatments during the experimental period (Figure 2c). It was found that as the density of tilapia stock increased, the alkalinity of the water decreased, and there was a gradual decrease with the progression of the aquaculture period in all treatments. Generally, the highest value of water alkalinity was in 3 kg/m^{-3}, followed by 6 kg/m^{-3}, while 9 kg/m^{-3} had the lowest value. The mean alkalinity of the current experiment ranged between 134.38 and 175.50 mg L^{-1} (Figure 2e). At 0.11 to 0.17 mg L^{-1}, the mean ammonia-N shows a significant difference ($p < 0.05$) across all treatments (Figure 2g). Nitrate-mean N's concentration ranged from 1.49 to 3.35 mg L^{-1} (Figure 2h). While comparing fish stock densities, the phosphate (PO_4-P) levels revealed a significant difference ($p < 0.05$). (Figure 2e). The content of phosphate in the current experiment ranged from 2.03 to 2.36 mg L^{-1} (Figure 2j).

Table 4. Water quality parameters in the aquaponic system stocked with *O. niloticus* at various stocking densities (mean ± standard error; *n* = 5) *.

Parameters	Aquaponic	*O. niloticus* Stock Density		
		3 kg/m^{-3}	6 kg/m^{-3}	9 kg/m^{-3}
T (°C)	Tilapia tank	25.88 ± 1.3	25.77 ± 1.6	25.63 ± 1.8
	NFT hydroponic	24.65 ± 1.1	24.37 ± 1.5	24.57 ± 1.7
pH	Tilapia tank	7.47 ± 0.005^{a}	7.24 ± 0.008^{b}	6.74 ± 0.006^{c}
	NFT hydroponic	7.35 ± 0.007^{a}	7.15 ± 0.007^{b}	6.64 ± 0.005^{c}
DO (mg L^{-1})	Tilapia tank	6.35 ± 0.004^{a}	5.43 ± 0.007^{b}	4.33 ± 0.006^{c}
	NFT hydroponic	6.73 ± 0.005^{a}	6.21 ± 0.002^{b}	5.22 ± 0.004^{c}
EC (ms cm^{-1})	Tilapia tank	0.354 ± 0.004	0.353 ± 0.008	0.355 ± 0.009
	NFT hydroponic	0.336 ± 0.001	0.335 ± 0.001	0.333 ± 0.007
TDS (g L^{-1})	Tilapia tank	0.236 ± 0.009^{a}	0.224 ± 0.004^{ab}	0.209 ± 0.002^{b}
	NFT hydroponic	0.204 ± 0.003	0.204 ± 0.006	0.203 ± 0.004
$CaCO_3$ mg L^{-1}	Tilapia tank	175.50 ± 2.70^{a}	160.34 ± 2.37^{b}	170.48 ± 4.25^{a}
	NFT hydroponic	136.83 ± 2.19	134.38 ± 1.65	135.12 ± 1.72
NH_3-N (mg L^{-1})	Tilapia tank	0.133 ± 0.009^{b}	0.143 ± 0.004^{b}	0.172 ± 0.003^{a}
	NFT hydroponic	0.113 ± 0.004	0.113 ± 0.003	0.117 ± 0.003

Table 4. *Cont.*

Parameters	Aquaponic	*O. niloticus* Stock Density		
		3 kg/m^{-3}	**6 kg/m^{-3}**	**9 kg/m^{-3}**
NO_2-N (mg L^{-1})	Tilapia tank	0.045 ± 0.007 ^{c}	0.075 ± 0.005 ^{b}	0.089 ± 0.004 ^{a}
	NFT hydroponic	0.043 ± 0.009 ^{c}	0.073 ± 0.009 ^{b}	0.085 ± 0.003 ^{a}
NO_3-N (mg L^{-1})	Tilapia tank	3.35 ± 0.029 ^{a}	2.44 ± 0.048 ^{b}	2.55 ± 0.039 ^{b}
	NFT hydroponic	2.72 ± 0.033 ^{a}	1.81 ± 0.037 ^{b}	1.49 ± 0.060 ^{c}
PO_4-P (mg L^{-1})	Tilapia tank	2.33 ± 0.013 ^{ab}	2.25 ± 0.037 ^{b}	2.36 ± 0.026 ^{a}
	NFT hydroponic	2.11 ± 0.041 ^{ab}	2.03 ± 0.031 ^{b}	2.14 ± 0.023 ^{a}

°C, temperature, DO, dissolved oxygen, EC, electric conductivity, TDS, total dissolved solids, $CaCO_3$, Alkalinity, NH_3-N, ammonia-nitrogen, (NO_2-N), nitrite-nitrogen, (NO_3-N) nitrate-nitrogen and PO_4–P, orthophosphate. * Mean value of 5 replicates ± SEM; Mean values sharing the same letters are insignificantly different ($p < 0.05$).

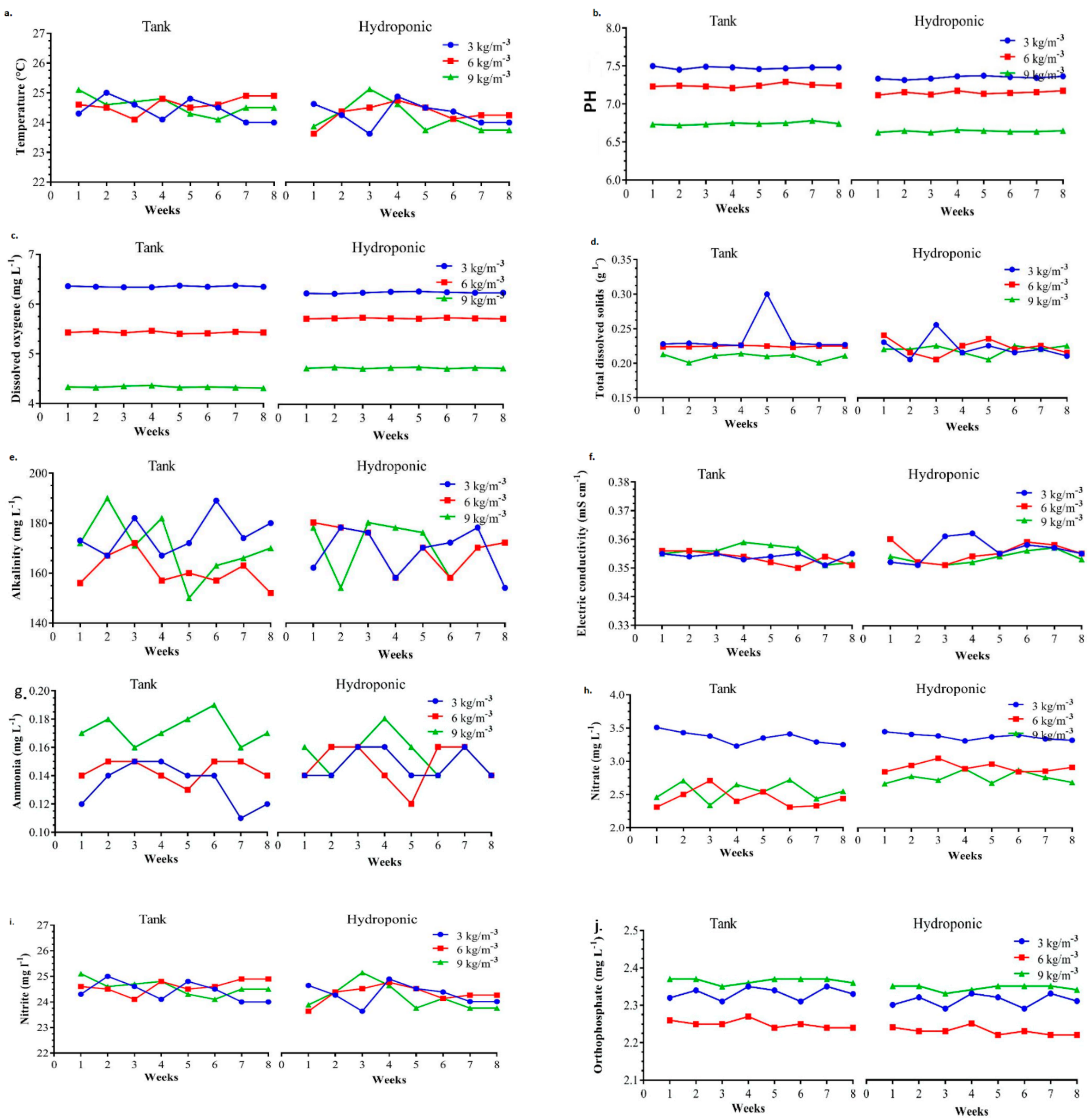

Figure 2. Effects of stocking density of Nile tilapia on water quality parameters during the experimental period (8 weeks) in aquaponic system: (**a**) temperature; (**b**) PH; (**c**) dissolved oxygen; (**d**) total dissolved solids; (**e**) alkalinity; (**f**) electric conductivity; (**g**) ammonia; (**h**) nitrite; (**i**) nitrate, and (**j**) orthophosphate.

Ammonia, nitrate, and nitrite had the highest percentages in extractor efficiencies across the three stocking densities in the hydroponic system during the experimental period (8 weeks) (Table 5). Ammonia, nitrites, nitrates, and orthophosphate extractor efficiency were all significantly ($p < 0.05$) impacted by fish stocking density. The densities of 3 kg/m^{-3} and 6 kg/m^{-3} and 9 kg/m^{-3} had the maximum ammonia, nitrites, and nitrates extractor efficiency, respectively. Orthophosphate was removed at the highest rates when stocking densities of 6 and 9 kg/m^{-3} were used compared to 3 kg/m^{-3} for the treatment.

Table 5. Percentage of extractor efficiency of the different nutrients in hydroponic system per different levels of Nile tilapia stocking density during the experimental period (8 weeks) in an aquaponic system.

Water Quality Parameters	*O. niloticus* Stock Density		
	3 kg/m^{-3}	6 kg/m^{-3}	9 kg/m^{-3}
NH_3-N (mg L^{-1})	18.3 ± 2.5 ^{a}	12.6 ± 3.1 ^{b}	6.7 ± 1.3 ^{c}
NO_2-N (mg L^{-1})	43.7 ± 5.7 ^{a}	29.6 ± 7.4 ^{b}	5.3 ± 1.2 ^{c}
NO_3-N (mg L^{-1})	17.3 ± 2.6 ^{a}	14.9 ± 3.2 ^{a}	4.9 ± 1.8 ^{b}
PO_4-P (mg L^{-1})	4.51 ± 0.78 ^{b}	9.34 ± 2.61 ^{a}	10.21 ± 2.11 ^{a}

NH_3-N, ammonia; NO_2-N, nitrite; NO_3-N, nitrate and PO_4-P, orthophosphate. Percentages in the same row with different superscripts are significantly different ($p < 0.05$).

4. Discussion

An appropriate balance is necessary between fish farmers with high stock intensification and the expectation of a high economic return and those who view the discharge of fresh water from high stock intensification as detrimental to environmental sustainability. To properly examine the sustainability of tilapia in an aquaponic system, this study provides vital evidence to determine the optimal stocking density to achieve the highest water utilization and the highest plant yield in the aquaponic system.

4.1. Performance of Tilapia- Spinach

In an aquaponic system, fish grow at various stocking densities [20,21]. Through this study, the growth of Nile tilapia was estimated, revealed by fish stocked at an initial average weight of 15.65 ± 1.52 g for 8 weeks. In comparison to fish that were stocked at 6 and 9 kg/m^{-3}, fish that were stocked at the lowest density of 3 kg/m^{-3} exhibited a faster rate of growth. According to [22–24], fish weight is adversely linked with stocking density. Our results support their findings; [25] concluded that silver catfish had the highest growth rate when stocked at the lowest density. The results showed that the survival rate of fish decreased with the increase in fish stocking density, probably because of the increased densities and crowded conditions and the ensuing competition for food and space. In addition, the fish may be fed until they are satisfied [26,27]. Furthermore, the FCR was lower significantly in the 3 kg/m stock density treatment, indicating efficient feed utilization compared to other treatments. This is consistent with previous findings that in the aquaponic system with low stocking density, Nile tilapia growth performance and feed utilization were higher [24]. Similarly, [28] discovered that in low-density rearing, African catfish had higher overall growth and feed utilization than the control.

In the current investigation, four harvests of marketable spinach production were recorded. The lowest yield was 0.88 kg/m^{-2} in the fourth harvest with 3 kg/m^{3} tilapia stocking density, while the highest yield was 2.58 kg/m^{-2} in the first harvest with 9 kg/m^{-3} tilapia stocking density. In this study, the higher the stocking density of fish, the higher the spinach yield ($p < 0.05$). It also turned out that the yield of the first harvest of spinach is the highest and the last harvest is the lowest, regardless of the stocking density of fish. Maybe the high growth of spinach yield is due to the availability of the nitrogen-containing nutrient mixture, which increases with the increase in the stocking density of fish or when the treatment is 9 kg/m^{-3} in this study. This were mentioned by [24,29] but on lettuce yield. With the increase in the fish stock density, the production of organic matter needed to feed the plant increases in the hydroponic system, where there is an increase in fish waste (fish excretion and unconsumed feed), on which anaerobic bacteria work to convert the ammonia produced in the system into nitrates for absorption by plants [30]. In the present study, the water quality, together with the availability of nutrients, affected the chlorophyll content of the plant. The higher chlorophyll values were recorded for the spinach with increased fish stock density, which is linked to the higher availability of nutrients (particularly N) [31].

4.2. Water Quality in Aquaponic System

Water quality parameters are the most crucial variables for the success of an aquaponic system, such as tank aeration, a pH level that is suitable for the system, efficient biological conversion of damaging ammonia to useful nitrate, and plant absorption of dissolved nutrients [32]. The main types of nitrogen used by plants are nitrate (NO_3^-) and ammonium (NH_4^+), whereas fish waste contains both ammonia (NH_3) and ammonium (NH_4^+), which together make up total ammonium nitrogen (TAN). NH_3 is toxic to fish and occurs at high concentrations. Biological membranes are often extremely permeable to NH_3 but impenetrable to NH_4^+ [33]. The pH of the system affects the NH3/NH4 + balance and, consequently, the level of toxicity of TAN, with NH_3 predominating at higher pH. Nitrite, which is produced when bacteria convert ammonia to nitrate, is likewise poisonous. It is poisonous because of its affinity for the Cl-binding system in fish gills [34]. It also affects the fish's osmoregulatory and endocrine systems and has the potential to produce methemoglobinemia [35]. In contrast, aquatic creatures that are exposed to high oxygen concentrations can detoxify nitrite [36]. An aquaponic system's potential toxicity can be avoided with careful design, planning, and maintenance. Critical parameters that must be frequently monitored and maintained include water temperature, pH, and excellent aeration to ensure adequate dissolved oxygen levels. In all tilapia stocking densities in this study, the T, pH, DO, EC, and TDS (physical metrics of water quality) were within the recommended range. The results showed that the average temperature of the aquaponic system was relatively consistent with [37,38] (27 to 30 °C) and is within the recommended range for Nile tilapia. The pH of the tilapia tanks during the experiment was 6.7–7.4, which is suitable for tilapia growth (6 to 7). This range of pH maintains ammonia in the form of NH^{+4} and, thus, reduces the production of toxic ammonia in the tanks [12,39]. In soilless agriculture methods, the pH of the water is essential for effective plant growth and development, in addition to ensuring that nutrients are available and permitting efficient nutrient uptake by plants. Overall, the pH levels in this study were ideal for optimal spinach growth [40]. Dissolved oxygen is one of the most important physiological factors associated with the physiological functions of tilapia and its survival rate. DO is essential to the process of nitrification and is inversely proportional to water temperature [24,41,42]. In general, it was observed in this experiment that the higher the tilapia stock density, the lower the dissolved oxygen level [43,44]. Despite this, the results of the dissolved oxygen level were in the recommended range, which ranged from 3.0 to 5.0 mg L^{-1} [45,46].

Dissolved ions and organic compounds created by the biological activities of nitrifying bacteria made up the chemical ingredient of the nutrition solution in the fish tanks. In this experiment, it was noted that higher stocking densities resulted in higher concentrations of total ammonia nitrogen (TAN), nitrite, and phosphate. This agrees with other studies by [47–49], whose ranges were 0.09–0.85 mg L-1 for NH_4^+ and 0.02–0.17 mg L-1 for NO_2, respectively. The concentration of nitrogen in the water will increase as the amount of fish waste (stock density and feed input) in the tank increases, which will have a negative effect on water quality and fish growth [44,50,51]. Lower nitrogenous compound levels in the NFT hydroponic portion compared to the tilapia tanks thus indicate the removal of TAN, nitrites, and nitrates from the water, which is likely caused by plant uptake [52]. Nitrate is absorbed by microflora in the hydroponic system or by plant cultivated roots with the help of biofilms [53]. These nutrients are important for the growth of spinach. Due to its unique ability to produce a very high yield in a relatively short period of time, spinach, *Spinacia oleracea*, stands out among vegetable crops. To develop quickly and to ensure a high and successful yield, this plant needs a lot of the mineral elements that are present in the soil, especially nitrogen (N). Nitrate is often the major source of N available to spinach [54]. The results indicate that the best performance under the aquaponic system was a stock density of 6 kg/m^{-3}, which results in the optimal balance between tilapia nutrient production and spinach uptake in NFT aquaponics system.

Finally, risks must be taken into account when applying the aquaponic system, such as an increase or decrease in the level of nitrates in the system being harmful to fish or plants, respectively. Therefore, the ratio between fish:plants must be adjusted. It is prohibited to add antibiotics, insecticides, and fungicides to treat fish or plants, respectively, because each of them is harmful to fish, plants, or the microflora of the biological filter [55].

4.3. Future Aquaponics Research

The foundation of aquaponics is a compromise between the requirements of plants and fish. There is currently a need for development to achieve the best conditions for both fish and plants, with either (1) a focus on the interdependent parameters of both system components (such as, combining fish and plant species that preferably require similar environmental conditions within the same range of temperatures and pH that ensure bacterial nitrification), or (2) the physical separation in two recirculating loops, i.e., an aquaculture and hydroponic loop, referred to as decoupled systems, where the optimal condition for each system is applied with periodic water exchange between them [55,56].

5. Conclusions

In the NFT aquaponic system, the growth parameters of Nile tilapia fingerlings were affected by the fish stocking density. The higher the fish stocking density, the lower the growth rate, while the spinach yield increased. All water quality traits were within the permissible limits for tilapia culture. However, it can be recommended to use the rearing density of Nile tilapia fingerlings within 6 kg/m^{-3} in the NFT aquaponic system. In addition, increasing stock densities of tilapia can be tested in future research.

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