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The Effect of Water Spinach on the Water Quality, Antioxidant System, Non-Specific Immune Response, Growth Performance, and Carbon Balance in Red Tilapia Production

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Abstract: In this study, the compound aquaculture model of red tilapia (*O. mossambicus albina* × *O. urolepis hornorum*) and water spinach (*Ipomoea aquatica*) was used to investigate the effect of water spinach rafts on the water quality, antioxidant system, non-specific immune response, and growth performance of red tilapia and the carbon balance of payments. Red tilapia is characterized by its high adaptability to different production environments and food sources, as one of the most productive fish in aquaculture, and is well accepted in the market due to its nutritional and organoleptic characteristics. The experiment lasted for nine weeks and included two systems: the red tilapia-water spinach raft aquaponics (AP) system with 10% cover ratio with water spinach floating beds, and the aquatic monoculture (AM) system with only red tilapia. The total phosphorus (TP), total nitrogen (TN), and nitrate nitrogen ($NO^-{}_3$-N) in the AM were higher than those in AP from the fifth to ninth week. On the second, third, fifth, and sixth weeks, the ammonia nitrogen ($NH_4{}^+$-N), in the AM was higher than those in the AP. From the seventh week, the pH of the AM was significantly lower than the AP, while the nitrite nitrogen ($NO^-{}_2$-N) was significantly higher than the AP. The water quality index of the AP was better than that of the AM, indicating that water spinach can remove the nutrients from aquaculture water bodies. The average daily gain and specific growth rate (SGR) of fish in AP were higher than those in the AM. The acid phosphatase (ACP), alkaline phosphatase (AKP), and catalase (CAT) activities in the hepatopancreas of red tilapia in the AP were also significantly higher than those in the AM, while the malondialdehyde (MDA) in the AP was lower than the AM. The serum ACP and CAT of red tilapia in the AP were also higher than those in the AM, while the MDA of fish in the AP was lower than the AM. The results showed that both the experimental group and the control group were carbon sources and released greenhouse gases into the atmosphere, but the total carbon emissions of the red tilapia and the water spinach symbiotic system in the experimental group was significantly lower than that of the control group ($p < 0.05$). These results demonstrated that the application of water spinach rafts in aquaponics can not only improve the water quality, but also improve the growth performance, antioxidant system and non-specific immune responses of red tilapia, while promoting the utilization of organic matter in the aquaculture system, improving the ecological benefits in terms of the carbon income and expenditure.

Keywords: aquaponics system; water purification; production performance; carbon balance of payments

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Key Contribution: In this study, on the basis of previous research, considering to the problems of traditional farming, an innovative red tilapia and water cabbage symbiotic breeding system was assessed, showing that water cabbage can reduce the total phosphorus (TP), total nitrogen (TN), and nitrate nitrogen (NO_3-N). It was also able to improve the fish growth performance compared to single aquaculture, and improve the ecological benefits in terms of the carbon income and expenditure.

1. Introduction

In China, freshwater aquaculture ponds are a traditional aquacultural system with a long history, and they account for about half of global aquaculture [1]. However, high stocking density and overfeeding not only leads to adverse health, but also causes feed and manure to accumulate in the ponds [2]. The large amount of carbon, nitrogen, and phosphorus in fish feed are utilized with low efficiency and deposited into the aquacultural wastewater [3]. These nutrients are then discharged into natural water bodies when the wastewater is discharged, causing eutrophication of those water bodies [4]. After discharge of eutrophicational water into the environment, nitrogen and phosphorus are enriched in the water and may support out-of-control growth of some plankton and algae, leading to water blooms, red tides, and other phenomena, resulting in insufficient dissolved oxygen in the water and the death of fish. Aquaponics systems may be a potential strategy to address these problems.

Aquatic systems play a major role in the global carbon cycle, and different aquatic systems can act as carbon sources or carbon sinks. Aquatic plants are important components of aquatic ecosystems and play important roles in maintaining ecosystem diversity and stability. Carbon is an indispensable element for plant growth. There are many sources of carbon for aquatic plants, including air, water, pore water of sediments, and so on. Green and low-carbon development is the strategic choice of China to achieve sustainable development and cope with the global greenhouse effects. In order to achieve this goal, reducing and controlling the content of greenhouse gases such as CO_2 (carbon dioxide) in the atmosphere is an important step. One strategy to manage greenhouse gas emissions is to collect greenhouse gases from the atmosphere and fix them, which can be achieved in two ways: industrial carbon fixation and biological carbon fixation. Fishery carbon sequestration is an important field in the research of biological carbon sequestration. The total output of aquaculture in China in 2000 exceeded the catch volume, so aquaculture has become an important part of the fishery industry. Aquaculture can not only provide high-quality protein to large numbers of people, but can also contribute to the income of fishermen and farmers and the structural adjustment of the fishery industry. Given the current situation of aquaculture and the need for sustainable development, a high-efficiency and low-carbon mode will be the inevitable choice for the development of the aquatic fishery industry, guaranteeing food safety and realizing the goal of energy conservation and emission reductions.

Aquaponics systems are a sustainable agricultural development model that repossesses waste from aquaculture as an input for crop production [5,6]. As a type of aquaponics system, raft aquaponics is a widely used aquaponics system with fewer environmental impacts [6,7]. In raft aquaponics systems, aquatic plants are fixed on floating rafts, absorbing of phosphorus and nitrogen by their roots from the water, resulting in an improvement in water quality and eutrophication [8,9]. Plants also release oxygen through their roots, providing favorable conditions for the microorganisms, especially nitrifying organisms. Bacteria accelerate the conversion of $NH^{+}{}_{4}$-N and $NO^{-}{}_{2}$-N into $NO^{-}{}_{3}$-N, which is not only conducive to the utilization of aquatic plants, but also reduces the threat of $NH^{+}{}_{4}$-N and $NO^{-}{}_{2}$-N to fish health.

Red tilapia (*O. mossambicus albina* × *O. urolepis hornorum*) belongs to the family of *Perciformes*, *Cichlidae*, and is a hybrid tilapia arising from Nile tilapia (*O. niloticus*) and the Mozambique tilapia (*O. mossambicus*) [10]. It has a bright and attractive body color, delicious meat, can be sold at lower price, and has become a widely cultivated species with both ornamental and economic value [11]. Red tilapia is adaptable to different environments, and is a widely salt-tolerant fish. Its suitable temperature range is 16 to 35 °C, and its optimal temperature is 25 to 32 °C. Red tilapia generally live in the bottom layer of ponds, lakes, rivers, and other water bodies, and swim to the upper layer to forage when the water temperature rises during the day. Red tilapia are an omnivorous fish, and the larvae usually feed on a variety of zooplankton and phytoplankton. Adult fish have a varied diet consisting of various zooplankton, algae, young aquatic plants, organic debris, and aquatic insects.

Aquatic plants can be divided into three categories: aquatic vascular plants, aquatic mosses, and higher algae. There have been many studies on the water purification effect of aquatic vascular plants. Many studies have shown that aquatic plants can be used in sewage treatment to effectively remove pollutants from the water. The effects of aquatic plants in water pollution control include absorption, enrichment, physical effects, biological sensory effects, transmission and oxygen release, and synergistic degradation. Following the growth of aquatic plants, the transfer of heavy metals, nitrogen and phosphorus and other nutrients can be realized by their removal from the water body. Aquatic plants can reduce the flow rate of water, which can reduce the impact of wind and waves on the bottom and have a positive effect on the settling of suspended solids on the surface. Moreover, insoluble colloidal material is also adsorbed by the root system to exchange, integrate, and precipitate with organic matter and other ions. Aquatic plants can also provide an environment for the surrounding microorganisms to attach to as well as releasing oxygen from their root system. The microenvironment formed by the oxidation state is conducive to the decomposition of organic matter and the growth and reproduction of nitrification bacteria. Water spinach used in this study is a semiaquatic tropical macrophyte with a well-developed root system that can even adapt to growth in swamp areas [12]. This plant is an important vegetable species in southern China and Southeast Asia [13].

Studies of tilapia-water spinach aquaponics systems have shown that the yield of water spinach can be increased by increasing the duration of light [14]. The survival rates of crayfish in crayfish-water spinach aquaponics system were better than in a crayfish monoculture system [15]. However, there are limited data reporting the impact of aquaponics systems on the growth performance, antioxidant system and non-specific immune responses of fish. The antioxidant system and immune system of fish are vital to their health [16]. Catalase (CAT) is a significant component of the primary antioxidant system and is often used to determine the antioxidant capacity [17]. Changes in $NH^{+}{}_{4}$-N in the water was shown to modify the CAT activity of crucian carp [18]. Malondialdehyde (MDA) is the end product of lipid peroxidation and is used as an indicator of oxidative damage [19]. Alkaline phosphatase (AKP) and acid phosphatase (ACP), critical indicators of non-specific immune responses, can reflect the metabolism and immunity of fish [20].

2. Materials and Methods

2.1. Pond and Tilapia-Water Spinach Raft Aquaponics System Design

All aquaculture processes in this study were carried out in Jinyang Aquaculture Co., Ltd., located in Guangzhou (China) (22°92′07″ N, 113°54′73″ E). An aquaponics system study based on *Ipomoea aquatica* and *O. mossambicus albina* × *O. urolepis hornorum*. was conducted in six concrete ponds (length × weight × height = 4 m × 4 m × 1.2 m). The roof was transparent polyethylene film, which removed the influence of precipitation while allowing in light. The mean air temperature in the experimental area was 23–28 °C, and the mean relative humidity in the greenhouse was 78.0%, with maximum 92.7% and minimum 42.1%. The average outdoor light intensity was 25,480 lx and the average indoor light intensity was 21,320 lx. The light transmittance in the greenhouse was 73–76%. Healthy red tilapia (*O. mossambicus albina* × *O. urolepis hornorum*) (body weight = 18.54 ± 1.78 g) were purchased from the Jinyang Aquaculture Co., Ltd. Guangzhou. The fish were kept for two weeks to adapt to the environment. Subsequently, 1200 healthy red tilapia were randomly allocated to six ponds; three were control ponds and three were for the experimental trials. Each pond held 200 red tilapia, which were fed twice a day with commercial feed (Fenghua tilapia compound feed, floating water particle type, crude protein $\geq$ 33%). The feed is provided by Qingfeng Feed Wholesale Commercial Bank from Foshan, Guangdong, China. The source of water for all ponds in the experiment was the same. The initial water level was 0.80 m, and the water level was 0.71 m at the end of the experiment. No supernumerary water was added during the experiment. In order to meet the fish demand for oxygen, air compressors (HG-200; Shenzhen, China) were continuously used in each pond. The Animal Care Committee of South China Agriculture University (Guangzhou, China) approved the current study, under the trial registration number G026, and the research was accomplished according to the Experimental Animal Management Law of China.

Water spinach (*Ipomoea aquatic*) is a common vegetable variety and was collected from the Jinyang Aquaculture Co., Ltd. The water spinach was fixed on floating rafts. The vegetable growing raft used in this experiment was independently developed by our research group (patent number: ZL2016 2 0287095.4). The floating raft was made of polyethylene, which is light and resistant to corrosion. There were also nets in the underwater part of the floating rafts to prevent the fish from harming the roots of the water spinach. The cover ratio of water spinach rafts in the experimental group was 10%. *Ipomoea* aquatic floating rafts was planted on the surface of each concrete pond. Water spinach was not planted in the concrete ponds of the control group (Figure 1).

Figure 1. The aquaponics system used in this study. The pictures were collected on the first day of the experiment. (**a**) Red tilapia, (**b**) water spinach, (**c**) red tilapia aquatic monoculture system, (**d**) red tilapia-water spinach raft aquaponics system with 10% plant cover.

The vegetable floating base used in this experiment was self-developed and created by the research group with a patented product. The vegetable floating base was divided into three parts: the upper column frame, which was cylindrical, hollow, and removable; the main part of the floating base, which was disc-shaped and had a circular cavity and which could be inlaid within the first part; and the underwater vegetable basket, which could be inlaid within the second part. The production process of the floating base is involved a polyethylene hot melt injection mold which was easy to disassemble, and because of its material, the floating base was easy to deform, had excellent performance in high temperature resistance and corrosion resistance, and could be reused.

2.2. Measurement of Water Quality

A water column sampler was used to collect water samples every Monday before feeding. Water samples (100 mL) from each pond were taken within 20 cm of the water surface at four locations, and were later mixed. All water quality indexes, including dissolved oxygen (DO), temperature, pH, ammonia nitrogen ($NH^{+}{}_{4}$-N), nitrite nitrogen ($NO^{-}{}_{2}$-N), nitrate nitrogen ($NO^{-}{}_{3}$-N), total nitrogen (TN), and total phosphorus (TP), were detected according to standard methods [21]. All analyses were carried out in triplicate.

2.3. Monitoring and Growth Performance of Red Tilapia

The aquaculture experimental period last from 29 August to 24 October. The health status and mortality of the red tilapia were monitored daily. On the first day of the experiment (29 August), fifty fish were randomly selected from each pond, and body weight was measured with an electronic balance (accurate to 0.01 g). During the experiment, the red tilapia were fed at 2% of their biomass, and the daily feeding amount of each pond was the same. At the end of the experiment (24 October), fifty red tilapia from each pond were randomly selected to measure their body weight. The fish were not fed for twenty-four hours before weighing. The average daily weight gain and specific growth rate of the fish were determined separately.

$$\text{Average daily gain} = (\text{Wt} - \text{W0})/\text{t}.$$

$$\text{Specific growth rate SGR } (\%/\text{day}) = \frac{\ln(\text{Wt}) - \ln(\text{W0})}{\text{t}} \times 100$$

Note: ln (Wt): average weight of fish on day t; ln (W0): average weight of fish on day 1, t: breeding days (day).

2.4. Antioxidant Status and Non-Specific Immune Responses

At the end of the experiment, thirty fish were randomly collected from each pond. After anesthesia with MS-222, a blood sample was collected from the caudal vein using a syringe and clotted at 4 °C overnight. Serum samples were collected and stored at −80 °C. The fish were dissected immediately with sterile scissors. The hepatopancreas, spleen, and kidneys were placed in liquid nitrogen, then stored at −80 °C. The tissue was rinsed three times in pre-cooled PBS, filter paper was used to absorb water on the surface of the sample, and 0.1 g of tissue was obtained for testing using an analytical balance. We added pre-cooled PBS in a ratio of 1:9, and used a homogenizer to make 10% tissue homogenate. After centrifugation, the supernatant was collected and stored at −80 °C for the immune and antioxidant assays.

The antioxidant status (MDA and CAT) and non-specific immune responses (ACP and AKP) were measured with commercial assay kits (Nanjing Jiancheng Institute, Nanjing, China) according to the instructions of the manufacturer. Catalase (CAT) was determined by the visible light method and promalondialdehyde (MDA) using the TBA method. Acid phosphatase (AKP) and alkaline phosphatase (ACP) were microlabeled. The kits were purchased from Nanjing Jiancheng Institute of Biology, and their operation was carried out in strict accordance with the instructions.

2.5. The Carbon Balance of Payments

2.5.1. Determination of the Solid Carbon Content

Red tilapia were collected before stocking and harvesting and dried at 110 °C to a constant weight to determine the moisture content. The oven-dried samples were ground and screened and the powder was used to determine the carbon content.

2.5.2. TOC Determination of the Water Body

First, the response values were measured with standard series solutions of different concentrations, and the calibration curve of organic carbon was drawn. The samples acidified to pH < 2 in a certain volume were added to the Total Organic Carbon(TOC) and Total Nitrogen German element liquiTOC Analyzers. The inorganic carbon was removed by aeration and then introduced into the high-temperature oxidation furnace, and the corresponding response values were recorded. According to the response values, the total organic carbon concentration was calculated from the calibration curve. The total organic carbon concentration was equal to the total carbon minus the total inorganic carbon concentration.

2.6. Statistical Analyses

The data in this study were expressed as the mean ± SEM (standard error of mean; the standard error of the sample mean was used to measure the gap between the sample mean and the population mean). All analyses were performed using Statistical Product and Service Solutions (SPSS 21.0, IBM, New York, NY, USA), a powerful statistical analysis tool widely used for data processing and statistical analysis in social science, business, medicine, education, and more. The statistical analysis was conducted by independent sample *t*-test. The *t*-value represented the difference between the sample mean and the population mean divided by the value of the standard error (SE), which was used to determine whether there was a significant difference between the mean of the two samples. In the independent sample *t*-test, the result was greater than the cut-off value, which indicated a significant difference between the means of the two samples.

3. Results

3.1. Modulation of Water Quality in the AP and AM System

The red tilapia-water spinach raft aquaponics system was treated as the experimental group, and red tilapia monoculture system was treated as the control group. The aquaculture experiment lasted for nine weeks. The water quality of each group was tested weekly, including the water temperature, dissolved oxygen, NH_4^+-N, NO_2^--N, NO_3^--N, TN, and TP. Throughout the experiment, due to continuous mechanical oxygenation, there was no significant difference in dissolved oxygen between the experimental group and the control group (Figure 2b). Since no warming equipment was used, the water temperature was between 23 °C and 30 °C (Figure 2a). These levels of dissolved oxygen and temperature ensured the growth of the red tilapia and water spinach. The results showed that from the third to the ninth week, the pH value of the control group was significantly lower than that of the experimental group ($p < 0.05$) (Figure 2c). At 2, 3, 5, and 6 weeks, the NH_4^+-N content of the experimental group was significantly lower than that of the control group ($p < 0.05$) (Figure 2d). From the third to the ninth week, the TN of the experimental group was significantly lower than that of the control group ($p < 0.05$) (Figure 2g). From the fourth week to the ninth week, the TP and NO_2^--N in the experimental group were significantly lower than in the control group ($p < 0.05$) (Figure 2e,h). These results indicated that the AP system significantly slowed down the acidification and deterioration of water quality.

3.2. The AP System Improved the Antioxidant and Non-Specific Immune Response of Red Tilapia

In order to further explore the effects of the aquaponics system on the antioxidation and non-specific immune indicators of red tilapia, the serum, hepatopancreas, kidneys, and spleen of red tilapia were collected after nine weeks. The results showed that the serum MDA of the AP system was significantly lower than that of the AM system (Figure 3d), and the serum CAT and ACP content of the AP system was significantly higher than that of the control group (Figure 3a,c). MDA in the hepatopancreas and spleen of the AP system was significantly lower than that of the AM system (Figure 3d). The CAT in the hepatopancreas and kidneys of the AP system was significantly higher than that of the AM system (Figure 3c). The ACP in the hepatopancreas and spleen of the AP system was significantly higher than that of the AM system (Figure 3a). The AKP in the hepatopancreas of the AP system was significantly higher than that of the AP system (Figure 3b). These results indicated that the antioxidant and non-specific immune indicators of red tilapia in the aquaponics system are better than those in the aquatic monoculture system.

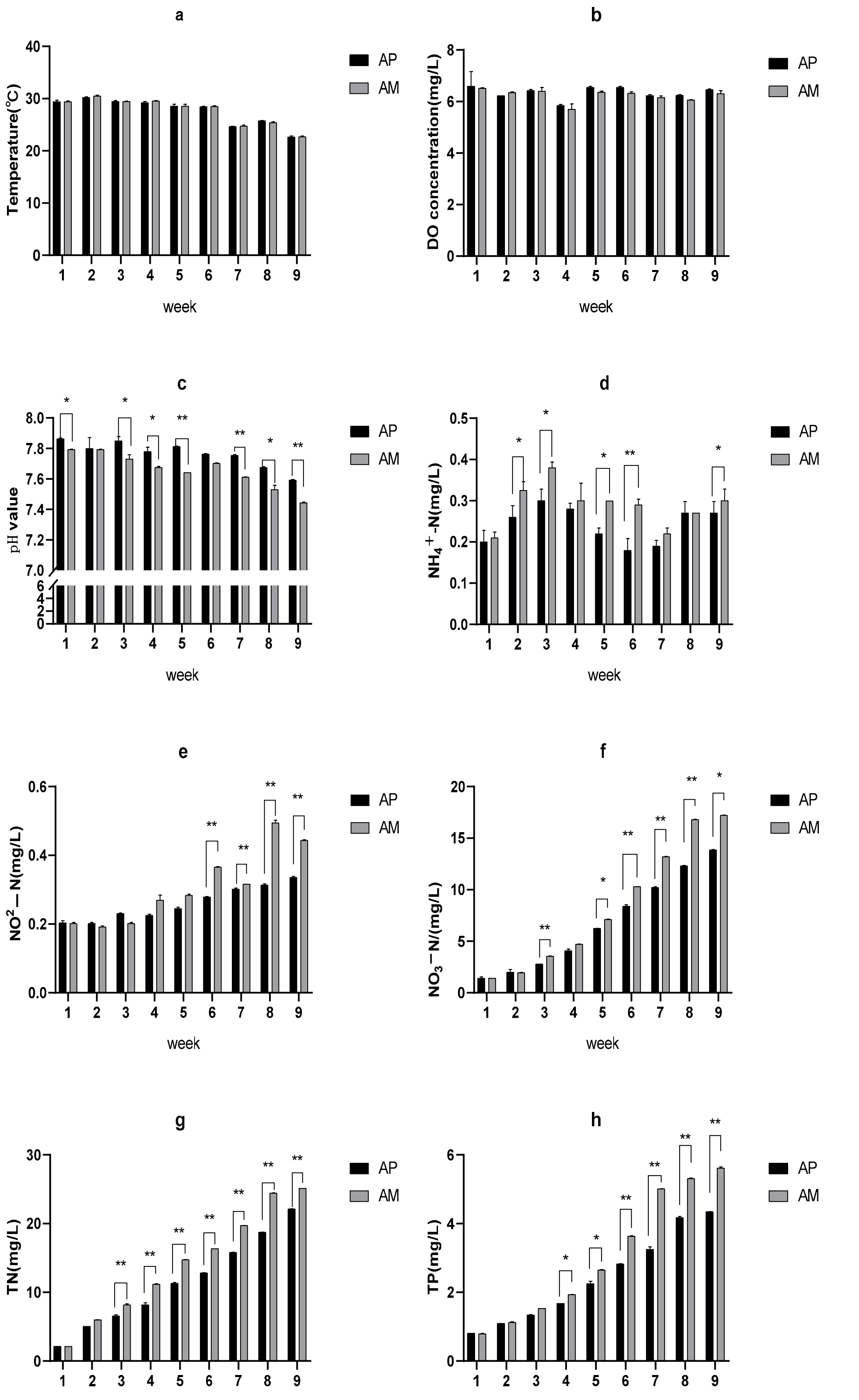

Figure 2. The modulation of the water quality, including the temperature, dissolved oxygen, pH, TN, NH_4^+-N, NO_2^--N, NO_3^--N, and TP in the AP and AM systems at different sampling weeks. The AP

system was covered at a 10% ratio with water spinach floating beds and the AM system included only red tilapia. (**a**) Temperature, (**b**) dissolved oxygen, (**c**) pH, (**d**) ammonia nitrogen (NH_4^+-N), (**e**) nitrite nitrogen (NO_2^--N), (**f**) nitrate nitrogen (NO_3^--N), (**g**) total nitrogen (TN), (**h**) total phosphorus (TP). (n = 3; * $p < 0.05$; ** $p < 0.01$). Note: There is no significant difference between the stage data. ($p > 0.05$). The bars in the graph represent standard deviations.

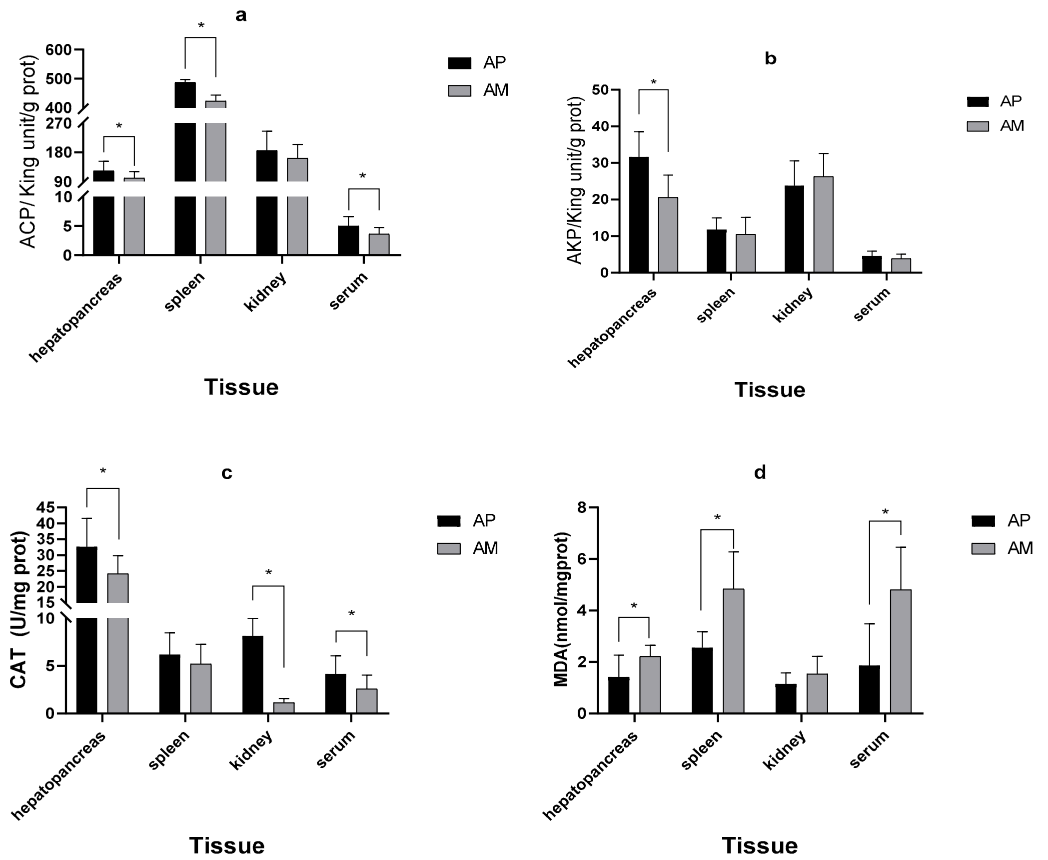

Figure 3. The antioxidant and non-specific immune response of red tilapia in AP and AM systems. The hepatopancreas, kidneys, spleen and serum of red tilapia from different systems were collected after nine weeks of aquaculture. (**a**) Acid phosphatase (ACP), (**b**) alkaline phosphatase (AKP), (**c**) catalase (CAT) and (**d**) malondialdehyde (MDA). (n = 3; * $p < 0.05$). Note: There is no significant difference between the stage data. ($p > 0.05$). The bars in the graph represent standard deviations.

3.3. The Red Tilapia-Water Spinach Raft Aquaponics System Improved the Production Performance of Red Tilapia

In order to further understand the impact of the AP system on the growth performance, the survival rate, average daily gain, and specific growth rate of red tilapia in the AP and AM systems were measured (Table 1). The results showed that the average weight, average daily gain and specific growth rate of red tilapia in the AP system were significantly higher than those in the AM system. The results also showed that there was no significant difference in the survival rate of tilapia in different experimental treatments. These results showed that the AP system improved the growth performance of red tilapia significantly.

Table 1. Fish growth.

	AP	AM
Initial stock (g)	600	600
Final stock (g)	581	573
Survival rate (%)	96.83 ± 2.05	95.50 ± 1.80
Initial body weight (g)	18.54 ± 1.78	18.54 ± 1.78
Final average body weight (g)	47.52 ± 1.93 *	40.56 ± 1.16
Average daily gain (g/d)	0.51 ± 0.003 *	0.39 ± 0.011
Specific growth rate (SGR)	1.65 ± 0.12 *	1.37 ± 0.09

The fish growth performance analysis in different treatments. In order to measure the final growth performance, ten tails of red tilapia were randomly sampled from each parallel at the ninth week. SGR = (lnWt − lnW0)/t(days) × 100%, Wt and W0 are the wet weight at the ninth week and at the first week. Note: The same line with * indicates a significant difference ($p < 0.05$), and the same line without * is not significant ($p > 0.05$).

3.4. Water Spinach Is a Carbon Sink in the Red Tilapia-Water Spinach Raft Aquaponics System

The dry matter and carbon contents of red tilapia, water spinach, and feed were determined in order to study the changes in the carbon budget in fish and vegetable co-culture (Table 2). The dry matter carbon content of red tilapia in the experimental group and control group was lower than that in the free range fish, but that of water spinach was higher than that in free range.

Table 2. Changes in the aquaculture carbon budget in the aquaponics system.

Items	Dry Matter/%		Carbon Content (Dry Weight)/%	
	Stocking	Harvesting	Stocking	Harvesting
red tilapia in experimental group	26.47	27.19	48.61	46.88
red tilapia in control group	26.47	28.26	48.61	45.96
water spinach	7.05	6.56	31.95	39.11
feed	91.25		45.72	

The carbon budget of aquaculture ponds helps to explain the role of the whole ecosystem in the carbon cycle in the environment, and can help us to understand the migration and fixation of carbon in this model.

4. Discussion

Temperature, dissolved oxygen, pH, TN, NH_4^+-N, NO_2^--N, NO_3^--N, and TP are the vital indexes to measure the water quality of aquatic systems [22]. Changes in NH4$^+$-N and pH in a water body can directly affect the health and production performance of fish [23–25]. The growth and development of bacteria, algae, and phytoplankton in the water body are affected at a lower pH [26,27]. When the pH value of the water is too high, it will corrode the fish body, and in serious cases dissolve the surface mucosa of the fish, resulting in the fish being unable to regulate its own osmotic pressure and dying [28]. In this study, the variation range of pH in the AP and AM systems was 7.6–7.9 and 7.4–7.8, which were within the normal growth range of red tilapia. However, the pH value of the AP system was significantly higher than that of the AM system from the seventh to the ninth week. Previously, studies have shown that the optimum pH for nitrification is 8.5; however, the optimum pH for aquaponics would likely be between 6.5 and 7.0 to maximize the performance of the fish and cash crops [29]. The AP system reveals the potential to slow down the water acidification. Approximately 25% of nitrogen and less than 30% of phosphorus in feed are absorbed and utilized by the fish, most of the phosphorus and nitrogen are released into the water in the form of uneaten pellets and feces [30]. The excess nitrogen in the system mainly exists in the form of NH_4^+-N, NO_2^--N, and NO_3^--N in the water. NH_4^+-N and NO_2^--N are toxic to fish, injuring the gill tissues, impairing respiration, and causing the fish to die [31]. Through nitrification, NH_4^+-N and NO_2^--N can be converted into NO_3^--N by nitrifying bacteria in the water. NO_3^--N is critical for plant growth [32]. The NO_3^--N in the AP system was significantly lower than that of the

AM system from the third week to the end of the experiment. The NH_4^+-N in the AP system was significantly lower than that of the AM system. The NO_2^--N in the aquaculture water of AP was significantly lower than that in the AM system from the sixth to the ninth week. These data suggested that the red tilapia-water spinach raft aquaponics system reduced accumulation of NH_4^+-N and NO_2^--N in the system. Studies have shown that a reduction in NH_4^+-N and NO_2^--N in water is beneficial to the production performance of the system [16,33]. The results indicated that the improvement in NH_4^+-N and NO_2^--N may be related to the improvement in red tilapia growth performance.

Nitrogen and phosphorus are the limiting factors for water eutrophication [34]. Plants absorb nitrogen and phosphorus from water to achieve water purification. Aquatic plants have been applied to eutrophication control of lakes, reservoirs and other polluted water bodies [35,36]. Studies have shown that water hyacinth (*Eichhornia crassipes*) can effectively remove nitrogen and phosphorus from wastewater from dairy farms [37]. A recent study found that aquaculture wastewater can be used as organic fertilizers for tomatoes planting, improving the utilization efficiency of nitrogen and phosphorus [38]. In this study, the TN and TP of the AP system were significantly lower than those of the AM system from the fourth week to the end of the experiment. The variation trend of TP and TN in the AP system showed that some of the nitrogen and phosphorus available in the water were absorbed by the water spinach, reducing the amount of nitrogen and phosphorus in the culture wastewater. More importantly, a reduction in TN and TP reduces eutrophication. Studies have found that adding microalgae to aquaponics systems seems to increase nitrogen utilization efficiency and improve water quality [39,40]. Investigating suitable algae and water spinach cultivation acreage should be further explored to optimize the red tilapia-water spinach raft aquaponics system.

Oxidative stress and reactive oxygen species (ROS)-mediated toxicity have been considered as the mechanism by which environmental conditions cause fish organ damage [41]. Under normal physiological conditions, there is a dynamic equilibrium between ROS and the antioxidant defense system. When the physiological antioxidant protection cannot counteract the elevated ROS levels, cellular oxidative stress will occur [42,43]. When oxidative stress exceeds the scavenging capacity of the antioxidant system, MDA, the final product of lipid peroxidation, will be generated [44]. MDA can be used as an indicator to reflect biological toxicity and cell structure damage [45]. The variation in MDA can reflect the damage degree of aquatic animals [46]. Catalase (CAT) is one of the vital enzymes in the antioxidant system, which can remove ROS and reduce its damage to cells [47]. Studies have shown that CAT activity is related to NH_4^+-N levels in aquaculture water [23]. For the non-specific immune system, AKP and ACP are both critical enzymes of macrophage lysosomes in organisms, as well as hydrolytic enzymes for non-specific immunity [20,48]. AKP and ACP help to accelerate the phagocytosis of phagocytes and the degradation of pathogenic microorganisms [49]. Related to the growth and development of aquatic animals, AKP plays an important role in protein synthesis and nutrient absorption and utilization [50]. AKP and ACP activities show a positive effect in the defense against external pathogenic bacteria and microbial invasion [51]. In research on *Houttuynia cordata* floating beds in a tilapia pond culturing system, the cultivation of Houttuynia cordata significantly increased the CAT and AKP activity of tilapia serum [6]. Similar studies have also found that tilapia in aquaponics systems contain higher level of interleukin-10 (IL-10), tumor necrosis factor α (TNF-α), and interleukin-8 (IL-8) in their serum [52]. In this study, the hepatopancreas, spleen, and serum ACP levels of red tilapia in the AP system were significantly higher than those in the AM system. The AKP concentration in the hepotopancreas of red tilapia in the AP system was significantly higher than that in the AM system. The CAT activity of the hepatopancreas, kidneys, and serum of red tilapia in the AP system was significantly higher than in AM system. In contrast, the hepatopancreas, spleen, and serum MDA level of red tilapia in the AP system was significantly lower than that in the AM system. These results suggested that water spinach rafts could improve the stress resistance of co-cultured red tilapia. The carbon expenditure in aquaculture ponds helps to illustrate the role of the whole ecosystem in the carbon cycle in the environment, and can help us to understand the migration and fixation of carbon in this mode. The results showed that both the experimental group were and the control group showed carbon sources and released greenhouse gases into the atmosphere, but the total carbon emissions of the red tilapia in the water spinach symbiotic system in the experimental group were significantly lower than that of the control group ($p < 0.05$). Water spinach absorbs fixed carbon from the water and environment through direct photosynthesis. Because it provides a relatively suitable microenvironment for microorganisms in the water, it also promotes the decomposition of organic matter and contributes to the growth of nitrifiers. The water spinach also purified the water quality, improving the utilization rate of feed and allowing more carbon to be fixed in the fish. Water spinach played an important role in this system and effectively improved the utilization rate of substances in the system. It is worth noting that deviations may have occurred in the water carbon content detection, fixed sampling of different aquaculture ponds gave the average of the water, but actually cannot completely truly represent the average of water carbon content, because of physical and chemical factors such as the temperature affecting the test results.

The improvement in water quality in aquaponics systems has been verified in fresh water and sea water [22,53]. The aquaculture water environment is a key factor in impacting fish growth [54]. In a recent study on Houttuynia cordata floating rafts, it was found that fish body weights from treatments with 10% and 15% floating raft coverage were both higher than that of fish from the control group [6]. Shilta et al. also reported that a reduction in NH_4^+-N and NO_2^--N in aquaculture water significantly improved the growth performance of fish [55]. In this study, the final average body weight, average daily gain, and specific growth rate of red tilapia in the AP system were better than those in the AM system. Water spinach floating rafts improved the fish production performance. The results indicated that this may have been related to the improvement in water quality. However, other factors may influence the process and needs to be further investigated.

5. Conclusions

The aquaponics system with water spinach floating rafts led to significant improvements in water quality. The acidification of aquaculture water and accumulation of ammonia nitrogen were both slowed. In addition, the antioxidant and non-specific immune responses of red tilapia were better than those in aquatic monoculture system. Interestingly, the water spinach floating rafts improved the growth performance of red tilapia in the system. These results provide a scientific basis for the application of water spinach and red tilapia aquaculture.

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