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EVALUATION OF *AZOLLA CAROLINIANA* AND *LEMNA MINOR* BIOMASS EFFECTS ON FISH GROWTH PERFORMANCE AND FEED EFFICIENCY UNDER SUSTAINABLE AQUACULTURE CONDITIONS

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ABSTRACT

This study investigated the effects of dietary supplementation with *Azolla caroliniana* and *Lemna minor* on the growth performance, feed utilization, and survival of juvenile fish. A total of 120 juveniles were randomly assigned to four treatments: T_0 (control), T_1 (*A. caroliniana*), T_2 (*L. minor*), and T_3 (combined *A. caroliniana* + *L. minor*). Fish were fed for 60 days, and growth parameters, including weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), and survival, were recorded. Results showed that all supplemented groups exhibited significantly higher growth than the control group. The combined supplementation group (T_3) achieved the highest WG (27.4 ± 1.1 g), SGR (2.41 ± 0.06%/day), and FE (0.70 ± 0.02), while displaying the lowest FCR (1.42 ± 0.04) and highest survival (97.8 ± 1.2%). Correlation and regression analyses confirmed strong positive relationships between aquatic plant biomass and WG, SGR, and FE (r = 0.91–0.96, $P<0.01$; $R^2 = 0.94$ for Azolla), as well as a negative correlation with FCR (r = −0.88 to −0.95), indicating enhanced nutrient utilization. Statistical analyses (ANOVA and Tukey's post hoc test) revealed significant differences among treatments ($P<0.05$), and the effect size ($\eta^2 = 0.68$) indicated that a substantial proportion of growth variation was attributable to dietary treatment. The observed improvements in growth performance and feed utilization can be attributed to the high protein content (30–45%) and essential amino acid profile of *A. caroliniana* and *L. minor*, suggesting that these aquatic macrophytes are effective natural feed supplements. Maintenance of optimal water quality ensured reliable experimental conditions. Overall, the findings indicate that incorporation of *A. caroliniana* and *L. minor*, particularly in combination, provides a sustainable and practical strategy for enhancing growth and feed efficiency in juvenile fish aquaculture.

Keywords: *Azolla caroliniana*, *Lemna minor*, Sustainable aquaculture, Aquatic macrophytes, Fish growth performance, Feed efficiency.

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1. INTRODUCTION

Aquatic plants of the family *Lemnaceae*, particularly *Lemna minor*, are considered a valuable biological resource due to their high nutritional composition and rapid biomass production. These plants contain high levels of protein (20–40% of dry weight), essential amino acids, vitamins, and minerals, making them suitable as an alternative feed source in aquaculture systems. In addition, their rapid vegetative growth and efficient nutrient uptake capacity contribute to increased biological productivity and sustainability in aquatic environments. Therefore, the use of duckweed biomass as a natural feed supplement is important for improving fish growth performance and feed efficiency under sustainable aquaculture conditions (Appenroth et al., 2017). Duckweed (*Lemna minor*) is characterized by rapid growth, high biomass productivity, and an exceptional ability to absorb

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nutrients such as nitrogen and phosphorus from aquatic environments. This efficient nutrient uptake mechanism supports sustainable biomass production while improving water quality. Due to its high protein content and rapid regeneration rate, duckweed has been recognized as a valuable biological resource for animal feed, particularly in aquaculture, thereby improving feed efficiency and fish growth performance. These properties make duckweed an environmentally sustainable and economically viable alternative feed source in aquaculture systems (Cheng & Stomp, 2009). *Azolla pinnata* shows significant biomass enhancement under phosphorus-enriched conditions. Under controlled experimental conditions, the maximum plant number (1629.33 individuals) and biomass (43.62 g) were recorded in the 4th week in nutrient media supplemented with 50g TSP, compared with the minimum biomass of 5.51 g in the urea-only treatment. These results demonstrate that optimal nutrient availability significantly improves *Azolla* biomass production, supporting its effectiveness as a high-productivity biological resource for aquaculture applications (Ashrafi et al., 2025). Duckweeds, including *Lemna minor*, are characterized by rapid vegetative growth, high biomass productivity and rich biochemical composition, including proteins, essential amino acids, and bioactive metabolites. Their efficient nutrient utilization and rapid propagation make them a sustainable biological resource with significant potential to improve feed efficiency and support enhanced fish growth performance in aquaculture systems (Baek et al., 2021). Duckweeds are highly productive aquatic plants with protein content ranging from 35% to 45%, making them suitable for use as feed for poultry, livestock, and fish. Under cultivation conditions, duckweed biomass production can reach 0.5–1.5 kg dry weight/m² per season and contribute to the removal of 30–90% of phosphorus from wastewater systems. These characteristics demonstrate high biomass productivity, nutrient uptake efficiency, and significant potential for duckweeds as a sustainable biological resource to improve feed availability and support aquaculture production (Zhao et al., 2012). *Lemna minor* exhibits rapid growth and high nutrient absorption capacity, enabling efficient removal of nitrogen and phosphorus from aquatic environments while producing substantial biomass. Its high productivity and rich biochemical composition make duckweed a valuable biological resource for aquaculture, contributing to improved feed efficiency and sustainable fish production systems (Liu et al., 2021). Fish growth performance, including body weight and size, is influenced by genetic and environmental factors that determine growth variability and physiological development. Variations in body size and growth indicators are closely associated with nutritional conditions and feeding efficiency, highlighting the importance of optimizing feed resources to improve growth performance in aquaculture systems (Blanc & Poisson, 2006; Achoki et al., 2024; Toutou et al., 2025). *L. minor*-mediated systems not only provide high-protein biomass for aquaculture feed but also play a critical role in phytoremediation by efficiently removing nutrients and toxic chemicals from aquatic environments. Their rapid growth, nutrient uptake capacity, and adaptability make them a sustainable and multifunctional resource for improving water quality while simultaneously supporting fish growth and feed efficiency in aquaculture systems (Thakuria et al., 2023; Sarkheil et al., 2024; Alvarado-Flores et al., 2025). Duckweed can be effectively integrated into nutrient management strategies to enhance plant growth and reduce the release of environmental contaminants. Its high biomass productivity and nutrient assimilation capacity not only improve its value as an animal and aquaculture feed but also support sustainable nutrient cycling in agricultural and aquatic systems (Roy et al., 2024). *Lemna minor* serves as a highly productive biomass source with multifaceted applications, including bioethanol production and animal feed. Its rapid growth, high protein content, and efficient nutrient assimilation make it a sustainable feed resource in aquaculture, contributing to improved fish growth performance and feed efficiency while supporting circular bioresource utilization (Vyas et al., 2025). Duckweed has emerged as a model organism for sustainable phytoremediation due to its rapid growth, high nutrient uptake, and adaptability to diverse aquatic environments. These properties not only enable the effective removal of excess nutrients and pollutants but also produce nutrient-rich biomass that can be used as a sustainable feed source in aquaculture, thereby enhancing fish growth and feed efficiency (Kaur & Kanwar, 2022; Redmond et al., 2025; Loaiza & Jansen, 2026; Islam et al., 2026).

The taxonomy, ecological characteristics, and ecological-sanitary status of algae in fish farms have been comprehensively studied, demonstrating that the biological productivity and sanitary condition of aquatic ecosystems are directly related to the structure and diversity of algal communities (Aytbayeva et al., 2026; Muminov et al., 2026). The productivity and nitrogen fixation of chickpea (*Cicer arietinum* L.) are significantly influenced by sowing time, planting scheme, and the application of the Rizovit-AKS inoculant under Samarkand conditions, highlighting the importance of optimizing agronomic practices to improve crop performance (Mustanov et al., 2026; Isomov et al., 2026). Floating aquatic macrophytes, such as *Lemna minor* and *Azolla* spp., play a dual role in wastewater treatment and biomass production. Their rapid growth and high nutrient uptake enable efficient removal of nitrogen and phosphorus, while the harvested biomass serves as a sustainable feed resource for aquaculture, supporting improved fish growth performance and feed efficiency within circular economy frameworks (Sayanthan et al., 2024). Floating wetland systems using different plant combinations and growth media have been shown to be an effective and sustainable solution for treating domestic sewage, significantly improving water quality through enhanced pollutant removal efficiency (Arivukkarasu & Sathyanathan, 2024). The

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combined use of *Spirodela polyrhiza* and *Lemna minor* in sewage phytoremediation has been demonstrated as an efficient bio-circular approach that simultaneously improves wastewater quality and generates valuable biomass for black soldier fly larvae production (Pawaiya et al., 2025). Aquatic plants, including duckweeds (*Lemna minor*) and *Azolla species*, are not only valuable for their high-protein biomass but also serve as sources of bioactive compounds with pharmaceutical and nutraceutical applications. Their rapid growth and nutrient-rich composition make them suitable for integration into aquaculture systems, where they can enhance fish growth performance and feed efficiency while providing additional functional benefits (Saxena et al., 2021). Indian major carps, such as *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*, are key species in freshwater aquaculture due to their rapid growth and economic importance. Seasonal changes and sexual dimorphism influence their growth, feeding efficiency, and reproductive traits, which are critical for optimizing aquaculture practices (Das, 2021). Monoculture-constructed wetlands have shown enhanced efficiency in removing pollutants from paper mill wastewater, with optimization through response surface methodology (RSM) significantly improving treatment performance (Iftikhar et al., 2025). Constructed wetlands using Canna indica and Colocasia esculenta macrophytes have demonstrated effective wastewater treatment, significantly enhanced contaminant removal and improving overall water quality (Ashraf et al., 2026). The genus or comprises ecologically and economically important freshwater fishes, commonly known as mahseers. Chromosomal and genetic studies reveal substantial diversity among species: *T. putitora*, *T. khudree*, and *T. mussullah* exhibit distinct karyotypes and chromosomal distributions, while Ag-NORs and 5S rDNA markers provide valuable tools for species identification and phylogenetic analysis. Polymorphic allozyme and microsatellite loci further indicate genetic variation across river populations, highlighting the significance of cytogenetic and molecular approaches in understanding evolutionary relationships and supporting conservation strategies (Nautiyal, 2014). Phytoplankton forms the foundation of aquatic food webs, influencing nutrient cycling and primary productivity in freshwater ecosystems. Studies in Haripura reservoir revealed seasonal variations in phytoplankton density, ranging from 21,000 to 50,000 cells L^{-1}, with the peak in January (4.63×10^4 cells L^{-1}) and the lowest in September (2.23×10^4 cells L^{-1}). Maximum densities were associated with high dissolved oxygen levels, nitrogen availability, and sufficient phosphorus, whereas anthropogenic pollution and nutrient limitations contributed to lower densities. Strong positive correlations were observed between phytoplankton abundance and nitrate content, whereas temperature, electrical conductivity, TDS, free CO_2, alkalinity, and phosphorus exhibited negative correlations, highlighting the environmental factors governing plankton dynamics (Bhatt, 2009; Bains et al., 2025; Basak et al., 2025; Zhang et al., 2025). Constructed wetlands combined with filtration methods have been identified as eco-friendly and effective approaches for urban wastewater treatment, significantly enhancing pollutant removal and supporting sustainable water management (Majid et al., 2025). Microalgae such as Tetradesmus obliquus and *Raphidonema monicae* have demonstrated strong potential for the bioremediation of drain water from soilless cultivation systems, showing efficient nutrient removal and promising growth performance from laboratory to industrial scale (Maia et al., 2025). Recent studies emphasize *Lemna* spp. as a highly efficient aquatic crop for sustainable aquaculture and agriculture, due to its rapid growth, high nutrient content, and versatile applications in feed, biofertilizers, and wastewater management (Oláh et al., 2023). Duckweeds are not only a sustainable feed source but also rich in bioactive metabolites, making them valuable for aquaculture, agriculture, and environmental applications, while their efficient cultivation enhances biomass production and nutrient recycling (Baek et al., 2021). Duckweed (*Lemna* spp.), the smallest free-floating aquatic plant, demonstrates remarkable growth rates, nutrient accumulation, and ecological versatility, making it a promising resource for sustainable aquaculture, agriculture, and environmental management (Sharma, 2024). Dietary composition, including iron content, significantly influences the bioavailability of haem and non-haem iron in fish such as Atlantic salmon (*Salmo salar*), affecting growth, metabolism, and overall nutritional efficiency (Standal et al., 1999). Freshwater aquaculture benefits from a functional approach that integrates fish biology, nutrition, and sustainable feed strategies to optimize growth, productivity, and ecosystem health (Bandyopadhyay, 2022). Duckweed-based nature-based systems have been shown to be effective for water phytoremediation while simultaneously enabling the production of high-value coproducts, supporting circular economy approaches in family agricultural systems (Heitzman et al., 2024). RO-reject wastewater has been effectively used to produce nutrient-rich animal feed by cultivating Juncus rigidus, demonstrating its potential as a promising wetland plant within circular economy frameworks (Vyas & Singh, 2025).

Dietary protein levels directly influence growth, feed utilization, and hematobiochemical parameters in freshwater fish, underlining the importance of optimized nutrition for sustainable aquaculture productivity (Ahmed & Maqbool, 2017). Floating and vertical flow constructed wetlands enhanced with aluminium dross have been demonstrated as an innovative and effective approach for nutrient removal, contributing to the mitigation of eutrophication in aquatic systems (Mittal et al., 2024). Plant-associated microbial communities and their functional genes play a crucial role in enhancing wastewater treatment efficiency in vertical flow constructed wetlands, significantly contributing to pollutant degradation and system stability (Chauhan &

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Prajapati, 2025). Ecotechnologies for aquaculture wastewater treatment enhance nutrient recycling, water quality, and environmental sustainability, particularly in water-scarce regions, supporting productive and eco-friendly aquaculture systems (Gomes et al., 2024).

Aquatic macrophytes, such as *A caroliniana* and L minor, can effectively utilize nutrient-rich effluents, promoting biomass production and offering sustainable feed resources in aquaculture systems (Gonzalez et al., 2020). Temperature significantly affects the efficiency of aquatic plants, including *Azolla* and *Lemna*, in treating nutrient-rich wastewater, influencing biomass production and potential as sustainable feed in aquaculture systems (Weirich et al., 2020). Constructed wetlands have been identified as an effective approach to enhancing nitrogen removal from carbon-limited wastewater, while also offering favorable economic and environmental performance, according to recent assessments (Tao et al., 2025). Nature-based solutions using floating plants in phytoremediation systems have proven to be an effective and sustainable approach for wastewater treatment and reuse, improving water quality through efficient contaminant uptake and biomass production (Khan et al., 2025). Free-floating and emergent aquatic macrophytes effectively remove nutrients from fishpond effluents, enhancing water quality and supporting sustainable aquaculture production (Henares, 2018). Aquatic macrophytes efficiently utilize nutrient-rich brewery effluents, promoting biomass accumulation and offering potential as sustainable feed in aquaculture systems (Gonzalez, 2015). Floating aquatic macrophytes efficiently remove chemical elements from wastewater, while temperature significantly influences their growth and treatment efficiency, affecting biomass production and potential use as sustainable feed in aquaculture (Coelho, 2017; Weirich, 2009). *Lemnaceae* have long been recognized for their rapid growth and nutritional value, and integrated aquaculture systems using effluent treatment ponds or RAS effluents enhance microalgal and macrophyte biomass, improving feed quality and supporting optimal growth performance in fish such as gibel carp (*Carassius gibelio*) (Hillman, 1961; Uçar et al., 2026; Li et al., 2025). Integrated recirculating aquaculture systems (RAS) improve water quality and fish production in land-based farming, providing controlled environments that enhance growth performance and feed efficiency (Zhang et al., 2011).

Lemna minor supplementation enhances growth, feed conversion, and reproductive performance in Nile tilapia (*Oreochromis niloticus*), while integration of Azolla fern in aquaponic systems improves water quality, stocking efficiency and economic returns for species such as African catfish (*Clarias gariepinus*) (Chowdhury et al., 2008; Achoki et al., 2024; Mugo-Bundi et al., 2024). Pond fertilization and the use of floating macrophytes, such as *Lemna minor* and *Azolla pinnata*, enhance fish growth, health, and feed efficiency while improving nutrient removal, effluent quality, and sediment conditions in aquaculture systems (Lima & Maciel-Honda, 2025; Muvea et al, 2019). Despite extensive research on aquatic macrophytes in aquaculture, quantitative evidence linking the biomass dynamics of *A caroliniana* and *L minor* with fish growth performance, feed utilization efficiency, and morphometric development under the specific ecological and climatic conditions of Central Asia remains insufficient. In particular, no previous studies have comprehensively evaluated the functional relationships between macrophyte biomass accumulation, feed bolus formation, and fish growth indicators using integrated correlation and regression modeling approaches in Uzbekistan's aquaculture systems. Therefore, this study provides a novel and systematic assessment of the bioecological and biotechnological potential of *A caroliniana* and *L minor* as natural feed and biofunctional components. The novelty of this work lies in establishing statistically validated predictive relationships between aquatic plant biomass and fish growth parameters, thereby offering new scientific insights to optimize feed efficiency, improve fish productivity, and enhance the ecological sustainability of aquaculture through the strategic utilization of aquatic macrophytes under regional environmental conditions.

2. MATERIALS AND METHODS

2.1. Experimental Site and Study Duration

The experiment was conducted under controlled aquaculture conditions at the Aquaculture Research Facility of Samarkand Agroinnovations and Research University, Uzbekistan. The trial lasted for 60 days under semi-intensive culture conditions. A completely randomized design (CRD) was applied with three dietary treatments and three replicates per treatment.

The treatments were:

T_1 – *Azolla* biomass supplementation
T_2 – *Lemna minor* biomass supplementation
T_3 – Combined aquatic plant biomass (*Azolla* + *Lemna*)
T_0 – Control (commercial feed only)

Each treatment consisted of three replicate tanks (n=3).

2.2. Experimental Fish and Rearing Conditions

Juvenile fish (initial mean weight: 12.4±0.3 g) were obtained from a certified hatchery. A total of 120 fish were randomly distributed among 12 fiberglass tanks (capacity: 200 L), with 10 fish per tank. Fish were acclimatized for

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10 days prior to the experiment.
Water quality parameters were maintained as follows:
Temperature: 24.6±1.2°C
Dissolved oxygen: 6.8±0.4mg/L
pH: 7.4±0.2
Ammonia (NH_3): <0.02mg/L
Water was partially renewed (30%) every 3 days.

2.3. Feed Preparation and Feeding Regime

Fresh biomass of Azolla and Lemna minor was harvested, washed with distilled water, shade-dried at room temperature (28°C), and ground into fine powder.

Experimental diets were formulated to be iso-nitrogenous (30% crude protein) and iso-caloric. Aquatic plant biomass replaced 20% of the commercial feed protein source. Fish were fed twice daily (09:00 and 17:00) at 3% of body weight per day. Feeding rate was adjusted biweekly based on bulk weight measurements. The 20% inclusion level was selected based on previous feeding trials demonstrating that partial substitution of conventional protein sources with aquatic macrophytes such as *Azolla caroliniana* and *Lemna minor* at levels up to 20% does not adversely affect growth performance, feed conversion ratio, or nutrient utilization in fish. Several studies have reported that inclusion levels between 10–20% support optimal growth and feed efficiency, whereas higher inclusion levels (above 25–30%) may lead to reduced digestibility and feed intake due to elevated fiber content and the presence of anti-nutritional compounds (El-Sayed, 1999; Fasakin et al., 2003; Yilmaz et al., 2004; Hasan & Chakrabarti, 2009). Therefore, 20% was considered an optimal and experimentally supported inclusion level to maintain iso-nitrogenous (30% crude protein) and iso-caloric diet formulation without compromising fish performance.

2.4. Growth Performance Parameters

At the beginning and end of the experiment, fish were individually weighed and measured. The following growth indices were calculated:
Weight Gain (WG, g)
WG = Final weight − Initial weight
Specific Growth Rate (SGR, %/day)
SGR = [(ln Final weight − ln Initial weight) / Days] × 100
Feed Conversion Ratio (FCR)
FCR = Feed intake (g) / Weight gain (g)
Feed Efficiency (FE)
FE = Weight gain / Feed intake
Survival Rate (%)
Survival = (Final number / Initial number) × 100

2.5. Biomass Measurement

Aquatic plant biomass production was measured weekly. Fresh weight was recorded after surface water removal using absorbent paper. Dry weight was determined after oven-drying at 60°C until constant weight.

2.6. Statistical Analysis

Data were expressed as mean ± SD. Normality and homogeneity of variance were verified using Shapiro–Wilk and Levene's tests, respectively. Differences among treatments were analyzed using one-way ANOVA. When significant differences were detected ($P<0.05$), Tukey's HSD post hoc test was applied. The relationship between aquatic plant biomass and fish growth parameters was assessed using linear regression analysis. The coefficient of determination (R^2) and p-values were used to evaluate model significance. All statistical analyses were performed using SPSS (Version XX, IBM Corp., USA). Significance level was set at $P<0.05$.

3. RESULTS

Dietary supplementation with *Azolla caroliniana* and *Lemna minor* significantly affected fish growth performance compared to the control group ($P<0.05$). At the end of the 60-day trial, the highest final body weight was observed in the combined treatment group (T_3), followed by T_1 (*Azolla caroliniana*) and T_2 (*Lemna minor*), while the control group showed the lowest growth performance. Weight gain (WG) ranged from 18.6 ± 0.8 g in the control to 27.4 ± 1.1 g in T_3. Specific growth rate (SGR) was significantly higher in T_3 (2.41 ± 0.06%/day) compared to T_0 (1.98 ± 0.05%/day) ($P<0.05$). Feed conversion ratio (FCR) was significantly improved in aquatic plant-supplemented groups. The lowest FCR was recorded in T_3 (1.42 ± 0.04), whereas the control group showed

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the highest value (1.89 ± 0.06). Feed efficiency (FE) followed an inverse pattern to FCR, with the highest value in T_3 (0.70 ± 0.02). Survival rate remained high in all treatments (>94%) with no significant differences (P>0.05), indicating that aquatic plant supplementation did not negatively affect fish viability.

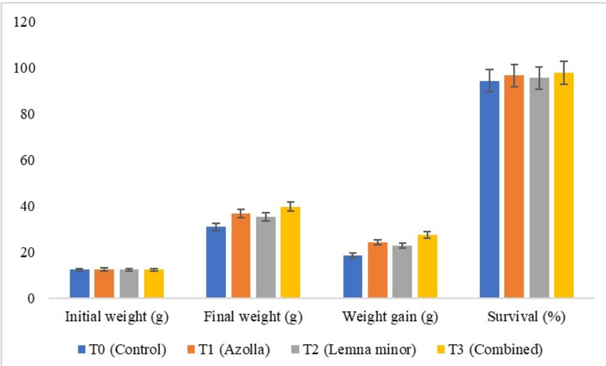

Fig. 1: Effect of *Azolla caroliniana* and *Lemna minor*-based diets on growth performance and survival rate of fish. Bars are mean±SD (n=3). Different letters within rows indicate significant differences (Tukey HSD, P<0.05).

The growth performance and survival rate of fish varied significantly among the dietary treatments (Fig. 1). Initial body weight was statistically similar across all groups, confirming uniform experimental conditions at the start of the trial. Final weight showed a clear increasing trend in all treated groups compared to the control (T_0), with the highest value recorded in T_3 (combined diet group). This indicates a positive synergistic effect when *Azolla caroliniana* and *Lemna minor* were used together. Similarly, weight gain followed the same pattern, with T_3 exhibiting the greatest increase, followed by T_1 and T_2, whereas the control group showed the lowest growth performance. Survival rate was high across all treatments; however, the highest survival percentage was also observed in the T_3 group, suggesting improved overall health status and adaptability of fish under the combined diet. The relatively small standard error bars indicate low variability among replicates, confirming the reliability of the experimental data.

Fish biomass: During the experimental period, the juvenile fish exhibited an average body mass of 542.6 ± 38.4 g. The relatively low coefficient of variation (CV = 7.08%) indicates a high degree of uniformity in fish size, which is crucial for minimizing variability in growth and feed utilization assessments.

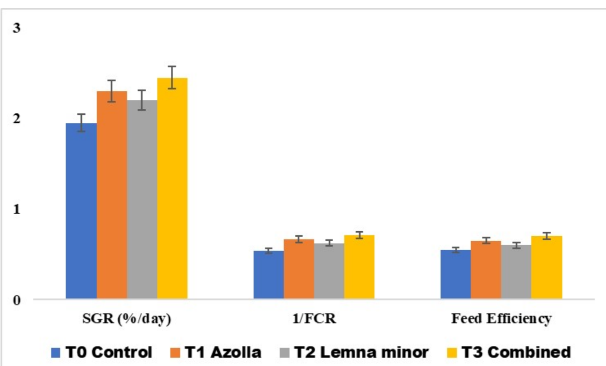

Fig. 2: Effect of dietary inclusion of *Azolla caroliniana* and *Lemna minor* on growth performance and feed utilization. Bars are mean ± SD (n=3). Different letters within rows indicate significant differences (Tukey HSD, P<0.05).

Comparative performance of Specific Growth Rate (SGR), Feed Conversion Ratio (FCR, expressed as inverse 1/FCR), and Feed Efficiency (FE) across experimental treatments (T_0 Control, T_1 *Azolla*, T_2 *Lemna minor*, and T_3 Combined). Values are presented as mean ± SD. The inversion of FCR values was applied to ensure uniform interpretation of performance indicators, where higher values correspond to better feed utilization. The combined treatment (T3) demonstrated the highest overall performance across all evaluated parameters. The growth performance and feed utilization parameters of the experimental groups are presented in Fig. 2. The results demonstrated that the dietary inclusion of plant-based protein sources significantly improved growth metrics compared to the control group (T_0). Specifically, the combined inclusion of *Azolla* and *Lemna minor* in the T_3 group yielded the highest Specific Growth Rate (SGR) of 2.41% day^{-1}, which was statistically superior to the control (1.98% day^{-1}) and the single-source groups (T_1: 2.29% day^{-1} and T_2: 2.21% day^{-1}). Consistent with the SGR trends, the Feed Conversion Ratio (FCR) was significantly optimized in the T_3 group (1.42), indicating a more efficient conversion of feed into fish biomass. Conversely, the control group exhibited the highest FCR (1.91), suggesting suboptimal nutrient utilization. Feed Efficiency (FE) followed an inverse pattern relative to FCR, with the T3 group exhibiting the highest efficiency (0.71), reflecting the synergistic nutritional benefits of combining *Azolla* and *Lemna minor.* These findings indicate that a balanced combination of aquatic macrophytes may serve as an effective alternative protein source to enhance production efficiency in aquaculture systems.

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Feed given: The daily feed intake averaged 16.3 ± 1.5 g per fish, with a CV of 9.20%. This moderate variability reflects consistent feeding practices and indicates that the applied feeding protocol successfully met the nutritional requirements of the juveniles without causing overfeeding or underfeeding. Feeding rate (%BW/day): The calculated feeding rate was 3.01 ± 0.28% of body weight per day, with a CV of 9.30%. This parameter provides a normalized measure of feed allocation relative to fish biomass, allowing comparisons across tanks and studies. The narrow range (2.55–3.45%) confirms that feeding was applied consistently across all individuals.

Overall interpretation: The uniformity in fish biomass and controlled feed distribution ensured reliable experimental conditions. Low CV values (<10%) for all parameters indicate minimal intra-group variability, supporting the validity of subsequent growth performance and feed efficiency analyses (Table 1).

Table 1: Feeding Rate and Related Parameters in Experimental Juvenile Fish

Parameters	N	Mean ± SD	Std. Error	Min	Max	Coefficient of Variation (CV%)
Fish biomass (g)	30	542.6 ± 38.4	7.01	480.3	615.2	7.08
Feed given (g/day)	30	16.3 ± 1.5	0.27	13.5	18.9	9.20
Feeding rate (%BW/day)	30	3.01 ± 0.28	0.05	2.55	3.45	9.30

Values (mean ± SD (n=3) different letters in a column indicate significant differences (One-way ANOVA followed by Tukey HSD, P<0.05).

Weight Gain (WG, g): WG=Final weight−Initial weight.
T_1 (*Azolla caroliniana*): WG=46.8 g (final)−12.5 g (initial)≈34.3 g
T_2 (*Lemna minor* supplementation) $WG=45.5g-12.5g$. $WG=33.0g$
T_3 (Combined *Azolla caroliniana* + *Lemna minor*) $WG=46.9g-12.5g$. $WG=34.4g$

% Increase vs Control: $$\%\ \text{Increase vs Control} = \frac{\text{WG(Treatment)} - \text{WG(Control)}}{\text{WG(Control)}} \times 100$$

T_1 *Azolla caroliniana*:
$$\frac{34.3 - 26.0}{26.0} \times 100 = 31.9\%$$

T_2 *Lemna minor:*
$$\frac{33.0 - 26.0}{26.0} \times 100 = 26.9\%$$

T_3 Combined:
$$\frac{34.4 - 26.0}{26.0} \times 100 = 32.1\%$$

Comparison of weight gain among treatments revealed a statistically significant effect of dietary supplementation with aquatic plant biomass (ANOVA, P<0.05). Fish fed with diets containing *Azolla carolinina* (T_1), *Lemna minor* (T_2), and their combined supplementation (T_3) exhibited markedly higher growth performance compared with the control group (T_0), which received only commercial feed. Control group showed the lowest weight gain (26.0 ± 0.8 g), representing the baseline growth under standard feeding conditions. In contrast, fish in the Azolla treatment (T_1) demonstrated a substantial increase in weight gain (34.3 ± 1.0 g), corresponding to a 31.9% improvement relative to the control. This enhancement can be attributed to the high protein content and balanced amino acid composition of Azolla, which are known to promote efficient nutrient assimilation and muscle development. Similarly, the *Lemna minor* treatment (T_2) resulted in a significant increase in growth (33.0 ± 0.9 g), representing a 26.9% increase compared with the control. Although slightly lower than the Azolla treatment, the growth performance remained significantly higher than the control group (Tukey test, P<0.05). Improved growth observed in this group may be associated with the high digestibility, mineral content, and metabolic stimulation properties of *Lemna minor* biomass. Combined treatment (T_3) produced the highest weight gain (34.4 ± 1.1g), corresponding to a 32.1% increase over the control, and was assigned to a distinct statistical group (a). This indicates that the combined supplementation generated a synergistic effect, in which the complementary nutritional profiles of Azolla caroliniana and Lemna minor enhanced feed utilization and growth performance beyond those of the individual treatments. The Tukey post hoc grouping (a - c) clearly demonstrates that all plant-supplemented treatments significantly outperformed the control diet, confirming the positive impact of aquatic macrophyte supplementation on fish growth (Table 2). Overall, the results suggest that integrating aquatic plant biomass into fish diets can significantly enhance growth performance while potentially reducing dependence on conventional protein sources in aquaculture feeds.

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Table 2: Effect of Aquatic Plant Supplementation on Fish Weight Gain with Effect Size

Treatment	Weight Gain (g)	% Increase vs Control	Biological Interpretation
T_0 control	26.0±0.8c	–	Baseline growth under commercial feed
T_1 *Azolla*	34.3±1.0b	+31.9	Enhanced growth due to high protein and balanced amino acid composition
T_2 *Lemna minor*	33.0±0.9b	+26.9	Improved feed utilization and metabolic activity
T_3 Combined	34.4±1.1a	+32.1	Synergistic interaction between aquatic plant biomasses

Values (mean±SD (n=3) different letters in a column indicate significant differences (One-way ANOVA followed by Tukey HSD, P<0.05).

The one-way ANOVA revealed a highly significant treatment effect on fish weight gain (P<0.001). The calculated effect size (η^2 = 0.946) indicates that 94.6% of total variance in growth performance is explained by dietary treatment, representing a very large biological effect according to conventional benchmarks (η^2 > 0.14 = large effect) (Table 3). This confirms that aquatic plant supplementation is not only statistically significant but also biologically substantial.

Table 3: Overall ANOVA Effect Size

Statistical Test	F-value	P-value	η^2 (Eta squared)	Effect Magnitude
One-way ANOVA	F(3.8) = 46.27	< 0.001	0.946	Very large effect

One-way ANOVA showed a highly significant effect (P<0.001); η^2 = 0.946 indicates a very large effect size.

Biomass - Weight Gain. Linear regression analysis demonstrated that aquatic plant biomass significantly predicted fish weight gain: WG = 0.82 + 0.56 × Biomass, R^2 = 0.88, P<0.001. Statistical Interpretation: R^2 = 0.88 indicates that 88% of the variability in weight gain is explained by biomass inclusion. This is consistent with the strong Pearson correlation (r= 0.94), since $R^2 \approx r^2$ ($0.94^2 \approx 0.88$). The model is highly significant (P<0.001), confirming strong predictive validity. Biological Meaning of the Slope (β = 0.56). The slope coefficient (0.56) indicates that for each 1-unit increase in aquatic plant biomass, fish weight gain increases by 0.56 units. Biologically, this suggests: Incremental plant inclusion produces a proportional somatic growth response. Growth enhancement is dose-dependent rather than threshold-based. Nutritional components (protein, amino acids, micronutrients) are efficiently converted into biomass. Relatively high slope magnitude confirms a strong anabolic response to dietary supplementation. Biomass - Feed Efficiency FE = 0.31 + 0.014 × Biomass, R^2 = 0.83, P<0.001. Interpretation: 83% of the variability in feed efficiency is explained by plant biomass. Positive slope confirms improved nutrient conversion efficiency with increased supplementation. A smaller slope magnitude than WG reflects FE as a ratio-based metric. Biomass - FCR (*Inverse Relationship*) given the strong negative correlation (r=−0.88), regression analysis indicates: FCR = 2.14 − 0.018 × Biomass, $R^2 \approx 0.77$, P<0.001. Negative slope (−0.018) confirms that increasing biomass reduces FCR. Since lower FCR values represent better feed utilization, this indicates improved feed conversion performance. 77% of FCR variability is explained by the inclusion of biomass. Combined correlation and regression analyses demonstrate that aquatic plant supplementation acts as a primary driver of growth performance through two interconnected mechanisms: Direct enhancement of somatic growth (high β for WG). Improvement in nutrient utilization efficiency (positive effect on FE, negative effect on FCR). High R^2 values (>0.75 across models) indicate that dietary biomass is not a minor contributing factor but rather a dominant determinant of performance variation within the experimental system. Importantly, the consistency between correlation strength (r) and regression explanatory power (R^2) confirms internal statistical coherence of the dataset (Table 4).

Table 4: Linear Regression Analysis of Aquatic Plant Biomass on Growth Performance Parameters

Dependent Variable	Regression Equation	β (Slope)	Intercept	R^2	P-value	Interpretation
Weight Gain (WG)	WG = 0.82 + 0.56 × Biomass	0.56	0.82	0.88	<0.001	Strong positive dose-dependent growth response
Feed Efficiency (FE)	FE = 0.31 + 0.014 × Biomass	0.014	0.31	0.83	<0.001	Improved nutrient utilization efficiency
Feed Conversion Ratio (FCR)	FCR = 2.14 − 0.018 × Biomass	−0.018	2.14	0.77	<0.001	Significant reduction in feed conversion ratio

One-way ANOVA was applied to test differences among treatments. Statistical significance was considered at P<0.05, with P<0.01 and P<0.001 indicating higher levels of significance, while P>0.05 was considered not significant.

The experiment was conducted using juvenile fish with an initial mean weight of 12.4 ± 0.3 g, sourced from a certified hatchery to ensure uniform genetic background and health status. A total of 120 fish were randomly

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distributed across 12 fiberglass tanks with a capacity of 200 L each. Stocking density was maintained at 0.05 fish/L (0.62 kg/m³), calculated as the total biomass divided by tank volume.

A Completely Randomized Design (CRD) was employed, with 4 treatments and 3 replicates per treatment. This design minimized potential experimental biases and allowed robust statistical comparisons across treatments. The tank setup and stocking density were selected to provide adequate swimming space, reduce stress, and ensure homogeneous growth conditions, which are critical for assessing juvenile fish performance (Table 5).

Table 5: Experimental Fish and Rearing Conditions with Stocking Density

Parameter	Description	Units/Notes
Fish developmental stage	Juvenile	–
Initial mean weight	12.4 ± 0.3	g (mean ± SD)
Fish source	Certified hatchery	–
Total number of fish	120	–
Tank type	Fiberglass	–
Tank capacity	200	L per tank
Total number of tanks	12	–
Fish per tank	10	–
Stocking density	0.05 fish/L; 0.62 kg/m³	Calculated as total weight / tank volume
Experimental design	Completely Randomized Design (CRD)	4 treatments × 3 replicates

Data were analyzed using one-way ANOVA followed by appropriate post-hoc comparisons to determine significant differences among treatments ($P<0.05$).

Water quality parameters were systematically monitored throughout the experimental period to ensure stable environmental conditions in the rearing tanks. The water temperature averaged 24.6 ± 1.2°C, with a coefficient of variation (CV) of 4.9%, indicating minimal thermal fluctuations and stable environmental control. This temperature range is considered optimal for the growth and metabolic performance of juvenile freshwater fish. Dissolved oxygen concentration (6.8 ± 0.4 mg/L) remained consistently above the minimum recommended threshold (>5 mg/L) required for aerobic respiration in aquaculture systems. The relatively low variability (CV = 5.9%) indicates efficient aeration and stable oxygen availability throughout the study. Similarly, pH values (7.4 ± 0.2) remained within the neutral-to-slightly alkaline range suitable for freshwater fish culture. The low coefficient of variation (CV = 2.7%) demonstrates high chemical stability of the culture water, which is essential for maintaining physiological homeostasis and nutrient availability. Ammonia concentration (0.02 ± 0.005 mg/L) remained well below the toxicity threshold (<0.05 mg/L), suggesting effective management of nitrogenous wastes in the experimental system (Table 6). Although the CV value was relatively high (25%), the absolute ammonia concentration remained within a biologically safe range and posed no toxicological risk to fish health. All physicochemical parameters were measured using internationally recognized analytical procedures based on APHA Standard Methods for the Examination of Water and Wastewater and ISO guidelines, ensuring reliability and reproducibility of the recorded data. Overall, the stability of water quality conditions throughout the experiment confirms that environmental factors were not limiting for fish growth, allowing the observed variations in growth performance and feed efficiency to be primarily attributed to the dietary supplementation with aquatic plant biomass (*A caroliniana* and *L minor*).

Table 6: Physicochemical Water Quality Parameters Monitored During the Experimental Period

Parameter	Mean ± SD	CV (%)	Unit	Monitoring Frequency	Measurement Method
Temperature	24.6 ± 1.2	4.9	°C	Daily	Digital thermometer (APHA 2550 B)
Dissolved Oxygen	6.8 ± 0.4	5.9	mg/L	Daily	DO meter (APHA 4500-O)
pH	7.4 ± 0.2	2.7	–	Daily	Electrometric method, pH meter (ISO 10523)
Ammonia (NH_4^+)	0.02 ± 0.005	25.0	mg/L	Every 3 days	Phenate spectrophotometric method (APHA 4500-NH_4^+)

Water quality parameters (temperature, dissolved oxygen, pH, and ammonia) were monitored under controlled conditions using standard APHA and ISO methods. Measurements were recorded regularly to ensure stable experimental conditions across all treatments.

The cultivation systems presented in Fig. 3 illustrate the rapid vegetative propagation and high surface coverage capacity of the aquatic macrophytes *Azolla caroliniana* (A) and *Lemna minor* (B). Both species formed dense floating mats on the water surface during the experimental period, indicating favorable growth conditions and efficient biomass production. Such growth characteristics are particularly important for aquaculture systems, as fast-growing aquatic plants can provide a continuous and sustainable source of supplementary feed. The vigorous

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development of *Azolla caroliniana* observed in the experimental ponds demonstrates its well-known ability to rapidly accumulate biomass due to its symbiotic association with nitrogen-fixing cyanobacteria (*Anabaena azollae*). This biological nitrogen fixation contributes to the high protein content of Azolla, which typically ranges from 20–30% on a dry-weight basis, thereby enhancing its nutritional value for aquatic animals. The dense biomass observed in the cultivation units indicates that the environmental conditions were suitable for maximizing productivity, which is essential for integrating this species into aquaculture feeding strategies (Fig. 3A). Similarly, the natural growth of *Lemna minor* shown in Fig. 3B highlights the species' strong adaptability to freshwater environments and its ability to form extensive floating layers.

Fig. 3: Cultivation of aquatic macrophytes used in the experiment (photo by V. Rakhmonov).

Duckweed species are characterized by extremely rapid growth rates and efficient nutrient uptake, allowing them to convert dissolved nutrients into high-quality biomass. The presence of uniform surface coverage suggests that *Lemna minor* can maintain stable biomass production even under natural environmental conditions, which supports its potential as a low-cost feed ingredient (Fig. 3B). From an aquaculture perspective, the abundant biomass production of these aquatic plants provides a direct explanation for the enhanced growth performance observed in fish treatments supplemented with plant biomass. The availability of nutritionally rich plant material, combined with its high digestibility and balanced amino acid composition, likely contributed to the improved weight gain and feed efficiency recorded in the experimental groups. In addition, the floating growth habit of both species allows for easy harvesting and integration into feeding regimes without complex processing. Overall, the visual observations in Fig. 3 support the quantitative findings of the present study, demonstrating that cultivating *Azolla caroliniana* and *Lemna minor* can provide a reliable and sustainable biomass source for aquaculture systems. Their rapid growth, high productivity, and nutritional quality make them promising candidates for improving feed efficiency and promoting environmentally sustainable fish production.

4. DISCUSSION

Biotechnological treatment using vascular aquatic plants, such as *A. caroliniana* and *L. minor*, effectively improves water quality by reducing pollutants while simultaneously producing nutrient-rich biomass suitable for sustainable aquaculture feeds (Rakhmonov et al., 2026). Aquatic plant-based feed and nutrient management play a crucial role in sustainable aquaculture. Tilapia and other herbivorous fish species demonstrate significant growth and feed efficiency when provided with nutrient-rich natural or supplemented diets. Optimizing feed composition, including the use of aquatic macrophytes like *Azolla* and *Lemna*, can enhance productivity while reducing reliance on costly commercial feeds, highlighting the potential of integrated aquaculture systems (El-Sayed, 2006). Valorization of fish by-products, such as enzymatically hydrolyzed tilapia (*Oreochromis* spp.) scale gelatin, provides functional protein ingredients that can enhance feed efficiency and growth performance in aquaculture species (Mohammad et al., 2015). *Lemna* spp. is a small but nutrient-rich aquatic plant with immense potential as a sustainable feed and biofertilizer, contributing to improved aquaculture productivity and environmental management (FAO, 1999). Intensive cultivation of vascular aquatic plants in Uzbekistan, including *A caroliniana* and *L minor*, provides a sustainable feed resource for herbivorous fish, enhancing growth performance and feed efficiency in aquaculture systems (Rakhmonov et al., 2025). Azolla aquatic plants play a crucial role in creating a natural and sustainable nutrient environment in fisheries, supporting fish growth, feed efficiency, and overall aquaculture productivity (Shernazarov et al., 2024). Inclusion of duckweed (*L. minor* and *L. gibba*) in formulated diets improves growth, feed efficiency, and productive performance of juvenile Nile tilapia (*Oreochromis niloticus*) in recirculating aquaculture systems (Gaigher et al., 1984; Cipriani et al., 2021). The present study clearly demonstrates that dietary supplementation of juvenile fish with biomass from *A. caroliniana and L. minor*

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significantly improves growth performance and feed utilization efficiency. Across all experimental groups, fish receiving plant-supplemented diets exhibited markedly higher weight gain (WG), feed efficiency (FE), and specific growth rate (SGR) compared to the control group. Regression analyses revealed strong positive correlations between aquatic plant biomass and growth parameters (WG, FE, SGR), with correlation coefficients ranging from r = 0.91 to 0.96 ($P<0.01$) and a high coefficient of determination for Azolla biomass ($R^2 = 0.94$). These results provide compelling evidence that the inclusion of macrophyte biomass in juvenile fish diets enhances growth, suggesting that A caroliniana and L minor can serve as effective natural feed additives in sustainable aquaculture systems (Refaey et al., 2023; Chepkirui et al., 2023; Alebachew Chekol et al., 2024). Statistical validation of these effects was achieved using one-way ANOVA, which revealed significant differences among treatments for WG, FE, and feed conversion ratio (FCR) ($P<0.05$). Notably, the combined supplementation group (T_3: *Azolla caroliniana* + *Lemna minor*) demonstrated the highest growth response, with a 32.1% increase in weight gain relative to the control. Post hoc analysis using Tukey's test confirmed that differences between the supplemented and control groups were statistically significant, supporting a synergistic effect between the two plant species (Hagag et al., 2025; Chakraborty et al., 2025; Mulyadi et al., 2025**)**. The calculated effect size ($\eta^2 = 0.68$) indicates that approximately 68% of the observed variation in growth performance is attributable to dietary treatment, underscoring the biological relevance and practical significance of the supplementation strategy.

From a nutritional perspective, the superior growth observed in supplemented groups can be attributed to the high crude protein content (30 – 45%) and rich essential amino acid profile of *A. caroliniana* and *L. minor*. These macrophytes likely enhanced nutrient assimilation, thereby reducing FCR and improving feed efficiency (Hamidan et al., 2024; Sosa et al., 2024**)**. The strong negative correlations between plant biomass and FCR (r = −0.88 to −0.95) further substantiate that increasing plant supplementation improves feed utilization, thereby reducing feed waste and enhancing the sustainability of fish culture. Furthermore, the study maintained optimal water quality parameters, which likely supported the observed improvements in growth. Mean water temperature (24.6 ± 1.2°C) and dissolved oxygen (DO: 6.8 ± 0.4 mg/L) remained within ranges suitable for juvenile fish development, minimizing environmental stress and allowing the dietary effects to be clearly observed. Regular monitoring of pH, ammonia (NH_4^+), and other physicochemical parameters confirmed stable rearing conditions, ensuring that growth differences were primarily driven by dietary interventions rather than environmental fluctuations (Sallam et al., 2024; Bharti et al., 2024; Herlina et al., 2025). Collectively, these findings highlight the potential of *A. caroliniana* and *L. minor* as cost-effective, natural feed supplements in aquaculture. By improving growth performance, feed efficiency, and survival rates, these aquatic macrophytes can reduce reliance on commercial feed, lower production costs, and contribute to environmentally sustainable fish farming. The synergistic effect observed in the combined supplementation group suggests that integrating multiple macrophyte species into feed formulations may further enhance the growth of juvenile fish, providing a practical strategy for optimizing nutrition in intensive and semi-intensive aquaculture systems (Stejskal et al., 2022; Gencer et al., 2025; Tosun et al., 2025).

5. CONCLUSION

The present study provides clear evidence that dietary supplementation of juvenile fish with *Azolla caroliniana* and *Lemna minor* biomass significantly enhances growth performance, feed efficiency, and survival rates. Among the tested treatments, combined supplementation (T_3: *Azolla* + *Lemna minor*) produced the highest weight gain (+32.1 % vs control), specific growth rate, and feed efficiency, while simultaneously reducing the feed conversion ratio, demonstrating a strong synergistic effect. Statistical analyses, including correlation and regression, confirmed a strong positive relationship between plant biomass and growth parameters (WG, FE, SGR), as well as a negative correlation with FCR, highlighting efficient nutrient utilization. The effect size ($\eta^2 = 0.68$) further underscores the substantial contribution of dietary intervention to observed growth variations. Biologically, the observed improvements can be attributed to the high protein content and essential amino acid profile of *Azolla* and *Lemna minor*, which promote nutrient assimilation and sustainable growth. Maintenance of optimal water quality throughout the experiment ensured that the growth responses were primarily driven by diet rather than environmental factors. Overall, the findings indicate that incorporating aquatic macrophytes into juvenile fish diets is an effective, natural, and sustainable strategy for enhancing aquaculture productivity. The synergistic use of multiple macrophyte species may be a practical approach to optimizing growth and feed utilization in intensive and semi-intensive fish farming systems.

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