Effects of fish stocking density on water quality and the growth of red tilapia and vegetables in microalgae-aquaponic systems

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Abstract

This study investigates the impact of fish stocking density on water quality, red tilapia (*Oreochromis sp*.) growth, and mustard green (*Brassica integrifolia*) growth in microalgae-aquaponics systems. Three densities were tested: D1 (90 fish/m³), D2 (70 fish/m³), and D3 (50 fish/m³). Water quality monitoring showed that TAN, NO_2^-, and NO_3^- levels increased with fish density, while phosphate levels remained relatively stable. D1 had the highest total fish weight gain but a lower weight gain rate (91.9 %) compared to D2 (105.6 %) and D3 (134.1 %). D3 had the highest average weight gain per fish (67.78 g) and the best survival rate (100 %), whereas D1 had the lowest survival rate (94 %). Mustard greens grew best in D1. Microalgae density increased during experimental time, with D3 showing the highest biomass in both the algal and fish tanks. The results suggest that higher fish densities promoted plant growth, while lower densities benefited fish and microalgae, thus offering insights for optimizing recirculating microalgae-aquaponics systems.

Received 30/09/2024
Accepted 30/11/2024
Published 28/12/2024

Keywords

microalgae-aquaponics, *Chlorella vulgaris*, fish stocking density, red tilapia, mustard greens

1 Introduction

The rapid increase in global population has led to challenges such as water scarcity, land degradation, climate change, and salinization, thus affecting food production [1]. To address the rising food demand due to resource limitations, a shift from unlimited growth-based farming models to balanced and sustainable practices is necessary [2]. In aquaculture, wastewater discharge from traditional intensive farming methods poses serious environmental and ecological threats, as well as risks to human health and the sustainability of aquatic resources [2]. To mitigate these issues, environmentally friendly and sustainable farming practices are essential.

Aquaponics, which integrates fish farming with hydroponic vegetable cultivation in a recirculating water system, is a sustainable and eco-friendly food production method [1, 3]. It combines aquaculture and hydroponics in a symbiotic environment, where nutrients from fish wastewater are used to nourish plants, and water is recycled back into the fish tanks [4]. Aquaponics offers a nearly waste-free solution for treating aquaculture wastewater, efficiently using limited land and water resources, while providing

https://doi.org/10.55401/7k3bpd04

economic benefits through sustainable production of both fish and plants [5].

Due to the low nitrogen utilization efficiency in aquaponics systems, especially in high fish stocking densities, most nitrogen remains in the system, causing significant environmental impacts and requiring technical improvements to optimize nutrient retention [1]. To address this issue and enhance the efficiency and sustainability of aquaponics systems, integrating microalgae cultivation into the system has been proposed [6, 7]. Microalgae, which can convert light energy into biomass and fix carbon dioxide through photosynthesis, also assimilate nutrients like nitrogen and phosphorus from wastewater. This allows microalgae to improve nutrient removal or recycling, thereby aiding in the treatment of fish wastewater in aquaponics systems [5].

Recently, microalgae have been explored as a valuable addition to aquaponics systems. Microalgae are known for their ability to remove excessive nutrients, such as nitrogen and phosphorus, from aquatic environments, which makes them suitable for aquaponic systems that need to manage fish waste efficiently [5]. In addition, microalgae generate oxygen through photosynthesis, and further improve water quality by supporting aerobic processes in the system. The integration of microalgae in aquaponics has been shown to enhance nutrient recycling and stabilize water quality, thus supporting both plant growth and fish health [7]. Several studies have applied microalgae, specifically *Chlorella sp.*, in aquaponics systems to remove nutrients from fish tanks and improve water quality in the system [7, 8]. Different algae species exhibit various nutrient absorption abilities, and their compatibility with specific plants is crucial to maximize nutrient recycling and system productivity. Addy et al. (2017) [7] demonstrated that algae can enhance water quality in aquaponics systems by helping to control pH reduction associated with nitrification, generating dissolved oxygen in the system, and serving as a supplemental fish feed due to their high content of unsaturated fatty acids.

Fish stocking density is one of the most critical variables in aquaponics systems, as it directly affects nutrient levels, water quality, and the overall system balance [5, 9]. Stocking density refers to the amount of fish biomass per unit volume of water. As stocking density increases, the amount of fish waste, primarily in the form of ammonia (NH_3/NH_4^+), also increases. Without proper management, high ammonia levels can lead to toxic conditions for fish and reduce plant nutrient uptake [4]. In addition, increased stocking densities may deplete dissolved oxygen levels, resulting in poor fish growth and increased stress [9]. Several studies have examined the effects of fish stocking density on aquaponics systems, with various results. In traditional aquaponics systems, higher fish densities can lead to higher nutrient availability for plants, as more fish waste is converted into plant-usable forms such as nitrate (NO_3^-) [5]. However, extremely high fish densities can lead to nutrient imbalances, where excessive ammonia or nitrate concentrations become detrimental to plant growth [9]. This balance between fish waste production and plant nutrient requirements is crucial to the successful operation of an aquaponics system.

While significant research has been conducted on aquaponics systems, the role of microalgae and the effects of fish stocking density on water quality and plant growth remain underexplored. Most aquaponics research has focused on traditional systems, without integrating microalgae as a functional component. This study will investigate the impact of fish stocking density on water quality, growth performance of red tilapia, and growth performance of mustard greens cultivated in microalgae-aquaponics systems.

2 Methodology

2.1 Experimental setup

The research was conducted over 7 weeks in the Environmental Ecology Laboratory of the Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Viet Nam. Three treatments were carried out in with varying fish stocking densities: (1) treatment D1 with 90 fish/m³, (2) treatment D2 with 70 fish/m^3, and (3) treatment D3 with 50 fish/m^3 [9]. The aquaponics system, as shown in Figure 1, included key components such as a 380 L rectangular glass fish tank (holding 350 L water for fish rearing), a 200 L plastic biological filter with Kalnes media and aeration, a 200 L plastic sedimentation tank, and a 200 L plastic automatic water supply tank. The hydroponics system, consisting of three plastic containers (size of 105 cm × 43 cm × 16 cm), was arranged using nutrient film technique (NFT) method. Each hydroponic container plants 12 mustard greens. For algal tank, a 65 L rectangular glass was used, which was connected before the fish tank, was used. Air pumps and circulation pumps were installed to ensure continuous aeration and water flow all compartments across systems.

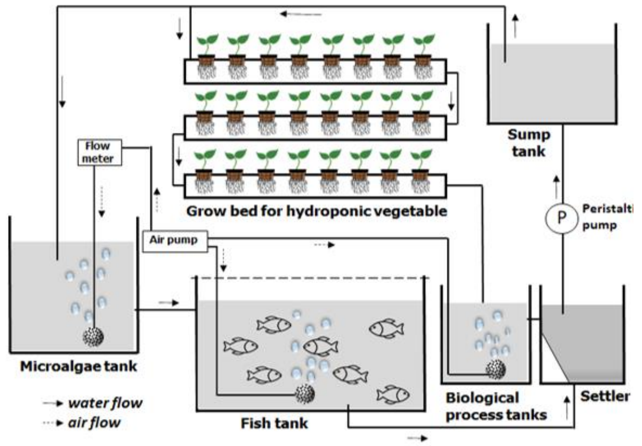

Figure 1 Illustrated diagram of the microalgae - aquaponics system in this study.

Red tilapia (*Oreochromis* sp.), with an average fresh weight of about 52.0 g per fish was used in the experiment. *Oreochromis* sp. was used in this aquaponics system because it possesses an air-breathing organ, enabling it to tolerate low oxygen conditions in the event of aeration system failures [9]. Additionally, this species is known to feed on plants and algae, with *Chlorella vulgaris* microalgae serving as a supplementary food source [10]. Fishes were fed at a rate of about 2.5 % of their biomass per day using 2 mm pellet feed containing 28 % protein [11], specifically formulated for tilapia under 200 g produced by Cargill (USA). The fish were acclimated for about 10 days in laboratory tanks to prevent transport shock before the experiment. Water was recirculated continuously in all three systems, with periodic water addition to compensate for evaporation losses. Total fish weight used in each system was measured at the beginning and after 45 days at the end of this experiment.

The plant grown in the hydroponic systems was mustard greens (*Brassica integrifolia*). Twelve healthy seedlings, grown on coconut coir for 15 days, were transplanted into each hydroponic container. Sunlight was the primary light source for the plants, supplemented with 8 hour/day of LED lights in the afternoon when there was no direct sunlight. The addition of LED lights was essential because natural sunlight was limited in indoor environments of the experiment, which can restrict photosynthesis and hinder plant development. Fish were introduced to the tanks 7 days before the plants and microalgae were added, with the plants being introduced 14 days later to ensure nutrients sufficiency for plant and algal growth. The plants were harvested after 45 days of growth. Throughout the experiment, feed intake, and overall health were observed and adjustments to feed amounts were made as necessary. Fish mortality was documented during the study for calculating survival rate of each treatment.

In the microalgae tank, *Chlorella vulgaris*, commonly used in aquaculture for nutrient absorption and environmental improvement, was utilized. The microalgae stock was from the Environmental Microbiology Lab at Nguyen Tat Thanh University. Prior to its use in the experimental systems, *C. vulgaris* was cultured in BG11 medium and grown in a 5 L plastic container under continuous aeration, with (2000-3000) lux light intensity, at a temperature of (25-28) °C. After (10-14) days, when the microalgae reached peak growth, they were transferred to the microalgae tanks of the aquaponics systems at 5 % of the tank volume. The microalgae were introduced into the aquaponics system on day 7, following the introduction of fish into the system. Microalgal growth in both the algae and fish tanks was monitored to evaluate factors influencing algal survival.

Microalgae cell density was tracked using spectrophotometric measurements at 680 nm (OD680), with higher OD680 values indicating greater microalgal density in the tanks. It should be noted that the wavelength of 680 nm is commonly used to measure microalgal density because it corresponds to the absorption peak of chlorophyll-a, the primary pigment involved in photosynthesis in most microalgae. Therefore, it is the ideal wavelength for estimating microalgal density. The microalgal density was measured spectrophotometrically using a UV-Vis spectrophotometer at a wavelength of 680 nm (OD680) [12]. Samples (10 mL each) were collected from three distinct locations within the fish tank and the microalgal tanks to ensure representative data. Measurements were conducted in triplicate for each sample, and the average OD680 value was reported.

2.2 Sampling and analysis method

To evaluate the fish growth, several parameters of the fish were observed, including survival rate, weight gain, and initial biomass and biomass at the time of harvest. For the plants, fresh weight was measured during harvest. Algal and water samples were collected once a week at around 10 a.m. from the algal and fish tanks of the systems. Water quality parameters in the fish tanks such as water temperature, pH, and dissolved oxygen (DO) were monitored using water testing equipment. Total ammonia nitrogen (TAN), nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}) were analyzed using standard methods [18]. The fish survival rate and weight gain were calculated using the following formulas:

(1) *Survival rate*: $SR (\%) = (Nt / No) \times 100$

where SR is survival rate (%); Nt is the number of fish at the end of the experiment; No is the initial number of fish

(2) *Weight gain*: $\Delta W = Wt - Wo$

where ΔW is the weight gain (g); Wt is the average weight at time t (g); Wo is the initial average weight (g);

The average fish weight, plant weight, and the minimum and maximum values of water quality parameters were calculated using Microsoft Excel.

3 Results and discussions

3.1 Water quality in the fish tanks

The ranges of water quality parameters including pH, temperature, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}) – for fish tanks with three different stocking densities (90 fish/m³, 70 fish/m³, and 50 fish/m³) in a microalgal-aquaponics system over 45 days were shown in Table 1. The results show that pH, temperature, and DO remained within appropriate ranges for tilapia growth and development across all systems. Maximum concentrations of TAN, NO_2^-, and NO_3^- in fish tanks increased with increased stocking density, while PO_4^{3-} levels slightly decreased.

Table 1 The ranges of water quality parameters, including pH, temperature, concentrations of DO, TAN, NO_2^-, NO_3^-, and PO_4^{3-} for fish tanks with three different stocking densities in microalgal-aquaponics systems over 45 days of experimentation and the appropriate values for tilapia found in other studies

Parameters	**D1 (90 fish/m³)**	**D2 (70 fish/m³)**	**D3 (50 fish/m³)**	**Appropriate ranges for tilapia in other studies**
Water temperature (°C)	26.3-28.8	26.6-29.2	25.7-29.1	18-32.9 [11]
pH	6.73-7.67	6.87-7.53	6.96-7.58	6-9 [11]
DO (mg/L)	3.26-5.41	3.55-5.87	3.41-6.04	≥ 3 [18]
TAN (mgN/L)	3.72-11.98	1.60-9.32	1.72-5.80	0.17-3.78 [19], 11-33 [3]
NO_2^- (mgN/L)	0.14-0.54	0.04-0.32	0.03-0.44	0.02-0.12 [20], 6.0-8.0 [7], < 5.0 [11]
NO_3^- (mgN/L)	6.45-31.92	4.80-26.12	4.94-23.98	0.2-219 [21], 1 6-31 [22], 26.7-54.7 [23]
PO_4^{3-} (mgP/L)	1.84-5.32	2.18-5.65	2.34-6.25	11.8-14.5 [24], 7.6-9.0 [23], 2.5-4.4 [25]

Water quality across treatments was similar except for TAN and NO_3^-, which increased with higher fish densities. Maximum TAN and NO_3^- concentrations were highest in D1 (11.98 mgN/L and 31.92 mgN/L), followed by D2 (9.32 mgN/L and 26.12 mgN/L) and D3 (5.80 mgN/L and 23.98 mgN/L). Ammonium (NH_4^+/NH_3), produced by fish waste and decomposing feed, can be toxic at elevated levels. TAN levels of 11-33 mgN/L are tolerable for red tilapia [3], TAN levels should be below 3.78 mgN/L in aquaculture systems [15]. In this study, the TAN levels across all treatments were generally within safe limits.

The range of PO_4^{3-} levels recorded in D1 treatment (1.84–5.32 mgP/L) was similar to those in D2 (2.18–5.65 mgP/L), while that range of D3, from 2.84 to 6.25 mgP/L, was slightly higher. Compared with other researches, range of PO_4^{3-} levels found in all the three microalgae-aquaponics systems of this study are similar to those values in polyculture aquaponics systems found in other studies, i.e. (2.5-4.4) mgP/L [41], (0.6-9.0) mgP/L [42].

The range of PO_4^{3-} levels in D1 (1.84-5.32) mgP/L was comparable to those in D2 (2.18-5.65) mgP/L, while D3 recorded a slightly higher range (2.34-6.25) mgP/L. These values are consistent with other studies, reported PO_4^{3-} levels of (11.8-14.5) mgP/L [18], observed levels ranging from 7.6 mgP/L to 9.0 mgP/L in polyculture aquaponics systems [17]. This demonstrates that the PO_4^{3-} concentrations observed in this study fall within typical ranges for such systems.

The findings show that water quality parameters, including TAN, NO_2^-, and NO_3^- concentrations tended to increase with higher fish stocking densities. This result is consistent with previous studies that have demonstrated higher fish biomass contributes to elevated nutrient levels in aquaponic systems, as fish waste generates higher levels of NH_4^+/NH_3, which is then converted into NO_2^- and NO_3^- through nitrification processes [4]. However, reductions in TAN and NO_2^- were less efficient in higher-density treatments (D1), suggesting that the biological filtration capacity of the system may have been overwhelmed at these higher densities. This finding is also supported by previous research, which suggests that increased stocking density can impair the efficiency of the biological filtration system [20]. PO_4^{3-} concentrations were not significantly different between treatments, possibly indicating that phosphate uptake by plants did not fully correlate with fish stocking density or that the system reached a threshold for PO_4^{3-} removal.

3.2 Fish and plant growth performance

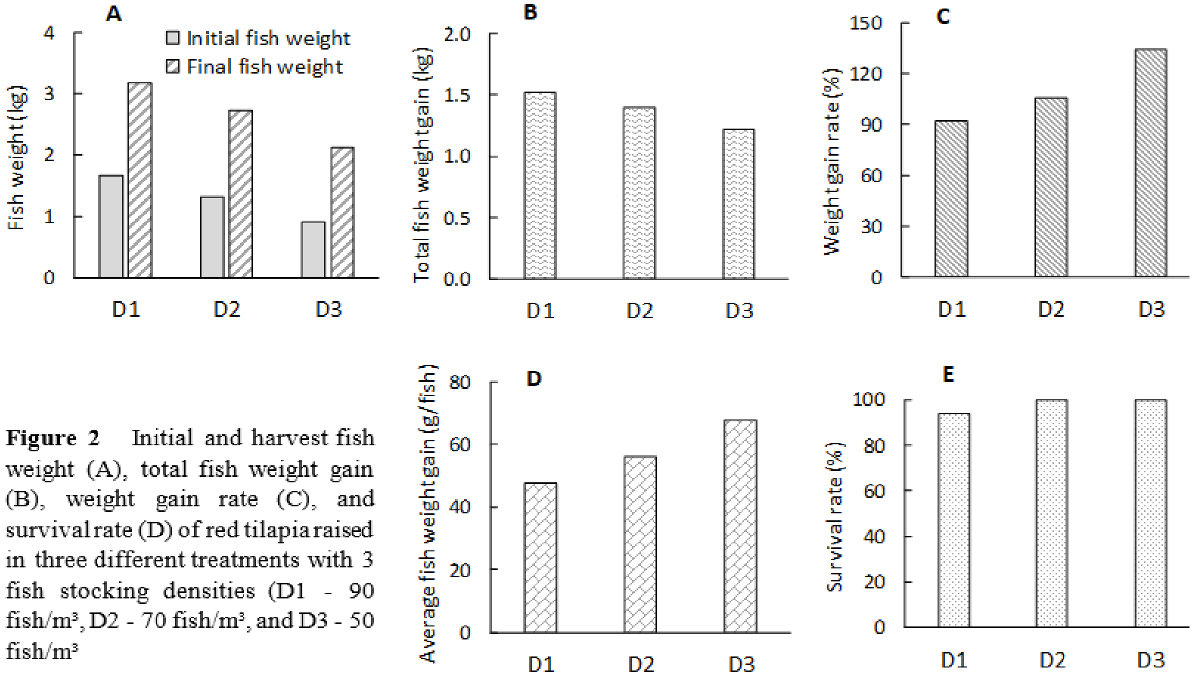

Figure 2 Initial and harvest fish weight (A), total fish weight gain (B), weight gain rate (C), and survival rate (D) of red tilapia raised in three different treatments with 3 fish stocking densities (D1 - 90 fish/m³, D2 - 70 fish/m³, and D3 - 50 fish/m³

The data of initial and harvest fish weight, total fish weight gain, weight gain rate, and survival rate of red tilapia raised in three different treatments with 3 fish stocking densities was shown in Figure 2. The growth performance of tilapia varied across the three microalgae-aquaponics systems with different stocking densities. D1 had the highest total weight gain (1.52 kg), but the lowest weight gain rate (91.9 %) and average weight gain per fish (47.6 g). D3 showed the highest average weight gain (67.78 g/fish) and survival rate (100 %), while D1 had a lower survival rate (94 %). Poorer water quality in D1, especially higher TAN and NO_2^- levels, was likely contributed to its reduced growth and survival rates, highlighting the impact of stocking density on fish performance.

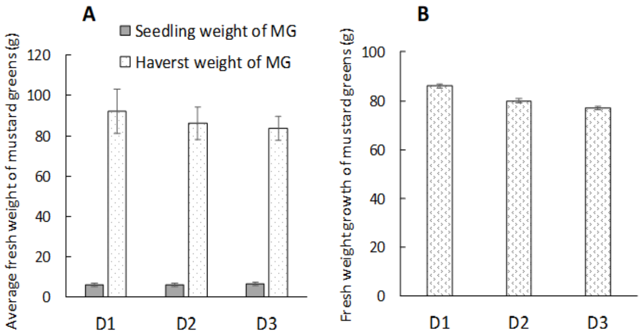

Figure 3 Average fresh weight (A) and fresh weight growth (B) of mustard greens cultivated in hydroponic sections of three microalgae-aquaponics systems with varying fish stocking densities: D1 (90 fish/m^3), D2 (70 fish/m^3), and D3 (50 fish/m^3)

The lower weight gain rate of tilapia in the D1 treatment, despite the highest overall biomass, may indicate that higher stocking densities can lead to increased competition for food and space, thereby reducing individual growth rates. Previous research has shown that high stocking density can lead to stress in fish, which may reduce their growth performance and feed conversion efficiency [21, 22]. The highest average weight gain was observed in D3 treatment, where fish density was the lowest, suggesting that more space and resources per fish lead to better growth performance. The lower survival rate (94 %) in D1 compared to D2 and D3 (100 %) may also indicate increased mortality risk under high stocking density, which has been widely documented in aquaculture settings [9, 22]. This reduced survival rate could be attributed to higher stress levels, increased disease susceptibility, or competition among the fish.

The results of average fresh weight and biomass of mustard greens at harvest (after 45 days of growth) for the treatments are shown in *Figure 3*. The average weight of harvested mustard greens was (92.12 ± 9.79) g/plant in treatment D1, compared to (86.20 ± 7.85) g/plant in D2, and (83.60 ± 6.02) g/plant in D3. This indicates that the biomass of harvested vegetables increases with higher fish stocking density. However, compared to data from other studies and cultivation guidelines [9, 23], these weights are not the maximum for this vegetable. Under optimal conditions, this type of vegetable can reach approximately 150 g/plant after (40 to 50) days of growth.

The increase in mustard green biomass with higher fish densities aligns with the hypothesis that higher nutrient availability, especially nitrogen compounds (TAN, NO_2^-, NO_3^-), enhances plant growth. Nitrogen is essential for plant development, and its abundance in the system possibly boosts nutrient uptake by plants. Although PO_4^{3-} levels remained stable across treatments, its sufficient presence likely met plant growth needs, showing no significant differences in uptake. This is consistent with other studies which suggest that phosphorus can reach a saturation point where additional amounts no longer significantly boost growth [24].

3.3 Microalgae growth

Optical density (OD_{680}) refers to the measurement of absorbance or turbidity of a microalgal culture at a wavelength of 680 nm using a spectrophotometer. The higher the OD_{680} value, the greater the concentration of microalgae in the culture. The data of OD_{680} over the operation time of the aquaponics – microalgae system, measured in the algae tanks and fish tanks of the three treatments was shown in Figure 3. It showed that the density of *Chlorella vulgaris* in both algal tanks and fish tanks generally increased in all three treatments over (3 to 4) weeks after being introduced into the aquaponic systems. Comparing the three treatments, the algae density in the fish and algae tanks was similar in treatments D1 and D2, but the corresponding values were generally higher in treatment D3 throughout the experiment. Additionally, the growth of microalgae biomass in all the algae tanks were not as high as in the pure culture environment (BG11 medium used during the preparation stage, where OD_{680} reached 1 to 2 after about 10 days of cultivation). This could be due to the microalgae not fully adapting to the system's water conditions as compared to the initial BG11 culture.

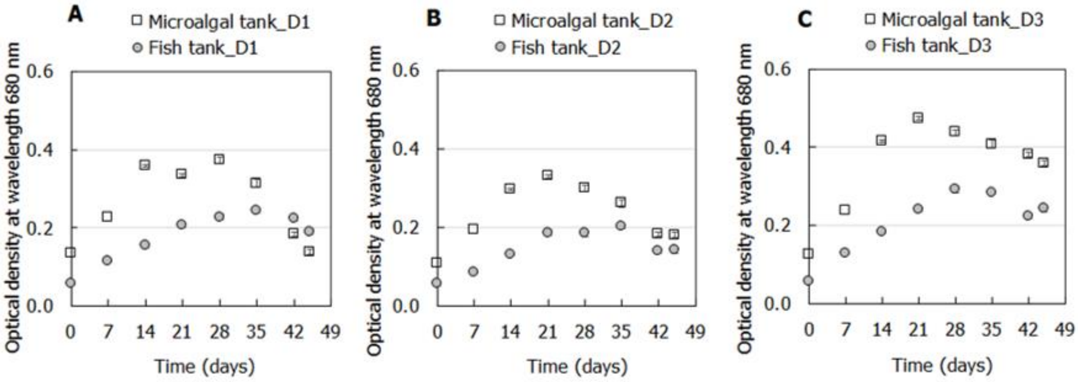

Figure 4 Optical density measurements at a wavelength of 680 nm (OD 680) over the operation time of the aquaponics–microalgae system, measured in the algae tanks and fish tanks of the three treatments with different fish stock densities including D1: 90 fish/m^3, D2: 70 fish/m^3, and D3: 50 fish/m^3.

Previous studies highlighted that light, temperature, and nutrients such as nitrogen, phosphorus, and carbon are key factors in microalgae growth [25]. Microalgae in D3 likely thrived due to better water quality, including lower TAN and NO_2^- levels, supporting healthier growth. Phosphorus is essential for microalgae, aiding cellular functions like enzyme production and energy regulation [7]. Another factor that can affect to microalgal growth is pH. According to various studies, pH is a crucial factor for *Chlorella vulgaris*, influencing several biological processes, such as nutrient uptake, photosynthesis, and enzyme activity [7, 26]. The optimal pH range for the growth of *Chlorella vulgaris* is from 7.0 to 8.5 [26]. In this study, pH levels were the lowest value in D1 pH = (6.73-7.67), while D3 pH = (6.96-7.58) had the highest algal density. This pattern indicates that pH may play a role in influencing the growth of *Chlorella vulgaris* under these conditions.

The results of this study suggest several practical implications for the design and management of microalgae-aquaponics systems. First, optimizing stocking density is critical to maintain water quality and promote both fish and plant growth. Higher densities may increase nutrient availability for plants, but this comes at the cost of lowered fish growth performance and survival. Secondly, the introduction of microalgae into aquaponic systems may benefit nutrient cycling and water quality, yes requires careful management of water chemistry to ensure optimal algal growth.

4 Conclusion

This study demonstrates the considerable influence of fish stocking density on water quality, fish growth, and plant productivity in microalgae-aquaponics systems. Higher stocking densities (D1 - 90 fish/m^3) led to increased nutrient concentrations, foster improved mustard green growth and reduce fish weight gain and survival rates. Conversely, lower densities (D3 - 50 fish/m^3) enhanced fish growth and microalgae biomass. The results indicate that moderate densities (D2 - 70 fish/m^3 or D3) offer an optimal balance between maximizing both fish and plant yields while maintaining favorable water quality. These findings provide valuable insights for designing and optimizing microalgae-aquaponics systems.

Acknowldgement

This research was funded by the Scientific Research Grant from Nguyen Tat Thanh University (project number 50/QD-NTT) and the Professor Le Tri Vien Research Fund. The research team sincerely extends their gratitude.

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Ảnh hưởng của mật độ cá nuôi đến chất lượng nước và tăng trưởng của cá điêu hồng và rau trong hệ thống thủy canh kết hợp vi tảo

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Tóm tắt Nghiên cứu này khảo sát ảnh hưởng của mật độ cá nuôi đến chất lượng nước, sự phát triển của cá điêu hồng (*Oreochromis sp*.) và rau cải ngọt (*Brassica integrifolia*) trong hệ thống thủy canh kết hợp vi tảo. Có 3 nghiệm thức mật độ cá bao gồm: D1 (90 con/m^3), D2 (70 con/m^3), và D3 (50 con/m^3). Theo dõi các chỉ tiêu chất lượng nước cho thấy hàm lượng TAN, NO_2^-, và NO_3^- tăng lên khi mật độ cá càng tăng, trong khi hàm lượng PO_4^{3-} có khuynh hướng ổn định. Nghiệm thức D1 có tổng trọng lượng cá tăng cao nhất nhưng tỷ lệ tăng trọng thấp hơn (91,9 %) so với D2 (105,6 %) và D3 (134,1 %). D3 có trọng lượng tăng trung bình mỗi con cao nhất (67,78 g) và tỷ lệ sống tốt nhất (100 %), trong khi D1 có tỷ lệ sống thấp hơn (94 %). Rau cải ngọt phát triển tốt nhất ở D1. Mật độ vi tảo tăng ở tất cả các nghiệm thức, trong đó D3 có sinh khối tảo cả trong bể tảo và bể cá cao nhất. Có thể thấy mật độ cá cao thúc đẩy sự tăng trưởng của rau thủy canh trong hệ thống, trong khi mật độ cá thấp thì cá và vi tảo tăng trưởng tốt. Điều này cung cấp những hiểu biết giá trị giúp tối ưu hóa hệ thống tuần hoàn aquaponics - vi tảo.

Key words hệ thống aquaponics – vi tảo, *Chlorella vulgaris*, mật độ cá nuôi, cá điêu hồng, cải ngọt