EFFECT OF STOCKING DENSITY ON THE GROWTH OF RAINBOW TROUT (*Oncorhynchus mykiss*) AND WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEMS

Tran Thi Kim Chi[1,*], Nguyen Thanh Hai[1],
Nguyen Khac Lam[2], Nguyen Duc Thong[1], Pham Thai Giang[1]
[1] *Research Institute for Aquaculture No.1*
[2] *Center for Agricultural Development of Hanoi*
**Email: tkchi@ria1.org*

ABSTRACT

Farming rainbow trout (*Oncorhynchus mykiss*) in Vietnam started in 2005. Farming in water flowing through rearing systems is applied as the main farming technique with low density. Floods and water shortages cause great damage to rainbow trout farming in the Northwest of Vietnam. This study aims to evaluate the impact of stocking density on water quality and the growth performance of rainbow trout cultured in a low-head recirculating aquaculture system (LH-RAS). Two stocking densities, including 45 fish/m^3 and 55 fish/m^3 were raised in a 150 m^3 LH-RAS separately. The LH-RAS has two raceway farming sections combined with a rough filtration and biological filtration treatment section. The system was supplemented with new water from 0.5 - 1l/second to compensate for the wastewater discharged from the system. The initial body weight of fish was 450 ± 5 g/fish and fed daily with industrial pellets containing 40 - 45% crude protein. The total body weight of the fish was measured every 25 days. Water temperature, DO, and pH were measured daily. N-NO_2^-, CO_2, H_2S, Alkalinity, TSS and N-NO_3^- were measured weekly. The results indicated that water parameters were significant differences between the two groups, specifically DO (6.09 ± 0.68 vs. 5.73 ± 0.55 mg/l), N-NO_2^- (0.014 ± 0.005 vs. 0.035 ± 0.0027 mg/l), N-NO_3^- (3.46 ± 1.22 vs. 8.63 ± 4.42 mg/l), CO_2 (6.82 ± 0.89 vs. 7.83 ± 1.25 mg/l) and TSS (4.11 ± 1.18 vs. 5.63 ± 1.10 mg/l) ($P < 0.05$). At day 125, the weights of the fish in 45 fish/m^3 and 55 fish/m^3 groups started to diverge with the size of 861 ± 28 g and 800 ± 39 g per fish, respectively ($P < 0.05$). After 180 days, the fish at a density of 45 fish/m^2 reached 1,340 ± 82 g per fish, while those at a density of 55 fish/m^3 reached 1,170 ± 47 g per fish ($P < 0.05$). The DWG indices for these two groups were 4.72 and 3.75 g/day, respectively. The better water quality contributed to better growth of the 45 fish/m^3 group compared to 55 fish/m^3.

Keywords: *Rainbow trout, RAS, stocking density, growth, water quality.*

Received: 25 November 2024; revised: 5 December 2024; accepted: 9 December 2024.

1. INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) was first introduced to Vietnam in 2005 and has undergone over 20 years of research and development. With an annual production of approximately 1,000 tons and a market price ranging from 220,000 to 250,000 VND/kg [1], rainbow trout has become a high-value aquaculture species. The Ministry of Agriculture and Rural Development (MARD) has identified it as a potential species for future aquaculture development due to growing domestic and international market demands. Currently, rainbow trout in Vietnam is commonly cultured in water flow - through systems, where water is used only once. This method relies heavily on a constant supply of clean, cold water to maintain optimal conditions. However, water shortages during the dry season and flooding in the rainy season pose significant challenges to large-scale commercial farming, as trout require a clean and stable water environment. These challenges necessitate advanced technological solutions to optimize productivity and sustainability.

Recirculating aquaculture systems (RAS) have proven to be an effective solution for aquaculture, particularly in regions with unsuitable environmental conditions. RAS technology recycles and reuses water after mechanical and biological filtration, removing suspended solids and metabolic compounds. This approach supports high - density farming, reduces land use and water requirements, and is particularly beneficial in water-scarce regions. A low-head recirculating aquaculture system (LH-RAS) has been implemented at the Research Center for Cold water Aquaculture (RCCA), under the Research Institute for Aquaculture No. 1, designed to suit Vietnam's topographical conditions. This system is cost - effective, energy - efficient, and adaptable. According to Pfeiffer and Riche (2011), LH-RAS consumes significantly less energy than traditional high - head RAS, improving economic efficiency and increasing profitability [2]. The LH-RAS system at RCCA has a total system length of 27.5 meters and a width of 8 meters. The fish culture area comprises two raceway tanks, each measuring 20 meters in length and 4 meters in width, with a water treatment area of 7.5 x 8 meters. The treatment area accounts for 37.5% of the total system area. The fish tanks have a total volume of 170 m^3 (85 m^3 per tank) with a designed capacity of 8.5 - 15 tons/year and a productivity of 88 kg/m^3. Maximum feed usage is 70 kg/day, and the water supply requirement is 1 - 2 liters/second. The water treatment area is equipped with two aeration systems to create water flow, oxygenate, and remove CO_2.

This study examines the effects of stocking density on the growth performance of rainbow trout and water quality in LH-RAS. The findings contribute to determining the optimal stocking density for rainbow trout in LH-RAS and provide practical insights into utilizing RAS for fish farming, particularly in contexts of water shortage during the dry season and degraded water quality during the rainy season.

2. MATERIALS AND METHODS

2.1. Materials

Rainbow trout (*Oncorhynchus mykiss*) juveniles were produced at the Coldwater Aquaculture Research Center, Sa Pa town, Lao Cai province. The fish were healthy, uniform in size, with an average weight of 450 ± 5 g.

2.2. Experimental design and rearing protocol

2.2.1. Experimental Setup and Feeding

Two stocking densities were tested: 45 fish/m^3 and 55 fish/m^3.

The experiment was conducted over 180 days:

45 fish/m^3: June to December 2023.

55 fish/m^3: January to June 2024.

Feeding regime: Fish were fed twice daily at 7:30 AM and 2:00 PM using commercial pellet feed containing >45% protein and 15% lipid. The feeding rate ranged from 0.8 – 1.1% of the total fish biomass per day, depending on fish size and daily water temperature.

Fish activities and daily mortality were recorded.

The design of the low-head recirculating aquaculture system (LH-RAS) is presented in figure 1.

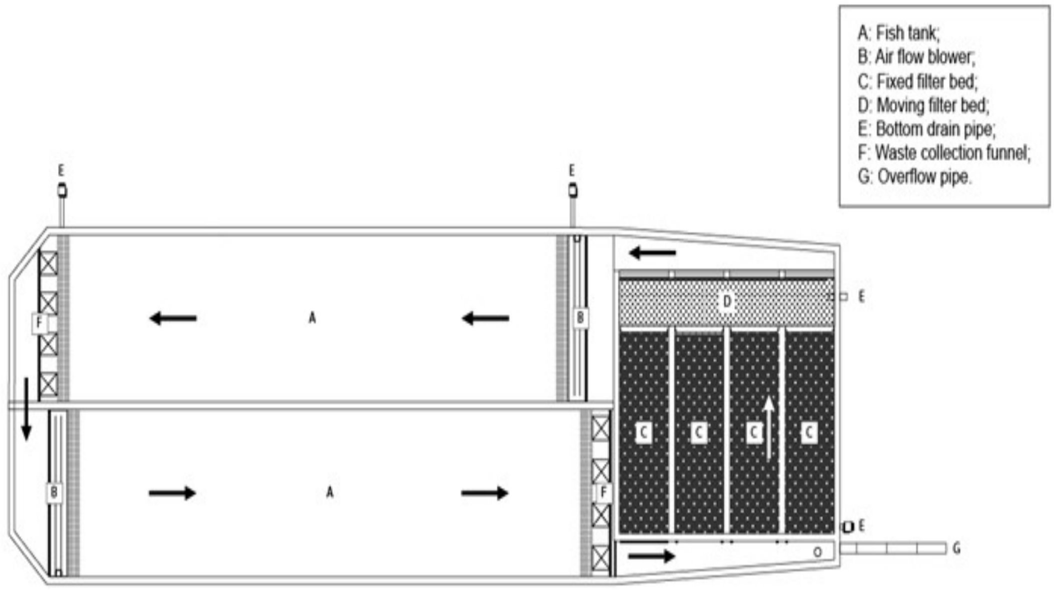

Figure 1. Low - head recirculating aquaculture system (LH-RAS) design

2.2.2. Evaluation Criteria

- Growth Monitoring: Fish size (length and weight) was measured every 25 days. For each measurement, 30 fish per tank were randomly selected and measured individually.

- Final Growth and Rearing Performance: At the end of the experiment, fish were harvested, and the following indicators were assessed: survival rate (SR), daily weight gain (DWG), daily length gain (DLG), specific growth rate (SGR), and the coefficient of variation (CV). These parameters were calculated using the following formulas:

  - Survival Rate (SR) (%) = (Total harvested fish/Total initial stocking) × 100.

  - Specific Growth Rate (SGR) (%) = [100 × (Ln final weight – Ln initial weight)]/Rearing days.

  - Daily Weight Gain (DWG) (g/day) = (Final weight – Initial weight)/ Rearing days.

  - Coefficient of Variation (CV) (%) = (Standard deviation of fish weight/Mean fish weight) × 100.

2.2.3. Environmental Monitoring and Analysis

- Dissolved oxygen (DO), temperature, and pH were monitored twice daily (7:00 AM and 2:00 PM) using a digital multiparameter instrument (YSI 1020).

- N-NO_2^-, COD, TSS, CO_2, N-NO_3^-, and alkalinity were analyzed using the methods described by Baird & Bridgewater (2017) [3], with the details of each method provided below:

  - N-NO_2^-: SMEWW 4500-NO_2^-
  - COD: SMEWW 5220 B,C:2017
  - TSS: SMEWW 2540D:2017
  - CO_2: SMEWW 4500-CO_2-C:2017
  - N-NO_3^-: SMEWW 4500-NO_3^-.E:2017
  - Alkalinity: SMEWW 2320:2017

2.3. Statistical Analysis

Data were presented as mean ± standard deviation (SD). Differences between the two stocking densities (45 and 55 fish/m^3) were analyzed using SPSS software version 23. Prior to analysis, data were checked for normal distribution and homogeneity of variance. For normally distributed data, a t-test was applied; otherwise, the nonparametric Mann-Whitney U test was used. Statistical significance was evaluated at $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

3.1. Water Quality

Table 1. Average Values of Water Quality in the LH-RAS

Environmental Parameter	Stocking density		TCVN 13952:2024
	45 fish/m^3	55 fish/m^3	
Temperature (°C)	15.26 ± 2.27	15.15 ± 3.04	10 - 20
DO (mg/l)	6.09 ± 0.68	5.73 ± 0.54*	≥ 4,0
pH	6.60 ± 0.33	6.66 ± 0.21	6,5 - 9
$N\text{-}NO_2^-$ (mg/l)	0.01 ± 0.00	0.04 ± 0.03*	≤ 0,5
$N\text{-}NO_3^-$ (mg/l)	3.47 ± 1.12	8.58 ± 4.35*	≤ 0,1
H_2S (mg/l)	0.13 ± 0.19	0.08 ± 0.05	≤ 0,1
CO_2 (mg/l)	6.83 ± 0.82	7.79 ± 1.24*	≤ 10
Alkalinity (mg/l)	11.02 ± 3.50	12.51 ± 2.43*	30 – 200
TSS (mg/l)	4.12 ± 1.08	5.59 ± 1.09*	≤ 100

*Note: Data are presented as mean ± standard deviation (mean ± SD). The symbol * indicates a statistically significant difference between the two stocking densities (P < 0.05, n = 30), TCVN 13952:2024 [4].*

There was no significant differences (P > 0.05) in temperature and pH levels between the two stocking densities. Both parameters were within the permissible limits defined by TCVN 13952:2024 [4], confirming that the initial environmental conditions were similar for both experimental treatments. Although the trials were conducted during different time periods, the consistency in temperature and pH suggests that these factors did not influence the experimental design. Hydrogen sulfide (H_2S) concentrations were not significantly different (P > 0.05) between the two treatments, with levels of 0.13 ± 0.19 mg/L at 45 fish/m^3 and 0.08 ± 0.05 mg/L at 55 fish/m^3. However, the higher density and relatively low pH range (pH = 5.73 – 6.09) contributed to elevated H_2S levels, exceeding the acceptable limit in the 45 fish/m^3 treatment. Maintaining aeration and adequate dissolved oxygen (DO) levels and minimized H_2S formation in both cases.

Significant differences in Dissolved oxygen, Carbon dioxide, nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$), and total suspended solids were observed between the two stocking densities. Dissolved oxygen levels were lower in the 55 fish/m^3 treatment (5.73 ± 0.54 mg/L) compared to the 45 fish/m^3 treatment (6.09 ± 0.68 mg/L), reflecting the higher oxygen demand associated with increased stocking density. DO is a critical parameter in intensive aquaculture systems, supporting metabolic processes and interacting with biological filtration [5]. In recirculating aquaculture systems (RAS), low DO can lead to increased fish stress, impaired biofiltration (nitrification), and potential fish mortality. Factors such as stocking density, feeding rates, temperature, and species tolerance to hypoxia can affect oxygen demand. Carbon dioxide (CO_2) concentrations (7.79 ± 1.24 mg/L vs. 6.83 ± 0.82 mg/L), alkalinity (12.51 ± 2.43 mg/L vs. 11.02 ± 3.50 mg/L), and total suspended solids (TSS) (5.59 ± 1.09 mg/L vs. 4.12 ± 1.08 mg/L) were higher in the 55 fish/m^3 treatment. These increases reflect higher oxygen consumption, elevated CO_2 production, and greater accumulation of suspended particles in the water. Excessive CO_2 can damage fish respiratory systems, reduce biofiltration efficiency, and even cause fish mortality [6, 7, 8]. Additionally, nitrate ($N\text{-}NO_3^-$) and nitrite ($N\text{-}NO_2^-$) levels were significantly higher in the 55 fish/m^3 treatment ($N\text{-}NO_3^-$: 8.58 ± 4.35 mg/L vs. 3.47 ± 1.12 mg/L;

$N\text{-}NO_2^-$: 0.04 ± 0.03 mg/L vs. 0.01 ± 0.00 mg/L). This may be attributed to increased feeding, leading to higher nitrogen input and accumulation of nitrate and nitrite in the system. Nitrite is toxic to fish, with recommended levels below 0.5 mg/L [9]. Growth inhibition in rainbow trout due to nitrite exposure has been reported, with NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) at 0.01 mg/L and 0.2 mg/L $N\text{-}NO_2^-$, respectively. Even at 0.01 mg/L $N\text{-}NO_2^-$, localized hyperplasia in gill secondary lamellae, increased blood glucose and decreased potassium were observed [10]. Nitrate is less toxic, with most fish tolerating up to 200 mg/L [11]. However, elevated nitrate levels can suppress growth and increase fish mortality [12].

Despite differences observed, water quality in both treatments generally met the requirements of TCVN 13952:2024 [4]. This indicates that the system operated stably and was capable of supporting both stocking densities. However, the 45 fish/m³ treatment demonstrated better water quality. Previous studies have shown that higher stocking densities can degrade water quality, but effective management, such as maintaining DO levels and controlling parameters like $N\text{-}NO_3^-$ and TSS, can sustain acceptable water conditions for fish growth.

3.2. Fish growth

3.2.1. Growth in fish length

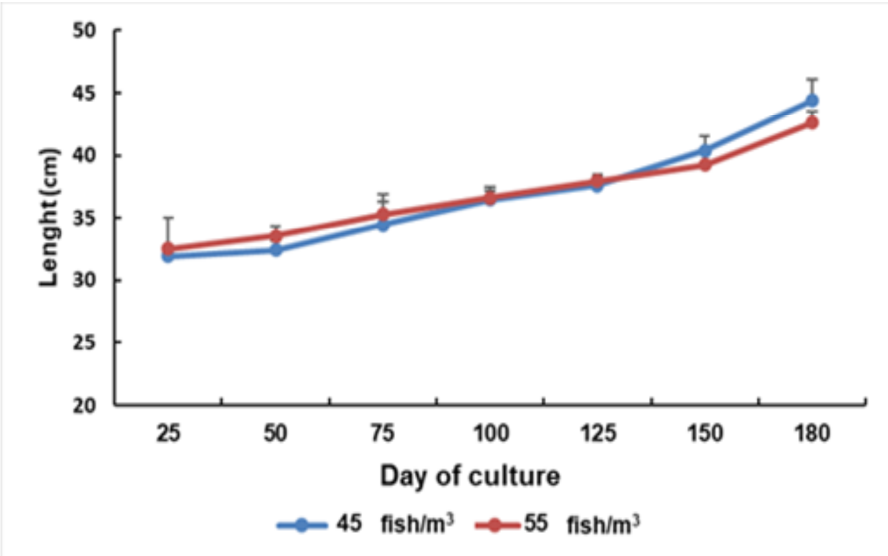

Figure 2. Length growth of fish at stocking densities of 45 fish/m³ and 55 fish/m³. *: Significant statistical difference between the two stocking densities ($P < 0.05$, n = 30).

After 180 days of rearing, rainbow trout stocked at densities of 55 fish/m³ and 45 fish/m³ achieved average lengths of 42.61 ± 0.89 cm and 44.37 ± 1.76 cm, respectively. This corresponds to daily length growth rates (DLG) of 69 mm/day and 56 mm/day. The specific growth rate (SGR) was 0.183% and 0.150% for the respective densities. Although fish reared at the lower density (45 fish/m³) tended to achieve a slightly greater average length than those at the higher density (55 fish/m³), the difference was not statistically significant ($P > 0.05$). This was primarily attributed to greater size variation among individuals in the 45 fish/m³ treatment compared to the 55 fish/m³ treatment. There were no clear differences in length growth between the treatments throughout the rearing cycle, suggesting that both stocking densities adequately supported the growth requirements of rainbow trout under the experimental conditions.

3.2.2. Growth in fish weigh

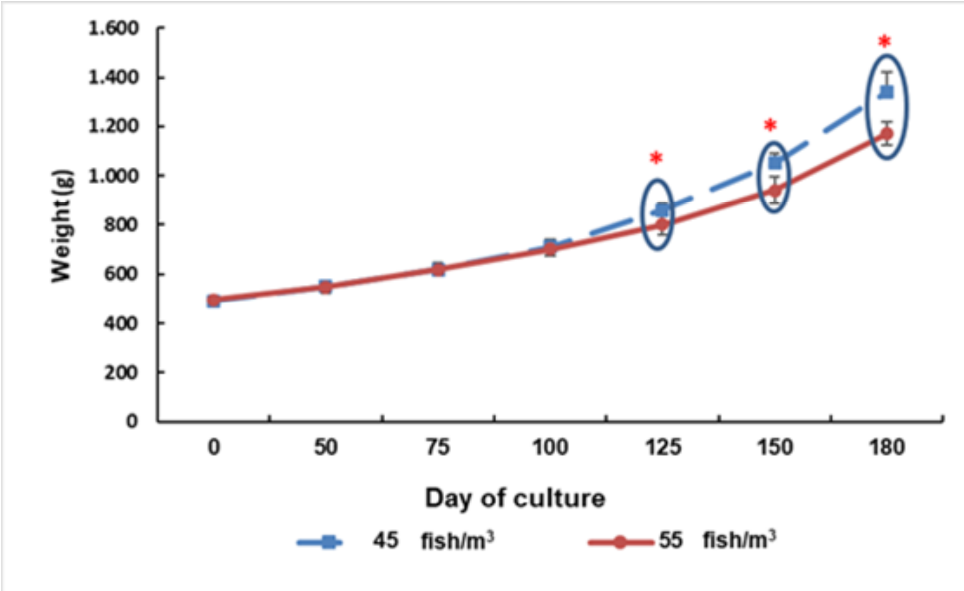

Figure 3. Weight growth of rainbow trout at stocking densities of 45 fish/m³ and 55 fish/m³. Asterisk (*) indicates a statistically significant difference between the two stocking densities ($P < 0.05$, $n = 30$).

In both rearing densities (45 and 55 fish/m³), the average weight of rainbow trout increased steadily over time. After 180 days of rearing, the average weight reached 1,340 ± 82 g at 45 fish/m³ and 1,170 ± 47 g at 55 fish/m³. The daily weight gain (DWG) was 4.32 g/day and 3.75 g/day, respectively, while the specific growth rate (SGR) was 0.558% and 0.478%.

The differences in fish weight between the two treatments became statistically significant from day 125 onward ($P < 0.05$), when the fish reached an average size of approximately 800 g per individual. Specifically, at a density of 45 fish/m³, the average weights on days 125, 150, and 180 were 860 ± 28 g, 1,053 ± 37 g and 1,340 ± 82 g, respectively. In contrast, at a density of 55 fish/m³, the corresponding weights were 800 ± 28 g, 941 ± 37 g and 1,170 ± 47 g. These differences suggest that higher stocking densities began to affect growth rates as the fish reached larger average sizes.

This finding aligns with studies conducted by Li *et al.* (2024) [13] and Liu *et al.* (2016) [14] on the effects of stocking density on fish growth, which reported slower growth rates at higher densities (36 and 40 kg/m³) compared to that at lower densities (26.3 kg/m³). The primary causes were resource competition, waste accumulation, and a decline in water quality (e.g., increased COD and N-NO_2^- levels). Moreover, growth differences tend to become more pronounced as fish reach larger sizes, due to fish increasing oxygen and space requirements.

3.2.3. Coefficient of Variation, Survival Rate, FCR and Productivity

Table 2. Coefficient of Variation of fish weight at Stocking Density at 45 fish/m³ and 55 fish/m³

Day of Culture	45 fish/m³	55 fish/m³
Day 0	1.82	1.74
Day 50	4.01	4.31
Day 75	4.75	4.00
Day 100	3.95	3.66
Day 125	3.27	4.89
Day 150	3.56	5.62
Day 180	6.09	4.01

The results in table 2 indicate a significant increase in the coefficient of variation (CV) for size disparity starting from day 50, with CV values of 4.01 and 4.31 for the two stocking densities, compared to initial values of 1.82 and 1.74. By day 180, the CV for the 45 fish/m^3 treatment showed a notable difference compared to the 55 fish/m^3 treatment, with values of 6.09 and 4.01, respectively.

Table 3. Survival Rate, FCR and Productivity

Parameter	Stocking Density	
	45 fish/m^3	55 fish/m^3
Survival Rate (%)	88	82
FCR	1.47	1.44
Productivity (kg/m^3)	53.1	54.4

The period from days 150 to 180 marked a faster growth phase compared to the period from days 125 to 150. The average daily growth rate (DWG) during days 150 - 180 was 9.59 g/day and 7.63 g/day for the two densities, respectively, compared to 7.69 g/day and 5.64 g/day during days 125 - 150. During this accelerated growth phase, less competitive individuals were unable to compete effectively for food resources, leading to a greater size disparity in the 45 fish/m^3 treatment.

As shown in Table 3, the stocking density of 45 fish/m^3 achieved a higher survival rate (88%) compared to 55 fish/m^3 (82%). These results indicate that higher stocking density negatively impacts the survival rate of rainbow trout reared in a recirculating aquaculture system (RAS). The feed conversion ratio (FCR) showed no significant difference between the two treatments, with values of 1.47 and 1.43 for 45 fish/m^3 and 55 fish/m^3, respectively. Consequently, the production yield at 55 fish/m^3 (54.4 kg/m^3) was not significantly different from that at 45 fish/m^3 (53.1 kg/m^3). From an economic perspective, however, rearing at 45 fish/m^3 offers higher efficiency (Table 4).

Table 4. Estimated costs and income at stocking densities of 45 fish/m^3 and 55 fish/m^3

Farming costs (vnd)	Groups	
	45 fish	55 fish
Labor cost	40.000.000	40.000.000
Electricity cost	100.000.000	100.000.000
Cost of fish feed	526.527.540	526.046.400
Cost of fingerling	507.870.000	578.407.500
Cost of medicines and vitamines	117.440.000	124.440.000
Others	50.000.0000	60.000000
Total input cost	1.341.837.540	1.428.893.900
Total Income	1.671.516.000	1.704.780.000
Interest	329.678.460	275.886.100

According to Fatima *et al.* (2020), high stocking density stress can reduce survival rates, growth performance, reproductive capacity, and water quality, especially when it exceeds the species' optimal threshold [15]. In this study, while water quality and growth performance tended to be affected at the higher density of 55 fish/m^3, the FCR did not show statistically significant differences. This suggests that rainbow trout can maintain feed utilization efficiency even at higher densities within the experimental range. Further studies are necessary to optimize RAS management for densities of 55 fish/m^3. Person *et al.* (2008) also reported that intensive aquaculture systems for rainbow trout could sustain growth and feed efficiency at stocking densities up to 50 - 60 kg/m^3, provided water quality and feeding regimes are well-managed [16]. This study demonstrates that our results can achieve productivity comparable to published outcomes of rainbow trout farming in RAS systems worldwide. The survival rate and productivity indicate that this model is feasible for practical application in production.

Due to limitations in infrastructure, we had only one system available for the study, requiring the two stocking densities to be tested at different times. This posed challenges in comparing the two treatments, as experimental conditions were not consistent. However, since there were no significant differences in water quality inputs and water temperature between the two experiments, this comparison is considered acceptable. Nevertheless, further studies should be conducted under conditions where treatments can be run in parallel to validate these findings. Additionally, testing a wider range of stocking densities and adjusting management practices to enhance stocking density could further improve the efficiency of farming in RAS systems.

4. CONCLUSION

Our findings suggest that a stocking density of 45 fish/m^3 is better than 55 fish/m^3 in low-head recirculating aquaculture systems, as it supports better water quality and promotes the growth of rainbow trout. The increasing stock density of rainbow leads to the decreasing growth rate.

Further research is needed to enhance water quality management and improve overall system productivity.

ACKNOWLEDGEMENTS

This study is part of the project titled "Optimization of the Commercial Farming Process for Rainbow Trout (Oncorhynchus mykiss) in Recirculating Aquaculture Systems (RAS) in Vietnam" funded by the Ministry of Agriculture and Rural Development (MARD).

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