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Growth and Hematological Response of Common Carp (*Cyprinus carpio*) to Dietary Supplementation with Water Hyacinth (*Eichhornia crassipes*) Extract

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ABSTRACT

This study examined the effects of dietary supplementation with *Eichhornia crassipes* leaf extract on the growth performance and hematological parameters of common carp (*Cyprinus carpio*). Phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, alkaloids, and saponins, with moderate antioxidant activity (50.89% DPPH inhibition). Experimental treatments showed that fish receiving moderate levels of the extract had significantly higher erythrocyte and leukocyte counts, indicating improved oxygen transport and immune function. Additionally, the group with the highest absolute weight gain suggests better nutrient absorption. All treatments maintained a 100% survival rate and stable water quality, confirming the extract's safety and environmental compatibility. These results support the use of *E. crassipes* as a sustainable, cost-effective, and environmentally friendly alternative to synthetic growth promoters in aquaculture. Further research is recommended to investigate its long-term effects and mechanisms of action.

INTRODUCTION

The common carp (*Cyprinus carpio*) is one of the most economically significant freshwater species in global aquaculture, renowned for its rapid growth, high feed efficiency, and exceptional adaptability to a wide range of environmental conditions (Nedoluzhko et al., 2020). With an annual global production exceeding four million tons, *C. carpio* ranks among the top three most farmed fish species worldwide (Eljasik *et al*., 2022; Su *et al*., 2023). As the demand for sustainable aquaculture intensifies, there is a growing emphasis on optimizing nutritional strategies—particularly the use of functional feed additives—to enhance growth performance, feed utilization, and physiological health in cultured fish (Dong *et al*., 2022; Kwasek *et al*., 2020).

Among these strategies, plant-based feed additives, also known as phytogenic, have emerged as promising alternatives to synthetic growth promoters and antibiotics. Derived from herbs, spices, and aquatic plants, phytogenics have been reported to improve fish growth, nutrient absorption, immune competence, and stress resilience (Kuebutornye *et al*., 2024). Previous studies have demonstrated the efficacy of plant-derived bioactive

compounds—such as those found in wheatgrass, barley, and ginger—in enhancing fish performance and survival under pathogenic challenges (Burducea *et al*., 2022; Zaman & Cho, 2023). Additionally, phytogenic substances like olive byproducts and green tea extract have shown the ability to modulate antioxidant status and lipid metabolism in several aquaculture species (Liu *et al*., 2022).

Eichhornia crassipes, commonly known as water hyacinth, is a fast-growing aquatic plant rich in phytochemicals such as flavonoids, phenols, tannins, and saponins. These compounds are known for their potent antioxidant, antimicrobial, and anti-inflammatory activities (Ben Bakrim *et al*., 2022; Ganorkar *et al*., 2023). In addition to its recognized potential in bioremediation and ecological applications, recent pharmacological research has highlighted the plant's promising biological activities against pathogens and oxidative stress *(Bhavsar et al*., 2020; Mueed *et al*., 2023). However, despite its phytochemical richness and global availability—often as an invasive species—its application in aquafeed, particularly in improving growth and physiological health in *C. carpio*, remains underexplored.

Existing studies on other plant-derived feed additives—such as tomato byproducts and *Beta vulgaris*—have shown improved feed conversion ratios and protein efficiency in common carp diets. Nevertheless, empirical evidence regarding the use of *E. crassipes* extract in aquaculture remains limited. More specifically, its effects on hematological parameters in fish are poorly understood, despite the critical role of hematological indices in assessing fish health, physiological adaptation, and immune function. Several plant extracts, such as olive leaf and *Laurencia caspica*, have demonstrated positive immunohematological effects, yet water hyacinth has not been sufficiently studied in this area. Effects, yet water hyacinth has not been sufficiently studied in this regard (Khanzadeh *et al*., 2023; Rajabiesterabadi *et al*., 2020).

Given these knowledge gaps, this study seeks to evaluate the effects of dietary supplementation with *E. crassipes* extract on the growth performance and hematological responses of *Cyprinus carpio*. The investigation focuses on key growth metrics such as specific growth rate, feed conversion ratio, and biomass gain, alongside hematological parameters including red and white blood cell counts, hemoglobin concentration, and hematocrit levels. The findings are expected to provide insights into the viability of *E. crassipes* as a functional and sustainable feed additive in modern aquaculture systems.

METHODS

Study Site and Experimental Duration

The experiment was conducted from April to May, 2025. Fish rearing trials and hematological observations were carried out at the Laboratory of Aquaculture Production and Reproduction, Faculty of Agriculture, University of Mataram. Hematological analyses were performed at the Fish Health Laboratory of the same faculty. Phytochemical screening, antioxidant analysis, and plant extraction procedures were conducted at the Analytical Chemistry Laboratory and the Basic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram.

Preparation of *Eichhornia crassipes* Leaf Extract

Fresh leaves of *Eichhornia crassipes* (water hyacinth) were collected, rinsed with clean water, and air-dried to remove surface moisture. The leaves were then chopped into smaller pieces and oven-dried at 42°C for approximately 72 hours until constant weight. The dried

material was ground into powder using a laboratory blender and sieved to obtain a fine powder.

Extraction was carried out using the maceration method. Approximately 300 grams of powdered leaves were soaked in 3 L of 96% ethanol for 72 hours with daily manual agitation. After maceration, the mixture was filtered through Whatman filter paper. The filtrate was concentrated using a rotary evaporator at 45–50°C to obtain a semi-solid extract. Phytochemical screening and antioxidant activity analysis were subsequently performed on the extract.

Experimental Design and Fish Rearing

A total of 120 healthy common carp (*Cyprinus carpio*) juveniles were acclimated in fiberglass tanks for 5 days and fed a control diet. Following acclimatization, fish were randomly distributed into 12 plastic container tanks (45 L each), with 10 fish per container, in a completely randomized design (CRD) with four treatments and three replicates.

Each tank was equipped with air stones connected to an air pump to maintain adequate dissolved oxygen levels. Water was partially renewed every 4–5 days. The experimental diets were prepared by spraying the fish feed with *E. crassipes* extract at different inclusion levels and drying at room temperature. Fish were hand-fed twice daily to apparent satiation for 30 days. Length and body weight were recorded at the beginning, every 10 days, and at the end of the trial. Feeding response and behavioral observations were monitored daily. Water quality parameters (temperature, pH, and DO) were measured at the start and end of the study using a digital thermometer, portable pH meter, and DO meter.

Blood Collection and Hematological Sampling

At the end of the feeding trial, one fish was randomly selected from each tank (n=12) for hematological analysis. Fish were anesthetized and placed on a moist cloth. Blood samples (1–2 mL) were collected from the caudal vein using 1 mL sterile syringes pre-flushed with 2% EDTA anticoagulant. Samples were immediately transferred to microtubes containing 2% EDTA and stored at 4°C for hematological analysis.

Experimental Parameters

Phytochemical Screening and Antioxidant Activity

Phytochemical analysis was performed to identify major secondary metabolites using standard qualitative tests. The presence of alkaloids was tested using Mayer's, Wagner's, and Dragendorff's reagents. Flavonoids were identified using 10% aluminum chloride ($AlCl_3$), while saponins were detected using distilled water and foam tests. Terpenoids and steroids were screened using chloroform, acetic anhydride, and concentrated sulfuric acid. Tannins and phenolic compounds were analyzed using 5% $FeCl_3$. Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Extract samples were reacted with DPPH solution, and absorbance was measured using a UV-Vis spectrophotometer at 517 nm.

Total Erythrocyte Count

Erythrocyte count was performed following Syawal *et al*. (2021). Blood was drawn using a Sahli pipette up to the 0.5 mark and diluted with Hayem's solution up to the 101 mark. The mixture was gently shaken in a figure-eight motion for 3–5 minutes. A drop of the mixture was placed on a Neubauer hemocytometer and observed under a light microscope (400× magnification). Cells were counted in five small squares. The erythrocyte count (cells/mm³) was calculated as:

$$\mathrm{RBC} = \left(\frac{\mathrm{a}}{\mathrm{n}}\right) \mathrm{x} \left(\frac{1}{\mathrm{v}}\right) \mathrm{x}\ \mathrm{DF}$$

Where:
a = number of cells counted
n = number of squares counted
v = volume of chamber
DF = dilution factor

Total Leukocyte Count

Leukocyte count followed a similar procedure using Turk's solution as the diluent. Blood was drawn into a capillary pipette to the 0.5 mark, then diluted to the 11 marks with Turk's solution. The mixture was shaken and placed on a hemocytometer. White blood cells were counted under 400× magnification in four large squares. The leukocyte count (cells/mm³) was calculated using the same formula as for erythrocytes.

Leukocyte Differential Count

Differential leukocyte count was conducted according to Civelekoglu *et al*. (2022). Blood smears were prepared by spreading a drop of blood on a glass slide at a 45° angle. Smears were air-dried, fixed in methanol for 5 minutes, and stained with Giemsa for 10 minutes. After rinsing and drying, slides were observed under oil immersion (1000× magnification). A total of 100 leukocytes was counted per slide to calculate the relative percentages of lymphocytes, monocytes, and neutrophils.

Weight

Absolute weight gain was calculated using:

$$W = Wt - Wo$$

Where:
W = weight gain (g)
W_o = initial weight (g)
W_t = final weight (g)

Length

Absolute length gain was determined by:

$$L = Lt - Lo$$

Where:
L = length gain (cm)
L_o = initial length (cm)
L_t = final length (cm)

Specific Growth Rate (SGR)

SGR was calculated using:

$$SGR = \frac{\ln(Wt) - \ln(Wo)}{T} \text{ x } 100$$

Where:
SGR = specific growth rate (%/day)
T = duration of the experiment (days)

Survival Rate

Survival rate was assessed using:

$$SR = \frac{Nt}{No} \text{ x } 100$$

Where:
SR = survival rate (%)
N_o = number of fish at the beginning
N_t = number of fish at the end

Water Quality Parameters

Water temperature, pH, and dissolved oxygen (DO) were measured at the beginning and end of the trial in all tanks using standardized instruments. Data were used to confirm suitability for carp culture and were presented descriptively.

Statistical Analysis

All quantitative data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at a 5% significance level using SPSS software (version 23.0). Data are presented as mean ± standard deviation. Water quality data were analyzed descriptively.

RESULTS

Phytochemical Screening and Antioxidant Activity

Phytochemical screening of *Eichhornia crassipes* leaf extract revealed the presence of alkaloids (+++), flavonoids (+++), saponins (++), and steroids/terpenoids (++), while tannins and phenolic hydroquinones were not detected. The antioxidant activity measured by DPPH method showed an average inhibition rate of 50.89%. The presence of high levels of flavonoids and alkaloids is indicated by the strong positive reactions in the qualitative tests, which are visually confirmed by distinct color changes such as reddish yellow in flavonoids and white precipitates in alkaloid tests. These compounds are known to exhibit strong free-radical scavenging activity, contributing to the antioxidant capacity of the extract. The DPPH inhibition data presented in Table 2 confirm that the extract had a consistent antioxidant effect across replicates, with values ranging from 50.05% to 51.42%, and an average of 50.89%. This indicates that *E. crassipes* extract possesses moderate antioxidant potential, which may contribute to the physiological benefits observed in the treated fish, especially in terms of hematological stability and cellular protection.

Table 1. Phytochemical Components of Water Hyacinth Leaf Extract

Compound Group	Result	Indicator Reaction Description
Alkaloids	+++	White precipitate (Mayer); Red (Wagner, Drag)
Flavonoids	+++	Yellow cloudy (NaOH); Reddish yellow (H_2SO_4)
Saponins	++	Persistent foam
Steroids/Terpenoids	++	Bluish green coloration
Tannins	-	No color change
Phenolic/Hydroquinone	-	No color change

Table 2. Antioxidant Activity of Water Hyacinth Extract (DPPH Method)

Sample Replicate	DPPH Absorbance	Sample Absorbance	Inhibition (%)
1	0.951	0.462	51.42
2	0.951	0.475	50.05
3	0.951	0.464	51.21
Average			50.89

Hematological Parameters

The hematological profile of *C. carpio* revealed marked differences in response to the dietary treatments. As shown in Figure 1, erythrocyte counts increased significantly with the addition of *E. crassipes* extract in the diet, with the highest count recorded in treatment P2

(3.74×10^6 cells/mm³), compared to the control group P0 (0.81×10^6 cells/mm³). Similarly, leukocyte counts followed a comparable trend (Figure 2), with P2 yielding the highest count (2.71×10^4 cells/mm³), suggesting enhanced hematopoiesis and possibly an upregulated immune response. Differential leukocyte analysis (Figure 3) indicated a higher proportion of lymphocytes and monocytes in the P2 group, with lymphocytes reaching 69.67%, while neutrophils were lowest in this group (12.67%), implying a more robust immune profile. These variations indicate that bioactive compounds in the extract, such as flavonoids and saponins, may have immunostimulatory effects, leading to increased blood cell production. Furthermore, the hematological improvements in P1, P2, and P3 treatments compared to the control reflect a dose-dependent physiological enhancement.

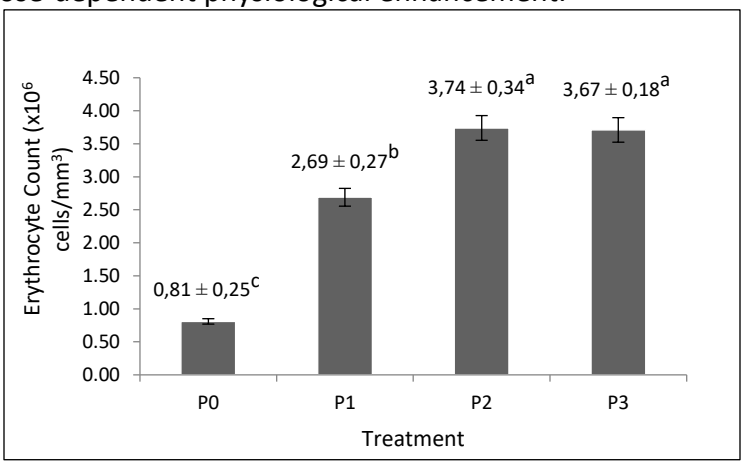

Figure 1. Erythrocyte Count in *C. carpio* (x10^6 cells/mm³)

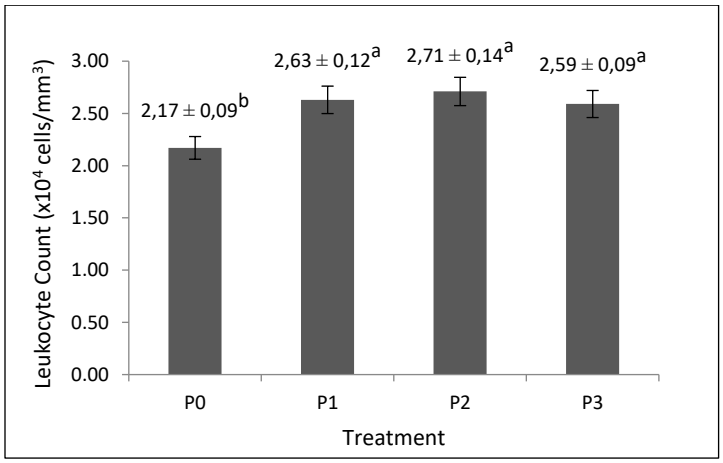

Figure 2. Leukocyte Count in *C. carpio* (x10^4 cells/mm³)

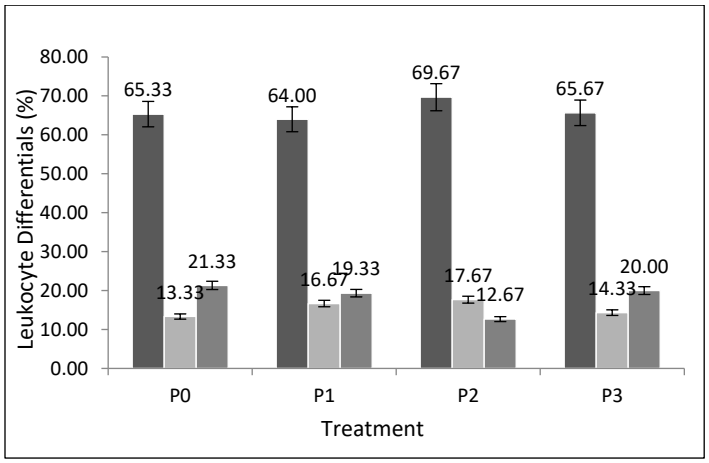

Figure 3. Leukocyte Differentials (%). Description: The sequence of differential leukocyte data starts from lymphocytes, monocytes, and neutrophils.

Growth Parameters

Growth performance data revealed a significant enhancement in absolute weight gain in fish fed diets containing *E. crassipes* extract. As illustrated in Figure 4, the P2 group showed the highest absolute weight gain (32.01 g), followed by P1 (28.04 g), P3 (27.15 g), and P0 (24.22 g). The increased weight gain in the extract-treated groups indicates improved nutrient assimilation and possibly enhanced metabolic efficiency, likely influenced by bioactive components in the extract. Although absolute length gain (Figure 5) and specific growth rate (Figure 6) did not show statistically significant differences among treatments, there were consistent upward trends in the treated groups compared to the control. All treatments achieved a 100% survival rate (Figure 7), demonstrating the safety and tolerability of the extract. These findings suggest that the inclusion of *E. crassipes* extract, particularly at moderate concentrations, supports growth and physiological development in *C. carpio*, likely through improved feed utilization and health status.

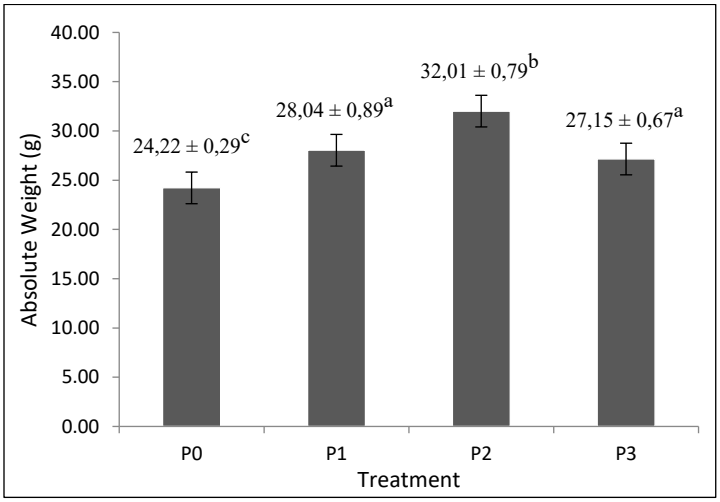

Figure 4. Absolute Weight (g)

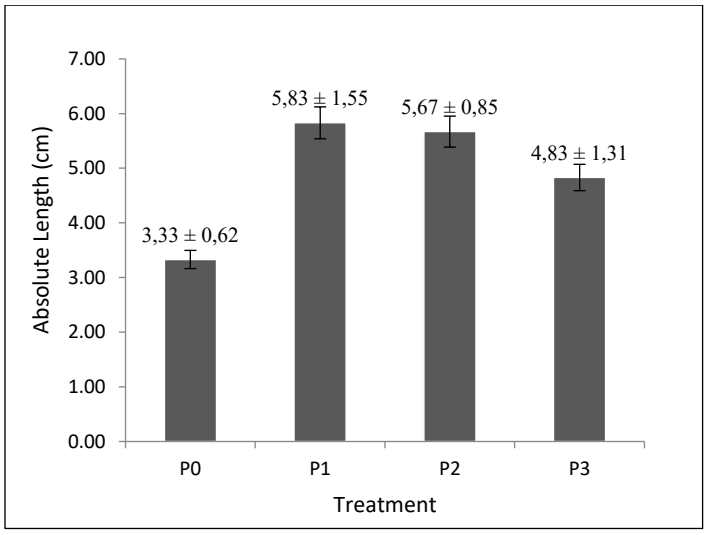

Figure 5. Absolute Length (cm)

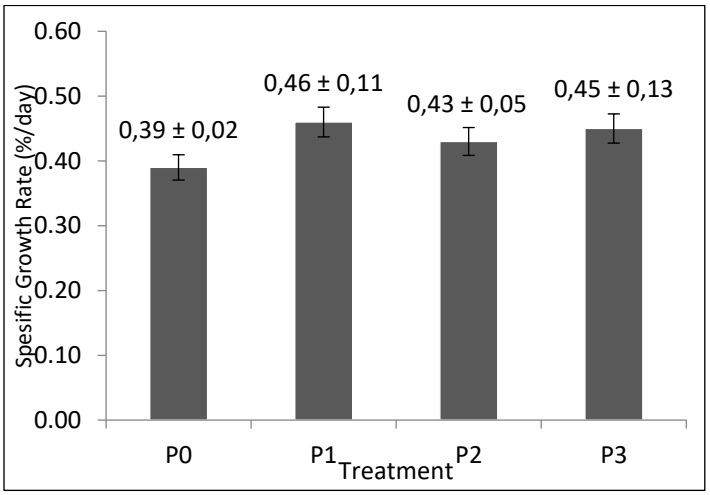

Figure 6. Specific Growth Rate (%/day)

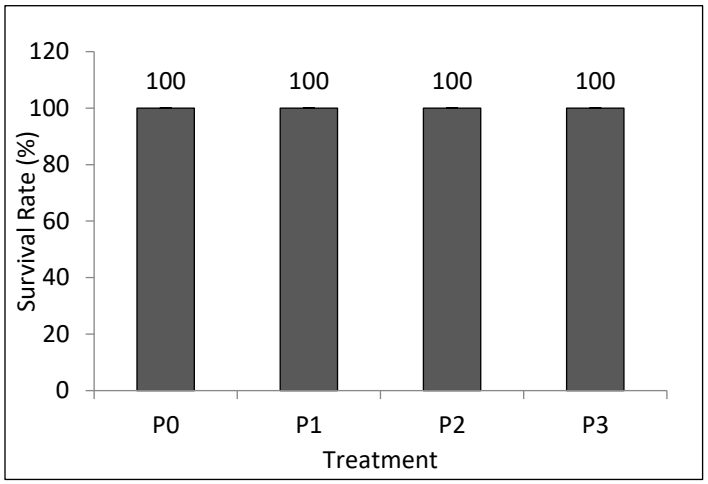

Figure 7. Survival Rate (%)

Table 3. Water Quality During Experiment

Parameter	Range	Optimal Range	Reference
Temperature	28.6-29.3°C	25-30°C	Rahmadiah (2013); Julianti *et al*. (2022)
pH	7.3-8.0	6.0-8.0	Silaban (2012); Hardhini (2018)
DO	5.3-7.7 mg/L	>5.0 mg/L	Mustofa *et al*. (2018)

DISCUSSION

Bioactivity of *Eichhornia crassipes* and Its Functional Role

The bioactive composition of *Eichhornia crassipes*—particularly its high content of flavonoids, alkaloids, saponins, and terpenoids—demonstrates its significant nutraceutical potential in aquaculture. These phytochemicals are widely known for their antioxidant, antimicrobial, and anti-inflammatory properties, which can be leveraged to improve fish health and performance (Awad & Awaad, 2017; Reverter *et al*., 2014). The results from the phytochemical screening in this study are consistent with previous research, indicating that *E. crassipes* contains potent antioxidant constituents such as luteolin, apigenin, and saponin glycosides, which may neutralize free radicals and reduce oxidative stress in fish (Ben Bakrim *et al*., 2022).

The antioxidant activity as determined by the DPPH assay, yielded an average inhibition rate of 50.89%, indicating moderate but stable free radical scavenging capacity. This is particularly relevant in aquaculture systems where oxidative stress—caused by handling, stocking density, or suboptimal water quality—can compromise immune competence and growth (Saurabh & Sahoo, 2008). Natural antioxidants in fish feed have been shown to reduce lipid peroxidation and improve mitochondrial function in hepatocytes and erythrocytes, thereby supporting systemic physiological functions (Monier *et al*., 2025; Zhang *et al*., 2019). Thus, the consistent antioxidant effect of *E. crassipes* may have contributed indirectly to the improvements in hematological parameters and weight gain observed in treated groups.

Moreover, the phytochemical richness of *E. crassipes* allows it to act not only as a protective agent but also as a growth promoter. Certain secondary metabolites, such as terpenoids and alkaloids, are known to modulate metabolic pathways, enhance protein synthesis, and stimulate digestive enzymes (Hoseinifar *et al*., 2015). This suggests that the extract could serve as a dual-function additive—both nutritionally and immunologically beneficial—making it a viable candidate for functional aquafeed development.

Hematological Modulation and Immune Responses

Hematological indices serve as sensitive biomarkers for assessing physiological and immunological responses in fish. In this study, supplementation with *E. crassipes* extract led to significantly increased erythrocyte and leukocyte counts, especially in the P2 treatment group. Elevated erythrocyte counts indicate enhanced hematopoiesis, which may be linked to the stimulation of erythropoietic centers in the kidney and spleen—a process commonly triggered by plant-derived immunostimulants (Kuebutornye *et al*., 2024). The improved erythrocyte production enhances oxygen transport capacity, which is vital for supporting growth and metabolic activity.

Leukocyte profile analysis further reveals the immunomodulatory effects of the extract. The increase in lymphocyte and monocyte percentages, accompanied by a reduction in neutrophil counts, suggests that fish in the P2 group were not undergoing acute inflammation or infection, but were instead in an elevated state of immune readiness. Lymphocytes, particularly T- and B-cells, play a crucial role in adaptive immunity, while monocytes serve as

precursors to macrophages involved in phagocytosis and tissue repair (Chen & Luo, 2023; Kuebutornye *et al*., 2024). These shifts are in line with other studies showing that plant-based feed additives, including ginger, garlic, and *Azadirachta indica*, can stimulate hematopoietic tissue and modulate leukocyte ratios in fish (Zhang *et al*., 2019).

The immunological benefits are likely driven by flavonoids and saponins, which have been shown to activate macrophages, increase complement activity, and upregulate proinflammatory cytokines under stress conditions (Harikrishnan *et al*., 2011). In addition, the stability of hematological parameters across treatments P1–P3 further supports the hypothesis that *E. crassipes* extract, at appropriate concentrations, can be safely used as an immuno-enhancing dietary supplement in aquaculture.

Growth Performance and Nutritional Enhancement

The observed improvement in absolute weight gain, especially in P2 (32.01 g), demonstrates the growth-promoting effect of *E. crassipes* extract. While specific growth rate (SGR) and length gain were not significantly different across treatments, the trend toward higher values in the extract-supplemented groups suggests enhanced feed utilization. Similar findings have been reported in *Cyprinus carpio* fed diets containing *Beta vulgaris* and tomato pomace extract, which also contain high levels of phenolics and flavonoids that improve digestive enzyme activity and nutrient absorption (Amiri *et al*., 2023; Kesbiç *et al*., 2022).

The mechanism behind the growth enhancement likely involves improved gut morphology and enzymatic function. Bioactive compounds such as flavonoids can stimulate the secretion of digestive enzymes (e.g., protease, lipase) and improve the integrity of intestinal villi, leading to better nutrient assimilation (Hoseinifar *et al*., 2015). Furthermore, alkaloids and saponins may reduce pathogenic bacterial load in the gut, promoting a favorable microbiota composition, which has been linked to better feed conversion ratios and metabolic efficiency in fish (Caipang, 2020; Citarasu, 2010).

Although no mortality was observed in any treatment, the superior performance of P2 indicates that there may be an optimal inclusion level for *E. crassipes* extract that balances immunological stimulation and nutrient bioavailability without inducing stress. This aligns with the concept of hormesis, where moderate exposure to bioactive compounds yields beneficial effects, while excessive dosages may be detrimental or ineffective (Calabrese & Mattson, 2017). Therefore, further dose-optimization studies are necessary to refine its application in commercial aquafeed.

Safety and Implications for Sustainable Aquaculture

The complete survival of fish in all treatment groups, alongside water quality parameters within optimal ranges, underscores the biosafety of *E. crassipes* extract as a feed additive. This finding is critical, given the concerns about the use of synthetic growth promoters and antibiotics in aquaculture, which may pose environmental and health risks (FAO, 2020). Natural, plant-based alternatives such as *E. crassipes* offer a sustainable and eco-friendly solution, especially when sourced from invasive species that otherwise pose ecological challenges.

The integration of *E. crassipes* into fish diets not only addresses issues of sustainability and waste valorization but also enhances circular bioeconomy models in aquaculture. The repurposing of aquatic weeds into functional feed ingredients has been increasingly supported by international efforts toward climate-smart aquaculture (Hasan & Soto, 2017). Moreover, its widespread availability and ease of harvesting make *E. crassipes* a low-cost resource with high potential for commercial application, particularly in regions with limited access to conventional feed ingredients.

Taken together, the findings of this study strongly advocate for further exploration into the functional roles of aquatic macrophytes in aquafeed development. Future research should focus on long-term feeding trials, gut histomorphology analysis, and immune gene expression profiling to fully understand the scope and limitations of *E. crassipes* extract as a phytogenic feed additive.

CONCLUSION

Eichhornia crassipes leaf extract is a promising, eco-friendly aquafeed additive that improves growth and hematological health in *Cyprinus carpio* by enhancing nutrient utilization, immune readiness, and antioxidant capacity, while offering a sustainable strategy for utilizing invasive aquatic plants. Dietary supplementation with *E. crassipes* extracts significantly improved hematological parameters, especially erythrocyte and leukocyte counts, indicating enhanced oxygen transport and immune readiness. Growth performance, particularly absolute weight gain, also improved in the treatment group receiving moderate extract levels (P2), suggesting better nutrient assimilation and feed efficiency. The 100% survival rate and stable water quality across treatments further validate the safety and environmental compatibility of the extract. Overall, *E. crassipes* extract shows promise as a sustainable, cost-effective, and eco-friendly alternative to synthetic growth promoters in aquaculture. Its use could help improve fish health and growth while promoting the valorization of an otherwise invasive aquatic plant. Future research should focus on dose optimization, long-term effects, and its role in modulating gut microbiota and immune gene expression to further validate its application in commercial aquafeed formulations.

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