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Article

Effect of Chitosan on The Growth Performance of Red Tilapia (*Oreochromis Mossambicus* × *Oreochromis Niloticus*) and Lettuce (*Lactuca Sativa* L.) in Aquaponic System

Yuniel Méndez-Martínez[1], Alba D. Zurita-Albán[2], Agustín N. Zambrano-Ostaiza[3], Juan J. Reyes-Pérez[4], Luiz Fernando de Sousa Antunes[5]

[1] Professor-Pesquisador. Universidad Técnica Estatal de Quevedo. ORCID: 0000-0002-5365-5794. Email: ymendezm@uteq.edu.ec

[2] Pós-graduando de Mestrado em Aquicultura. Universidad Técnica Estatal de Quevedo. ORCID: 0009-0000-6743-7184. Email: azuritaestrategiahh@gmail.com

[3] Professor-Pesquisador. Universidad Técnica Estatal de Quevedo. ORCID: 0009-0003-4862-9705. Email: azambranoo3@uteq.edu.ec

[4] Professor-Pesquisador. Universidad Técnica Estatal de Quevedo. ORCID: 0000-0001-5372-2523. Email: jreyes@uteq.edu.ec

[5] Doutor em Fitotecnia. Professor Pesquisador pela Universidade Federal Rural do Semi-Árido. ORCID: 0000-0001-8315-4213. Email: fernando.ufrrj.agro@gmail.com

RESUMO

Aquaponia é uma alternativa sustentável que integra o cultivo simbiótico de organismos e plantas aquáticas, cujo desempenho pode ser aprimorado por meio de bioestimulantes naturais como a quitosana. Este estudo avaliou a resposta bioprodutiva de tilápia híbrida (*Oreochromis mossambicus* × *O. niloticus*) e três variedades de alface (Lactuca sativa L.) em um sistema de aquaponia do tipo técnica de filme de nutrientes (NFT) sob diferentes concentrações de quitosana. No cultivo de alface, foi implementado um delineamento fatorial completo 3×3 (dose de quitosana × variedade de alface), com três tratamentos (A1: 0 ppm, A2: 500 ppm, A3: 1000 ppm) e três variedades de alface (B1: Grandes Lagos, B2: Regina 500, B3: Red Rock), com três repetições por combinação. Para os peixes, foi aplicado um delineamento unifatorial utilizando as mesmas concentrações de quitosana. Variáveis de desempenho de crescimento em tilápia (peso final, comprimento, taxa de crescimento específico, taxa de conversão alimentar e taxa de eficiência proteica) e parâmetros agronômicos em alface (peso fresco, número de folhas e altura) foram avaliados. Os resultados indicaram que o tratamento com 1000 ppm de quitosana (A3) melhorou significativamente ($P < 0,05$) o desempenho dos peixes, atingindo um SGR de 2,56, FCR de 1,05 e PER de 2,12. Em alface, as interações A2B2 e A3B1 produziram o melhor desempenho agronômico ($P < 0,05$). A análise de componentes principais e a correlação de Pearson revelaram associações positivas entre a aplicação de quitosana e a produtividade de ambas as espécies. Em conclusão, a quitosana melhorou o crescimento integrado de tilápia e alface no sistema de aquaponia, representando uma alternativa funcional para otimização do sistema.
Palavras-chave: aquicultura; eficiência alimentar; sistema de recirculação; crescimento; sobrevivência.

ABSTRACT

Aquaponics is a sustainable alternative that integrates the symbiotic cultivation of aquatic organisms and plants, whose performance can be enhanced through natural biostimulants such as chitosan. This study evaluated the bioproductive response of hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) and three varieties of lettuce (*Lactuca sativa* L.) in a nutrient film technique (NFT)-type aquaponic system under different concentrations of chitosan. In lettuce cultivation, a full factorial 3×3 design (chitosan dose × lettuce variety) was implemented, with three treatments (A1: 0 ppm, A2: 500 ppm, A3: 1000 ppm) and three lettuce varieties (B1: Grandes Lagos, B2: Regina 500, B3: Red Rock), with three replicates per combination. For the fish, a unifactorial design was applied using the same chitosan concentrations. Growth performance variables in tilapia (final weight, length, specific growth rate, feed conversion ratio, and protein efficiency ratio) and agronomic parameters in lettuce (fresh weight, number of leaves, and height) were evaluated. Results

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indicated that the 1000 ppm chitosan treatment (A3) significantly improved ($P < 0.05$) fish performance, reaching an SGR of 2.56, FCR of 1.05, and PER of 2.12. In lettuce, the A2B2 and A3B1 interactions yielded the best agronomic performance ($P < 0.05$). Principal component analysis and Pearson correlation revealed positive associations between chitosan application and the productivity of both species. In conclusion, chitosan enhanced the integrated growth of tilapia and lettuce in the aquaponic system, representing a functional alternative for system optimization.
Keywords: aquaculture; food efficiency, recirculating system; growth; survival.

Introduction

In recent years, global aquaculture has solidified its preeminence in aquatic food production. The combined total production of fisheries and aquaculture reached 223.2 million tonnes, representing a 4.4% increase compared to 2020 (FAO, 2024a). Of this total, aquaculture contributed 130.9 million tonnes when including both animals and algae (FAO, 2024b), while the specific production of aquatic animals was 94.4 million tonnes, equivalent to 51% of the total aquatic animals produced (185.4 million tonnes), confirming the predominance of this activity over capture fisheries. Moreover, inland aquaculture contributed 59.1 million tonnes, accounting for 62.6% of global animal aquaculture, underscoring its strategic role in food systems and nutritional security (FAO, 2024a).

Within this context of sustained global growth, Ecuador has followed a similar trajectory by expanding its aquaculture sector, particularly through the cultivation of red tilapia (*Oreochromis mossambicus* × *O. niloticus*). This hybrid species, known for its rapid growth, adaptability, and consumer preference, has become the second most economically important aquaculture product in the country (Méndez-Martínez et al., 2023). Red tilapia production in Ecuador has increased at an average annual rate of 7%, with approximately 10,000 hectares dedicated to farming and a reported annual yield of 35,000 tonnes (Veintimilla-Morán, 2023).

Keong and Romano (2013) note that tilapia is an essential, highly valuable, and beneficial species, particularly in intensive or extensive culture systems that integrate plant cultivation, contributing to beneficial outcomes. According to Ani *et al.* (2022), tilapia is a domesticated freshwater fish species that has been used in aquaponic systems for many years. Likewise, Lim *et al.* (2011) suggest that in addition to providing a reliable source of protein, tilapia is known to improve plant growth rates. When properly maintained, tilapia often graze on the plants' leaves and introduce nitrogen into the environment, thereby increasing crop yields (Delgado, 2020).

According to Tetreault et al. (2023), aquaponics is a highly productive alternative. Waller *et al.* (2025) explain that aquaponics is an efficient and modern production system integrating hydroponics and aquaculture, which maximizes water use, converts waste into nutrients, reduces space requirements through higher stocking densities, and minimizes energy use via gravity. Similarly, Atique et al. (2022) describe aquaponics as a bioregenerative system that uses fish waste, plant waste, and mineral nutrients in a closed-loop system to create fertilizers.

However, there is currently a need to improve techniques that can diversify production systems and promote water optimization using biopolymers, which enhance water quality and improve bioproductive parameters in both fish and agricultural crops (Pinho *et al.,* 2021). Furthermore, aquaponic systems are susceptible to diseases and pests affecting both fish and plants, and their prevention and control require specific strategies for each system. Research is needed to develop better methods for disease prevention and control in aquaponics (Chen *et al.,* 2014).

Chitosan, derived from crab and shrimp shell waste, has been studied for its potential use as a filter and biostimulant in tilapia and lettuce aquaponic systems, as it can improve water quality parameters and production performance in both aquaculture species and crops. One study found that the optimal concentration of chitosan as a filter was 50 mg, which resulted in significant increases in tilapia weight and improved water quality parameters (Tetreault *et al.,* 2023).

Another study examined the effect of commercial biofertilizers, including chitosan, on the production efficiency of tilapia and green beans in an aquaponic system. While the biofertilizers improved water quality, they did not significantly affect fish growth or the fruiting of the green beans (Saufie *et al.,* 2022).

Overall, chitosan has shown potential as a biostimulant that enhances the immune system and production performance in aquaponic systems, but further research is needed to determine its optimal concentration and its impact on plant growth and nutrient availability. Therefore, this study aims to evaluate the effect of chitosan levels in an aquaponic system cultivating juvenile red tilapia and lettuce.

Materials and Methods

Location

The research was conducted in 2024 at the Aquaculture greenhouse of the "La María" University Campus (Faculty of Animal and Biological Sciences, Universidad Técnica Estatal de Quevedo, UTEQ) in Quevedo, Los Ríos province, Ecuador. The facility is located at kilometer 7½ on the Quevedo–El Empalme road, at 1°06'13" S latitude and 79°29'22" W longitude, at an altitude of 73 m above sea level.

Experimental Design

The experimental design was unifactorial for tilapia culture, considering the effect of chitosan doses (A1: 0 "control", A2: 500 and A3: 1000 ppm L^{-1}). Meanwhile, for lettuce culture a bifactorial (A × B) design was used, where factor A represents chitosan doses and factor B represents lettuce varieties (B1: Grandes lagos, B2: Regina 500, and B3: Red rock). Under the fish experimental conditions, 270-L tanks were used with 20 juvenile tilapia per tank, yielding a total of 60 fish per treatment and 180 experimental fish. An NFT (nutrient film technique) system was used for lettuce cultivation. This system consisted of PVC tubes with planting holes, in which 20 plants were grown per treatment, totaling 180 experimental plants. Water from the lettuce system returned to the fish tanks via a submersible pump, establishing continuous water recirculation, and a blower was used to aerate the fish. The study was conducted over an eight-week period, during which bioproductivity indicators of both crops (lettuce and tilapia) were evaluated. Chitosan was applied directly to the culture water in different doses.

The choice of chitosan concentrations was based on previous studies in both fish and horticultural crops, where similar levels of chitosan have been shown to be safe and effective as immunostimulants and biostimulants, enhancing growth, immune response, and plant physiology without negatively affecting water quality (Méndez-Martínez et al., 2021; Xu and Mou, 2018; Ramírez-Rodríguez et al., 2024).

Chitosan Characteristics

The chitosan used in this study was obtained from crustacean shell waste, supplied in powdered form by Sigma-Aldrich®, St. Louis, MO, USA. According to the product's technical datasheet, it corresponded to a medium molecular weight (≈ 250 kDa), with a degree of deacetylation of 85% and a purity greater than 95%, features considered critical for its solubility and bioactivity in aquaculture and agricultural applications (Badawy and Rabea, 2011; Xu and Mou, 2018). The biopolymer was soluble in a 1% acetic acid solution, with the pH subsequently adjusted to neutrality prior to application. For each treatment, the chitosan was freshly prepared by dissolving the required dose in dechlorinated water under constant stirring for 30 minutes to ensure homogeneity before being directly incorporated into the aquaponic system tanks (Lyalina et al., 2023; Abu-Elala et al., 2025).

Biofilter Description

Each 270-L fish tank was connected to an independent biofilter r designed to treat waste from feces and uneaten feed. The biofilter consisted of a container with a working volume of approximately 40 L (15% of tank volume), which is within the recommended 10–20% range for small-scale aquaponic units to provide sufficient nitrification capacity (Graber and Junge, 2009; Rakocy, 2012). Inert plastic media (plastic caps and cut plastic bottles) were used inside the biofilter to increase the surface area for bacterial colonization. Water was continuously recirculated through the biofilter at a flow rate of 270 L h^{-1} (4.5 L min^{-1}), corresponding to a complete tank turnover approximately every hour. This turnover rate falls within the 0.5–2 h range reported as adequate for maintaining water quality and biofilter efficiency in aquaponic systems (Endut et al., 2011; Atique et al., 2022).

Prior to fish acclimation, each biofilter was inoculated for one week with Seachem Stability® (Seachem Laboratories, Inc., Madison, Georgia, USA), following the manufacturer's instructions. This product contains nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.), which convert ammonia derived from fish metabolism into nitrite and subsequently into nitrate, a form readily available for plant uptake through the hydroponic component (Graber and Junge, 2009). Chitosan doses were applied directly into the water of each treatment, ensuring homogeneity by gentle stirring prior to distribution.

Fish Culture Management

Strict management measures were implemented to ensure the health and well-being of the fish. A one-week acclimation period was established, during which fish were dewormed and their health and water quality parameters were monitored daily. The juvenile tilapia had an initial weight of 27.50 ± 3.0 g at the beginning of the study.

Regarding feeding, two specific schedules were established. In the morning (09:00), 60% of the daily feed ration was administered, reserving the remaining 40% for the afternoon (17:00). Juvenile tilapia were fed a commercial feed containing 32% protein to ensure a balanced and adequate diet. This careful approach to management and feeding contributed to the healthy growth of the fish during this stage.

Evaluated Parameters in Fish Culture

At the beginning and at the end of the experiment (eight weeks), all fish in each tank were fasted for 24 hours and then anaesthetised with 4-allyl-2-methoxyphenol ($1:10^{-4}$) prior to biometric evaluation. All fish existing in each biometrical sampling were individually weighed on digital scales (± 0.01 g) and measured for total length with a vernier caliper (± 0.001 mm). Mathematical formulae were subsequently applied to determine growth performance parameters (Méndez-Martínez et al., 2024).

- Weight gain (g) = Wf - Wi, where Wf is final weight (g) and Wi is initial weight (g);
- Specific growth rate (%/day) = ((ln Wf - ln Wi)/t) × 100, where t is the duration of the experiment (days);
- Length gain (cm) = Final length (cm) - Initial length (cm);
- Condition factor = (weight (g)/length $(cm)^3$) × 100;
- Survival (%): (final number of fish / initial number of fish) × 100.;
- Feed conversion ratio: feed intake (g) / weight gain (g);

- Feed efficiency = weight gain (g) / feed intake (g);

- Protein efficiency ratio = wet weight gain of the fish (g) / dry weight of protein consumed in the feed (g).

Lettuce Culture Management

The planting sponges for the lettuce were pre-soaked in water (air inside the sponge was expelled by squeezing) for approximately 2 hours, and then rinsed twice with clean water. Each sponge was placed in the lower net and submerged to a depth of about 2–3 mm. Lettuce seeds were sown in germination trays using a coconut fiber-based substrate at a depth of 0.5 cm. Seedlings were transplanted once they had developed the third true leaf; at this stage the plants were carefully removed from the trays and their roots were thoroughly washed to eliminate all traces of substrate. The seedlings were then placed in small plastic cups with a piece of sponge for support, spaced 20 cm apart within rows and 40 cm between rows. The crop was harvested manually when the leaves reached an optimal stage of development and were suitable for consumption.

Evaluated Parameters in Lettuce Culture

During the biometric evaluations, all experimental lettuce plants were measured. The following parameters were recorded for each plant: number of leaves, plant height (cm), root length (cm), and plant weight at harvest (g).

Water Quality Parameters Assessment

The physicochemical parameters of the water were monitored throughout the experiment. Temperature was measured with a mercury thermometer, dissolved oxygen was measured using a digital oxygen meter, and a colorimetric test kit (Salwater Master Test) was used to measure the remaining parameters: pH, nitrite (mg L^{-1}), ammonium (mg L^{-1}), and nitrate (mg L^{-1}). Additionally, leaf tip necrosis ("tip burn") in the plants was observed as an indicator of water quality stress. Leaves near the base of the plant began to become pale and yellow, and in some cases they fell off completely.

Statistical Analysis

The Kolmogorov–Smirnov test (P<0.05) was used to assess data normality, and Bartlett's test (P<0.05) was employed to verify the homogeneity of variances prior to performing analysis of variance (ANOVA). A one-way ANOVA was conducted for the tilapia dataset, while a two-way ANOVA was applied to the lettuce dataset. When significant F-values were observed, Tukey's multiple comparison test was used to identify differences among means at a significance level of P<0.05, using Minitab version 18 (Minitab LLC, Philadelphia, PA, USA). In addition, Pearson correlation analysis and principal component analysis (PCA) were performed using RStudio software (RStudio, PBC, Boston, MA, USA).

Results

In Table 1, the water quality parameters (temperature, dissolved oxygen, pH, ammonium, nitrite, and nitrate) are presented for three chitosan levels (0, 500, 1000 ppm) in a controlled aquaponic system. No significant differences (P<0.05) were observed among treatments for any of these water parameters.

Table 1. Water quality parameters in the culture system.

Parameters	Chitosan Level			*P-value*
	A1: 0 ppm	A2: 500 ppm	A3: 1000 ppm	
Temperature, °C	27.36 ± 0.58	27.60 ± 0.40	27.75 ± 0.47	0.0847
Dissolved oxygen, mg L^{-1}	4.45 ± 2.27	4.35 ± 0.25	89.27 ± 0.32	0.0934
Ph	7.48 ± 0.10	7.47 ± 0.12	7.46 ± 0.11	0.0729
Ammonium, mg L^{-1}	0.005 ± 0.0007	0.0028 ± 0.003	0.004 ± 0.006	0.0901
Nitrite, mg L^{-1}	0.001 ± 0.0001	0.001 ± 0.001	0.001 ± 0.001	0.0624
Nitrate, mg L^{-1}	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.0930

Mean ± standard deviations of the replicates. Source: Authors' data (2024).

Table 2 shows the productive parameters of red tilapia (*Oreochromis mossambicus* × *O. niloticus*) under the same conditions. All measured parameters differed significantly among treatments (P<0.05). For final weight, the highest mean value (128.71 g) occurred in treatment A3 (1000 ppm chitosan), compared to 108.69 g in the control (0 ppm). Similarly, final length differed significantly: the greatest length (213.69 mm) was at A3 (1000 ppm) versus 191.36 mm in the control.

Table 3, summarizes the growth of different lettuce varieties under the chitosan treatments. Overall, chitosan application increased all measured morpho-agronomic traits (number of leaves, root length, plant weight, plant height) as well as yield. For number of leaves, there was a significant chitosan×variety interaction (P<0.05). The highest count (45.40 leaves) was in I7 (1000 ppm on Regina 500), and the lowest (14.67) in I3 (0 ppm on Red Rock). Considering factors separately, A3 (1000 ppm) averaged 34.16 leaves vs. 19.13 in the control, and Regina 500 (B2) averaged 32.31 leaves vs. 17.16 for Red Rock (B3).

Table 2. Productive parameters of tilapia in aquaponic system with chitosan application

Parameters	Chitosan Level			*P-value*
	A1: 0 ppm	A2: 500 ppm	A3: 1000 ppm	
FWT (g)	108.6 ± 5.76ª	122.86 ± 5.83b	128.71 ± 7.60c	0.0027
FLT (mm)	191.36 ± 17.62ª	204.85 ± 17.42b	213.69 ± 21.80b	<0.0001
WGT (g)	81.15 ± 1.02ª	95.06 ± 0.94b	100.96 ± 1.74c	<0.0001
SGR	2.29 ± 0.02ª	2.48 ± 0.02b	2.56 ± 0.02c	<0.0001
TGL (mm)	101.91 ± 4.24ª	115.25 ± 3.59b	124.42 ± 5.58b	0.0027
CFT (mm)	1.55 ± 0.09b	1.43 ± 0.06ab	1.32 ± 0.08a	0.0344
SUR (%)	88.89 ± 3.85ª	100.00 ± 0.00b	97.78 ± 3.85b	0.011
FCR	1.23 ± 0.03c	1.11 ± 0.01b	1.05 ± 0.02a	0.0002
FER	0.61 ± 0.01ª	0.70 ± 0.01b	0.74 ± 0.01c	0.0001
PER	1.74 ± 0.05ª	1.99 ± 0.03b	2.12 ± 0.04c	0.0001

Each value given above is a mean ± standard deviations of the replicates. abc: Significant differences among treatment means are noted (P<0.05). FWT: Final Weight, FLT: Final Length, WGT: Weight Gain, SGR: Specific Growth Rate, TLG: Total Length Gain, CFT: Condition Factor, SUR: Survival Rate, FCR: Feed Conversion Ratio, FER: Feed Efficiency Ratio, PER: Protein Efficiency Ratio. Source: Authors' data (2024).

Table 3. Growth parameters of lettuce varieties (Lactuca sativa L.) an aquaponic system with chitosan application.

Interaction: Chitosan and Lettuce variety		**Number of leaves (U)**	**Root length (cm)**	**Plant weight (g)**	**Plant height (cm)**
I1:	$A_1 B_1$	18.93 ± 0.70 b	15.80 ±1.08 b	67.80 ±2.43 c	11.87 ±0.74 a
I2:	$A_1 B_2$	23.80 ± 1.37 c	19.33±0.98 c	94.00 ±1.60 f	21.60 ±0.99 c
I3:	$A_1 B_3$	14.67 ± 0.90 a	12.87 ±0.83 a	25.13 ±1.06 a	11.07 ±0.80 a
I4:	$A_2 B_1$	25.47 ± 1.19 c	15.67 ±1.23b	79.53 ±1.19e	15.27 ±0.70b
I5:	$A_2 B_2$	35.07 ± 1.53 d	28.53 ±1.19 f	134.73 ±1.33g	35.60 ±1.30 g
I6:	$A_2 B_3$	17.83 ± 1.06 b	18.40 ±0.91 c	50.40 ±1.35b	33.73 ±0.83 f
I7:	$A_3 B_1$	45.40 ± 2.06 f	18.20 ±1.08c	136.87 ±2.03g	29.20 ±1.57e
I8:	$A_3 B_2$	38.07 ± 2.81e	26.47 ±2.33e	165.20 ±3.43 h	33.73 ±1.87 f
I9:	$A_3 B_3$	18.93 ± 0.96 b	21.53 ±0.92 d	74.07 ±2.72 d	23.07 ±1.39 d
			Chitosan Levels		
A1:	0 ppm	19.13 ± 3.91 a	16.00 ± 2.83 a	62.31 ± 28.76 a	14.84 ± 4.91 a
A2:	500 ppm	26.13 ± 7.23 b	20.87 ± 5.70 b	88.22 ± 35.39 b	22.09 ± 9.71 b
A3:	1000 ppm	34.13 ± 11.47 c	22.07 ± 3.76 c	125.38 ± 38.61c	28.67 ± 4.70 c
			Lettuce variety		
B1:	Grandes lagos	29.93 ± 11.47 b	16.56 ± 1.62 a	94.73 ± 30.58 b	18.78 ± 7.66 b
B2:	Regina 500	32.31 ± 6.51 c	24.78 ± 4.28 c	131.31 ± 29.58 c	30.31 ± 6.43 c
B3:	Red rock	17.16 ± 2.07 a	17.60 ± 3.73 b	49.87 ± 20.29 a	16.51 ± 5.12 a
			P-value		
Interaction		0.0001	0.0011	0.0030	0.0022
Chitosan		0.0004	0.0021	0.0064	0.0040
Lettuce		0.0001	0.0065	0.0008	0.0001

Each value given above is a mean ± standard deviations of the replicates. abcdefgh: Significant differences among treatment means are noted (P<0.05). Source: Authors' data (2024).

For root length, the interaction was also significant (P<0.05). The longest roots (28.53 cm) were at I5 (500 ppm on Regina 500), and the shortest (12.87 cm) at I3 (0 ppm on Red Rock). By factor, A3 (1000 ppm) gave longer roots (22.07 cm) than A1 (16.00 cm), and Regina 500 had longer roots (24.78 cm) than Grandes Lagos (B1) (16.56 cm). For plant weight, I8 (1000 ppm on Regina 500) yielded the highest weight (165.20 g), significantly above the lowest (125.38 g) in I3 (0 ppm on Red Rock). Chitosan dose A3 (1000 ppm) averaged 115.38 g vs. 62.31 g in the control. Among varieties, Regina 500 plants (131.31 g) were significantly heavier than Red Rock (49.87 g).

For plant height, a significant interaction (P<0.05) was observed. The tallest plants (35.60 cm) were at I5 (500 ppm on Regina 500) and the shortest (11.07 cm) at I3 (0 ppm on Red Rock). By factor, A3 (1000 ppm) produced 28.67 cm versus 14.84 cm for A1; Regina 500 plants reached 30.31 cm versus 16.51 cm for Red Rock.

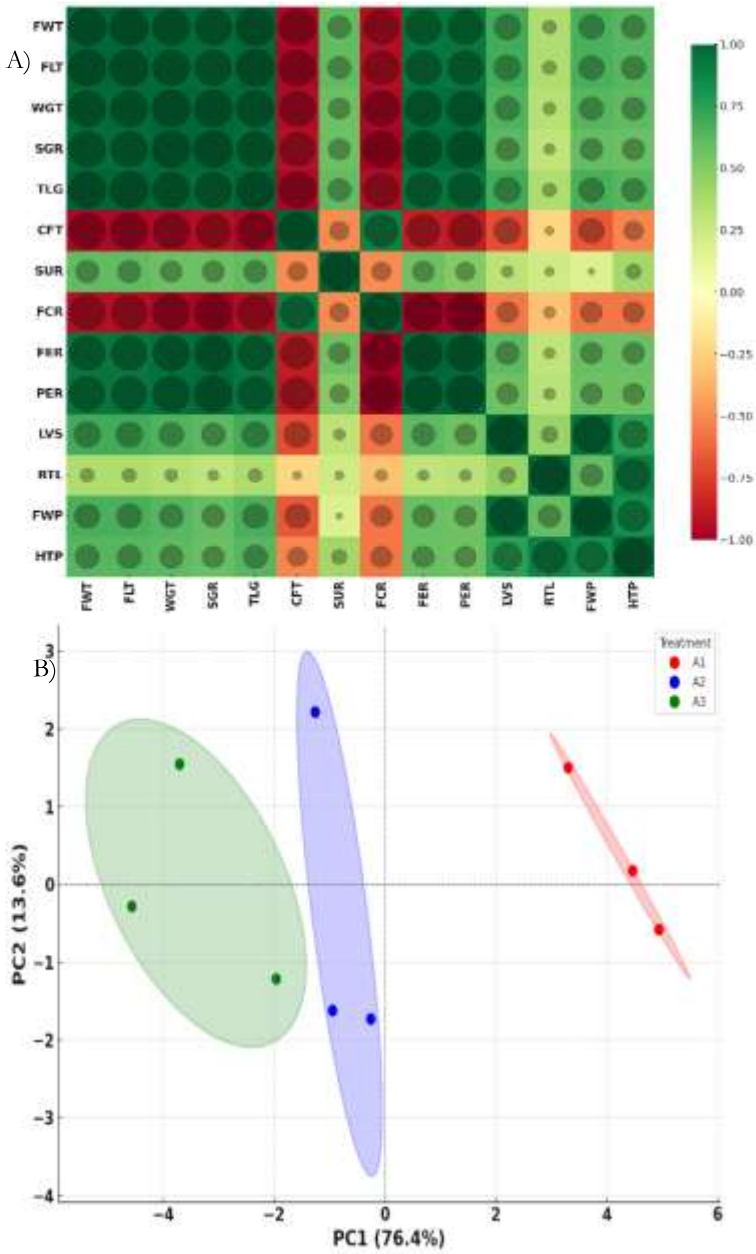

Fig. 1. A) Pearson's correlation heatmap among the different study variables. B) Biplot of Principal Component Analysis (PCA), which allows for dimensionality reduction and pattern visualization in the treatments with chitosan. FWT: Final Weight, FLT: Final Length, WGT: Weight Gain, SGR: Specific Growth Rate, TLG: Total Length Gain, CFT: Condition Factor, SUR: Survival Rate, FCR: Feed Conversion Ratio, FER: Feed Efficiency Ratio, PER: Protein Efficiency Ratio, LVS: Number of Leaves, RTL: Root Length, FWP: Fresh Weight of the Plant, HTP: Height of the Plant. Source: Authors' data (2024).

In contrast, the feed conversion ratio (FCR) showed moderate negative correlations with lettuce root length (RTL) ($R = -0.32$) and fresh weight ($R = -0.57$), suggesting that lower feed conversion (i.e., greater feed efficiency) in fish may benefit plant growth. The strongest overall positive correlation was between lettuce variables LVS and FWP ($R = 0.96$), while the strongest negative correlation was between FCR and FWP ($R = -0.57$). These results support the interconnection of fish and plant productivity in aquaponics and highlight the role of chitosan in enhancing system-wide performance. The principal component analysis (PCA) revealed clear separation among treatments based on the integrated performance of tilapia and lettuce under different chitosan doses (Fig. 1B).

The first two principal components (PC1 and PC2) explained a substantial portion of the total variance, allowing effective visualization of the multivariate data. The A3, associated with the highest chitosan dose, formed a distinct cluster along PC1, indicating superior performance in both fish growth (e.g., higher final weight and specific growth rate) and lettuce development (e.g., greater fresh weight and plant height). This grouping suggests a positive effect of chitosan on overall system productivity. The A1, representing the control or lowest chitosan dose, was located in the opposite quadrant, showing lower performance in most variables. The A2 occupied an intermediate position, suggesting a dose-dependent response to chitosan application. The clustering patterns and the non-overlapping ellipses indicate that the differences among treatments are consistent and multivariately significant

Discussion

The application of chitosan at different concentrations did not significantly alter key water quality parameters, including temperature, pH, dissolved oxygen, and nitrogenous compounds. These results are consistent with those reported by Abu-Elala et al. (2025), who observed that chitosan supplementation in biofloc systems did not affect pH or ammonia levels, suggesting limited disruption of microbial nitrification. Similarly, Abd El-Naby et al. (2019), found no significant effect of dietary chitosan on water quality in Nile tilapia (*Oreochromis niloticus*) tanks.

In our study, temperature values remained within the optimal thermal range for both tilapia and lettuce growth (25–30 °C), as suggested by Rakocy (2012) and Endut et al. (2011). Dissolved oxygen levels also remained adequate for aerobic respiration in fish and rhizospheric root zones, aligning with findings by Atique et al. (2022) in NFT aquaponic systems using lettuce and tilapia.

Nitrogenous compounds, including ammonium, nitrite, and nitrate, showed minimal fluctuation across treatments. These low values suggest efficient biofiltration and stable nitrifying bacterial activity, even with the presence of chitosan. This corroborates the findings of Abu-Elala et al. (2025), who demonstrated that chitosan at moderate levels does not suppress nitrifiers in aquaponic or recirculating systems (Bensalem et al., 2024). Notably, although chitosan is known for its antimicrobial properties, its selective action may spare autotrophic nitrifiers such as *Nitrosomonas* and *Nitrobacter*, as reported by Badawy and Rabea (2011).

Therefore, the absence of significant differences in water quality parameters across treatments supports the hypothesis that chitosan, even at 1000 ppm, is environmentally compatible with aquaponic systems and does not compromise system stability or microbial efficiency. This finding reinforces the potential of chitosan as a functional additive in integrated aquaculture without necessitating additional water quality management interventions.

In aquaponic systems, water quality is a key integrative factor, since it simultaneously affects fish metabolism, plant nutrient uptake, and microbial activity in biofilters. Optimal ranges are generally established at 25–30 °C for temperature, above 5 mg L^{-1} for dissolved oxygen, pH between 6.5 and 7.5, and nitrogenous compounds (ammonium, nitrite, nitrate) kept at low but detectable levels to sustain plant nutrition without causing toxicity to fish (Rakocy, 2012; Endut et al., 2011; Atique et al., 2022). Critical thresholds, such as nitrite levels exceeding 1 mg L^{-1} or ammonium above 2 mg L^{-1}, are known to impair gill function and fish survival (Graber and Junge, 2009). In the present study, the observed values remained well within these ideal ranges, with stable pH and negligible concentrations of ammonium, nitrite, and nitrate, indicating that the system operated under environmentally safe conditions. This suggests that the integration of chitosan supplementation did not exert stress on the coupled fish–plant environment, reinforcing its compatibility with sustainable aquaponic management.

The observed improvement in growth parameters with increasing dietary chitosan levels underscores its functional role in enhancing nutrient utilization and promoting somatic development in red tilapia. Specifically, fish receiving 1000 ppm of chitosan showed superior weight gain, growth rate, and feed efficiency, which suggests that chitosan may positively modulate digestive physiology and metabolic processes (Hossam-Elden et al., 2024). This trend is in agreement with the findings of Ani et al. (2022), who reported enhanced growth performance and protein utilization in *Oreochromis niloticus* fed chitosan-supplemented diets under recirculating aquaculture conditions.

Similar outcomes were documented by Oushani et al. (2020) and Hossam-Elden et al. (2024), who attributed these improvements to increased intestinal enzyme activity and better nutrient absorption associated with chitosan's bioadhesive properties. These effects are crucial in aquaponic systems, where the sustainability of the nutrient cycle depends heavily on optimal feed conversion and minimal waste output.

The reduction in feed conversion ratio (FCR) and increase in protein efficiency ratio (PER) observed in the present study reflect improved dietary protein assimilation, consistent with the mechanism proposed by Shi et al. (2020), where chitosan enhances gut morphology and microbial balance. Notably, our results are in line with those of Ovalı et al. (2024), who observed similar trends in common carp and related species when dietary chitosan was administered.

In addition to growth-related improvements, survival and condition factor also responded positively to chitosan inclusion, particularly at intermediate doses (500 ppm). This suggests a possible immunoprotective window where physiological resilience is enhanced without overstimulation. This aligns with Abdel-Tawwab et al. (2019), who found that low-to-moderate doses of dietary chitosan improved immune function and survival in Nile tilapia exposed to environmental stress.

While some studies have reported dose-dependent benefits of chitosan, others have highlighted potential saturation points beyond which no further improvement occurs or adverse effects may emerge (Chen et al., 2014). In our trial, the absence of adverse effects at 1000 ppm supports the suitability of this concentration for short-term productivity gains under aquaponic conditions. This reinforces the notion that chitosan can be used not only as a growth promoter but also as a system-compatible additive that fits within the integrative logic of aquaponic production.

In fish, chitosan exerts its immunomodulatory role through multiple interconnected physiological processes. At the intestinal level, its cationic nature allows interaction with the gut mucosa, strengthening epithelial integrity by enhancing tight-junction protein expression and reducing bacterial translocation (Shi et al., 2020). This protective effect is accompanied by stimulation of the innate immune system, reflected in increased lysozyme activity, complement activity, and leukocyte phagocytosis, which reinforce the first line of defense against aquatic pathogens (Oushani et al., 2020; Méndez-Martínez et al., 2021; Awad, 2025). Moreover, chitosan has been shown to upregulate genes related to both pro-inflammatory (IL-1β, TNF-α) and anti-inflammatory cytokines (IL-10), indicating a balanced immune modulation that prevents immunosuppression while avoiding chronic inflammation (Hossam-Elden et al., 2024). In parallel, its antioxidant capacity enhances the activity of enzymes such as superoxide dismutase and glutathione peroxidase, mitigating oxidative stress in hepatocytes and enterocytes (Abu-Elala et al., 2025). These mechanisms explain why dietary chitosan improves survival and feed efficiency, as immune reinforcement enables fish to allocate more metabolic energy to somatic growth rather than costly defensive responses.

The observed enhancement in lettuce growth across all morpho-agronomic traits in response to chitosan application clearly demonstrates its effectiveness as a plant growth promoter in aquaponic systems. The significant increases in number of leaves, root length, plant height, and fresh biomass at 500 and 1000 ppm suggest a dose-dependent response, with 1000 ppm showing the most pronounced effects. These findings are consistent with studies conducted by İkiz et al. (2024) and Ramírez-Rodríguez et al. (2024), who reported that foliar or root application of chitosan in lettuce significantly improved plant height, number of leaves, and yield under hydroponic and soil conditions.

Chitosan has been widely reported to stimulate plant growth through multiple mechanisms, including increased cell division, stimulation of phytohormone synthesis (e.g., auxins), enhanced mineral uptake, and activation of defensive pathways that indirectly boost vegetative growth (Xu and Mou, 2018; Li et al., 2021). In this study, the elongation of roots and the increase in aboveground biomass observed under chitosan treatments may be attributed to improved nutrient absorption and enhanced photosynthetic capacity, facilitated by better root architecture. According to Lyalina et al. (2023), chitosan-treated plants often exhibit longer and denser roots, which allow more efficient access to water and nutrients, thereby supporting greater shoot development and leaf production.

The differential response observed among the lettuce varieties tested (Grandes Lagos, Regina 500, and Red Rock) indicates that genotype plays a key role in modulating the effect of biostimulants like chitosan. The variety Regina 500 consistently outperformed the others in terms of root development, fresh weight, and plant height, especially under 1000 ppm, suggesting a higher physiological sensitivity or compatibility with the elicitor. Similar varietal responses to chitosan have been reported by Khater et al. (2024), Xu and Mou (2018), who emphasized that genotype-specific anatomical or metabolic traits can influence the uptake and action of biopolymers.

Moreover, the significant interaction between chitosan concentration and lettuce variety underscores the complexity of plant–elicitor responses in aquaponic systems. While the synergistic effect of chitosan and the nutrient-rich aquaponic medium likely contributed to the enhanced growth performance, the magnitude of this effect appears to be modulated by the genetic potential of each cultivar. This is supported by Waller et al. (2015), who demonstrated that leafy vegetables can exhibit highly variable responses to biostimulants when grown in recirculating systems, depending on their nutrient acquisition strategies and stress tolerance.

It is also worth noting that the application of chitosan did not negatively impact the water quality parameters of the aquaponic system, suggesting that the observed plant growth improvements were not due to environmental alterations but rather to the direct physiological effects of chitosan. This is in line with the conclusions of Abu-Elala et al. (2025), who reported that moderate chitosan concentrations can enhance crop performance without disturbing microbial balance or water chemistry in aquaponic setups. The number of leaves and plant height are critical indicators of photosynthetic potential and marketable yield in lettuce. The significant increases in these parameters, especially in Regina 500 treated with 1000 ppm chitosan, are promising from a production standpoint. Similarly, the substantial improvements in root length may offer advantages for post-transplanting resilience and nutrient scavenging efficiency, important traits in commercial hydroponic operations.

In lettuce, chitosan functions as a bio-stimulant by triggering physiological responses that extend beyond nutrient supply. Its application induces defense-like signaling pathways, particularly those mediated by jasmonic acid and salicylic acid, which enhance the accumulation of phenolics and phytoalexins and activate systemic acquired resistance (Xu and Mou, 2018; Ramírez-Rodríguez et al., 2024). Concurrently, chitosan stimulates the biosynthesis of auxins and gibberellins, promoting cell expansion and foliar development, which result in increased photosynthetic area and biomass accumulation (İkiz et al., 2024; Lyalina et al., 2023). At the root level, chitosan enhances cellular differentiation and lateral branching, improving water and mineral uptake efficiency in NFT and hydroponic systems (Khater et al., 2024). In addition, it has been reported to regulate stomatal conductance and water-use efficiency, thereby reducing symptoms of salinity and drought stress and contributing to plant resilience in intensive production environments (Ovalı and Ünlü, 2024). These combined processes explain the superior physiological plasticity observed in the Regina 500 cultivar under higher chitosan doses, highlighting the interaction between plant genotype and chitosan as a metabolic regulator in aquaponic production.

The observed correlations between fish and plant variables in the aquaponic system highlight the functional integration of both components. For instance, the positive association between fish growth parameters (e.g., final weight and specific growth rate) and lettuce development traits (e.g., leaf number and fresh weight) suggests that improved fish performance may enhance nutrient availability for plants, supporting the nutrient recycling principle in aquaponics. This is consistent with previous findings by Seawright *et al.* (1998) and Rakocy (2012), who emphasized the role of fish waste as a nutrient source for plant growth in recirculating systems.

Moreover, the inverse relationship between feed conversion ratio and plant development indicators implies that higher feed efficiency in fish may translate into better water quality and more accessible nutrients for plants. This reinforces the idea that fish metabolism and nutrient dynamics are interconnected within the system. The tight correlation among lettuce variables, particularly between leaf number and biomass, aligns with previous work by Delaide *et al.* (2016), highlighting the importance of nitrogen bioavailability in plant performance. Overall, the correlations validate the synergistic effects of aquaponic integration and underscore the role of biostimulants such as chitosan in enhancing both fish and plant productivity (Ani *et al.,* 2022).

The application of PCA in this study proved to be a valuable statistical tool to distinguish multivariate effects of chitosan doses on both fish and lettuce performance within an aquaponic system. The PCA evaluate treatment clustering, reduce data complexity in integrated aquaculture-hydroponics systems (Goddek *et al.,* 2016; Yep and Zheng, 2019). The distinct grouping of treatments observed in the PCA aligns with studies showing that biostimulants like chitosan influence multiple physiological variables simultaneously (El Hadrami *et al.,* 2010), which can be visualized through multivariate techniques.

Moreover, PCA has been widely used to reveal relationships among biological indicators, especially when comparing responses of plants and animals under shared environmental conditions (Delaide *et al.,* 2016; El-Naggar et al., 2022). The separation of treatments and tight clustering within groups reinforces the internal consistency of the experimental design and the potential of chitosan to simultaneously improve multiple components of aquaponic systems. These findings validate the inclusion of PCA as a complementary analysis to traditional univariate methods, particularly in studies where integrated system responses must be assessed.

Conclusions

A Chitosan supplementation in an aquaponic system improved the growth performance of both red tilapia and lettuce without affecting water quality parameters. The highest dose (1000 ppm) resulted in significantly better fish growth (weight gain, specific growth rate, feed and protein efficiency) and enhanced plant development (number of leaves, root length, plant weight, and height), particularly in the Regina 500 variety. Positive correlations between fish and plant variables confirmed the synergistic relationship within the system. PCA also showed clear separation of treatments, with 1000 ppm chitosan associated with the best overall performance. These results suggest that chitosan is a safe and effective additive to enhance productivity and integration in aquaponic systems.

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