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# DIETARY *MANGIFERA INDICA* LEAF POWDER ENHANCES HEMATOLOGICAL PARAMETERS AND SURVIVAL OF *CYPRINUS CARPIO* UNDER SALINITY VARIATION

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**Abstract:** Common carp (*Cyprinus carpio*) is a leading freshwater fish commodity adaptable to various water conditions. This study examined the effectiveness of mango leaf powder (*Mangifera indica*) as a phytoimmunostimulant on hematological profiles and survival rate of common carp in freshwater and brackish water over 30 days. Fish were fed commercial feed supplemented with 1.5 g/100 g mango leaf powder (treatment) or without supplementation (control), with three replicates. Blood samples collected on days 0 and 30 were analyzed for total erythrocyte, hematocrit, and hemoglobin. Supplementation significantly improved all hematological parameters in both media ($p < 0.05$), with freshwater values reaching $1.75 \times 10^6$ cells/mm³, 33.66%, and 8.10 g/dL, and brackish-water values reaching $1.63 \times 10^6$ cells/mm³, 30.66%, and 7.70 g/dL. Survival rates reached 94.67% (freshwater) and 92.00% (brackish water), surpassing the control group. Mango leaf powder supplementation effectively improves hematological parameters and survival, supporting its use as a safe antibiotic alternative.

**Keywords:** *Mangifera indica*, phytoimmunostimulant, hematology, immune response, carp.

## INTRODUCTION

Common carp (*Cyprinus carpio*) is among the most widely cultivated freshwater fish species worldwide and plays a crucial role in the blue revolution (Stanicuk *et al.,* 2024). It is also a strategic aquaculture commodity in supporting global food security (De Silva, 2016). One advantage of common carp in aquaculture is its ability to adapt to various water conditions, including salinities up to 5 ppt (Effendi *et al.,* 2025a; Effendi *et al.,* 2025b; Saputra *et al.*, 2023). This osmoregulation tolerance opens opportunities to diversify cultivation media for brackish water, address the limitations of freshwater cultivation land, and provide open access for cultivators in coastal areas. Raising common carp in brackish water environments poses a unique challenge for farmers that warrants scientific study. Exposure to salinity causes fish to allocate energy to osmoregulation, thereby reducing the energy available for growth and immune function (Lin and Hwang, 2024).

Another problem in intensifying common carp farming is the high incidence of disease and environmental degradation (Song and Zhang, 2025). According to Yusoff *et al.* (2024), intensive farming systems are characterized by high stocking densities, suboptimal water quality, and excessive feeding, which create chronic stress for fish, affect their health, and pose environmental risks. Additionally, Gundi *et al.* (2025) noted that the widespread use of antibiotics in aquaculture as a preventive measure has led to the development of resistant pathogen strains, complicating fish health management and posing risks to humans. Therefore, the development of herbal materials (phytoimmunostimulants) as an alternative to antibiotics is necessary.

The development of phytoimmunostimulants offers a promising approach by leveraging bioactive compounds from various herbs to boost immunity, improve growth performance, and confer antimicrobial benefits (Andriani and Aisyah, 2025; Effendi *et al.,* 2025b). Other advantages of phytoimmunostimulants include a broad spectrum of biological activity (Singh *et al.,* 2022), a relatively low risk of resistance, lower production costs (Mariappan *et al.,* 2023), and greater safety for humans, fish, and aquatic environments (Wang *et al.,* 2017). Additionally, Effendi *et al.* (2020) state that integrating medicinal plants into aquaculture practices not only improves fish health but also contributes to the bioremediation of polluted waters, addressing broader environmental issues.

Mango (*Mangifera indica*) is a tropical plant widely distributed in Indonesia that has various medicinal properties and contains bioactive compounds, such as alkaloids, saponins, tannins, flavonoids, steroids, phenolics, and mangiferin (Huang *et al.,* 2018; Carvalho *et al.,* 2024). These compounds have potential as antimicrobials, antioxidants, anti-inflammatories, and immunomodulators (Singhania and Bishnoi, 2024). These compounds make mango leaves a highly promising candidate for phytostimulants in aquaculture.

The indicator for determining the effect of phytoimmunostimulants is through hematological analysis. According to Satkar *et al.* (2024), fish hematology is one of the most sensitive indicators of fish health status. It provides information on immune responses and physiological stress, which is very

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important for early disease detection and management. According to Effendi *et al.* (2025b); Saputra *et al.* (2023), adding mango leaves to the diet can increase the erythrocyte count in carp reared in different salinity conditions, such as the total erythrocyte count from $1.28 \times 10^6$ cells/mm³ to $1.36 \times 10^6$ cells/mm³, hemoglobin from 4.2 to 6.73 g/dL, hematocrit from 20 to 30.33%, and survival rate to 100%.

Given the urgency of developing safe and effective antibiotic alternatives, the significant potential of the bioactive content of mango leaves, and the importance of exploring common carp farming in brackish water as an effort to diversify aquaculture, this study aims to examine the effectiveness of mango leaves as a phytoimmunostimulant on the hematological and physiological profiles of common carp.

This study is a direct development of our previous research (Effendi *et al.*, 2025b; Saputra *et al.*, 2023), which demonstrated that mango leaf powder supplementation improved erythrocyte count, hemoglobin, hematocrit, and survival rate of common carp reared exclusively in brackish water (5 ppt). However, that earlier work did not evaluate whether the same phytoimmunostimulant effect occurs in freshwater, which remains the primary and most widely used rearing medium for common carp. This gap raises an important question of whether the hematological benefits of mango leaf powder are consistent across salinity conditions or are specific to osmotically stressed environments. To address this, the present study extends our earlier findings by simultaneously evaluating the effects of mango leaf powder supplementation in both freshwater and brackish water. This comparative approach is expected to provide farmers with practical, medium-specific guidance on the application of mango leaf powder as a phytoimmunostimulant, supporting informed health-management decisions for common carp cultivated in both conventional freshwater ponds and brackish water areas increasingly used to diversify aquaculture production.

## MATERIALS AND METHODS

### Time and location

This study was conducted from April to June 2023 at the Floating Net Cage in the Koto Panjang Hydroelectric Power Plant (PLTA) Reservoir, Kampar Regency, Riau Province, Indonesia, and the Integrated Laboratory, Department of Marine Science, Faculty of Fisheries and Marine Sciences, Universitas Riau, Pekanbaru, Indonesia.

### Method

This study used a comparative experimental method with a comparative site design approach. The two maintenance locations used were the Koto Panjang Hydroelectric Power Plant Reservoir (representing natural freshwater conditions) and the laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Riau (representing controlled brackish water conditions with a salinity of 5 ppt). At each location, two treatments were applied: (1) positive control, namely fish fed commercial feed with the addition of 1.5 g of mango leaf powder per 100 g of feed, and (2) negative control, namely fish fed only Hi-Provit 781-2 commercial feed without the addition of mango leaf powder. Each treatment was repeated three times. This comparative site design was deliberately chosen as a direct extension of our previous work (Effendi *et al.*, 2025b; Saputra *et al.*, 2023), which evaluated mango leaf powder supplementation only in brackish water. By testing both media within a single, standardized experimental framework, this study enables a direct, farmer-relevant comparison of treatment efficacy, allowing the results to be applied confidently regardless of the type of water resource available to cultivators.

### Procedure

#### Preparation and production of phytoimmunostimulant feed

Mango leaves were selected based on uniform maturity. The leaves were washed thoroughly with running water, then air-dried for 7 days. The dried leaves were cut into small pieces, ground in a blender, and sieved through a 100-200 mesh sieve to obtain a fine, uniform powder. The treatment feed was prepared by weighing 1.5 g of mango leaf powder, then mixing it with ground, homogenized commercial Hi-Provit 781-2 pellets. After that, the feed mixture was recast using a pellet machine. The molded pellets were air-dried and, once dry, were ready for feeding to the fish (Effendi *et al.,* 2025b).

### Fish maintenance

Common carp maintenance was carried out at two different locations, namely in floating net cages at the Koto Panjang Hydroelectric Power Plant Reservoir measuring 2 m x 1 m x 1 m, with 6 units and a stocking density of 25 fish/container (freshwater). In the laboratory, six 1 m-diameter containers with a volume of 100 L were used, filled with brackish water at 5 ppt salinity. The fish used were common carp fry measuring 9.00±1.00 cm with a stocking density of 25 fish/container. Farmers at the Koto Panjang Hydroelectric Power Plant supplied the fish used. The fish used had clinical signs, including good movement, complete fins, and no body surface defects or ulcers. Before being used as test animals, the fish were first adapted to each medium for 7 days. In the 5 ppt salinity medium, the fish were gradually acclimated over one week to minimize osmotic stress. Feeding was carried out three times a day, at 08:00 AM, 12:00 AM, and 4:00 PM, with a feed dose of 5% of the total fish biomass weight per day. Sampling was conducted every 10 days to monitor common carp growth and survival.

### Fish blood sampling

Blood samples were collected at the beginning (day 0) and end (day 30) of the study. Three fish from each tank were used as blood samples. Before blood collection, the fish were anesthetized using clove oil at a dose of 0.01 mL/L of water. Blood was collected from the caudal vein using a 1 mL syringe rinsed with 10% EDTA as an anticoagulant to prevent blood


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clotting. The collected blood samples were stored in microtubes for further analysis of hematological parameters, including total erythrocyte count, hematocrit, and hemoglobin (Effendi *et al.,* 2025b; Saputra *et al.*, 2023).

### Data Analysis

The data obtained, including total erythrocytes, hemoglobin, hematocrit, and sedimentation rate, were tabulated and analyzed using an independent-samples t-test at the 95% confidence level ($p < 0.05$) to determine the effects of the treatment and control groups, as well as differences between the two maintenance media.

## RESULTS AND DISCUSSION

### Hematology Profile

Measurements of the hematological profile of common carp reared in freshwater and brackish water are shown in Table 1.

**Table 1.**

Total erythrocytes of common carp raised in freshwater and brackish water

| Parameter | Medium | Without mango leaves | | Addition of mango leaves | | Normal range |
|---|---|---|---|---|---|---|
| | | Initial | End | Start | End | |
| RBC (x$10^6$cells/mL) | Freshwater | 1.47±0.01 | 1.56±0.01$^b$ | 1.46±0.2 | 1.75±0.02$^b$ | 1.43–1.88* |
| | Brackish water | 1.43±0.01 | 1.51±0.01$^a$ | 1.45±0.01 | 1.63±0.01$^a$ | |
| Hematocrit (%) | Freshwater | 25.00±1.00 | 28.33±1.33$^b$ | 26.66±1.33$^b$ | 33.66±1.33 | 21-44%** |
| | Brackish water | 23.00±1.00 | 24.66±1.33$^a$ | 25.55±1.33$^a$ | 30.66±1.33 | |
| Hemoglobin (g/dL) | Freshwater | 6.5±0.20 | 7.03±0.50$^b$ | 6.70±0.30 | 8.10±0.10$^b$ | 5.2-8.2 |
| | Brackish water | 5.60±0.20 | 6.50±0.10$^a$ | 6.30±0.20 | 7.70±0.20$^a$ | g/dL*** |

RBC: Red Blood Concentration *Sezgin and Aydin (2021); **Shabirah *et al.* (2019); ****Effendi *et al*. (2025a)

The results of the study indicate that administering mango leaf powder as a phytoimmunostimulant significantly increases the total erythrocyte count of common carp in both rearing media. The average total erythrocyte count of common carp treated with mango leaf powder in freshwater reached 1.75 × $10^6$ cells/mm$^3$, whereas in brackish water (5 ppt salinity), it reached 1.63 × $10^6$ cells/mm$^3$. These values are still within the normal range of total erythrocytes in common carp reported by Sezgin and Aydin (2021), namely 1.43–1.88 × $10^6$ cells/mm³. An independent t-test showed a significant difference between the treatment and control groups in both culture media ($p<0.05$).

The increase in total erythrocytes in common carp fed mango leaf powder can be explained through several biochemical and physiological mechanisms. The secondary metabolites found in mango leaves can support erythropoiesis (the formation of new red blood cells). According to Acharya *et al.* (2025), mango leaves contain large amounts of iron (437 ppm), which is an essential element for hemoglobin synthesis in erythrocytes. This high iron content supports red blood cell production, thereby increasing the total erythrocyte count in common carp. Effendi *et al.* (2025b) phytochemical compounds, such as flavonoids, phenols, tannins, and saponins, have been shown to boost the immune system of fish and reduce stress and disease susceptibility, thereby indirectly supporting the production and maintenance of healthy erythrocytes.

The difference in total erythrocyte values between fish raised in freshwater and brackish water is a physiological response of fish. Fish raised in saline media experience higher osmotic pressure, which forces their physiological systems to allocate greater metabolic energy to osmoregulation. According to Martemyanov and Vasiliev (2018), changes in erythrocyte values and blood profiles are observed in fish as they adapt to different salinities. For example, the crucian common carp showed an increase in erythrocyte osmotic concentration when exposed to higher salinity, which affected cell volume. However, the administration of mango leaf powder was found to compensate for this osmotic pressure and maintain erythrocyte counts within the normal range, indicating the effectiveness of this phytoimmunostimulant.

The hematocrit levels of crucian carp in all treatment groups were within the normal range for this species, 21-44% (Shabirah *et al.,* 2019). Common carp fed with mango leaf powder supplements in freshwater media showed an average hematocrit level of 33.66%, while those in brackish water media showed 30.66%. Both values were significantly higher than those of the control groups in each medium. The independent t- test showed a significant difference between the treatment and control groups in both rearing media ($p<0.05$).

The increase in hematocrit in fish fed mango leaf powder reflects an increase in blood oxygen-carrying capacity, which ultimately supports fish metabolism and growth efficiency. Lin and Hwang (2024) stated that fish regulate their internal osmotic conditions through various physiological mechanisms, including changes in blood parameters such as hematocrit, hemoglobin concentration, and red blood cell count. These changes are crucial for maintaining homeostasis in different salinity environments. The results showed that hematocrit values remained within the normal range in both media, indicating successful adaptation of the fish with the support of phytoimmunostimulants. Hemoglobin levels in common carp in the treatment group of common carp increased significantly in both rearing media. Common carp fed mango leaf powder in freshwater media had an average hemoglobin level of 8.03 g/dL, while those in brackish water media had a level of 7.76 g/dL. Both values are considered normal according to Effendi *et al.* (2025a), who state that the hemoglobin level range for common carp is 5.2-8.2 g/dL.

The increase in hemoglobin levels in fish fed mango leaf powder was directly correlated with an increase in erythrocytes, considering that hemoglobin is contained in erythrocytes. The increase in hemoglobin levels due to mango leaf flour can be attributed to its potential to stimulate erythropoiesis, the process of producing new erythrocytes. This is supported by the increase in red blood cell count and hemoglobin levels observed in fish fed mango leaf extract (Obaroh *et al.,* 2014).

The hematological improvements observed in this study are consistent with, and extend, the findings of our earlier research conducted solely in brackish water (Effendi *et al.*, 2025b; Saputra *et al.*, 2023), which reported increases in total erythrocyte count from 1.28 × $10^6$ to 1.36 × $10^6$ cells/mm³, hemoglobin from 4.2 to 6.73 g/dL, and hematocrit from 20% to 30.33% following mango leaf powder supplementation. The present results confirm that these benefits are not restricted to osmotically stressed, brackish-water conditions but are equally, and in freshwater even more strongly, expressed under normal freshwater rearing conditions. This finding is practically important, as it demonstrates that mango leaf powder can serve as a reliable, low-cost phytoimmunostimulant for common carp farmers, regardless of the water medium used, thereby broadening its applicability across both conventional freshwater aquaculture and emerging brackish-water diversification efforts.

### Survival Rate

The results of measuring the survival rate of common carp raised in freshwater and brackish water are shown in Figure 1.

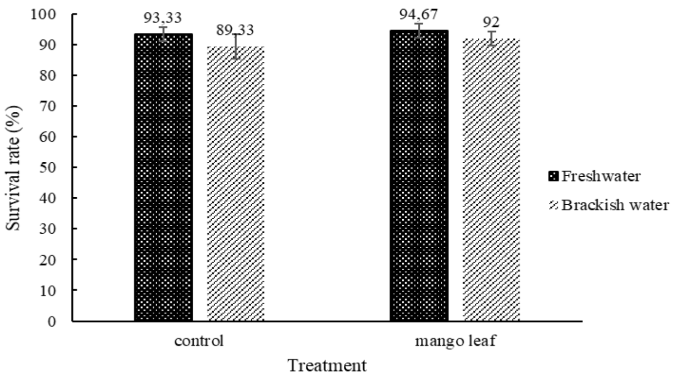


**Fig 1.** Common carp survival rate.

The survival rate of common carp over 30 days of rearing was satisfactory in both media. In freshwater media, the survival rate of the treatment group reached 94.67%, while in brackish water media, it was 92%. These survival rates are considered good for common carp rearing in controlled containers, given the stress factors associated with stocking density and the adjustment to the new environment in the brackish water group. Survival in the treatment group was consistently higher than in the control group across both media, indicating that mango leaf powder supplementation improved common carp survival. Fontana *et al.* (2024) stated that in tilapia, mango powder supplementation at optimal doses can enhance immune responses, including increased skin mucus and serum lysozyme activity, which are crucial for disease resistance.

Mortality in the brackish water group was mainly due to osmotic adaptation stress, particularly in the early phase of transferring fish from freshwater to brackish water. According to Lin and Hwang (2024), euryhaline fish can adjust their osmoregulatory mechanisms to survive across a range of salinities. This adaptive ability is essential for survival during salinity transitions. Furthermore, the ability of fish to utilize feed efficiently depends on the type and quality of feed provided. Feed enriched with mango leaf phytoimmunostimulants can improve feed digestibility and acceptance in fish, ultimately supporting higher survival rates. This is in line with the immunomodulatory role of the bioactive compounds in mango leaves in strengthening the non-specific immune system of fish against various environmental stressors and pathogens (Effendi *et al.,* 2023).

## CONCLUSIONS

Supplementation with mango leaf powder (1.5 g/100 g feed) significantly increased total erythrocytes, hematocrit, hemoglobin, and survival of common carp in both freshwater and brackish water (5 ppt) media. The bioactive compounds in mango leaves are effective in strengthening the immune and physiological resistance of fish, making them a safe and sustainable alternative to antibiotics in aquaculture systems.

These findings extend our previous research, which was limited to brackish water conditions, by confirming that the hematological and protective benefits of mango leaf powder are consistently expressed in freshwater as well. This broader evidence base allows common carp farmers to adopt mango leaf powder as a practical, low-cost phytoimmunostimulant strategy regardless of the type of water resource they use, supporting more sustainable and antibiotic-free aquaculture practices across both conventional freshwater ponds and brackish water farming systems.

## AUTHORS CONTRIBUTIONS

Conceptualization: RK and IE.; Methodology: IE and FF.; data collection: AS.; data validation: RK, IE, FF, NIS.; data processing: RK and AS.; writing-

original draft preparation: RK, IE, and NIS.; writing-review and editing: RK, IE, FF, and NIS.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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