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Original Research

Evaluating the Impact of Diverse Types of Green Vegetables on Snakehead Fish (*Channa Striata*) Nursery through an Eco-Friendly Aquaponic Approach

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Abstract

The increasing demand for snakehead fish *Channa striata* consumption and the efforts to sustain its production has driven the need to develop efficient and environmentally friendly aquaculture technologies. As an innovative farming technique, aquaponics achieves these goals by mitigating emissions, optimizing resource efficiency through water reuse, and fostering economic benefits through sustainable cultivation of fish and plants. This study assesses the impact of lettuce (A), pak choi (B), and mustard greens (C) on water quality, hemato-biochemical parameters, and production performance of snakehead fish cultured in aquaponic systems. The research findings conclude that using different plant species significantly impacted water quality, nitrogen-oxidizing bacteria abundance, hemato-biochemical parameters, and production performance during the study. Treatment A resulted in the highest production performance of snakehead fish with a survival rate (SR), weight-specific growth rate (SGR_W), length-specific growth rate (SGR_L), and feed conversion ratio (FCR) measuring 90.33±2.08%, 3.37±0.22% day^{-1}, 1.23±0.10% day^{-1}, and 0.79±0.05, respectively. Treatment A also yielded lower stress levels and improved water quality. The better nitrogen retention (NR) and phosphorus retention (PR) by lettuce plants, reaching 87.08±0.56% and 11.75±0.19%, respectively, led to the highest plant productivity.

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The results indicate lettuce's potential as a promising phytoremediation plant for future snakehead fish nursery cultivation.

Keywords: snakehead fish, aquaponic, production performance, hemato-biochemical, water quality

Introduction

The production of Channidae fish (e.g., snakehead fish, *Channa argus*) in 2020 constituted a significant share of 10.5 percent of the overall output of air-breathing fish, reaching a notable quantity of 6.2 million tons [1]. The snakehead fish, *Channa striata*, represents a high-value commodity suitable for cultivation due to its significant economic potential. This fish possesses advantages such as an excellent growth rate and the ability to thrive in high stocking densities [2]. Moreover, researchers recognize the snakehead fish as a freshwater fish with medicinal attributes due to its significant content of bioactive constituents like albumin, amino acids (glycine, lysine, and arginine), and fatty acids. These components play crucial roles in various physiological processes, including antinociception, gastroprotection, disease resistance, antioxidant mechanisms, and the facilitation of wound healing [3].

The considerable natural yield of snakehead fishing raises apprehensions regarding the potential for overfishing, resulting in a decrease in wild stocks. To tackle this concern, cultivating snakehead fish emerges as a viable solution, guaranteeing consistent and sustainable availability, thus alleviating the adverse effects of overfishing on natural populations [4]. Nonetheless, the effluents released directly into open water from conventional aquaculture activities constitute a significant peril to aquatic ecosystems, giving rise to pollution, nutrient enrichment, and habitat modification, among other concerns [5]. Thus, addressing these issues necessitates adopting comprehensive, ecologically responsible, and sustainable approaches to harvested goods [6, 7].

The aquaponics system, characterized as an almost nonexistent discharge system, presents a potential remedy by enhancing nutrient and wastewater reutilization within the framework. This approach curtails detrimental emissions from aquaculture locales, optimizes land and water usage (with water reuse efficiency of 95–99%), and concurrently generates economic gains from sustainable fish and plant cultivation [6, 8, 9]. The aquaponics system guarantees nutrient recycling and increased profitability by cultivating multiple profitable harvests within the same production process [6, 9].

Preliminary research on the snakehead fish nursery phase using the Recirculating Aquaculture System (RAS) highlights the influence of varying fish densities on production performance response, fish hemato-biochemical profiles, and water quality within the system [4, 10]. Moreover, this prompts the need to further optimize snakehead fish cultivation in a RAS-aquaponic hybrid system, including exploring different plant species. Understanding the impact of diverse plant species on snakehead fish cultivation can offer insights into enhancing aquaponics efficiency and productivity [11]. As a result, each plant species possesses unique nutrient uptake traits, root structures, and growth rates, affecting nutrient cycling, water filtration, and oxygenation, consequently influencing fish growth, health, and production outcomes [6, 11].

Therefore, investigating the use of various plant species in snakehead RAS-aquaponic hybrid systems is crucial. Thus, using the RAS-aquaponic hybrid system, this study evaluates the effects of different leafy green species on production performance, hemato-biochemical parameters, and water quality in snakehead fish nursery cultivation. The outcomes contribute to refining sustainable RAS-aquaponic practices for snakehead fish cultivation, addressing the demand for eco-friendly food production methods amid global challenges, and emphasizing system sustainability.

Material and Methods

Experimental Design

This study employed a completely randomized design with three treatments involving distinct types of leafy greens: lettuce *Lactuca sativa* (A), pak choi *Brassica rapa* (B), and mustard green *Brassica juncea* (C), and each replicated three times. The snakehead fish and leafy greens were cultured within a Recirculating Aquaculture System – an aquaponic hybrid system.

Preparation of a Recirculating System and Test Fish

The containers employed in this study consisted of vegetable containers, fish tanks, and filter containers, each comprising nine units. The vegetable containers and fish tanks were constructed from fiber, exhibiting a rectangular shape measuring 60 × 50 × 40 cm. The filter containers were cylindrical and made of plastic, with a diameter of 30 cm and a height of 45 cm, and the filters were filled with bioballs, limestone, and crushed stone as the filter media.

The Recirculating Aquaculture System – Aquaponic Hybrid System Installation is illustrated in (Fig. 1). The installation is kept outdoors to receive direct sunlight and is sheltered with transparent plastic fiber to protect the system from rainfall. Snakehead fish used in the experiment had an average initial weight of 1.70±0.04 g and an average initial length of 5.67±0.07 cm.

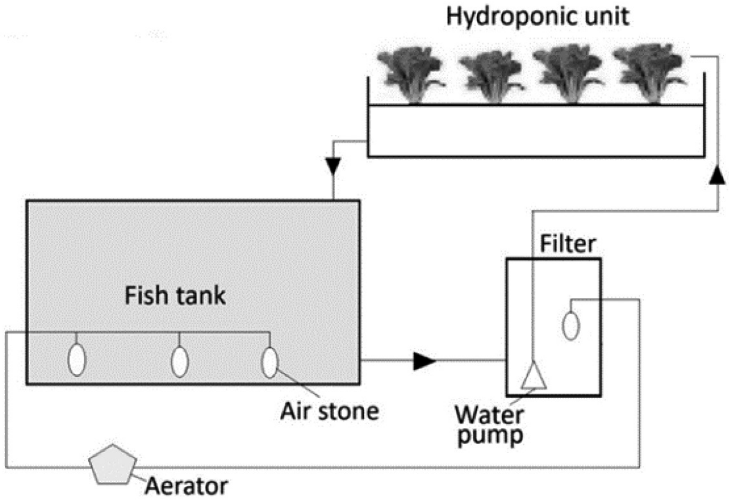

Fig. 1. The Recirculating Aquaculture System - Aquaponic Hybrid installation.

Fish and Leafy Greens Maintenance

Snakehead fish were selected and transferred to the experimental tanks at a density of 4 individuals per liter [10]. Throughout the maintenance period, the juvenile snakehead fish were fed a commercial diet containing 35% protein [4] at a rate of 5% of their biomass per day. The leafy green plant density in the aquaponic system was 50 plants per square meter [11]. Leafy greens were introduced into the system on the 15th day after the snakehead fish were introduced to the aquaponic system.

Parameters, Observation, and Data Analysis

Water quality parameters, including temperature, pH, dissolved oxygen, total ammonia nitrogen, nitrite, nitrate, and orthophosphate, were measured every 15 days. The sample collection and preservation procedures followed the guidelines outlined in APHA [12]. The production performance of snakehead fish is assessed through the survival rate (SR), Weight-specific growth rate (SGRW), and Length-specific growth rate (SGRL) calculated using the Hien methodology [13]. Feed conversion ratio (FCR) was calculated using Zehra's formula [14], while total biomass determination followed Srivastava's method [15]. Fish body weight and length measurements were taken every 15 days to adjust feed quantity based on fish biomass.

Blood collection for snakehead fish followed Michael & Priyadarshini's guidelines [16], with hemato-biochemical parameters analyzed on the 60th day. Glucose levels were determined spectrophotometrically [17], while red and white blood cell counts were quantified using a hemocytometer [18]. Hemoglobin concentration was assessed using the Sahli method [17], and hematocrit levels were analyzed using the microhematocrit method [19]. The abundance of ammonification bacteria (AB), ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB) in the water was measured every 15 days using the Most Probable Number (MPN) method [20, 21].

Production performance data, fish hemato-biochemical parameters, the abundance of AB, AOB, and NOB, and the removal of TAN, nitrite, and nitrate were tabulated using Excel in Microsoft 365 and analyzed statistically. ANOVA and F-test analyses were conducted in SPSS Version 25 with a 95% confidence level to evaluate treatment effects. Significant differences among treatments were further analyzed using the Duncan test for post hoc analysis. Descriptive analysis was applied to water quality data.

Results and Discussion

Water Quality

Water quality profoundly impacts a fish's growth, development, survival, physiology, and reproduction. The degradation of water quality parameters and the escalating contamination levels present formidable challenges to the efficacy and sustainability of aquaculture systems [7, 22, 23]. The study's water quality assessment data is presented in Fig. 2. The concentrations of Total Ammonia Nitrogen (TAN), nitrite, and nitrate in the nursery fish tank during this study exhibited variations within the ranges of 0.16-20.27, 0.00-0.13, and 2.83-35.84 mgL^{-1}, respectively. The established standards for TAN, nitrite, and nitrate levels in aquaculture are less than 120.00 [24, 25], 5.00, and 100.00 mgL^{-1} [26], respectively.

Throughout the study, Dissolved Oxygen (DO) concentrations and temperatures fell within the range of 0.22–3.87 mgL^{-1} and 24.7-29.40°C, respectively. The recommended DO concentration and temperature for snakehead fish cultivation are 0.20-8.6 mgL^{-1} and 26.8–32.5°C

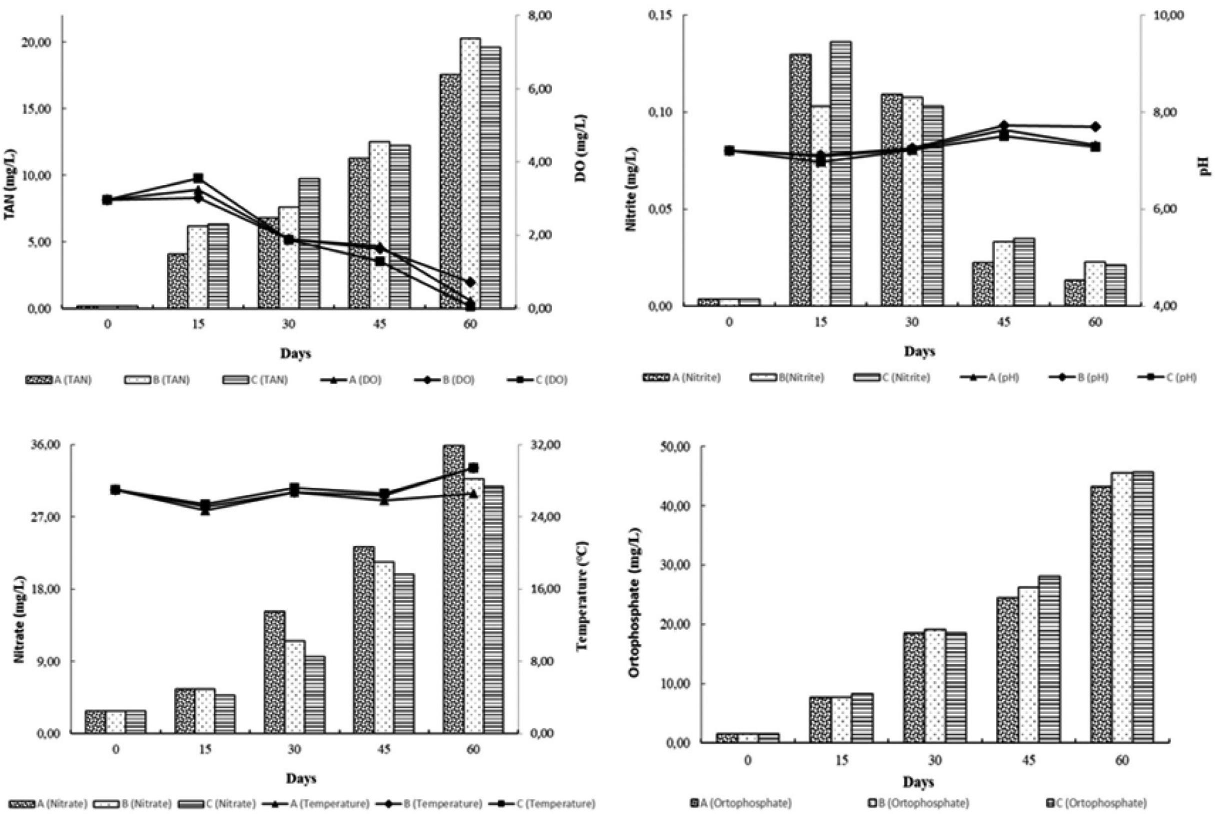

Fig. 2. The physicochemical water quality parameters in the fish cultivation tank during the study with lettuce (A), pak choi (B), and mustard greens (C) treatment.

[4]. Observed pH values ranged from 6.65 to 7.73, while orthophosphate concentrations spanned from 1.50 to 45.65 mgL^{-1}. As per Puspaningsih's study [4], the prescribed pH range is 4.00–9.00, with the suggested orthophosphate concentration being less than 80.00 mgL^{-1} [27]. In summary, the water quality parameters in the cultivation tank meet the criteria for optimal fish culture.

In aquaponic systems, fish feed serves as the primary nitrogen input source, with a significant proportion of the feed being excreted by the fish as ammonia, accounting for over 90% [28]. Consequently, ammonia excreted by fish is one of the essential nutrient supplies for plant development. Within aquaponic systems, nitrogen is assimilated by plants in the form of nitrate and ammonium [28, 29]. Orthophosphate (PO_4^{3-}), in the form of phosphorus, is also a crucial essential nutrient vital for the growth and development of plants in aquaponic systems. Phosphorus is pivotal in biological processes and represents one of the macronutrients plants require [30].

Based on Fig. 2, the TAN, nitrite, and orthophosphate levels during the study in treatment A tended to be lower than the other treatments. Low nutrient levels in water suggest a nitrification process by bacteria and better nutrient uptake by lettuce plants than pak choi and mustard greens. These findings are further supported by Fig. 3, where the abundance of ammonification bacteria (AB), ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB) and the nutrient absorption capability of lettuce treatment during the study tended to be better than other treatments. Nitrification is a series of biochemical reactions that convert TAN, predominantly composed of ammonium (NH_4^+) and ammonia (NH_3), into nitrite (NO_2^-) and subsequently into nitrate (NO_3^-) [31, 32]. The initial stage of nitrification involves AOBs such as *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio* sp., among others [28, 33, 34]. Researchers investigating aquaponic systems [11, 29] have identified the presence of AOB and NOB, such as Nitrobacter sp. and Nitrospira sp., on the surfaces of plant roots.

The nutrient retention capacity varies among plant species under identical agronomic conditions, showing species-specific traits [33, 35]. Moreover, the choice of plant species significantly influences the distribution of both nitrogen (N) and phosphorus (P) [36]. As illustrated in Fig. 2, lettuce (Treatment A) led to lower concentrations of total ammonia, nitrogen, nitrite, and orthophosphate during the study. Additionally, lettuce has demonstrated the best nutrient absorption capabilities and a higher quantity of AB, AOB, and NOB compared to other treatments (Fig. 3). Several factors influencing nutrient removal rates in plants within aquaponic systems include growth rate, root nutrient absorption capacity, efficient nutrient utilization,

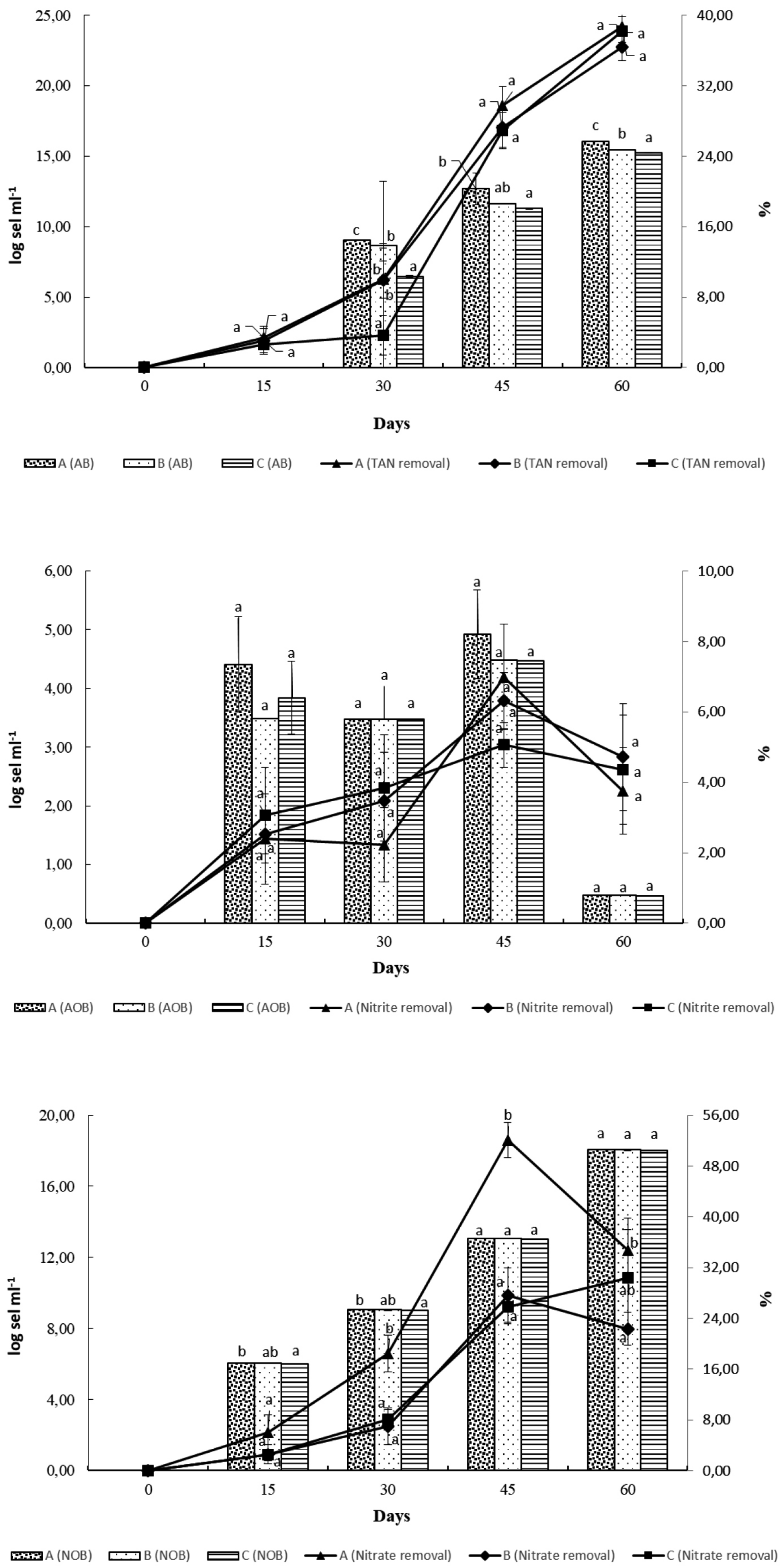

Fig. 3. The abundance of ammonification bacteria (AB), ammonia-oxidizing bacteria (AOB), and nitrite oxidizing bacteria (NOB), as well as the removal of TAN, nitrite, and nitrate with lettuce (A), pak choi (B), and mustard greens (C) treatment.

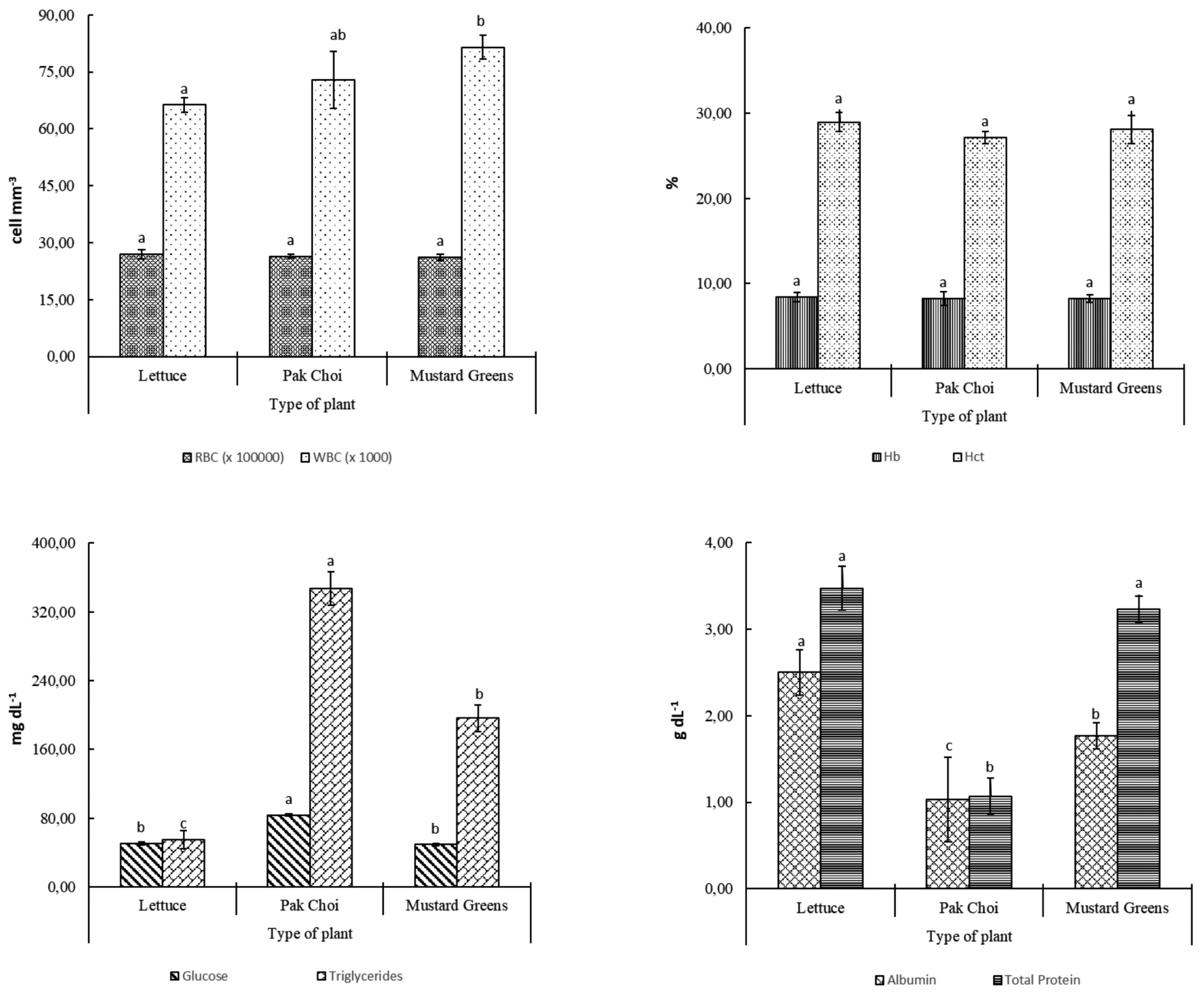

Fig. 4. Hemato-Biochemical Parameters Data of Snakehead Fish Reared in an Aquaponic Cultivation System with Varied Vegetables.

and microbial-root interactions [6, 28, 33, 37]. Fast-growing plants typically exhibit higher nutrient requirements to support their rapid growth, making them more efficient at nutrient uptake from the water.

Lettuce (*Lactuca sativa*) was selected for its adaptability to aquaponic systems and short growth cycle, typically around six weeks [38]. The root system is the plant's primary organ for nutrient and water absorption [39–41]. Furthermore, the plant root's ability to absorb nutrients relies on factors such as root quantity, root structure, and active root surface area availability. Typically characterized by shallow root systems, lettuce plants exhibit efficient nutrient uptake from water. Despite their relatively short roots, their root type features numerous active root hairs, enhancing their nutrient absorption capacity. Lettuce plants cultivated in hydroponic systems exhibit better phosphorus uptake capabilities than tomato and basil plants [36]. The nitrogen uptake by lettuce reaches 110 mg of nitrogen per plant, with a growth rate of 0.0562% day^{-1} [42]. Notably, the nitrification rate on the surface of lettuce roots reached 0.20 g m^{-2} day^{-1}[43].

Hemato-Biochemical Parameters

Blood-based assays are routinely employed to assess fish health for predictive and diagnostic purposes. Fluctuations in blood hemato-biochemical parameters are valuable indicators of physiological shifts in response to environmental changes [44]. Specific hematological parameters, notably red blood cell (RBC) count, hematocrit (Hct), hemoglobin (Hb) concentration, and white blood cell (WBC) count, hold significant importance in the examination of fish physiology [45–47]. Furthermore, hemato-biochemical parameters on metabolism, immunity, hormones, ions, and ionic regulation, such as glucose levels, total protein (TP) concentration, and triglyceride (TG) levels, are considered pivotal markers reflecting the wellbeing and physiological state of fish [26, 44, 48]

As observed in Fig. 4, the hemato-biochemical parameters (RBC, WBC, Hb, and Hct) of snakehead fish under various vegetable plant treatments in the aquaponic system exhibited no statistically significant differences ($P > 0.05$). Only the WBC count of snakehead fish under the pak choi

Table 1. Snakehead Fish's and Plant's Production Performance in Aquaponic Cultivation System.

Production performance	Treatment of Green Vegetables		
	Lettuce	Pak Choi	Mustard Greens
Fish:			
Survival Rate (%)	90.33±2.08[a]	83.67±1.15[b]	85.67±0.58[b]
SGR_W (% day^{-1})	3.37±0.22[a]	2.93±0.04[b]	3.09±0.10[b]
SGR_L (% day^{-1})	1.23±0.10[a]	0.99±0.06[b]	0.99±0.08[b]
FCR	0.79±0.05[b]	1.24±0.10[a]	1.10±0.13[a]
Final Weight (g)	11.26±1.32[a]	8.73±0.18[b]	9.58±0.56[b]
Final Length (cm)	11.28±0.62[a]	9.92±0.32[b]	9.86±0.46[b]
Nitrogen Retention (%)	39.02±0.61[a]	19.73±0.89[c]	26.67±1.29[b]
Phosphorus Retention (%)	76.26±2.72[a]	65.75±0.54[b]	67.47±1.77[b]
Vegetables:			
Final weight (g)	679.4±3.48[a]	642.72±8.72[b]	650.12±4.76[b]
Nitrogen Retention (%)	87.08±0.56[a]	68.95±0.98[c]	75.43±0.89[b]
Phosphorus Retention (%)	11.75±0.19[a]	9.89±0.01[c]	10.65±0.09[b]

Significant differences ($p < 0.05$) among treatments are indicated by distinct lowercase letters in the table.

treatment significantly differed from that under the mustard green treatment ($P < 0.05$). This finding aligns with the research conducted by Puspaningsih [10], which reported a significant difference ($P < 0.05$) in white blood cell counts among snakehead fish under different stocking densities.

White blood cell count is a hematological parameter employed to assess a fish's immune response to environmental stressors, including hypoxia, heavy metal exposure, and endocrine disruptor compounds. An increase in WBC count indicates an immune response to environmental toxicity, while a decrease suggests immune system impairment due to stress [44]. In this study, the lettuce treatment (A) resulted in snakehead fish's highest blood albumin and total protein levels. Meanwhile, glucose and blood triglyceride levels in snakehead fish were lower in the lettuce treatment (A) compared to the pak choi (B) and mustard green (C) treatments.

Fish maintain their physiological equilibrium through precise regulation of blood osmoregulation, ion concentrations, and glucose levels, reflecting their adaptation to dynamic environmental conditions [44, 49]. Environmental fluctuations also impact fish hemato-biochemical levels, influencing glucose and cortisol levels. Fish respond to stress by elevating their glucose and cortisol levels, resulting in a simultaneous reduction in serum protein and triglyceride concentrations [50–52].

A study of environmental stressors could elevate glucose, triglycerides, and cortisol levels while concurrently reducing plasma total protein in the *European seabass*, *Dicentrarchus labrax* [50, 51, 53]. Fish exposed to stressful environmental conditions can interpret these metabolic shifts as adaptations to meet increased energy demands. During oxygen deficiency stress, fish initially prioritize using glucose and triglycerides as energy sources, leading to a subsequent decline in serum protein content. Research on sockeye salmon (*Oncorhynchus nerka*) and yellow croaker (*Larimichthys crocea*) has demonstrated that proteins become the primary source of cellular energy during severe oxygen deficiency and after other energy sources are unavailable. Additionally, the reduction in total protein (TP) levels constitutes an adaptive mechanism employed by fish to fulfill energy demands during stress [54]. Similarly to our finding, treatment A effectively maintained better water quality than other treatments throughout the cultivation period, thus proving more efficient in reducing stress levels in fish.

Production Performance

The results of the snakehead fish's biological performance revealed that Treatment A yielded the best production performance with survival rate, weight's specific growth rate (SGR_W), length's specific growth rate (SGR_L), and feed conversion ratio measuring 90.33±2.08%, 3.37±0.22% day^{-1}, 1.23±0.10% day^{-1}, and 0.79±0.05, respectively (Table 1). The results of this study are improved compared to previous research, where the SR, SGR_W, and FCR were 84±4.00%, 2.80±0.03% day^{-1}, and 0.80±0.09, respectively [10]. Among the treatments, lettuce treatment displayed the highest final biomass at 679.4±3.48 g, followed by mustard green at 650.12±4.76 g and pak choi at 642.72±8.72 g. Treatment A, characterized as lettuce, also demonstrated the highest nutrient retention among all treatments, with nitrogen retention (NR) and phosphorus retention rates (PR) reaching 87.08±0.56% and 11.75±0.19%, respectively.

Conclusions

This study's findings conclude that lettuce plant application (Treatment A) significantly and positively influenced the production performance of juvenile snakehead fish. The lettuce plants effectively maintained optimal water quality conditions by having better nitrogen removal ability and nitrogen-oxidizing bacteria abundance, which, in turn, positively influenced the hemato-biochemical parameters of the fish. Optimal environmental conditions in Treatment A can reduce fish stress levels, resulting in improved production performance with SR, SGR_W, SGR_L, and FCR measuring 90.33±2.08%, 3.37±0.22% day^{-1}, 1.23±0.10% day^{-1}, and 0.79±0.05, respectively. Additionally, the lettuce plants exhibited substantial NR and PR, reaching 87.08±0.56% and 11.75±0.19%, respectively, and achieved the highest plant productivity values. These attributes signify the significant potential for developing lettuce cultivation in snakehead fish nurseries with the Recirculating Aquaculture System - an aquaponic hybrid system.

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Conflict of Interest

The authors declare no conflict of interest.

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