

## ORIGINAL ARTICLE OPEN ACCESS

# Laligurans (*Rhododendron arboreum*) Enhances Growth, Enzyme Profiles, and Immune Response of Grass Carp *Ctenopharyngodon idella* (Val. 1844)

Anil Kumar Jha<sup>1</sup> | Monowar Alam Khalid<sup>1</sup> | Shyam Narayan Labh<sup>2</sup> 

<sup>1</sup>Department of Environmental Science, Integral University, Lucknow, Uttar Pradesh, India | <sup>2</sup>Department of Zoology, Amrit Science Campus, Tribhuvan University, Thamel, Kathmandu, Nepal

**Correspondence:** Monowar Alam Khalid ([makhalid@iul.ac.in](mailto:makhalid@iul.ac.in))

**Received:** 11 September 2024 | **Revised:** 5 December 2024 | **Accepted:** 6 December 2024

**Funding:** This research received no external funding.

**Keywords:** antioxidant | carp | enzyme | growth | immunity | protein | *Rhododendron arboreum*

## ABSTRACT

This study evaluates the effects of Laligurans (*Rhododendron arboreum*) flowers on the growth performance and health parameters of grass carp (*Ctenopharyngodon idella*). Known for their anti-inflammatory properties and medicinal uses, Laligurans flowers were incorporated into diets as an ethanol extract (LGE) and fine powder (LGP) to test their efficacy. A total of 630 grass carp fingerlings ( $3.07 \pm 0.76$  g to  $3.95 \pm 0.85$  g) were randomly distributed into 21 nylon cages ( $1.5 \text{ m} \times 1 \text{ m} \times 2 \text{ m}$ ) placed in an earthen pond. Seven dietary treatments were administered: a control diet (T1) with standard commercial feed, LGE-supplemented diets (T2, T3, T4, and T5) at 0.20%, 0.40%, 0.80%, and 1.60% inclusion levels, respectively, and LGP-supplemented diets (T6 and T7) at 0.20% and 0.40%, respectively. Results demonstrated significant improvements ( $p < 0.05$ ) in liver, kidney, and blood serum protein levels (total protein, albumin, globulin, and AG ratios) in treated groups compared to the control. Reductions in SGOT, SGPT, and ALP levels were observed in the liver and blood serum of treated groups, while control fish exhibited elevated blood urea nitrogen and creatinine. Fish fed LGE diets showed superior blood profiles compared to those receiving LGP. The T4 group (0.80% LGE) achieved the highest final weight gain, specific growth rate, and survival rate ( $p < 0.05$ ), highlighting the potential of Laligurans flower extract as a natural immunological and growth-enhancing supplement in aquaculture diets.

## 1 | Introduction

The aquaculture industry is among the fastest growing areas in animal food production, experiencing rapid growth and is anticipated to expand by 3.2% by 2030 (Khanal et al. 2021). The grass carp (*Ctenopharyngodon idella*) is the fish species with the highest documented aquaculture production globally, exceeding five million tons annually. It is a sizable herbivorous freshwater fish species belonging to the Cyprinidae family, indigenous to eastern Asia and other nations, and cultivated with artificial diets. The widespread application of antibiotics in aquaculture for

growth promotion, immune enhancement, and bacterial illness treatment has considerable dangers. The dangers encompass the emergence of resistant infections, the accumulation of antibiotic residues in grass carp, and detrimental environmental effects (Shakya and Labh 2020).

The use of antibiotics in fish culture is discouraged due to their transmission through the food chain and their impact on the diversity of environmental bacteria. As a result, there is an increasing need for natural alternatives, especially plant-based goods, which provide safer and more economical chemical

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Author(s). *Aquaculture, Fish and Fisheries* published by John Wiley & Sons Ltd.

compounds with diverse advantageous features. Numerous efforts are being made to utilize phytobiotics as dietary supplements to enhance fish development, health, and immunity (Labh and Shakya 2020).

Plants with historical therapeutic uses are promising feed additives with negligible adverse consequences. Recently, numerous alternative pharmaceuticals derived from medicinal plant components have been investigated in aquaculture (Phinyo et al. 2024). Herbal flowers possess bioactive chemicals obtained from plants, including glycosides, organic acids, polysaccharides, alkaloids, tannins, and flavonoids (Jha, Khalid, and Labh 2024a). Numerous studies have examined the impact of dietary medicinal herb supplementation on fish growth.

The Laligurans *Rhododendron arboreum*, a member of the Ericaceae family, is recognized for its clusters of deep red to pink flowers. The national flower of Nepal is celebrated for its medicinal properties and has been referenced in traditional medicine in other nations. The flowers of *Rhododendron* are utilized locally in many forms and employed in the treatment of serious ailments, including inflammation, diarrhoea, headaches, and infections (Labh and Shakya 2020). The flowers comprise substances such as quercetin-3-rhamnoside, coumaric acid, rutin, quercetin, and several phenolic compounds and amino acids, with over 208 chemicals identified from distinct *Rhododendron* species, where phenols and flavonoids are the most bioactive constituents (Sharma, Chaudhary, and Harchanda 2022). Antioxidant qualities of rhododendron flowers were previously investigated (Jha, Khalid, and Labh 2024b), and an experiment was conducted on common carp (Jha, Khalid, and Labh 2024a) to assess the impact of this flower on carp growth and immune system.

Although extensive research has been conducted on the nutritional needs of grass carp, there is a paucity of information regarding the physiological functions and antioxidant properties of flowers, rendering this study essential for the advancement of aquaculture practices. This study seeks to assess the dietary needs of Laligurans flower extract and examine its effects on growth performance, antioxidant capacity, and serum biochemical parameters in juvenile grass carp, building upon the prior experiment. This study investigates the impact of Laligurans (*Rhododendron arboreum*) flower extract on the growth and health of juvenile grass carp, considering the promising potential of plant extracts.

## 2 | Materials and Methods

### 2.1 | Collection of Flowers

Chandragiri Hill, situated in the south-west of Kathmandu Valley, is the premier hill station in Nepal (Madhvi et al. 2020). It offers unparalleled views of the Kathmandu Valley and the Himalayan Mountain ranges, spanning from Annapurna to Mt. Everest. This location combines convenience and natural beauty, providing thrilling adventure activities, cultural importance, luxurious lodging, diverse cuisine, and a tranquil atmosphere. Combining affordability with luxury, this place is perfect for a brief and wonderful getaway. The flowers of Laligurans (*Rhododendron*

*arboreum*) were gathered from the Chandragiri Hills (Madhvi et al. 2020) in March 2023. The collected flower samples were then carefully sealed in a plastic bag, dried in the shade, and subsequently sent to the National Herbarium and Plant Laboratories in Godavari, Kathmandu for taxonomic identification.

### 2.2 | Extraction of Phytochemicals From Flower Petals

The flower petals of *R. arboreum* were air-dried for 10 days at room temperature, finely powdered in a blender, and stored in a container. Twenty grams of dried Laligurans powder was combined with 2 mL of 70% ethanol and inserted into cotton plugs coated in aluminium foil before incubating in an orbital shaker with a magnetic stirrer for 48 h (Figure 1). The mixture was centrifuged at 10,000 revolutions per minute for 5 min to separate the solids. The resultant liquid (supernatant) was then filtered via Whatman filter paper No. 1. The solvent was subsequently evaporated using nitrogen gas at room temperature, and the ethanol extract of Laligurans flowers' petal (LFE) was stored at 4°C for later use (Labh 2020a).

### 2.3 | Examination of Ethanol Extract for Antioxidant Activity

The capacity of ethanol extracts derived from the flowers of *Rhododendron arboreum* to eliminate hydrogen peroxide was assessed using the methodology outlined by Chitrakar, Khalid, and Labh (2024). A 40 mM hydrogen peroxide solution was produced in a phosphate buffer with a pH of 7.4. The addition of extracts (100 µg/mL) in distilled water was made to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at a wavelength of 540 nm (Figure 2) was measured after a 10-min interval, using a blank solution that contained the phosphate buffer but did not contain hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both flowers extracts and standard compounds was calculated:

$$\% \text{ Scavenged } [\text{H}_2\text{O}_2] = [(\text{AC} - \text{AS}) / \text{AC}] \times 100, \quad (1)$$

where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of *R. arboreum* extracts or standards.

### 2.4 | Selection of Fish and Identification

The grass carp (*C. idella*) is a species of large herbivorous freshwater fish in the family Cyprinidae and found in lakes, ponds, pools, and in rivers, preferring large, slow-flowing or standing water bodies with abundant vegetation (Dani and Baniya 2022). Its body is moderately compressed laterally, mouth is terminally located on a wide head, and eyes are small on the head. It is olive-brown on the dorsal side, with silver sides and a white belly and lacks barbels. The fish is locally available; hence, the fingerlings of minimum  $3.07 \pm 0.76$  g to maximum  $3.95 \pm 0.85$  g were brought from the local hatchery of Madhesh, Janakpur, Nepal.

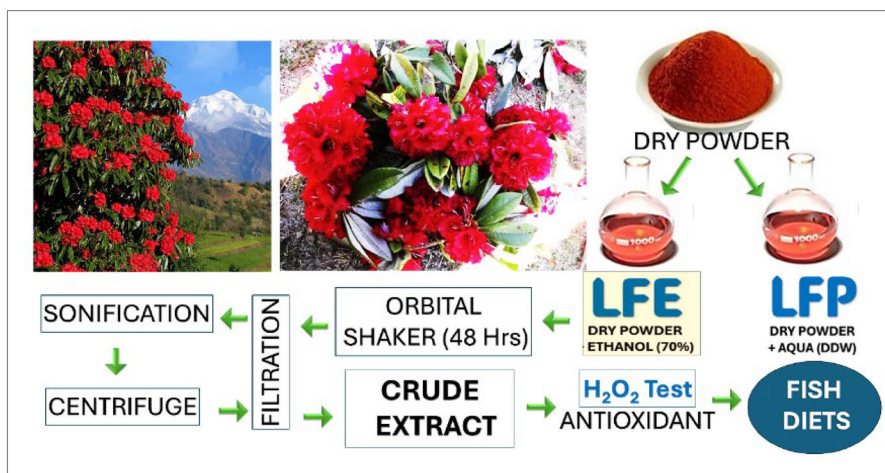


**FIGURE 1** | Extraction techniques for the preparation of LGE and LGP from the flowers of *Laligurans R. arboreum*.

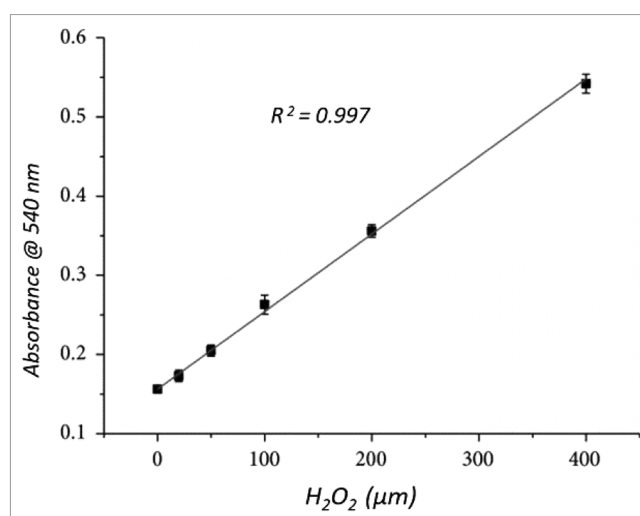


**FIGURE 2** | Standard for the quantification of hydrogen peroxide from the flower extract.

## 2.5 | Preparation of Experimental Diets

For this experiment, three different fish diets with 40% protein each were created. Diet 1 (T1) was the control diet; it did not contain *Laligurans* flower extract. *Laligurans* flower extract (LGE) (0.2%), LGE (0.4%), LGE (0.8%), and LGE (1.6%) were added to diets T2, T3, T4, and T5. On the other hand, *Laligurans* powder (LGP) (0.2%) and LGP (0.4%), which were made of dry LGP, were added to diets T6 and T7. The components are listed in Table 1.

## 2.6 | Study Design and Feeding

A total of 21 nylon and bamboo cages, measuring 1m × 1m × 2m, were set in an earthen pond for this experiment. The cages were arranged in groups of three, for a total of seven treatments. The experiment was carried out within the natural system in a confined culture area constructed by the Aquaculture Research Unit of the Provincial Research and Training Center, under the Government of Nepal, in Province Madhesh, located in

Janakpurdham. A total of 630 fingerlings of grass carp, weighing between  $3.07 \pm 0.76$  g and  $3.95 \pm 0.85$  g, were evenly and randomly distributed into 21 nylon cages, with each cage containing 30 fish. The fish were provided with a daily diet consisting of an amount of food equal to 3% of their body weight, which was divided into two feedings at 9 a.m. and 4 p.m. The net weight increase was measured every 15th day using an electronic balance, and the amount of feed was changed accordingly after each 15-day period. Temperature, pH, and dissolved oxygen levels were observed and documented daily. The temperature fluctuated between  $27 \pm 0.23^\circ\text{C}$  and  $31 \pm 0.11^\circ\text{C}$ ; the pH ranged from  $6.12 \pm 0.47$  to  $7.39 \pm 0.21$ , and the dissolved oxygen levels varied from  $6.13 \pm 0.46$  to  $6.78 \pm 0.31$ . The trial lasted for a period of 90 days. Fish mortality was monitored to determine the survival rate during the experimental periods.

## 2.7 | Sampling, Collection of Blood and Tissue

After the experiment was over, the weight of each fish was measured using a digital balance in order to evaluate their growth performance and biochemical analysis.

The fish were exposed to a concentration of 50 mg/L of tricaine methane sulfonate (MS 222; Sigma Chemical Co. St. Louis, MO, USA). To adequately collect blood from fish, it is necessary to anesthetize them for a few minutes; hence, the fish were treated with 50 mg/L of tricaine methane sulfonate (MS 222). A quantity of 1–2 mL of blood was obtained from the vena caudalis using a disposable hypodermic needle with a gauge size of 26. Randomly selected fish from each cage were captured and anesthetized to acquire blood samples. Half of the blood samples were immediately transferred to a sterile container that contained a small quantity of heparin powder. The vial was delicately agitated and kept at a temperature of  $4^\circ\text{C}$  in order to perform haematological profiling. In order to isolate the serum, the remaining blood samples were transferred to sterile Eppendorf tubes devoid of any anticoagulant and thereafter stored at a temperature of  $-20^\circ\text{C}$  for immunological examination. Further analysis was conducted using stored blood serum and blood plasma. Similarly, the liver and kidney were taken by a thorough dissection technique. Afterwards, a 5% tissue homogenate was

**TABLE 1** | Diet formulation and preparation of varied proportion for the experiment.

| Ingredients                   | T1   | T2   | T3   | T4   | T5   | T6   | T7   |
|-------------------------------|------|------|------|------|------|------|------|
|                               | C    | LGE  | LGE  | LGE  | LGE  | LGP  | LGP  |
|                               | 0.0% | 0.2% | 0.4% | 0.8% | 1.6% | 0.2% | 0.4% |
| Casein <sup>a</sup>           | 33   | 33   | 33   | 33   | 33   | 33   | 33   |
| Gelatin <sup>a</sup>          | 7.3  | 7.3  | 7.3  | 7.3  | 7.3  | 7.3  | 7.3  |
| Dextrin <sup>a</sup>          | 16.8 | 16.8 | 16.8 | 16.8 | 16.8 | 16.8 | 16.8 |
| Starch <sup>a</sup>           | 19   | 19   | 19   | 19   | 19   | 19   | 19   |
| Cellulose <sup>a</sup>        | 11   | 11   | 11   | 11   | 11   | 11   | 11   |
| Cod liver oil <sup>b</sup>    | 4    | 4    | 4    | 4    | 4    | 4    | 4    |
| Sunflower oil <sup>b</sup>    | 4    | 4    | 4    | 4    | 4    | 4    | 4    |
| Vitamin premix <sup>b</sup>   | 1.5  | 1.5  | 1.5  | 1.5  | 1.5  | 1.5  | 1.5  |
| Mineral premix <sup>b</sup>   | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  |
| CMC <sup>a</sup>              | 2.8  | 2.6  | 2.4  | 2    | 1.2  | 2.6  | 2.4  |
| Choline chloride <sup>a</sup> | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| LGE <sup>c</sup>              | 0    | 0.2  | 0.4  | 0.8  | 1.6  | 0    | 0    |
| LGP <sup>d</sup>              | 0    | 0    | 0    | 0    | 0    | 0.2  | 0.4  |
| Total (g)                     | 100  | 100  | 100  | 100  | 100  | 100  | 100  |

<sup>a</sup>Hi-Media Lab Pvt Ltd., India.

<sup>b</sup>Procured locally.

<sup>c</sup>Laligurans flower's extract.

<sup>d</sup>Laligurans flower's powder.

created using a chilled sucrose buffer that consisted of 0.25 M sucrose (with a molecular weight of 342), 3 mM CaCl<sub>2</sub>, 1 mM Tris at a pH of 8.0, and 0.5% NP-40. The entire surgical procedure was performed in extremely cold settings. The samples underwent centrifuged at a speed of 10,000 revolutions per minute for 10 min at a temperature of 4°C. The liquid fraction obtained, referred to as the supernatant, was gathered in 10-mL vials and preserved in a deep freezer at a temperature of -20°C for further examination.

where W1 and W2 are the initial and final body weight in grams, respectively, and *t* describes the duration of the trial in days.

$$\begin{aligned} \text{FCR} &= \text{Food intake/weight gain} \\ \text{Survival \%} &= \text{Number of fish/initial number of fish} \times 100 \quad (4) \end{aligned}$$

(Note: All weights were calculated in grams).

## 2.8 | Examination Procedure

### 2.8.1 | Growth Performance

Fish growth was assessed in terms of weight gain (WG), percent weight gain (PWG), specific growth ratio (SGR) and food conversion ratio (FCR) followed by percentage survival on regular monitoring.

$$\text{WG} = \text{Mean final weight} - \text{mean initial weight}, \quad (1)$$

$$\begin{aligned} \text{PWG} &= [\text{Mean final weight} - \text{Mean initial weight}] \\ &/\text{Mean initial weight} \times 100, \end{aligned} \quad (2)$$

$$\text{SGR (\%)} = [\ln W_2 - \ln W_1] \times 100/t, \quad (3)$$

### 2.8.2 | Enzyme Profiles (Tissues and Blood Serum)

The biuret method (Labh, Shakya, and Kayastha 2015) was used to assess the total protein concentrations in the liver, kidney, and blood serum. The albumin levels were measured using a commercially available kit from Labh. The globulin levels and the ratio of albumin to globulin were approximated using simple calculation procedures. The UV-Kinetic method devised by Chitrakar and Parajuli (2017) was used to quantify serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) in blood serum and liver tissue samples from grass carp. The kidney function test involved diagnosing blood urea nitrogen (BUN) and creatinine (CREAT) using the I-Lab Aries Fully Automated Chemical Analyzer, manufactured by Instrumentation Laboratory S.P.A. in Milan, Italy.

### 2.8.3 | Blood Profiles and Differential Counts

Haemoglobin levels were determined using the method described by Labh (2024) and Reitman and Frankel (1957). The erythrocyte

count was performed following the protocol outlined by Larsen and Snieszko (1961a). Packed cell volume was measured with a microhematocrit reader, as explained by Larsen and Snieszko (1961b). For leukocyte counts, blood samples were diluted in Shaw's solution at a 1:100 ratio. The diluted samples were then counted using the same procedure as for the erythrocyte count. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated (Labh, Chakrabarti, and Fawole 2017).

## 2.9 | Statistical Analysis

Statistical analyses were performed using the SPSS software, version 22. For post hoc comparisons of means across different variables, Duncan's multiple range test (DMRT) was utilized to identify significant differences at the 5% significance level ( $p < 0.05$ ). The results are expressed as means accompanied by their respective standard errors (SE), and each experiment was replicated three times ( $n = 3$ ) to ensure the reliability of the data.

## 3 | Results

### 3.1 | Hydrogen Peroxide Scavenging Capacity

The scavenging capability of powder (LGP) and ethanol extracts (LGE) from the flower of *Laligurans* (*R. arboreum*) on hydrogen peroxide was investigated. Butylated hydroxyanisole (BHA), a synthetic phenol widely used as a preservative in the food and cosmetic industries, possesses notable antioxidant properties. These properties allow BHA to serve as a standard in scientific studies to demonstrate the involvement of reactive oxygen species (ROS) in various cellular processes, including cell death. Consequently, the *Laligurans* extracts were compared to BHA and  $\alpha$ -tocopherol, another well-known antioxidant. The extracts from *R. arboreum* showed a dose-dependent ability to scavenge hydrogen peroxide. When tested at a concentration of 100  $\mu$ g, the powder and ethanol extracts exhibited scavenging activities of 17.51%–29.33% on hydrogen peroxide. In comparison, BHA and  $\alpha$ -tocopherol demonstrated higher scavenging activities of 49.17% and 37.53%, respectively, at the same concentration. The order of decreasing scavenging activity is as follows: BHA (49.17%) >  $\alpha$ -tocopherol (37.53%) > LGE (29.33%) > LGP (17.51%). The results demonstrate that at a concentration of 100  $\mu$ g, the scavenging activity of *R. arboreum* extracts against hydrogen peroxide is inferior to that of BHA and  $\alpha$ -tocopherol.

### 3.2 | Growth Performances

Grass carp (*C. idella*) were fed a diet supplemented with flower extracts (LGE and LGP) from *R. arboreum* for 90 days, after which their growth performance was recorded (Table 2). The study observed 100% survival and significant weight gain ( $p < 0.05$ ) in all fish fed the treated diets. The mean final weight gain was the highest in fish fed the T4 diet, reaching  $48.42 \pm 0.48$  g, compared to  $14.77 \pm 0.12$  g in fish fed the T1 diet. Additionally, a higher specific growth rate (SGR) of  $3.01 \pm 0.36$  was recorded in the T4 group, compared to  $1.50 \pm 0.19$  in the control group (T1). An

inverse relationship was observed in the FCR. The FCR value in the control group was  $0.94 \pm 0.19$ , which progressively decreased with higher doses of LFE. The FCR values were  $0.54 \pm 0.13$  in T2,  $0.27 \pm 0.73$  in T3, and  $0.20 \pm 0.13$  in T4 (Table 2).

### 3.3 | Protein and Enzyme Profiles

Significant ( $p < 0.05$ ) differences were observed in the protein profiles of grass carp fed with *Laligurans* (*R. arboreum*) extracts (LGE and LGP) after a 90-day feeding trial (Table 3). The levels of total protein, albumin, globulin, and the albumin-to-globulin ratio in the liver, kidney, and blood serum are presented in Table 3. The total protein levels were significantly higher ( $p < 0.05$ ) in the T4 diet-fed group compared to the control group, with values recorded at  $27.45 \pm 3.09$  in the kidney,  $25.34 \pm 1.30$  in the liver, and  $24.24 \pm 1.55$  in the blood serum. In contrast, the control group had total protein levels of  $3.85 \pm 0.46$  in the kidney,  $4.72 \pm 0.42$  in the liver, and  $6.70 \pm 0.31$  in the blood serum. Similar trends were observed for albumin and globulin levels, with the LGE and LGP groups showing higher values compared to the control group (Table 3).

Inverse trends (Table 4) were noted for SGOT, SGPT, and alkaline phosphatase (ALP) in both the liver and blood serum of all treated groups compared to the control. The SGOT, SGPT, and ALP levels were the highest in the control group (T1) and decreased progressively with higher doses of the flower extract. Specifically, the control group exhibited the highest SGOT levels in the liver ( $84.49 \pm 2.60$  IU/L) and blood serum ( $116.62 \pm 0.85$  IU/dL), SGPT levels in the liver ( $44.69 \pm 0.89$  IU/L) and blood serum ( $93.28 \pm 1.05$  IU/L), and ALP levels in the liver ( $63.47 \pm 0.62$  IU/L) and blood serum ( $83.56 \pm 0.78$  IU/L).

In contrast, the T4 diet group showed the lowest levels: SGOT at  $59.48 \pm 2.69$  IU/L in the liver and  $76.62 \pm 1.70$  IU/dL in the blood serum, SGPT at  $13.28 \pm 0.89$  IU/L in the liver and  $66.52 \pm 1.17$  IU/L in the blood serum, and ALP at  $46.12 \pm 0.82$  IU/L in the liver and  $75.94 \pm 1.76$  IU/L in the blood serum (Table 4). Furthermore, BUN was significantly higher in the control group ( $13.80 \pm 1.38$  mg/dL), whereas the lowest BUN level was recorded in the T4 diet group ( $2.94 \pm 0.42$  mg/dL). Creatinine levels were the highest in the T6 diet group ( $1.83 \pm 0.06$  mg/dL) and lowest in the T4 diet group ( $0.82 \pm 0.15$  mg/dL) (Table 4).

### 3.4 | Blood Biochemistry

Subsequent to the 90-day feeding trial utilizing *R. arboreum* flower extracts (LGE and LGP), an extensive blood examination was performed (Table 5). This encompassed a complete blood count (CBC), haematological indices, and differential leukocyte counts. Significant differences ( $p < 0.05$ ) were noted between the groups administered *Laligurans* extracts and the control group (Table 3). Although all measured values remained within the normal range, the treated group exhibited superior blood profiles compared to the control diet group. Minor discrepancies in erythrocyte counts were seen among the experimental groups relative to the control.

**TABLE 2** | The effects of different dosages of the rhododendron flower extracts LGE and LGP on the growth performance of grass carp (*Ctenopharyngodon idella*) were investigated.

| Treatments                  | T1                | T2                | T3                | T4                  | T5                 | T6                | T7                 |
|-----------------------------|-------------------|-------------------|-------------------|---------------------|--------------------|-------------------|--------------------|
|                             | Control           | LGE (0.2%)        | LGE (0.4%)        | LGE (0.8%)          | LGE (1.6%)         | LGP (0.2%)        | LGP (0.4%)         |
| Initial weight (g)          | 3.83<br>± 0.231a  | 3.83<br>± 0.231a  | 3.83<br>± 0.231a  | 3.83<br>± 0.231a    | 3.83<br>± 0.231a   | 3.83<br>± 0.231a  | 3.83<br>± 0.231a   |
| Final weight (g)            | 14.77<br>± 0.129a | 23.79<br>± 0.178b | 33.58<br>± 0.197c | 48.42<br>± 0.485d   | 43.15<br>± 0.143d  | 25.60<br>± 0.354b | 24.06<br>± 0.313b  |
| Final weight gain (g)       | 10.94<br>± 0.173a | 19.84<br>± 0.125b | 30.51<br>± 0.192c | 45.23<br>± 0.182d   | 39.84<br>± 1.155d  | 22.17<br>± 0.133b | 20.51<br>± 1.312b  |
| Weight gain (%)             | 285.41<br>± 3.15  | 501.55<br>± 26.27 | 992.45<br>± 24.62 | 1416.23<br>± 108.34 | 1202.78<br>± 39.98 | 645.77<br>± 6.28  | 577.58<br>± 40.64  |
| Specific growth rate (SGR)  | 1.50<br>± 0.194a  | 1.99<br>± 0.462b  | 2.65<br>± 0.263d  | 3.01<br>± 0.366e    | 2.85<br>± 0.433d e | 2.23<br>± 0.192c  | 2.12<br>± 0.717b c |
| Feed conversion ratio (FCR) | 0.94<br>± 0.193d  | 0.54<br>± 0.113c  | 0.27<br>± 0.735a  | 0.20<br>± 0.133a    | 0.23<br>± 0.197a   | 0.42<br>± 0.334b  | 0.47<br>± 0.425b c |

Superscripts in the results indicate a statistically significant difference compared to the control group ( $p < 0.05$ ).

**TABLE 3** | The effects of different dosages of the rhododendron flower extracts (LGE and LGP) on the protein profile of grass carp (*Ctenopharyngodon idella*) were investigated.

| Treatments       |        | T1           | T2              | T3            | T4            | T5            | T6             | T7              |
|------------------|--------|--------------|-----------------|---------------|---------------|---------------|----------------|-----------------|
| Tissues          |        | Control      | LGE (0.2%)      | LGE (0.4%)    | LGE (0.8%)    | LGE (1.6%)    | LGP (0.2%)     | LGP (0.4%)      |
| Total            | Liver  | 4.72 ± 0.42a | 7.32 ± 0.09a b  | 14.11 ± 2.24c | 25.34 ± 1.30d | 22.89 ± 0.36d | 5.97 ± 0.92a b | 10.61 ± 0.59b c |
| Protein (µg/mg)  | Kidney | 3.85 ± 0.46a | 5.69 ± 0.24a    | 10.44 ± 1.43a | 27.45 ± 3.09b | 22.54 ± 2.43b | 4.91 ± 0.26a   | 10.27 ± 0.56a   |
|                  | Blood  | 6.70 ± 0.31a | 10.18 ± 0.71a b | 13.88 ± 1.76c | 24.24 ± 1.55c | 21.01 ± 1.50c | 7.48 ± 0.50a   | 11.01 ± 0.88a b |
| Albumin (µg/mg)  | Liver  | 1.98 ± 0.32a | 3.64 ± 0.05a b  | 6.61 ± 1.23c  | 11.46 ± 0.34d | 10.71 ± 0.31d | 2.49 ± 0.35a b | 4.77 ± 0.19b c  |
|                  | Kidney | 1.54 ± 0.33a | 2.38 ± 0.13a    | 4.89 ± 0.63a  | 12.37 ± 1.73b | 10.37 ± 1.39b | 2.13 ± 0.01a   | 4.43 ± 0.44a    |
|                  | Blood  | 3.03 ± 0.30a | 4.76 ± 0.32a b  | 6.42 ± 0.90b  | 10.19 ± 0.63c | 9.61 ± 0.34c  | 3.48 ± 0.22a   | 4.94 ± 0.42a b  |
| Globulin (µg/mg) | Liver  | 2.73 ± 0.16a | 3.67 ± 0.13a    | 7.49 ± 1.02b  | 13.88 ± 1.00c | 12.19 ± 0.52c | 3.49 ± 0.60a   | 5.84 ± 0.44a b  |
|                  | Kidney | 2.31 ± 0.16a | 3.31 ± 0.26a    | 5.55 ± 0.82a  | 15.07 ± 1.38b | 12.17 ± 1.04b | 2.78 ± 0.26a   | 5.84 ± 0.15a    |
|                  | Blood  | 3.67 ± 0.07a | 5.42 ± 0.39a b  | 7.46 ± 0.86b  | 14.05 ± 0.94c | 11.40 ± 1.16c | 4.00 ± 0.35a   | 6.07 ± 0.49a b  |
| A/G ratio        | Liver  | 0.72 ± 0.09a | 1.00 ± 0.05a    | 0.87 ± 0.04a  | 0.83 ± 0.05a  | 0.88 ± 0.06a  | 0.72 ± 0.06a   | 0.82 ± 0.04a    |
|                  | Kidney | 0.66 ± 0.11a | 0.73 ± 0.08a    | 0.89 ± 0.05a  | 0.81 ± 0.04a  | 0.85 ± 0.04a  | 0.78 ± 0.08a   | 0.75 ± 0.06a    |
|                  | Blood  | 0.83 ± 0.08a | 0.88 ± 0.02a    | 0.86 ± 0.03a  | 0.73 ± 0.01a  | 0.86 ± 0.06a  | 0.88 ± 0.06a   | 0.81 ± 0.03a    |

Superscripts in the results indicate a statistically significant difference compared to the control group ( $p < 0.05$ ).

## 4 | Discussion

Plants as dietary supplements in aquaculture offer cost-effective, eco-friendly, and sustainable solutions by enhancing fish health and productivity while reducing dependency on synthetic drugs and mitigating environmental challenges (Branfield et al. 2021). The global shift towards organic aquaculture emphasizes producing safe, high-quality fish, conserving resources, and accessing premium markets, particularly in South Asia, where reduced input costs and ecological sustainability are prioritized (Bain et al. 2016). Medicinal plants such as *Choerospondias axillaris* have improved growth, nutrient digestibility, and immunity in

*Oncorhynchus mykiss* (rainbow trout) (Labh 2020b), while *Nigella sativa* enhanced growth, haematology, and immunity in *Labeo rohita* (Subba et al. 2020). Similarly, *Allium sativum* improved antimicrobial defences and feed efficiency in *O. mykiss* (Shakya and Labh 2019), *Mentha piperita* promoted immunity and reduced oxidative stress in *Rutilus caspicus* (Mishra, Khalid, and Labh 2019), *Allium cepa* elevated antioxidant capacity and growth in *Oreochromis niloticus* (Mustafa and Dikel 2022), and *Mespilus germanica* enhanced growth and disease resistance in *O. mykiss* (Paknejad et al. 2020). These plant-based supplements align with sustainable aquaculture by offering local, cost-effective, and environmentally benign alternatives. Their bioactive compounds

**TABLE 4** | The effects of different dosages of the rhododendron flower extracts (LGE and LGP) on the enzyme profile of grass carp (*Ctenopharyngodon idella*) were investigated.

| Treatments    | T1             | T2             | T3             | T4            | T5             | T6             | T7             |
|---------------|----------------|----------------|----------------|---------------|----------------|----------------|----------------|
| Tissues       | Control        | LGE (0.2%)     | LGE (0.4%)     | LGE (0.8%)    | LGE (1.6%)     | LGP (0.2%)     | LGP (0.4%)     |
| SGOT in liver | 84.49 ± 2.60b  | 69.45 ± 1.90ab | 68.19 ± 1.62ab | 59.48 ± 2.69a | 81.42 ± 3.99b  | 76.24 ± 2.52ab | 79.32 ± 7.83b  |
| SGOT in Serum | 116.62 ± 0.85d | 102.50 ± 2.15c | 97.27 ± 0.85bc | 76.62 ± 1.70a | 88.81 ± 3.44b  | 113.94 ± 1.88d | 95.80 ± 1.68bc |
| SGPT in liver | 44.69 ± 0.89c  | 38.28 ± 1.64c  | 24.27 ± 2.36b  | 13.28 ± 0.89a | 18.59 ± 0.65ab | 40.77 ± 3.36c  | 35.84 ± 1.67c  |
| SGPT in serum | 93.28 ± 1.05d  | 80.68 ± 1.80bc | 67.04 ± 4.99a  | 66.52 ± 1.17a | 70.92 ± 0.56ab | 90.53 ± 1.22cd | 80.28 ± 2.65bc |
| ALP in liver  | 63.47 ± 0.62c  | 55.93 ± 1.24bc | 46.48 ± 3.46a  | 46.12 ± 0.82a | 49.16 ± 0.39ab | 62.76 ± 0.85c  | 55.65 ± 1.84bc |
| ALP in serum  | 83.56 ± 0.78a  | 83.26 ± 6.14a  | 79.59 ± 2.43a  | 76.28 ± 1.95a | 75.94 ± 1.76a  | 87.49 ± 1.50a  | 78.89 ± 4.00a  |
| Serum BUN     | 13.80 ± 1.38e  | 9.15 ± 0.40cd  | 6.38 ± 0.72abc | 2.94 ± 0.42a  | 5.39 ± 0.67ab  | 11.90 ± 0.82de | 8.42 ± 0.50bcd |
| Serum CREAT   | 1.13 ± 0.10a   | 1.06 ± 0.20a   | 0.82 ± 0.07a   | 0.82 ± 0.15a  | 1.31 ± 0.11ab  | 1.83 ± 0.06b   | 1.17 ± 0.07a   |

Superscripts in the results indicate a statistically significant difference compared to the control group ( $p < 0.05$ ).

**TABLE 5** | The effects of different dosages of the rhododendron flower extracts (LGE and LGP) on the complete blood profile of grass carp (*Ctenopharyngodon idella*) were investigated.

| Treatments          |            | T1             | T2              | T3              | T4              | T5             | T6               | T7              |
|---------------------|------------|----------------|-----------------|-----------------|-----------------|----------------|------------------|-----------------|
| Parameters          |            | Control        | LGE (0.2%)      | LGE (0.4%)      | LGE (0.8%)      | LGE (1.6%)     | LGP (0.2%)       | LGP (0.4%)      |
| Complete haemogram  | Hb         | 10.72 ± 0.76a  | 12.90 ± 1.67a   | 15.12 ± 0.28ab  | 20.71 ± 0.35c   | 19.07 ± 0.30bc | 12.73 ± 1.08a    | 14.80 ± 1.68ab  |
|                     | RBC        | 1.99 ± 0.05a   | 2.78 ± 0.14abc  | 3.18 ± 0.27bcd  | 3.65 ± 0.14cd   | 3.94 ± 0.18d   | 2.61 ± 0.36ab    | 2.93 ± 0.17abc  |
|                     | WBC        | 22.68 ± 0.93ab | 26.00 ± 0.88bc  | 28.68 ± 0.86c   | 38.03 ± 0.58d   | 35.23 ± 0.40d  | 23.75 ± 0.58ab   | 22.17 ± 0.46a   |
|                     | PCV        | 35.52 ± 0.70a  | 33.97 ± 0.75a   | 38.48 ± 1.76a   | 45.68 ± 2.08b   | 38.93 ± 0.23ab | 36.66 ± 1.30a    | 37.14 ± 2.09a   |
|                     | MCV        | 179.15 ± 1.55c | 123.15 ± 7.80ab | 121.94 ± 5.60ab | 125.04 ± 3.55ab | 99.39 ± 4.78a  | 144.24 ± 15.10bc | 127.06 ± 7.18ab |
|                     | MCH        | 33.38 ± 1.68b  | 27.21 ± 3.40ab  | 25.50 ± 1.51ab  | 22.11 ± 1.35ab  | 20.42 ± 0.21a  | 29.36 ± 3.30ab   | 25.87 ± 3.75ab  |
|                     | MCHC       | 30.12 ± 1.57a  | 38.10 ± 5.41ab  | 39.51 ± 2.46ab  | 45.61 ± 2.98ab  | 48.98 ± 0.51b  | 35.03 ± 4.31ab   | 40.31 ± 5.80ab  |
|                     | C-Index    | 0.46 ± 0.02a   | 0.56 ± 0.08a    | 0.52 ± 0.05a    | 0.44 ± 0.02a    | 0.51 ± 0.03a   | 0.47 ± 0.02a     | 0.58 ± 0.01a    |
| Differential counts | Eosinophil | 0.46 ± 0.02a   | 0.56 ± 0.08a    | 0.52 ± 0.05a    | 0.62 ± 0.01a    | 0.44 ± 0.02a   | 0.51 ± 0.03a     | 0.52 ± 0.00a    |
|                     | Basophil   | 0.05 ± 0.02a   | 0.17 ± 0.15a    | 0.49 ± 0.21a    | 0.87 ± 0.40a    | 0.80 ± 0.40a   | 0.24 ± 0.11a     | 0.76 ± 0.02a    |
|                     | Neutrophil | 36.15 ± 0.25a  | 34.85 ± 0.25a   | 48.47 ± 1.46c   | 42.89 ± 0.27b   | 35.62 ± 0.23a  | 44.64 ± 1.91bc   | 44.14 ± 0.20bc  |
|                     | Leucocytes | 13.48 ± 0.45a  | 19.79 ± 0.46b   | 22.16 ± 0.46b   | 38.03 ± 0.58e   | 35.24 ± 0.41d  | 32.03 ± 0.57c    | 42.08 ± 0.84f   |
|                     | Monocytes  | 1.24 ± 0.08ab  | 1.67 ± 0.40abc  | 3.77 ± 0.08d    | 5.02 ± 0.08e    | 1.87 ± 0.21bc  | 0.93 ± 0.08a     | 2.43 ± 0.00c    |

Abbreviations: Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin; PCV, packed cell volume; RBC, red blood corpuscles; WBC, white blood corpuscles.

Superscripts in the results indicate a statistically significant difference compared to the control group ( $p < 0.05$ ).

modulate gut microbiota, enhance nutrient absorption, and strengthen immune pathways, making them critical for fish health management. However, challenges such as optimizing dosages, addressing anti-nutritional factors, and assessing long-term impacts remain. Future research should explore synergistic combinations, advanced processing techniques, and eco-certifications to promote wider adoption of sustainable practices in global aquaculture systems.

#### 4.1 | Discussion on Growth Performance of Grass Carp Fed Laligurans Flowers

In this experiment, grass carp (*C. idella*) were fed diets supplemented with varying doses of lapsi fruit extract (LGE) and lapsi fruit powder (LGP), resulting in significant differences in growth performance metrics, including WG, SGR, and FCR, compared to the control group. Among all dietary treatments, the T4 group

exhibited the highest WG and SGR, indicating a clear dose-dependent improvement in growth performance. Conversely, FCR, which reflects feed efficiency, improved as the dietary dose of floral extract increased, showing an inverse correlation between FCR and the dose of LGE or LGP. This suggests enhanced nutrient utilization at higher supplementation levels.

Previous studies have shown that plant-based supplements generally support or enhance fish growth without adverse effects. For instance, dietary flower extracts were found to positively influence the growth performance of grass carp in multiple studies (Younes et al. 2021). However, exceptions exist, such as in largemouth bass (*Micropterus salmoides*), where increasing dietary levels of *Chlorella* meal reduced final body weight and SGR (Patra et al. 2023). Similarly, studies on rainbow trout (*O. mykiss*) observed a decline in growth performance when dietary protein profiles in liver and kidney were altered beyond optimal ranges (Wildhaber et al. 2023). Excessive dietary levels of alternative feed ingredients, such as black soldier fly (*Hermetia illucens*) meal above 40% inclusion, also resulted in reduced growth in rainbow trout (Xi et al. 2022). Likewise, in spotted seabass (*Lateolabrax maculatus*), growth and weight gain decreased significantly when dietary zinc supplementation exceeded recommended levels (Mishra, Khalid, and Labh 2020).

Grass carp fed with the amphibious plant *Zizania latifolia* demonstrated remarkable growth rates (1.65 g/fish/day), with a total production of 185.76 kg achieved over 150 days, further supporting the potential of plant-based dietary supplements to enhance aquaculture productivity (Kumar et al. 2021). Yuan et al. (2023) highlighted the antioxidant properties of plant-based feeds, while growth studies in grass carp have demonstrated daily growth rates ranging from 1.7 to 14.7 g/day, consistent with the present study. Notably, diets supplemented with *N. sativa* were also shown to significantly enhance growth performance in major carp, as observed by Subba et al. (2020). Additionally, supplementation of lapsi fruit (*C. axillaris*) in carp diets led to increased final body weight, weight gain, and SGR in a dose-dependent manner (Hemlata, Singh, and Devi 2016), aligning closely with the findings of this study. Our previous research also corroborates these trends, affirming the growth-promoting effects of lapsi supplementation (Jha, Khalid, and Labh 2024).

These results collectively highlight the efficacy of plant-based dietary supplements in enhancing growth performance in aquaculture species, while also emphasizing the need to optimize dosage to prevent negative outcomes observed in certain cases with excessive inclusion levels.

## 4.2 | Discussion on Immune Response of Grass Carp Fed Laligurans Flowers

### 4.2.1 | Protein Profile

In this study, the incorporation of varying levels of Laligurans flower extract (LGE and LGP) into the diets of grass carp (*C. idella*) resulted in significant enhancements in the levels of total protein, albumin, globulin, and the albumin-to-globulin ratio across their blood serum, liver, and kidneys compared to the control group (T1). Among the experimental groups, the

T4 diet group exhibited the highest concentrations of these protein profiles, highlighting the dose-dependent effects of the supplementation. The findings suggest that Laligurans flowers, a high-altitude herbal resource from the Himalayas, significantly bolster the immune system of grass carp by stabilizing the endoplasmic reticulum, thereby enhancing protein synthesis and immune functionality.

This observed elevation in protein levels aligns with the role of floral extracts as potent sources of antioxidants and immunostimulants. These compounds, present in Laligurans flowers, may mitigate oxidative stress, protect cellular integrity, and promote overall health in fish. The results corroborate earlier findings where the ethanol extract of Laligurans flowers similarly enhanced total protein, albumin, globulin, and the albumin-to-globulin ratio in common carp (*Cyprinus carpio*) (Jha, Khalid, and Labh 2024). Comparable outcomes have been reported in studies utilizing other plant-based supplements. For instance, the synergistic effects of moringa leaf, ginger, and garlic powder on the growth and innate immune responses of *Clarias gariepinus* juveniles demonstrated marked improvements in health indices (Mishra et al. 2019). Similarly, dietary supplementation with vitamin C enhanced protein profiles and immune responses in major carp rohu (*L. rohita*) (Shakya and Labh 2018). Such findings underscore the critical role of natural bioactive compounds in promoting fish health.

The mechanisms underlying these effects can be attributed to the rich antioxidant content of floral extracts, which not only act as immunostimulants but also support cellular metabolism and protein synthesis (Megbowon et al. 2024). The significant improvements in protein profiles in the liver, kidneys, and blood serum of grass carp suggest enhanced metabolic activity and immune resilience. These results affirm the potential of Laligurans flower extract as a functional dietary additive in aquaculture, offering a natural and sustainable approach to improving fish health and productivity.

### 4.2.2 | Liver Enzyme Profile

Immunological markers, such as SGPT, SGOT, and ALP, were analyzed to assess liver functionality in grass carp (*C. idella*) fed diets supplemented with Laligurans flower extract (LGE and LGP). These markers were measured using enzymatic UV kinetic techniques based on the International Federation of Clinical Chemistry's reference methods. Results showed a dose-dependent decrease in SGPT, SGOT, and ALP levels in the blood serum and liver of grass carp, with the lowest values recorded in the T4 diet group. The significant reduction in these markers compared to the control group indicates that LGE and LGP supplementation likely preserved hepatocyte cell membrane integrity and supported the regeneration of damaged cells. This stabilization is particularly evident from the normalization of SGPT and SGOT levels, which are indicators of liver health and cellular damage (Labh 2012).

The observed decrease in ALP levels further supports the hypothesis that Laligurans extract stabilized biliary function, which is often impaired during toxicological challenges, such as carbon tetrachloride (CCl<sub>4</sub>)-induced liver damage. SGPT (alanine

aminotransferase, ALT) and SGOT (aspartate aminotransferase, AST) are enzymes found within liver, heart, kidney, gill, and muscle cells, and their release into blood serum under normal conditions is minimal. Elevated levels of these enzymes in serum are typically associated with pathological conditions, such as tissue damage or organ dysfunction, making them critical diagnostic markers in fish health assessments (Fawole et al. 2021; Labh 2023).

Previous studies have highlighted the detrimental impact of environmental stressors, such as high stocking densities or declining water quality, on liver enzyme levels. Elevated AST and ALT levels under such stress conditions indicate the extent of hepatic damage, underscoring their role as reliable biomarkers (Bhat et al. 2022). Additionally, environmental toxicology studies have frequently used SGOT and SGPT levels to evaluate the impact of pollutants on various fish species, including carp (Fawole et al. 2021). Hossain et al. (2021) demonstrated that *C. axillaris* fruit extract significantly enhanced phagocytic and respiratory burst activities in Nile tilapia (*O. niloticus*), suggesting immunomodulatory properties. Similarly, in a previous study on common carp (*C. carpio*), fish fed diets supplemented with Laligurans ethanol extract exhibited significantly lower SGOT, SGPT, and ALP levels compared to the control group, confirming the hepatoprotective effects of the extract (Jha, Khalid, and Labh 2024).

The hepatoprotective action of Laligurans extract can be attributed to its rich bioactive compounds, such as flavonoids and antioxidants, which protect hepatocytes from oxidative stress and support cellular regeneration. These compounds also contribute to stabilizing enzyme levels, suggesting enhanced liver function and resilience against stressors. The findings of this study provide compelling evidence for the use of Laligurans extract as a functional dietary additive in aquaculture, offering both immunomodulatory and hepatoprotective benefits.

#### 4.2.3 | Kidney Function Tests

Elevated BUN levels in grass carp (*C. idella*) are indicative of impaired kidney function, often resulting from excessive protein metabolism or renal damage. In this study, fish fed the control diet exhibited the highest BUN levels, while a gradual decrease was observed with increasing doses of LGE and LGP. The lowest BUN levels were recorded in the T4 diet group, suggesting a protective role of the extract in kidney function. BUN, a byproduct of protein catabolism, can accumulate in fish blood due to diets rich in protein or kidney dysfunction, making it a reliable biomarker for renal health (Evalde et al. 2019).

Similarly, creatinine (CREAT), another critical indicator of renal health, followed a comparable trend. Creatinine levels decreased progressively with increasing dietary LGE and LGP, reaching their lowest values in the T4 group. Elevated creatinine levels in the bloodstream are commonly associated with renal impairment, structural damage, postrenal obstruction, or leakage, highlighting the importance of maintaining optimal kidney function for overall health. In this study, the reduction in both BUN and creatinine levels suggests that Laligurans flower extract may

enhance renal function, likely by protecting kidney structures and improving filtration efficiency (Labh 2015).

Previous research corroborates these findings, as common carp (*C. carpio*) fed diets supplemented with LGE and LGP also exhibited higher BUN and creatinine levels in the control group compared to the treatment groups, further emphasizing the renal benefits of Laligurans extract (Jha, Khalid, and Labh 2024). The plant's bioactive compounds, including antioxidants and anti-inflammatory agents, may contribute to the observed improvements by mitigating oxidative stress and promoting tissue repair, thereby supporting kidney health.

The reduction in protein breakdown products, as indicated by lower BUN and creatinine levels, also implies a more efficient metabolic balance in fish fed with Laligurans-supplemented diets. Improved kidney function may enhance nitrogen excretion, reducing the metabolic load on renal tissues and promoting better physiological homeostasis. This enhanced metabolic efficiency could positively influence overall health and growth performance in grass carp.

The findings underscore the potential of natural plant extracts, such as those derived from Laligurans flowers, in aquaculture to alleviate organ dysfunction and improve critical physiological parameters. The integration of such functional additives in fish diets not only supports renal health but also aligns with sustainable aquaculture practices by reducing reliance on synthetic supplements and enhancing fish welfare. These results highlight the importance of exploring natural bioactive compounds as viable tools for promoting health and productivity in aquaculture systems.

#### 4.2.4 | Complete Blood Profile

Fish haematopoiesis, the production of blood cells, occurs primarily in the spleen and, in some species, the head kidney, particularly for white blood cells in bony fish. Compared to other vertebrates, fish generally have a lower blood volume, with bony fish (*Osteichthyes*) having 2–4 mL/100 g body weight and cartilaginous fish (*Chondrichthyes*) ranging from 6 to 8 mL/100 g (Baldișsera and Baldișserotto 2023; Emam et al. 2024). Fish blood comprises plasma and blood cells, which include red blood cells (RBCs or erythrocytes) and white blood cells (WBCs or leukocytes). The size of red blood cells differs between groups, with bony fish having erythrocytes measuring ~12–14  $\mu\text{m}$  and cartilaginous fish ranging from 20 to 27  $\mu\text{m}$ . RBCs are abundant in fish, averaging 101 million cells/ $\text{cm}^3$ , whereas WBCs are significantly fewer, at around 150,000 cells/ $\text{mm}^3$  in most species, although these counts can vary based on species, environmental factors, and physiological states (Witeska, Kondera, and Bojarski 2023). Among WBCs, lymphocytes often dominate in marine fish, making up about half of the total leukocyte count. WBCs include four main types: granulocytes, thrombocytes, lymphocytes, and monocytes. Granulocytes, which consist of neutrophils, eosinophils, and basophils, play a critical role in immune defence, particularly during bacterial infections, by acting as phagocytes. These granule-bearing cells are instrumental in the body's response to pathogens, with their numbers increasing during disease outbreaks (Jeamah et al. 2023).

In this study, the haematological profiles of grass carp were evaluated following dietary supplementation with varying doses of ethanol extract of *Laligurans* flowers (LGE) and conventional *Laligurans* flower powder (LGP). Results indicated that carp fed with LGE exhibited significantly improved blood parameters compared to those receiving LGP or the control group fed with a standard artificial diet. This improvement highlights the potential immunostimulatory and physiological benefits of the ethanol extract. Enhanced blood profiles, including optimized counts of RBCs and WBCs, suggest better overall health and immune competence in the LGE-fed groups, underscoring the extract's role in bolstering fish well-being. Fish haematological markers are valuable indicators of health, as they reflect responses to internal and external factors, such as sex, seasonal changes, environmental conditions, and management practices (Fazio 2019; Witeska et al. 2022). These parameters can detect both normal physiological fluctuations and pathological alterations caused by disease or stress (Chen and Luo 2023). For instance, environmental stressors and poor management can adversely impact blood profiles, leading to compromised immunity and metabolic imbalances.

In the context of aquaculture, assessing blood parameters offers a comprehensive view of fish health and is pivotal for disease prevention, optimizing diets, and ensuring sustainable practices. The findings from this study reinforce the utility of natural plant extracts, such as *Laligurans*, in enhancing fish resilience and overall health. By improving key haematological markers, these extracts provide a natural and effective means of promoting fish welfare and productivity. The study's primary objective of evaluating diverse haematological markers in grass carp was achieved, offering critical insights into the benefits of *Laligurans* supplementation in aquaculture systems.

## 5 | Conclusion

Disease outbreaks are increasingly prevalent in intensive aquaculture, prompting the use of natural and medicinal herbs as sustainable alternatives to manage fish health. Plants, rich in bioactive compounds, offer eco-friendly and safe substitutes for chemicals and antibiotics, enhancing immune function and combating infections. Among these, *Laligurans* (*R. arboreum*), an evergreen tree in the Ericaceae family, is notable for its vibrant red or pink flowers and cultural significance, being the national flower of Nepal and the state flower of Himachal Pradesh, India. Traditionally, its flowers, rich in phenolics and flavonoids, have been utilized for treating ailments such as rheumatism, headaches, diabetes, and infections, owing to their anti-inflammatory and antioxidant properties. Beyond medicinal applications, these flowers are also prized in hilly regions for preparing pickles, juices, jams, and syrups.

In a 90-day study on grass carp (*C. idella*), fish were reared in earthen ponds using bamboo-supported nylon cages and fed diets supplemented with *Laligurans* flower ethanol extract (LGE) or double-distilled water extract powder (LGP). Key parameters evaluated included growth performance, total protein, liver enzymes, kidney function markers (urea and creatinine), and haematological profiles. Results showed that LGE outperformed LGP and the control diet, demonstrating significant health and

growth benefits. LGE enhanced immune response, metabolic efficiency, and overall well-being, making it a superior additive for aquaculture. In conclusion, this study strongly recommends incorporating *Laligurans* flowers into artificial diets, offering fish farmers a natural and effective approach to sustainable aquaculture development.

---

## Author Contributions

**Anil Kumar Jha:** formal analysis, project administration, writing—original draft, investigation. **Monowar Alam Khalid:** investigation, validation, formal analysis, supervision. **Shyam Narayan Labh:** conceptualization, investigation, methodology, software, data curation, supervision, resources, project administration, formal analysis, writing—review and editing, visualization, validation, writing—original draft, funding acquisition.

## Acknowledgements

Prof. M. A. Khalid, Prof. S. N. Labh and Mr. A. K. Jha would want to thank Mrs. Sabita Jha, FDO at Fisheries Human Resource Development and Technology Validation Centre, Janakpurdham, Nepal, for providing research facilities, and Prof. T. Usmani, Dean of Doctoral Studies, Integral University, Kursi Road, Lucknow, (UP), India, for allowing to publish this review article by supplying MCN No. IU/R&D/2024-MCN0029.

## Ethics Statement

The guidelines for the use and treatment of experimental animals were supplied by the Fisheries Human Resource Development and Technology Validation Centre, a government organization in Janakpurdham, Nepal. The Institute of Science and Technology (IOST) of Tribhuvan University authorized the study protocol, which was created in accordance with these requirements.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1002/aff2.70029>

## References

- Bain, B. J., I. Bates, M. A. Laffan, and S. M. Lewis. 2016. *Dacie and Lewis Practical Haematology: Expert Consult: Online and Print*. Elsevier Health Sciences. <https://shorturl.at/YX0HA>.
- Baldissera, M. D., and B. Baldisserotto. 2023. "Creatine Kinase Activity as an Indicator of Energetic Impairment and Tissue Damage in Fish: A Review." *Fishes* 8, no. 2: 59. <https://doi.org/10.3390/fishes8020059>.
- Bhat, R. A., C. Saoca, C. Cravana, et al. 2022. "Effects of Heavy Pollution in Different Water Bodies on Male Rainbow Trout (*Oncorhynchus mykiss*) Reproductive Health." *Environmental Science and Pollution Research* 30, no. 9: 23467–23479. <https://doi.org/10.1007/s11356-022-23670-w>.
- Branfield, S. L., X. D. Valez, J. A. G. Feliciano, et al. 2021. "Deciphering the TLT-1 Fibrinogen Ligand Interaction." *Blood* 138: 4207. <https://doi.org/10.1182/blood-2021-153648>.

- Chen, H., and D. Luo. 2023. "Application of Haematology Parameters for Health Management in Fish Farms." *Reviews in Aquaculture* 15, no. 2: 704–737. <https://doi.org/10.1111/raq.12753>.
- Chitrakar, P., M. A. Khalid, and S. N. Labh. 2024. "Spatiotemporal Variability in Water Quality, Ichthyofaunal Diversity, and Its Conservation in the Rivers of Nepal: A Mini." *Iranian Journal of Fisheries Sciences* 23, no. 4: 633–648. <https://doi.org/10.22092/ijfs.2024.131390>.
- Chitrakar, P., and K. Parajuli. 2017. "Length and Weight Relationship Studies of Alimentary Canal Compared to the Total Body Weight of Grass Carp *Ctenopharyngodon idella* (Valenciennes, 1844) at Balkhu Live Fish Market of Kathmandu, Nepal." *International Journal of Fisheries and Aquatic Studies* 5, no. 6: 185–190. <https://shorturl.at/Hrgx9>.
- Dani, R. S., and C. B. Baniya. 2022. "Tree Species Diversity and Regenerating Potential Along Disturbance Gradient in Chandragiri Hill, Kathmandu, Central Nepal." *Journal of Institute of Science and Technology* 27, no. 2: 109–124. <https://doi.org/10.3126/jist.v27i2.49614>.
- Emam, M. A., R. M. Shourbela, W. N. El-Hawarry, et al. 2024. "Effects of *Moringa oleifera* Aqueous Extract on the Growth Performance, Blood Characteristics, and Histological Features of Gills and Livers in Nile Tilapia." *Aquaculture and Fisheries* 9, no. 1: 85–92. <https://doi.org/10.1016/j.aaf.2021.12.011>.
- Evalde, N., P. O. James, O. Onuorah, et al. 2019. "Clinical Diagnosis of Disease States Using Enzymes and Proteins." *Asian Journal of Biochemistry, Genetics and Molecular Biology* 1, no. 3: 1–6. <https://doi.org/10.9734/ajbgmb/2018/v1i329642>.
- Fawole, F. J., S. N. Labh, M. S. Hossain, et al. 2021. "Insect (Black Soldier Fly larvae) Oil as a Potential Substitute for Fish or Soy Oil in the Fish Meal-Based Diet of Juvenile Rainbow Trout (*Oncorhynchus mykiss*)." *Animal Nutrition* 7: 1360–1370. <https://doi.org/10.1016/j.aninu.2021.07.008>.
- Fazio, F. 2019. "Fish Hematology Analysis as an Important Tool of Aquaculture: A Review." *Aquaculture* 500: 237–242. <https://doi.org/10.1016/j.aquaculture.2018.10.030>.
- Hemlata, S., K. R. Singh, and W. S. Devi. 2016. "Studies on the Growth of Grass Carp, *Ctenopharyngodon idella* (Cuvier and Val.) Fed on Amphibious Plant *Zizania latifolia* Under Intensive Fish Culture." *International Journal of Research and Scientific Innovation* 3, no. 11: 28–31. <https://shorturl.at/y47xs>.
- Hossain, M. S., F. J. Fawole, S. N. Labh., B. C. Small, K. Overturf, and V. Kumar. 2021. "Insect Meal Inclusion as a Novel Feed Ingredient in Soy-Based Diets Improves Performance of Rainbow Trout (*Oncorhynchus mykiss*)." *Aquaculture* 544: 737096. <https://doi.org/10.1016/j.aquaculture.2021.737096>.
- Jeamah, A., S. Senarat, S. Kong-Oh, et al. 2023. "Hematological Evaluation of Three Common Teleosts in Relation to the Environmental Changes From Trang Province, Thailand." *Tropical Life Sciences Research* 34, no. 3: 113–127. <https://doi.org/10.21315/tlsr2023.34.3.6>.
- Jha, A. K., M. A. Khalid, and S. N. Labh. 2024a. "Himalayan Flower Lalligurans (*Rhododendron arboreum*) Improve Growth and Enhance Immunity in Common Carp (*Cyprinus carpio*) Under Recirculating Culture System." *Journal of Experimental Zoology India* 27, no. 2: 1801. <https://doi.org/10.51470/jez.2024.27.2.1801>.
- Jha, A. K., M. A. Khalid, and S. N. Labh. 2024b. "In Vitro Antioxidant and Antibacterial Activities of Medicinal Flower Lalligurans *Rhododendron arboreum* Collected From Kathmandu Valley, Nepal." *International Journal of Food Science* 2024, no. 1: 6073042. <https://doi.org/10.1155/2024/6073042>.
- Khanal, M., S. Lamichhane, A. Bhattarai, B. L. Kayastha, and S. N. Labh. 2021. "Extract of Aloe Vera (*Aloe barbadensis* Miller) Enhances the Growth, Protein Contents, and Gastrointestinal Index (GaSI) of Common Carp *Cyprinus Carpio*." *Journal of Nutrition and Metabolism* 2021: 8029413. <https://doi.org/10.1155/2021/8029413>.
- Kumar, V., F. J. Fawole, N. Romano, et al. 2021. "Insect (Black Soldier Fly, *Hermetia illucens*) Meal Supplementation Prevents the Soybean Meal-Induced Intestinal Enteritis in Rainbow Trout and Health Benefits of Using Insect Oil." *Fish & Shellfish Immunology* 109: 116–124. <https://doi.org/10.1016/j.fsi.2020.12.008>.
- Labh, S. N. 2012. "Dietary Supplementation of Vitamin C, L-Ascorbate 2-Triphosphate Calcium (L-ATP-Ca) on Oocyte Development of Major Carp Rohu (*Labeo rohita*) Reared in a Natural Concrete Pond; Ecoprint." *An International Journal of Ecology* 19: 7–15. <https://doi.org/10.3126/eco.v19i0.9849>.
- Labh, S. N. 2015. "RNA: DNA Ratio and Growth Performance of Rohu *Labeo rohita* (Hamilton) Fed Varied Proportion of Protein Diet During Intensive Aquaculture." *International Journal of Life Sciences* 9, no. 6: 113–122. <https://doi.org/10.3126/ijls.v9i6.11585>.
- Labh, S. N. 2020a. "Expression of Immune Genes and Stress Enzyme Profiles of Rainbow Trout (*Oncorhynchus mykiss*) Fed *Moringa oleifera* Leaf Meal (MLM)." *International Journal of Biological Innovations* 2, no. 2: 155–164. <https://doi.org/10.46505/IJBI.2020.2212>.
- Labh, S. N. 2020b. "Effects of Lapsi *Choerospondias axillaris* on Growth and Immune-Related Genes in Silver Carp (*Hypophthalmichthys molitrix*)." *SciMedicine Journal* 2, no. 2: 86–99. [10.28991/SciMedJ-2020-0202-6](https://doi.org/10.28991/SciMedJ-2020-0202-6).
- Labh, S. N. 2023. "Applications of Biotechnology in Aquaculture Nutrition and fisheries." In *Frontiers in Aquaculture Biotechnology*, 163–170. Academic Press. <https://doi.org/10.1016/B978-0-323-91240-2.00007-5>.
- Labh, S. N. 2024. "Vitamin C (L-ascorbate 2-Triphosphate Calcium) Enhances the Growth, Immunobiochemical, and Haematological Performance of Common Carp *Cyprinus carpio*." *Advances in Obesity, Weight Management & Control* 141: 8–18. <https://shorturl.at/kWdnz>.
- Labh, S. N., R. Chakrabarti, and F. J. Fawole. 2017. "Effects of Feeding Higher Level of Vitamin C L-Ascorbate-2-Triphosphate Calcium (L-ATP) on Growth and Haemato-Biochemical Indices of Common Carp (*Cyprinus carpio*)." *World Journal of Pharmaceutical Research* 6, no. 6: 904–921. <https://doi.org/10.20959/wjpr20176-85291>.
- Labh, S. N., and S. R. Shakya. 2020. "Ethanol Extract of *Choerospondias axillaris* Fruit Pulp Enhances Haematological Parameters in *Oncorhynchus mykiss* Cultured in Nepal." *Nepal Journal of Biotechnology* 7, no. 1: 21–29. <https://doi.org/10.3126/njb.v7i1.26947>.
- Labh, S. N., S. R. Shakya, and B. L. Kayastha. 2015. "Extract of Medicinal lapsi *Choerospondias axillaris* (Roxb.) Exhibit Antioxidant Activities During in Vitro Studies." *Journal of Pharmacognosy and Phytochemistry* 4, no. 3: 194–197. <https://shorturl.at/2Ql7F>.
- Larsen, H. N., and S. F. Snieszko. 1961a. "Modification of the Microhematocrit Technique With Trout Blood." *Transactions of the American Fisheries Society* 90, no. 2: 139–142. [https://doi.org/10.1577/1548-8659\(1961\)90\[139:MOTMTW\]2.0.CO;2](https://doi.org/10.1577/1548-8659(1961)90[139:MOTMTW]2.0.CO;2).
- Larsen, H. N., and S. F. Snieszko. 1961b. "Comparison of Various Methods of Determination of Hemoglobin in Trout Blood." *The Progressive Fish-Culturist* 23, no. 1: 8–17. [https://doi.org/10.1577/1548-8659\(1961\)23\[8:COVMOD\]2.0.CO;2](https://doi.org/10.1577/1548-8659(1961)23[8:COVMOD]2.0.CO;2).
- Madhvi, S. K., M. Sharma, J. Iqbal, M. Younis, and R. Sheikh. 2020. "Phytochemical Analysis, Total Flavonoid, Phenolic Contents and Antioxidant Activity of Extracts From the Leaves of *Rhododendron arboreum*." *Research Journal of Pharmacy and Technology* 13, no. 4: 1701–1706. <https://doi.org/10.5958/0974-360X.2020.00307.8>.
- Megbowon, I., E. A. Ukenye, O. R. Oguntade, et al. 2024. "Individual and Combined Effects of Moringa Leaf, Ginger and Garlic Powder on Growth and Innate Immune Response of *Clarias gariepinus* Juveniles." *Cogent Food & Agriculture* 10, no. 1: 2328427. <https://doi.org/10.1080/23311932.2024.2328427>.
- Mishra, B. K., M. A. Khalid, and S. N. Labh. 2019. "Assessment of the Effect of Water Temperature on Length Gain, Feed Conversion Ratio (FCR) and Protein Profile in Brain of *Labeo rohita* (Hamilton 1822) Fed *Nigella sativa* Incorporated Diets." *International Journal of Fisheries and Aquatic Studies* 7, no. 3: 06–13. <https://shorturl.at/GMxsC>.

- Mishra, B. K., M. A. Khalid, and S. N. Labh. 2020. "Assessment of Water Temperature on Growth Performance and Protein Profiles in Liver and Kidney of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum, 1792)." *Journal of Experimental Zoology India* 23, no. 2: 1511–1520. <https://shorturl.at/E6Zj5>.
- Mishra, C., M. A. Khalid, N. Fatima, et al. 2019. "Effects of Citral on Oxidative Stress and Hepatic Key Enzymes of Glucose Metabolism in Streptozotocin/High-Fat-Diet Induced Diabetic Dyslipidemic Rats." *Iranian Journal of Basic Medical Sciences* 22, no. 1: 49. <https://doi.org/10.22038/ijbms.2018.26889.6574>.
- Mustafa, Ö. Z., and S. Dikel. 2022. "Effect of Garlic (*Allium sativum*)-Supplemented Diet on Growth Performance, Body Composition and Fatty Acid Profile of Rainbow Trout (*Oncorhynchus mykiss*)." *Cellular and Molecular Biology* 68, no. 1: 217–225. <https://doi.org/10.14715/cmb/2022.68.1.26>.
- Paknejad, H., S. P. Hosseini Shekarabi, M. Shamsaie Mehrgan, A. Hajimoradloo, Z. Khorshidi, and S. Rastegari. 2020. "Dietary Peppermint (*Mentha piperita*) Powder Affects Growth Performance, Hematological Indices, Skin Mucosal Immune Parameters, and Expression of Growth and Stress-Related Genes in Caspian Roach (*Rutilus caspicus*)." *Fish Physiology and Biochemistry* 46: 1883–1895. <https://doi.org/10.1016/j.fsi.2015.10.005>.
- Patra, I., A. P. Dewi, M. Fawzi, et al. 2023. "Effects of Dietary Medlar (*Mespilus germanica* L.) Extract on Growth Performance, Innate Immune Characteristics, Antioxidant Status, and Responses to Crowding Stress in Rainbow Trout (*Oncorhynchus mykiss*)." *Aquaculture Nutrition* 2023, no. 1: 7613330. <https://doi.org/10.1155/2023/7613330>.
- Phinyo, M., P. Sangarun, E. Wangkahart, and K. Sujipuri. 2024. "Effects of Banana Flower Powder (*Musa* sp.) Supplementation on Growth Performance, Whole Body Composition, Antioxidant and Immune Responses, Gene Expression and Liver Histology in Nile Tilapia (*Oreochromis niloticus*)." *Animal Feed Science and Technology* 308: 115882. <https://doi.org/10.1016/j.anifeedsci.2024.115882>.
- Reitman, S., and S. Frankel. 1957. "A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases." *American Journal of Clinical Pathology* 28, no. 1: 56–63. <https://doi.org/10.1093/ajcp/28.1.56>.
- Shakya, S. R., and S. N. Labh. 2018. "Himalayan Lapsi, *Choerospondias axillaris* (Roxb.) Enhances Concentration of Vitamin C in Tissues of Rohu (*Labeo rohita* H) Cultured at Chitwan (Nepal)." *International Journal of Biosensors & Bioelectronics* 4, no. 3: 152–156. <https://doi.org/10.15406/ijbsbe.2018.04.00116>.
- Shakya, S. R., and S. N. Labh. 2019. "Ethanol Extract of *Choerospondias axillaris* Fruit Pulp Enhances Haematological Parameters in *Oncorhynchus mykiss* Cultured in Nepal." *Nepal Journal of Biotechnology* 7, no. 1: 21–29. <https://doi.org/10.3126/njb.v7i1.26947>.
- Shakya, S. R., and S. N. Labh. 2020. "Effects of Fruits of Lapsi (Nepali Hog Plum) *Choerospondias axillaris* (Roxb.) Bl Burt & Aw Hill on Some Blood Parameters of Major Carp Rohu *Labeo rohita* (H) During Intensive." *Nepal Journal of Science and Technology* 19, no. 1: 186–195. <https://doi.org/10.3126/njst.v19i1.29801>.
- Sharma, S., S. Chaudhary, and A. Harchanda. 2022. "Rhododendron Arboreum: A Critical Review on Phytochemicals, Health Benefits and Applications in the Food Processing Industries." *Current Nutrition & Food Science* 18, no. 3: 287–304. <https://doi.org/10.2174/1573401317666210921104622>.
- Subba, S., V. K. Mahaseth, B. R. Subba, and S. N. Labh. 2020. "Fecundity, Histomorphology of the Ovary and Size at First Maturity of *Neolissochilus hexagonolepis* (McClelland) in Tamor River, Nepal." *Journal of Applied and Natural Science* 12, no. 3: 401–410. <https://doi.org/10.31018/jans.v12i3.2338>.
- Wildhaber, M. L., B. M. West, K. K. Ditter, A. P. Moore, and A. S. Peterson. 2023. "A Review of Grass Carp and Related Species Literature on Diet, Behavior, Toxicology, and Physiology Focused on Informing Development of Controls for Invasive Grass Carp Populations in North America." *Fishes* 8, no. 11: 547. <https://doi.org/10.3390/fishes8110547>.
- Witeska, M., E. Kondera, and B. Bojarski. 2023. "Hematological and Hematopoietic Analysis in Fish Toxicology—A Review." *Animals* 13, no. 16: 2625. <https://doi.org/10.3390/ani13162625>.
- Witeska, M., E. Kondera, K. Ługowska, and B. Bojarski. 2022. "Hematological Methods in Fish—Not Only for Beginners." *Aquaculture* 547: 737498. <https://doi.org/10.1016/j.aquaculture.2021.737498>.
- Xi, L., Q. Lu, Y. Liu, et al. 2022. "Effects of Fish Meal Replacement With Chlorella Meal on Growth Performance, Pigmentation, and Liver Health of Largemouth Bass (*Micropterus salmoides*)." *Animal Nutrition* 10: 26–40. <https://doi.org/10.1016/j.aninu.2022.03.003>.
- Younes, A. M., A. Y. Gaafar, A. E. D. Z. Abu-Bryka, et al. 2021. "Effects of Onion (*Allium cepa*) in Diets of *Oreochromis niloticus*: Growth Improvement, Antioxidant, Anti-Inflammatory and Disease Resistance Perspectives." *Aquaculture Research* 52, no. 5: 2324–2334. <https://doi.org/10.1111/are.15084>.
- Yuan, J., X. Li, K. Song, et al. 2023. "Effects of Dietary Zinc Levels on Growth, Intestinal Health, Antioxidant and Non-Specific Immunity of Spotted Seabass (*Lateolabrax maculatus*) Reared at Two Temperatures." *Aquaculture* 577: 739959. <https://doi.org/10.1016/j.aquaculture.2023.739959>.